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Earthworms and rice straw enhanced soil bacterial diversity and promoted the degradation of phenanthrene

Ali Mohamed Elyamine^{1,2,3} and Chengxiao Hu^{1,2*}

Abstract

Background: Since the industrial revolution, the contamination of agricultural soils by polycyclic aromatic hydrocarbons (PAHs) has increasingly become of serious global environmental concern and poses a huge threat to human beings and natural ecosystems. Microbial degradation is a proved technology mostly used to depollute polycyclic aromatic hydrocarbon (PAH) in the environment. However, very limited information is available regarding the interaction of earthworms with rice straw on the soil microbial community and the degradation of phenanthrene. This study was performed to enlighten the rice straw and earthworms' interaction on soil bacterial abundance and structure and phenanthrene removal.

Results: Result about functional gene information revealed that both rice straw and earthworm enhanced phenanthrene degradation. Subsequently, both Shannon diversity index ($r^2 = -0.8807$, $p < 0.001$) and bacterial 16S rRNA genes ($r^2 = -0.7795$, $p < 0.001$) negatively correlated with the remaining phenanthrene concentration in soil. The application of both rice straw and earthworms in soil had the lowest ratio of soil remaining phenanthrene concentration (0.16 ± 0.02), the highest Shannon diversity index (6.45 ± 0.2) and the highest bacterial 16S rRNA genes. This implied that both earthworms and rice straw might improve the phenanthrene metabolism by increasing soil bacteria diversity. The abundance of genera *Pseudomonas*, *Luteimonas*, *Rhodanobacter*, *Sphingomonas*, *Gemmatimonas*, *Flavobacterium*, and *Leifsonia* was significantly increased in the presence of both earthworms and rice straw and was found to negatively correlate with the remaining phenanthrene concentration in soil.

Conclusion: Based on these results, this study offers clear and strong evidences that the positive interaction between earthworms and rice straw could promote phenanthrene degradation in soil. These finding will improve our understanding on the importance of the natural resources forsaken and how they can interact with the soil macro- and microorganisms to change soil structure and enhance PAH degradation in soil.

Keywords: Rice straw, *Eisenia fetida*, Microbiome, Phenanthrene–bacterial metabolism, *Aporrectodea caliginosa*

Background

The anthropologic activities such as mining activities [14], vehicle circulation and urbanization [1], industrialization, and heat generation by moderate industrial

incineration [21] constitute the main source of polycyclic aromatic hydrocarbons (PAHs). PAHs are a class of hazardous chemicals well known with carcinogenicity, teratogenicity, mutagenicity and toxicity properties [46]. Among the polycyclic aromatic hydrocarbon, phenanthrene is the simplest composed by three-fused ring compound [40], present in PAH-contaminated soil, and used as model by several researchers for remediation process [21].

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Given the benefits of simple technology without secondary pollution, microbial degradation has become the primary means to depollute the environment polluted by organic pollutant. This technology not only proved to be effective, but also a low-cost and eco-friendly strategy [7, 30]. Microorganisms contain degrading genes that can effectively degrade and catabolize the target pollutant [8, 17, 28]. For efficient and profitable bioremediation of PAHs, both bio-augmentation involving resistant microorganism strains capable of degrading various pollutants in soil [39] and bio-stimulation which attempts to increase exogenous and indigenous soil microbial activity [24] are commonly used.

Subsequently, another low-cost efficient and environmentally friendly remediation technology is the use of earthworms. They were reported to accelerate the removal of organic contaminant such as herbicides [34], polychlorinated biphenyl (PCBs) [36], PAHs [9] and crude oil [35]. Earthworms enhance the transference of soil microorganisms within different soil layers and are implicated in the degradation of soil organic matter. Truly, during the feeding process of earthworms, organic and mineral composites are mingled in their gut and turned down as casts on the soil surface or along burrows [26]. This phenomenon might increase the microbial population and improve the connection between soil microorganisms and pollutants [22]. However, although the approach integrating earthworm–soil microbial interaction to remove PAHs in soil appeared to be compromising, most of PAH-removal study such as [3, 4, 7] involved plant system and very limited information is available regarding the integration of rice straw in the microbial community change and the degradation of PAHs.

Rice straw, as a dissimilar material, was reported to be effective, not only as biosorbent to reducing heavy metals in aquatic environment [19, 33], but also as organic fertilizer, which can be used for PAH removal. The use of organic fertilizers to remove PAH in the soil was reported to be of great success [41] since there is an adjacent correlation between soil microbial community and the degradation of pollutant [25]. Furthermore, through the humification and the mineralization effect of microorganisms, the abundance of micro-fauna attached to plant residues improved with decomposition of organic matter [48, 49].

Thus, the use of earthworms and rice straw could be an effective technology to promote phenanthrene degradation in the soil by enhancing phenanthrene-degrading bacteria abundance and activities. The main goal of this study was to explore how earthworms and rice straw interaction could change the structure of soil bacterial community and enhance phenanthrene degradation

in the contaminated soil. The hypothesis of the present study is that the application of rice straw and earthworms could create different distinctive phenanthrene-degrading bacteria community and would consequently improve the removal of phenanthrene in soil. Result of this study will improve our understanding on the importance of the neglected natural resources and how the rice straw can interact with the soil macro- and microorganisms to change soil structure and enhance PAH degradation in soil.

Materials and methods

Soil properties

Soil from 0 to 20 cm was collected at Huazhong Agricultural University (HZAU) test site (30° 28' 26" N, 114° 20' 51" E) in Wuhan, China, during the autumn season (October 2017), by removing the litter layer. Soil samples were then transferred to the greenhouse, air dried and sieved (2 mm). The characteristics of the used soil can be found in our previous studies [12, 13].

