Journal of Environmental Management 384 (2025) 125562

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Earthworms attenuate antibiotic resistance genes and mobile genetic elements during vermicomposting of sewage sludge

María Gómez-Brandón [a,*], Manuel Aira [a], Maraike Probst [b], Ning Liu [c,d], ZhiJian Zhang [e], Yong-Guan Zhu [c,d,f], Jorge Domínguez [a]

[a] Grupo de Ecología Animal (GEA), Universidade de Vigo, Vigo, 36310, Galicia, Spain
[b] Universität Innsbruck, Department of Microbiology, University of Innsbruck, Technikerstrasse 25d, Innsbruck, A-6020, Austria
[c] Key Laboratory of Urban Environment and Health, Ningbo Urban Environment Observation and Research Station, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, 361021, China
[d] Zhejiang Key Laboratory of Urban Environmental Processes and Pollution Control, CAS Haixi Industrial Technology Innovation Center in Beilun, Ningbo, 315830, China
[e] College of Environmental and Natural Resources, Zhejiang University, YuHangTang Ave 866, HangZhou, 310058, ZheJiang Province, China
[f] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

ARTICLE INFO

Keywords:
Antimicrobial resistance
Bioremediation
Eisenia andrei
Gut-associated processes
Earthworm casts
Vermicompost

ABSTRACT

Sewage sludge is among the richest reservoirs of antibiotic resistance genes (ARGs) that may spread to urban environment. Further investigation is warranted for removal of sludge-borne ARGs in large-scale vermicomposting systems. Under this scenario, there is the necessity to unveil the role of the widely-used earthworm species *Eisenia andrei*, since the current body of literature mostly focuses on *E. fetida*. The present study sought to evaluate the changes in sludge-borne ARGs and mobile genetic elements in a pilot-scale vermireactor in the presence of *E. andrei* in response to both gut- and cast-associated processes (GAPs and CAPs, respectively), by coupling high-throughput quantitative PCR and Illumina sequencing. After gut transit, large decreases in the relative abundances and number of the genes conferring resistance to major antibiotic classes, including some specific genes classified as of potentially high risk to human health, were recorded in the fresh casts. Likewise, genes encoding resistance to heavy metals were about nine-times lower in the egested materials than in the initial sludge. Genes coding for integrases or insertional sequences also exhibited reduced abundance as a result of GAP and CAP processes, suggesting that vermicompost appears to be less prone to horizontal gene transfer than untreated sludge. These findings provide evidence about the capacity of the earthworm *E. andrei* to diminish the risk of ARG spread during vermicomposting, reinforcing its potential for bioremediation purposes by transforming large quantities of waste into an improved fertiliser. This is crucial to propel vermicomposting technology forward and achieve transition toward net zero-waste process.

1. Introduction

Antimicrobial resistance (AMR) has become a threat from the One Health perspective, representing a health priority worldwide (White and Hughes, 2019). In this context, wastewater treatment plants (WWTPs) are potential hotspots for antibiotic-resistance genes (ARGs), with sewage sludge (SS) acting as a reservoir of ARGs that may spread to the urban environment (Su et al., 2015; Wang et al., 2023). Moreover, ARGs can potentially assemble close to one another in a process involving mobile genetic elements (MGEs). This is of particular interest when the MGEs carry important virulence genes and can potentially confer a multidrug resistance phenotype to the host bacterium that acquires them (Nguyen et al., 2021).