Collection and treatment of earthworms and rice straw

Two ecologically different earthworm species, i.e. *Eisenia fetida* and *Aporrectodea caliginosa* were used for the experiment. The first one is an epigeic species, well known for its fast growth, rapid productivity and organic waste conversion efficiency [23]. In contrast, the second specie is endogeic, known with its higher bioturbation property [11]. The two species were selected in the installed earthworms breeding site at HZAU described in our previous studies [12, 13]. Straw of *Oryza sativa* was also collected at the agricultural field of HZAU. Once in the laboratory, distilled water was used to wash the collected straw three times before drying it at 60 °C and crushing it with grinder to obtain a fraction with particle size between 1 and 2 mm [13].

Experimental design

Three kilogram of air-dried soil was placed in ceramic pots (22 × 21 cm) for the test experiment. An analytically pure acetone was used to dissolve phenanthrene (97% purity) as described by [10] and [51]. A final concentration of 50 mg/kg of phenanthrene was produced by spiking the obtained solution thoroughly with the soil. To assess whether the solvent acetone presents or not any effect on soil microbial population, the negative control treatment (+Ck/–Ck) was prepared using clean soil and acetone only.

The experiment had four treatments with three replicates, including the positive control treatment (P) prepared with neither rice straw nor earthworms; PS treatment with rice straw only; PW treatment with earthworms only; and PSW treatment with both earthworms

and rice straw. Crushed straw (to 2 mm particle size, 50 g/3 kg soil) was applied on the soil surface of the PS and PSW treatments as described in [13]. Ten epigeic and endogeic adults and healthy individuals' species were randomly selected and treated for the experiment. To void the gut content of earthworms, the selected individual's earthworms were regrouped, rinsed with DI water and placed on moist paper for 24 h before placing them on the soil of PW and PSW treatments for 30 experimental days.

Soil collection after 30 experimental days and analysis

The soil samples were collected after scattering the first top 2-cm level to eliminate any possible surfaces influences.

Determination of phenanthrene concentration

Phenanthrene concentration in soil was determined using the protocol described in our previous study [12]. Brief, soil sample was ground and mixed with 1.5 times its wet weight of Na_2SO_4 to a fine powder. The detail of the protocol can be found in [51]. High-performance liquid chromatography (HPLC) (HITACHI Chromaster 5300, Hitachi Beijing Tech Information Systems Co., Ltd) was used to measure phenanthrene concentration. The certified standard material NIST1647 Priority Pollutant Polycyclic Aromatic Hydrocarbon (PPAH) with the recovery rate of $95 \pm 3\%$ was used for the accuracy and analysis quality of phenanthrene measurements.

Quantification of soil bacterial abundance

The quantification of soil bacterial abundance was performed by following the protocol in [44] with slight modification. Total genomic DNA of soil sample was extracted using a PowerSoil Kit (MoBio Laboratories, Solana Beach, CA, United States) strictly following the manufacturer's instruction. High-quality DNA were amplified by polymerase chain reaction (PCR) amplification as described in [18, 44], using 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGCTGCTGG-3') universal primers. PCR amplifications were performed in a PRISM[®] 7500 rapid real-time PCR system.

Determination of bacterial community

Illumina MiSeq sequencing and universal primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGA CTACHVGGGTWTCTAAT-3') were used to amplify the 16S rRNA genes V4–V5 region of the 16S rRNA gene for the analysis of soil microbial community by Sangon Biotec Institute (Shanghai, China). Purified PCR amplicons were pooled in equimolar and paired-end sequenced (2 × 250 bp) on the Illumina MiSeq platform

in Shanghai, China. The detail of the protocol used for the whole process can be found in [2].

Computational analysis

Quantitative Insights into Microbial Ecology (QIIME Version 1.8.0) was used to demultiplex and quality-filter the raw fastq files. Then the filtered sequences were grouped into Operational Taxonomic Units (OTUs) clustered with a 97% similarity cutoff using UPARSE software (version 7.0.1001). UCHIME 4.2.4.0 was used to identify and remove chimeric sequences. The RDP Classifier (version 2.12) 16S rRNA (ribosomal) database was employed to analysis the taxonomy of each 16S rRNA gene sequence using a confidence threshold of 97%. A bioinformatics technique, PICRUSt was used to predict and generate a table of functional genes from 16S rRNA data. Prior to metagenome prediction, the OTUs of 16S rRNA sequences were normalized by PICRUSt. PCoA of the UniFrac distances in QIIME Version 1.8.0 was performed to visualize the distance matrix.

Statistical analysis

Bacterial abundance was log₁₀-transformed to produce a normal distribution. ANOVA using SPSS 20 statistical software was used for statistical analysis. To evaluate similarities among different treatments, ANOSIM was used. Permutational analysis of variance (PERMANOVA) was used to evaluate the effects of compartment, rice straw and earthworm's effects on bacterial communities. To discover the specific genes which can explain the different abundance in rice straw and earthworm treatments, linear effect size (LEfSe) method was used to identify significant genes belonging to phylum to genus. Then linear discriminant analysis (LDA) was used to reduce the dimensions and estimate the effect sizes of significant different of specific bacteria (expressed by LDA score). Relative abundance was calculated by the following equation and the Shannon index was calculated to describe α diversity and the richness of the microbial community.

$$RA = \frac{\text{OTUs allied to the same phylogenetic group}}{\text{Total number of OTUs}}$$

Results

Degradation of phenanthrene and its products/metabolites

The result of the negative control (+ Ck/– Ck) showed that the inoculation of acetone does not present any effect on soil microbial composition (Additional file 1: Figs. S1, S2 and S3). After 30 days of incubation, the phenanthrene concentration in soil was 17.76 mg/kg, 9.77 mg/kg and

8.46 mg/kg in straw (PS), earthworms (PW) and earthworms + straw (PSW) treatments, respectively (Table 1). Phenanthrene concentration in rice straw treatment (PS) was significantly different ($p < 0.05$) compared to the control (P). Moreover, the introduction of earthworms, considerably ($p < 0.001$) enhanced phenanthrene reduction. Out of a total of 50 mg/kg of phenanthrene initially added in the soil, 64%, 80% and 83% was removed in rice straw (PS), earthworms (PW) and in earthworms + straw (PSW) treatments, respectively. Additionally, PW and PSW treatments showed a phenanthrene remaining ratio significantly ($p < 0.001$, Table 1) lower compared to the control (P).

Predicted functional genes from 16S rRNA data were carried out to determine the presence of possible degradation products/metabolites or other functional genes involved in the degradation/metabolism of organic pollutants. Therefore, a quantitative comparison of COGs among treatments was performed to identify functions among the treatment (Fig. 1). Almost all the significantly changed individual COGs fitting to the categories of lipid metabolism, metabolism of cofactor, carbohydrate metabolism, amino acid metabolism and vitamins, and energy metabolism were enriched in all treatments. Compared to the control (P) genes involved in carbohydrate metabolism were enriched remarkably in the three others treatments. However, the application of rice straw (PS) or earthworms, either in single (PW) or dual (PSW) treatment did not manifest any significance difference regarding to the functional diversity.

Figure 2 shows different organic products identified in the different soil samples. Although the profile of all the treatments appeared to be similar, the abundance of genes involved in the dual (PSW) treatment was more pronounced compared to that in the single treatment. Compared to the control treatment (P), naphthalene degradation, phallic and salicylic acid metabolism, bio-phenol, fluorobenzene, benzoate, and ethylbenzene degradation were more enriched in PS, PW and PSW.

Changes of soil microbial

Abundance and diversity of soil bacteria

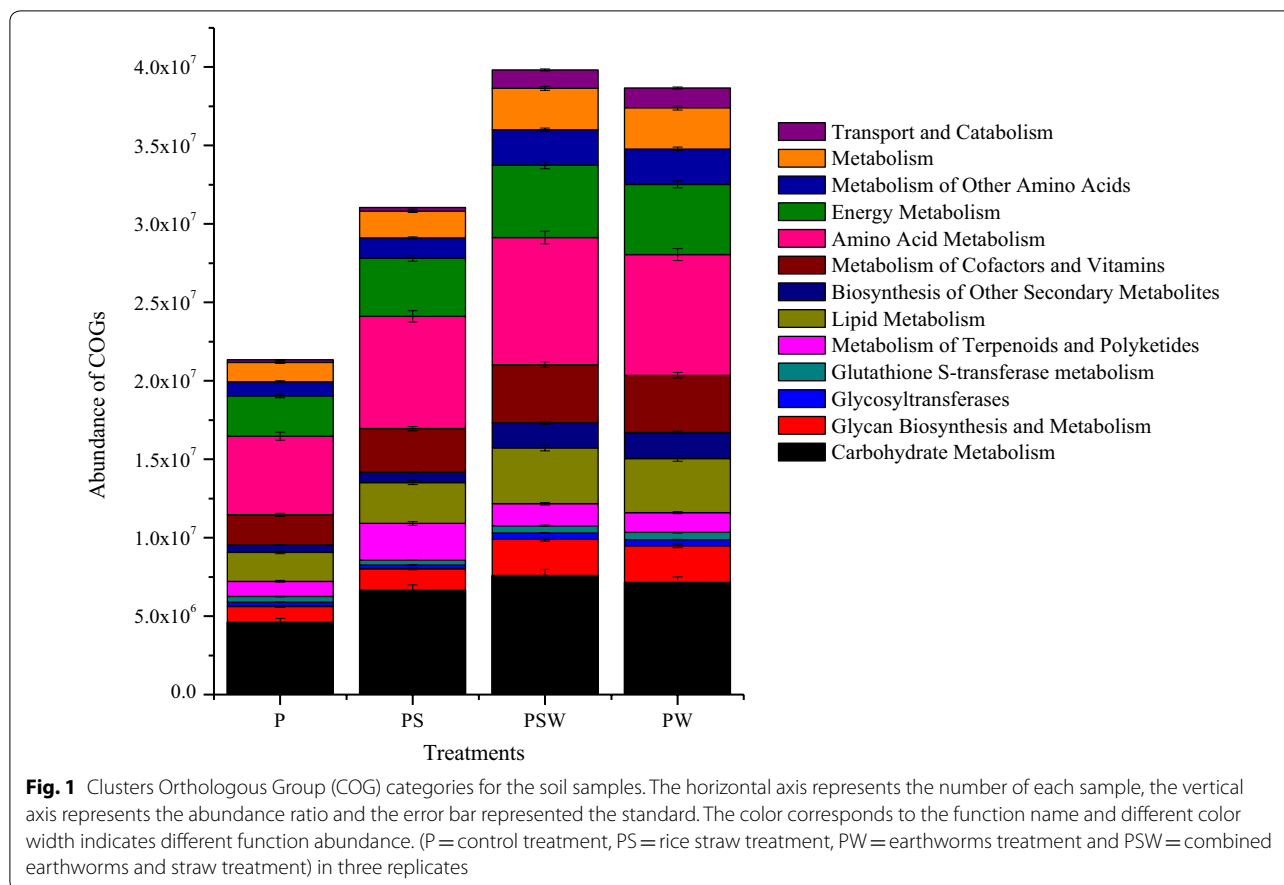
Throughout the 16S rRNA genes sequencing, a total of 518,488 bacterial sequences and 41,936 OTUs were identified in the present work. OTUs which differed among the four treatments was significantly ($p < 0.01$) higher in PW treatment and highest ($p < 0.001$) in PSW compared to the control (Table 1). A Venn diagram for OTU number ranging from 5740 (P treatment) to 7067 (PSW treatment) among the four treatments is presented in Fig. 3. 3522 OTUs over 25,840 were shared by all the four treatments. However, in 7067 OTUs identified in PSW treatment 4111, 4035 and 3684 were shared with PW, PS and P, respectively. The richness estimated by Shannon diversity index indicated that the bacterial diversity increased significantly ($p < 0.05$) in PSW and PW treatments in the phenanthrene-contaminated soil (Table 1). Soil microbial population increased significantly in earthworms (PW) treatment; however, the increase was exceptionally more important in the combined (PSW) treatment. Although bacterial diversity in straw treatment (PS) was not significantly different compared to the control, the numbers of OTUs were significantly increased. Yet, compared to straw treatment (PS), bacterial community was 1.12- and 1.37-fold higher in earthworm (PW) and combined (PSW) treatment, respectively. The highest soil bacterial diversity was observed in PSW treatment.