Sewage sludge is rich in nutrients and organic matter (Seleiman et al., 2020), but its direct application as a soil amendment may lead to phytotoxic and antimicrobial effects, with possible consequences for human health via the uptake of ARGs and other emerging environmental contaminants by plants or their subsequent release into water bodies via run-off (Chen et al., 2016; Wolters et al., 2019). In line with the circular economy principles (European CommissionDirectorate-General for Communication, 2020), sustainable and profitable methods of treating SS prior to application on land must be considered in order to minimize

* Corresponding author.
E-mail addresses: mariagomez@uvigo.es, mariagomezbrandon@gmail.com (M. Gómez-Brandón).

https://doi.org/10.1016/j.jenvman.2025.125562
Received 11 December 2024; Received in revised form 14 April 2025; Accepted 25 April 2025
Available online 30 April 2025

the spread of ARGs in agricultural soils. The use of vermicomposting has proliferated over the last twenty years, and the procedure has become a well-established option that ensures the circular management of a wide variety of types of organic waste in a biologically-based, low-impact manner (Domínguez, 2023). Indeed, a marked decrease in the abundance of sludge-borne ARGs throughout this process has been reported, with reduction rates up to 95 % depending on the type of ARG and the vermicomposting conditions (reviewed by Li et al., 2023). Nonetheless, up to now most vermicomposting trials have primarily focused on the attenuation effects of the earthworm species *Eisenia fetida* on ARG dissemination (Fang et al., 2023). This calls for the necessity to further investigate the potential role of other species such as *E. andrei*, which is one of the most widespread and common earthworms in vermicomposting companies and farms worldwide (Domínguez, 2023). Bearing this in mind, the current study will focus on this earthworm species to shed light onto its impact on the antibiotic resistome during vermicomposting. Both *E. fetida* and *E. andrei* are considered different biological species with distinct life histories (Domínguez et al., 2005). This feature may lead to species-specific effects in response to stress factors and/or the presence of contaminants (Junior et al., 2022; Wu et al., 2023) and, likely on the diffusion or inhibition intensity of ARGs and MGEs over the course of the process.

In previous trials with *E. andrei*, it has been shown that more than 90 % of bacterial and fungal taxa in sewage sludge are eliminated during vermicomposting, primarily through the gut-associated processes (GAPs; Domínguez et al., 2021; Gómez-Roel et al., 2024). Supporting this, Aira et al. (2022) demonstrated that earthworm casts from *E. andrei* are mainly populated (>90 %) by bacteria of earthworm origin, i.e., bacteria that are not found in the undigested material. In a latter work, the same authors provided additional evidence of a reduction and/or absence of human pathogenic bacteria (HPB) such as *Escherichia coli* and *Listeria monocytogenes*, which are considered as potential ARG hosts (Nguyen et al., 2021), in the fresh casts from *E. andrei* compared to the initial sludge (Aira et al., 2024). Altogether, the compositional shifts in bacterial communities and the attenuation of pathogenic load after the gut transit of this earthworm species are expected to affect the fate of ARGs at the earlier stages of the process (Guo et al., 2024). However, despite all the aforementioned evidence, the impact of the earthworm *E. andrei* on the presence and abundance of ARGs and MGEs throughout the process of vermicomposting has not been thoroughly studied yet.

Once GAPs are completed, the egested materials will undergo a period of ageing during which the associated microbial communities experience a turnover through the cast-associated processes (CAPs; Domínguez et al., 2021). Although already reported for *E. fetida* (Huang et al., 2018, 2020; Cui et al., 2018, 2019, 2022; Guo et al., 2024), it is still necessary to unravel whether and to which extent the reduction or elimination of ARGs and MGEs with *E. andrei* occur during the active (earthworm casts) or maturation (vermicompost) stages of vermicomposting. In order to fill in this knowledge gap, such changes in the presence of *E. andrei* will be evaluated at a pilot-scale reactor designed to handle large amounts of substrate and performed at the maximum earthworm density capacity. This may have important implications for the usefulness of vermicompost as an organic amendment because if a reduction in ARGs/MGEs takes place at the earlier stages, that is after the transit of the material through the earthworm gut, this could be an indication that the process will be density-dependent and easily optimizable.