To assess whether there was correlation between both bacterial Shannon diversity index and soil bacterial 16S rRNA genes with the remained phenanthrene concentration in soil, correlation test was performed. It was found that both Shannon biodiversity index ($r^2 = -0.8807$, $p < 0.001$) and bacterial 16S rRNA genes ($r^2 = -0.7795$, $p < 0.001$) negatively correlated with the remained phenanthrene concentration in soil (Fig. 4 and Additional file 1: Table S2). Since the dual rice straw and earthworms introduction in soil (PWS) had the lowest remained phenanthrene concentration ratio (0.16 ± 0.02 ; tableau 1), the highest Shannon index (6.45 ± 0.2) and the highest

Table 1 Phenanthrene remained concentration in soil, phenanthrene remained ratio, estimates of bacterial abundance and diversity indices in different treatments

Treatment	PHE concentration remained	PHE remained ratio r	Seq_sequences	OTUs_num	Shannon index
P	29.52 ± 0.35	0.59 ± 0.06	101,650	9429	5.8 ± 0.7
PS	17.76 ± 0.85*	0.35 ± 0.04*	130,882	9695*	6.12 ± 0.8
PW	9.77 ± 0.46***	0.19 ± 0.08***	142,878	10,906**	6.38 ± 0.7*
PSW	8.46 ± 0.86***	0.16 ± 0.09***	143,078	11,906***	6.45 ± 0.5*

Data are the mean of three replicate ± SD and were compared by Duncan's multiple range tests. Seq_num is the quality number of samples reads and OTU_num is the number of 16S rRNA sequences OTUs obtained by sample clustering and normalized PICRUST. Different asterisks *, ** and *** in the same column (4 values) indicate significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively



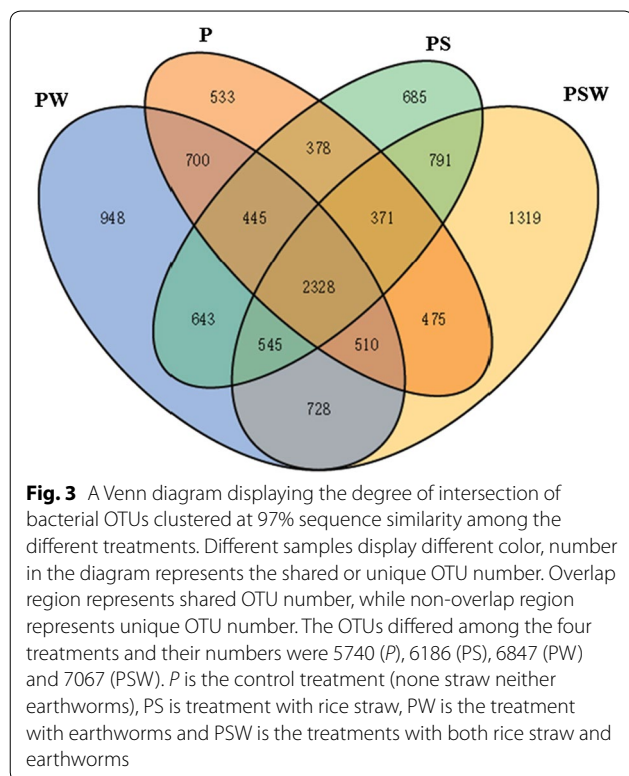
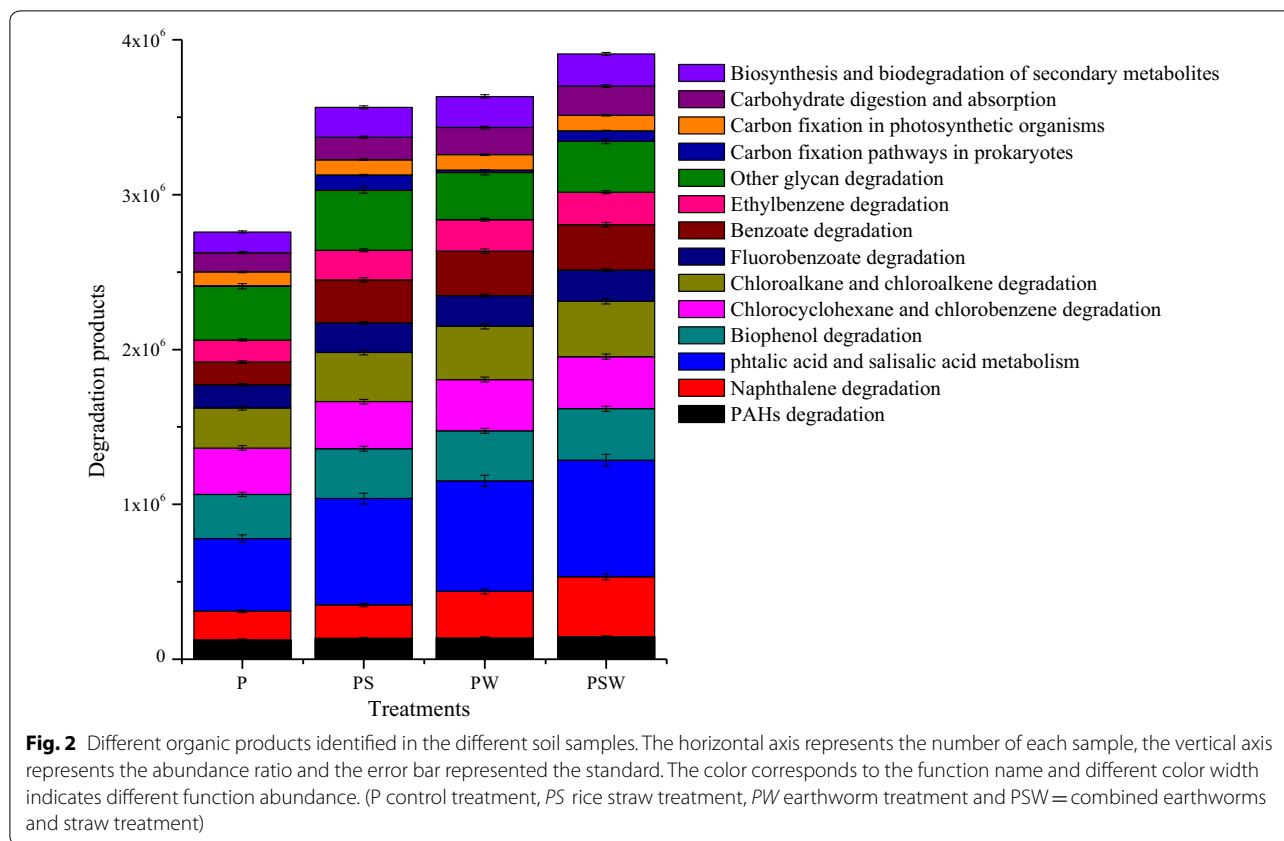
bacterial reads for 16S rRNA genes, this implied that both earthworms and rice straw might improve the phenanthrene metabolism by increasing soil bacteria biomass and diversity.

Community composition

The relative abundance of specific bacteria was explored at class level (Table 2). The richness index was similar in the control (P) and straw (PS) treatments and differed to that in earthworms (PW) and combined (PSW) treatments. Shannon index revealed that the population repartition in PW and PSW was similar. In a global view, no significant difference ($p > 0.05$) in bacterial OTUs and species richness were observed between the four treatments. However, in relative abundance, earthworms significantly increased the abundance of *Proteobacteria* which was represented by alpha, gamma, beta and delta-*proteobacteria* counting more than 50% of the total represented classes. *Sphingobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Flavobacteria* and *Cytophagia* were the next represented classes with 25%, 20%, 12%, 7% and 7%, respectively. The abundance of *Gamma-proteobacteria* ($r^2 = -0.81$, $p < 0.01$), *Alpha-proteobacteria*

($r^2 = -0.74$, $p < 0.01$), *Gemmatimonadetes* ($r^2 = -0.61$, $p < 0.05$), *Flavobacteria* ($r^2 = -0.581$, $p < 0.05$) and *Actinobacteria* ($r^2 = -0.52$, $p < 0.05$) classes was found to negatively correlate with the remained concentration of phenanthrene in soil.

The bacterial community was further analyzed at the genus level (Table 2). Earthworms and rice straw addition significantly increased the relative abundance of *Sphingomonas* (34.7%), *Luteimonas* (24.63%), *Rhodanobacter* (21.34%), *Gemmatimonas* (15.65%), *Pseudomonas* (9.87%), *Flavobacterium* (9.43%), *Leifsonia* (8.98%) and *Sediminibactium* (8.76%), which were the dominant genus of the OTUs in each treatment. In addition, for all represented genus, relative abundance in PSW and PW treatments was particularly marked ($p < 0.05$). Pearson correlation coefficient among these most represented genus showed that the abundance of *Pseudomonas* ($r^2 = -0.93$, $p < 0.01$), *Luteimonas* ($r^2 = -0.87$, $p < 0.01$), *Rhodanobacter* ($r^2 = -0.75$, $p < 0.01$), *Sphingomonas*, ($r^2 = -0.68$, $p < 0.05$), *Gemmatimonas* ($r^2 = -0.63$, $p < 0.05$), *Flavobacterium* ($r^2 = -0.54$, $p < 0.05$), and *Leifsonia* ($r^2 = -0.56$, $p < 0.05$) were negatively correlated with the remained concentration of phenanthrene in soil.



Principal component analysis (PCoA) expounded bacterial community structure

Based on the presence or absence of phylotype in the four treatments, the unweighted principal component analysis (PCoA) was performed and the result is reported in Fig. 5. More particularly, earthworms (PW) and combined (PSW) treatments presented PC1 values much higher than those of the control (P) and straw (PS) treatments. The community structure of bacteria in earthworm treatment (PW and PSW) was distinguished and clustered together. Thus, the result of this PCoA strongly accentuated that both earthworms and straw and especially earthworms are a booster factor in the formation of the bacterial community, responsible for the removal of phenanthrene.

Relative abundance between straw and earthworm treatments

To further investigate the bacterial taxa distinct among earthworms and straw groups, LEfSe analysis and LDA was performed (Fig. 6). The circle, from internal to external, indicates bacteria from phylum to genus, respectively. Yellow points represent bacteria that have no significant differences from each other, and specific