Specifically, by coupling high-throughput quantitative PCR (384 primer sets targeting resistance genes covering all major antibiotic classes) and Illumina sequencing of bacterial 16S rRNA gene, this study sought to (i) evaluate the changes in the relative abundance and number of sludge-borne ARGs and MGEs in response to both GAPs and CAPs; and (ii) investigate the associations between ARGs and MGEs and their potential bacterial hosts in the egested casts and the final vermicompost. These findings may help to pave the way towards more efficient utilization of resources and to further generate recommendations for the development of the vermicomposting industry in terms of bioremediation and fertilizer production to generate safe bio-based products in a circular, green economy.

2. Material and methods

2.1. Sewage sludge, vermicomposting and sampling

Sewage sludge was kindly provided by the manager of a wastewater treatment plant in Caldas de Reis (9775 inhabitants), in Pontevedra (Galicia, northwestern Spain). The main properties of the initial feedstock are summarized in Table 1. Briefly, raw sewage sludge was vermicomposted in a rectangular plastic pilot-scale vermireactor (1.1 m long, 1.05 m wide, 70 cm high) placed in the greenhouse facilities (without any temperature control) of the Animal Ecology Group at the University of Vigo (Galicia, northwestern Spain). The vermireactor consisted of a vermicompost layer of 12 cm high (as a bed for the earthworms) and a density of 12,000 individuals per m^2 of *Eisenia andrei*, as previously described (Domínguez et al., 2021). Raw sewage sludge (120 kg, fresh weight, fw) was placed on a plastic mesh (aperture, 5 mm) on top of the vermicompost bedding. To maintain the moisture level at about 85 %, the vermireactor was covered with a cloth throughout the experiment. Fresh earthworm cast and vermicompost (3 months old) samples (n = 5 each) were collected for further analysis, as previously described (Domínguez et al., 2021). To collect fresh cast samples, i.e. those due to gut associated processes (GAP), adult individuals of the earthworm species *E. andrei* were removed from the vermireactor, washed three times with sterile distilled water and placed in clean, sterile Petri dishes on moistened sterile filter paper (20 individuals per dish, 5 dishes). Sampling dishes were then placed in an incubation chamber in darkness for 24 h. Afterwards, fresh earthworm casts were collected from each sampling dish with a sterile spatula (flame sterilized between samples). Casts were then stored in 1.5 mL Eppendorf tubes at − 80 °C.

2.2. Physico-chemical and nutrient analyses

Moisture and organic matter contents of the initial sewage sludge were determined by oven-drying the samples 24 h at 105 °C and combusting for 5 h at 550 °C in a muffle furnace (Carbolite, CWF 1000), respectively. The electrical conductivity (EC) and pH of the initial sludge

Table 1
Characterization of the initial sewage sludge used in this study. Values are means ± standard error. Nutrient data are expressed on a dry weight (dw) basis.

	Raw sludge
Moisture (%)	84 ± 1.0
Organic matter (%)	76 ± 3.8
pH	8.54 ± 0.02
Electrical conductivity (μS cm^{-1})	1215 ± 109
Total C (%)	36.59 ± 0.32
Total N (%)	6.52 ± 0.05
C to N ratio	5.62 ± 0.02
Ca (mg kg^{-1})	7919 ± 127
K (mg kg^{-1})	13,892 ± 495
Mg (mg kg^{-1})	7786 ± 176
P (mg kg^{-1})	38,854 ± 899
S (mg kg^{-1})	9542 ± 170
Al (mg kg^{-1})	14,278 ± 150
B (mg kg^{-1})	23 ± 0.53
Cr (mg kg^{-1})	32 ± 1.4
Cd (mg kg^{-1})	3.0 ± 0.1
Cu (mg kg^{-1})	356 ± 12
Fe (mg kg^{-1})	5511 ± 132
Mn (mg kg^{-1})	80 ± 1.3
Mo (mg kg^{-1})	5.4 ± 0.08
Ni (mg kg^{-1})	19 ± 0.6

were measured in aqueous extracts (1:10 mass/volume), with a conductivity meter (Crison CM35) and a pH meter (Crison MicropH 2000) respectively. Total C and N contents were determined in oven-dried (60 °C) samples, in an elemental analyzer (CHNS–O analyzer, Carlo Erba, EA 1108). The total contents of macro- and micronutrients were determined, in extracts from dried sludge samples subjected to nitric-perchloric digestion and then optical emission spectrometry with inductively coupled plasma (ICP-OES), following the USEPA 3050 B method (USEPA, 1996).