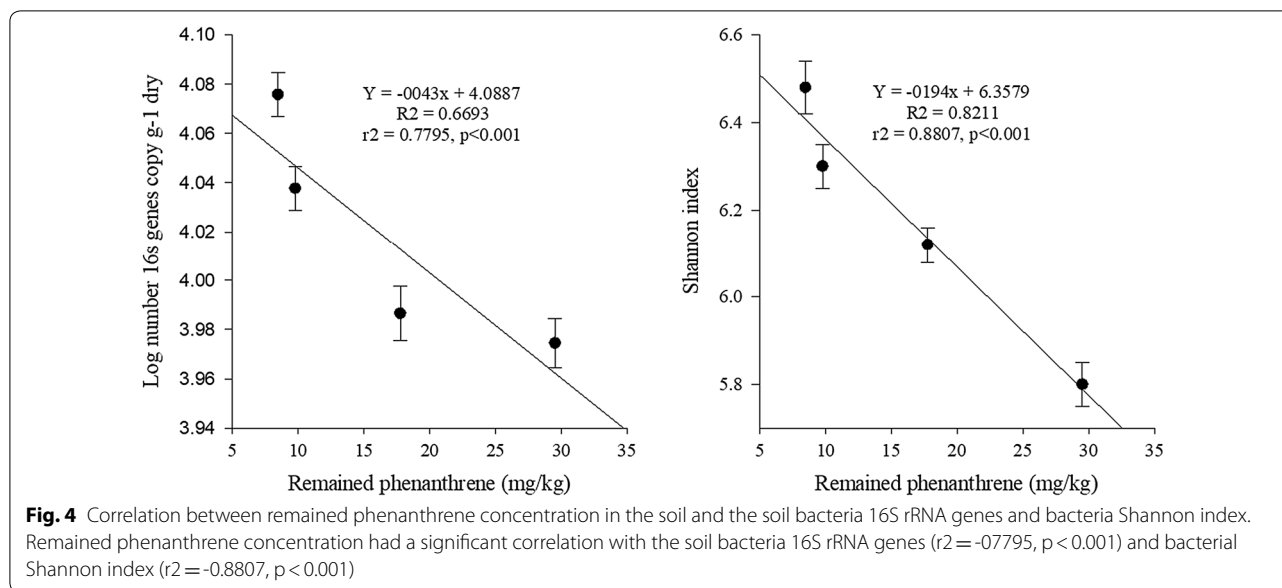


Table 2 Pearson’s correlation coefficient relating to phenanthrene and the relative abundance of bacterial groups at class and genus level, respectively

Classification level	Bacterial group	Percentage	Person’s correlation coefficient
Class	<i>Gamma-proteobacteria</i>	30	$r^2 = -0.81$, $p < 0.01$
	<i>Alpha-proteobacteria</i>	15	$r^2 = -0.74$, $p < 0.01$
	<i>Sphingobacteria</i>	25	$r^2 = -0.56$, $p < 0.05$
	<i>Actinobacter</i>	20	$r^2 = -0.52$, $p < 0.05$
	<i>Gemmatimonadetes</i>	12	$r^2 = -0.61$, $p < 0.05$
	<i>Flavobacteria</i>	7	$r^2 = -0.581$, $p < 0.05$
	<i>Cytophagia</i>	7	$r^2 = -0.54$, $p < 0.05$
Genera	<i>Sphingomonas</i>	34.7	$r^2 = -0.68$, $p < 0.05$
	<i>Luteimonas</i>	24.63	$r^2 = -0.87$, $p < 0.01$
	<i>Rhodanobacter</i>	21.34	$r^2 = -0.75$, $p < 0.01$
	<i>Gemmatimonas</i>	15.65	$r^2 = -0.63$, $p < 0.05$
	<i>Pseudomonas</i>	9.8	$r^2 = -0.93$, $p < 0.01$
	<i>Flavobacterium</i>	9.43	$r^2 = -0.54$, $p < 0.05$
	<i>Leifsonia</i>	8.98	$r^2 = -0.56$, $p < 0.05$

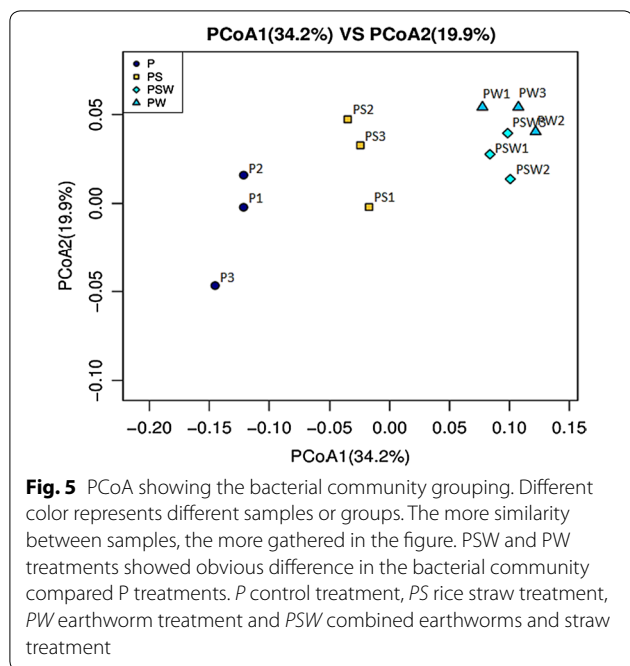
bacteria are colored by their corresponding class color. The LDA score of different specific bacteria and LEfSe applied to the microbiota data of rice straw and earthworms’ samples found 13 differentially abundant taxonomic clades with an LDA score higher than 3.5. It was revealed that the higher taxonomic levels of *Bacteroidetes* and *Proteobacteria* (including *Sphingobacteria/Alpha-proteobacteria* and *Betaproteobacteria*, respectively) were significantly less abundant in straw groups, whereas the *Flavobacteria* class, *Rhodobacterales* order, *Firmicutes* and *Actinobacteria* phylum were less abundant in earthworm groups.

Discussion

The investigation of the impacts of earthworms and rice straw on phenanthrene degradation and soil microbial community was conducted in this present study. The results showed that rice straw and earthworms not only improve the soil structure and phenanthrene degradation, but also interacted each other and changed the soil microbial community and structure.

Phenanthrene degradation

Phenanthrene concentration decreased when earthworms were applied (Table 1), indicating that the



degradation, sorption and/or transformation of phenanthrene could produce. Information about functional genes disclosed that carbohydrate metabolism including bisphenol, benzoate and ethylbenzene degradation was more enriched in soil samples, indicating that both rice straw and earthworm enhanced phenanthrene

degradation (Figs. 1 and 2). Xenia and Refugio [43] reported that the interactions between enzymes that degrade phenanthrene and modify its structure as well as its toxicity are determining for microbial phenanthrene degradation. Specific microorganisms, through multi-enzyme complex, oxidize the carbon molecules and incorporate an oxygen molecule, resulting in a hydroxylated carbon to alcohol [52]. Subsequently, the alcohol group is oxidized to aldehyde and finally to carboxylic acid [38]. This could explain the enrichment of phenol and benzoate degradation in this present study. Earthworms are reported to stimulate phenanthrene removal by promoting soil phenanthrene degrading microorganism [9, 34]. Similarly, in the present study, in the presence of earthworms, it was found that phenanthrene degradation correlated with the increase of soil microbial community. The ratio of phenanthrene remained in soil significantly decreased in PW and PSW treatments (Table 1), indicating to more efficiency of earthworm implication on the decrease of phenanthrene concentration. Thus, our results reaffirm previous reports that earthworms could enhance PAH degradation.

Changes in microbial community and phenanthrene degradation in the soil

Induced by earthworms

Earthworms were reported to accelerate the removal diver organic pollutant including PAHs [9]. Earthworms enhance PAH degradation by stimulating microbial

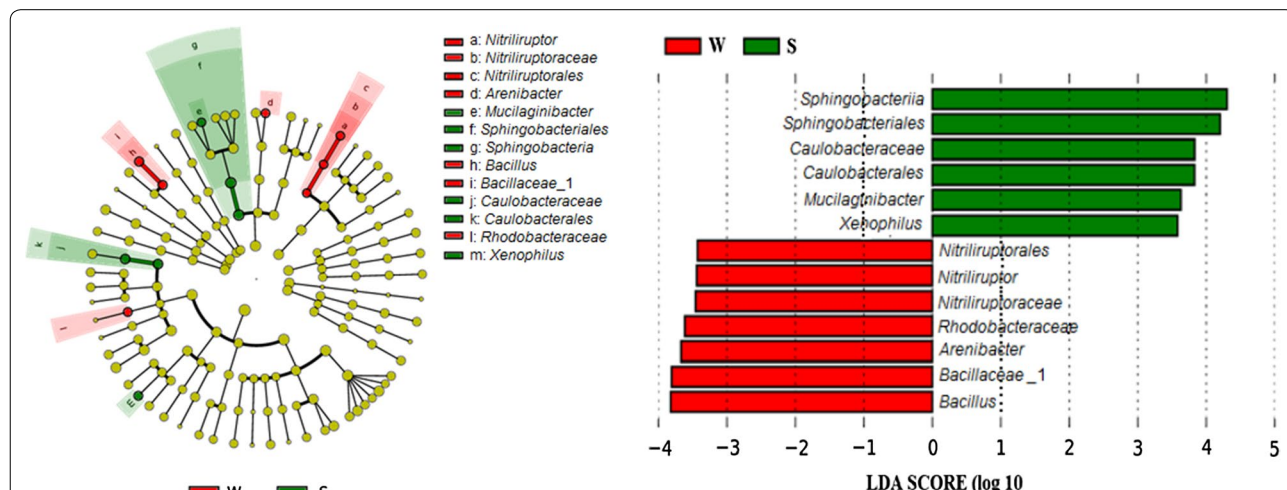


Fig. 6 Phylogenetic dendrogram of specific bacteria (in the left) between straw group (S) with earthworms groups (W) samples and their LDA score (in the right). In the figure at right, different colors represented different groups, the horizontal axis is LDA score after LDA analysis and the longitudinal is the significant microbial group. In the left, different colors represent different groups, and different color nodes in the tree represent the bacterial groups that play an important role in the corresponding grouping of colors. Yellow nodes represent bacteria that have no significant differences from each other. The name of the species represented by the letters in the graphic displayed in the right legend. Bacterial taxa significantly enriched in samples from earthworm to straw group and then the control. Taxa differentiating were detected by the LefSe analytic method ($p < 0.05$, log linear discriminant analysis [LDA] score > 2)

growth and thereby affect the soil microbial community [45]. The addition of earthworms either in separate (PW) or dual (PSW) treatments revealed to play a key role on the formation of the bacterial community in soil. This observation was confirmed by the significant difference observed in PCoA value in this present study (Fig. 5). Soil bacterial abundance was remarkably increased in PW and in PSW treatments. These results indicated that earthworms might improve the phenanthrene degradation by increasing soil bacteria biomass and diversity. The addition of earthworm strongly stimulated the growth of *Proteobacteria* phylum which was largely dominant. *Proteobacteria* composed by alpha, gamma, beta and delta-*proteobacteria* is one of the most frequently isolated phyla from the contaminated environments and reported able to degrade PAHs such as naphthalene, anthracene and phenanthrene by complete mineralization via similar pathways [6, 27, 29]. *Sphingomonas* strains such as *S. koreensis*, *Sphingobium* sp., *Novosphingobium pentaromaticivorans*, *Sphingopyxis* sp., *Rhizobiales* strains such as *Sinorhizobium* sp., *Ochrobactrum* sp. and *Pseudomonas* strains such as *P. aeruginosa*, *Pseudomonas* sp. and *P. putida* have been reported to degrade phenanthrene [37, 47]. Additionally, some *Actinobacteria* phylum strains such as *Mycobacterium* sp., *M. vanbaalenii* and *M. fortuitum* and some *Flavobacteria* strains were also shown with high efficiency to degrade phenanthrene [42]; [20]. In this present study, the abundance of *Pseudomonas*, *Luteimonas* and *Rhodanobacter* significantly increased in the PW and PSW treatment (Table 2). This suggested that earthworm's activities stimulated their growth. In addition, these genera negatively correlated with the remained phenanthrene in soil. This indicated that *Pseudomonas*, *Luteimonas* and *Rhodanobacter* were not only involved in the phenanthrene degradation but also were the most beneficial phenanthrene degraders.

Induced by rice straw

Many agricultural management practices such as fertilization, tillage, and straw return significantly influence soil organic matter which is the midpoint of soil function and quality [50]. Rice straw addition increased soil microorganisms, which in return play key roles in organic matter decomposition and soil nutrient biogeochemical cycling such as carbon and nitrogen in ecosystems [16, 53]. The litter alteration including fragmentation and consumption of associated microorganisms usually increases the microbial activity [15, 32]. However, soil microbial community structure was reported to vary with agricultural management practices and its application time [5]. Rice straw showed a positive influence on phenanthrene removal by increasing significantly the abundance of 16S rRNA genes while having no effect on bacterial Shannon

index (Table 1). Studies reported that the use of organic fertilizers likely played a critical role in the degradation and removal of organic contaminant in soil [41]. It can be speculated that the phenanthrene removal in rice straw treatment was strongly assessed by soil microbial stimulated by rice straw decomposition activities rather than rice straw itself. Rice straw as organic fertilizer influences microbial population in soil through stimulatory and suppressive effects on specific components and impacts the whole community structure [2]. Crop residue retention was reported to significantly affect bacterial community structure and increased the abundances of *Bacteroidetes*, *Betaproteobacteria*, and *Gemmatimonadetes* [31]. *Sphingomonas*, *Luteimonas*, *Gemmatimonas* and *Flavobacterium* showed significant negative correlation with the remained phenanthrene in soil (Table 2). This indicates that rice straw would accelerate the degradation of phenanthrene by stimulating the growth and the abundance of these bacteria in soil.

Induced by the combination of earthworms and rice straw

Several PAH bioremediation process, involving biostimulation application, focused on the complex interaction between earthworms and soil microbial community. However, the organic fertilizer rice straw can be involved in PAH removal. Very little information is available concerning the implication of crops straw on PAH removal in general and how earthworms interact with crops straw and affect the microbial degradation of PAHs in particular. Thus, in the present study, it is shown that earthworms and rice straw can increase the transformation rate of phenanthrene which may fulfil the aim of the bioremediation approach. The co-existence of earthworms and rice straw in this study significantly increased the abundance of bacteria 16S rRNA and bacterial Shannon index (Table 1). Additionally, the introduction of earthworms and rice straw in soil had the lowest phenanthrene remained ratio (Table 1), the lowest phenanthrene remained concentration (Table 1) and the higher abundance of some specific bacteria such as *Sphingomonas*, *Rhodanobacter* and *Pseudomonas* (Table 2). The dual rice straw and earthworms introduction in soil had the soil remained phenanthrene concentration negatively correlated with the soil bacterial 16S rRNA genes and bacterial Shannon index (Fig. 4). Considering these results, this study highlights that the ecological context of remediation should not be limited on soil–earthworms–plant interaction only, but should be extended by integrating the natural resources forsaken which can provide a positive influence on soil microorganisms' changes and enhance PAH degradation in soil.

Conclusion

This study demonstrates the positive interaction between earthworms and rice straw in soil. Their co-existence with soil microbial interactively improves the degradation of phenanthrene in soil. Both earthworms and rice straw stimulate the abundance of specific bacteria which showed strong and clear correlation with the phenanthrene concentration in soil. What it should be noted is that this study established that earthworms and rice straw could quicken phenanthrene degradation by changing bacterial growth and community configuration in the soil. The variation in soil nutrients and structure by earthworms and rice straw addition enable to modify the structure of the microbial community. From this present study, it can be seen, the importance of the neglected natural resources like rice straw and how this later can interact with the soil macro- and microorganisms to change soil structure and enhance PAH degradation in soil.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12302-020-00400-y>.

Additional file 1: Table S1. Estimation of bacterial abundance and diversity indices in different treatments extract control treatments. Seq_num is the quality number of samples reads and OTU_num is the number of 16S rRNA sequences OTUs obtained by sample clustering and normalized SPSS. **Figure S1.** Relative abundance of soil bacterial (grouping of the three replicates of each soil samples) at phylum level. The horizontal axis represents the treatment and the vertical axis represents the abundance ratio in percentage. The color corresponds to the phylum name and different color width indicates different phylum. (-Ck = control without acetone and +Ck = control with acetone). **Figure S2.** Relative abundance of soil bacterial (in replicate form of each soil samples) at phylum level. The horizontal axis represents the treatment and the vertical axis represents the abundance ratio in percentage. The color corresponds to the phylum name and different color width indicates different phylum. (-Ck = control without acetone and +Ck = control with acetone). **Figure S3.** Relative abundance of soil bacterial (grouping of the three replicates of each soil samples) at order level. The horizontal axis represents the treatment and the vertical axis represents the abundance ratio in percentage. The color corresponds to the phylum name and different color width indicates different phylum. (-Ck = control without acetone and +Ck = control with acetone). **Figure S4.** Relative abundance of soil bacterial (in replicate form of each soil samples) at order level. The horizontal axis represents the treatment and the vertical axis represents the abundance ratio in percentage. The color corresponds to the phylum name and different color width indicates different phylum. (-Ck = control without acetone and +Ck = control with acetone). **Table S2.** Data are the mean of three replicate \pm SD and were compared by Duncan's multiple range tests. Seq_num is the quality number of samples reads and 16S rRNA num is the number of 16S rRNA sequences obtained by sample clustering and normalized PICRUSt.

Abbreviations

HZAU: Huazhong Agricultural University; HPLC: High-performance liquid chromatography; PAH: Polycyclic aromatic hydrocarbons; PPPAH: Priority pollutant polycyclic aromatic hydrocarbons.

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Authors' contributions

AME designed and performed the study; CH supervised and funded the project. Both authors read and approved the final manuscript.

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Competing interest

Authors declare no competing financial interest.

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