2.3. DNA extraction and high-throughput quantitative polymerase chain reaction (HT-qPCR)

DNA was extracted from 0.25 g (fw) of each sample type (initial sewage sludge, earthworm casts and vermicompost) using the MO-BIO PowerSoil kit according to the manufacturer's protocols. DNA quality and quantity were assessed using BioTek's Take 3 Multi-Volume Plate. Prior to HT-qPCR, DNA templates were diluted to equimolar concentrations (30 ng μL^{-1}) using a Qubit 3.0 fluorimeter (Invitrogen) to ensure uniformity across reactions.

HT-qPCR was performed using the WaferGen SmartChip Real-time PCR system (WaferGen Biosystems Inc., Fremont, CA, USA), which enables the simultaneous assessment of large numbers of ARGs and MGEs (Zhu et al., 2013, 2020). A total of 384 primer pairs were used to target 308 ARGs, 8 taxonomic genes, 10 heavy metal resistance genes, 36 MGEs marker genes, 10 transposase genes, 3 integrase genes, 8 insertional sequences and the 16S rRNA gene (Table S1). The 100-nL reaction system was used to amplify the target genes, with all qPCR reactions conducted in triplicate for each primer set, including a non-template negative control. HT-qPCR mixtures consisted of 1 X LightCycler 480 SYBR Green I Master (Roche Applied Sciences, Indianapolis, IN), 1 mg/mL BSA (bovine serum albumin) (New England Biolaboratories, Beverly, MA), 500 nM of each primer and a DNA template of 50 ng μL^{-1}. The thermal cycle of HT-qPCR comprised 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 30 s and annealing at 60 °C for 30 s. Melting curve analyses were automatically generated by WaferGen software. The qPCR results were then subjected to analysis using SmartChip qPCR Software (2.7.0.1 version), and wells exhibiting multiple melting peaks were excluded from the analysis. The amplification efficiencies ranged from 1.8–2.2, with R^2 values consistently exceeding 0.99. A threshold cycle (Ct) of 31 was used as the detection limit, in accordance with the platform's sensitivity. The absolute copy number of each ARG was then normalized to the absolute 16S rRNA gene copy number (Zhu et al., 2020) to minimize errors caused by variations in the background 16S rRNA gene abundance across different sample types (Zhu et al., 2019, 2020). For each biological sample, the mean gene copy number of the three technical replicates was considered in further data analysis to reduce any false positive or negative detections. Detection of an ARG was regarded as positive when all three technical replicates were amplified. If no ARG was detected in any of the three technical replicates, the ARG was considered absent. Detection of an ARG in two out of three technical replicates was considered a positive result if the ARG was detected in ten out of the twelve HT-qPCR measurements (i.e. in three of the four biological replicates).

2.4. 16S rRNA amplification, sequencing and bioinformatic analysis

Amplicon sequencing of the 16S rRNA V4 gene region was performed in an Illumina MiSeq genome sequencer in the *Argonne National Laboratory* using the primers listed by Domínguez et al. (2021). The DADA2 pipeline (version 1.16) was used to infer the amplicon sequence variants (ASVs) present in each sample (Callahan et al., 2016) according to Github developers' protocol (version 1.16; https://benjjneb.github.io/dada2/tutorial.html). 16S forward/reverse read pairs were trimmed and filtered, with forward reads truncated at 140 nt and reverse reads at 130 nt Domínguez et al. (2021). By using the run-specific error rates, forward and reverse reads were separately corrected for potential sequencing errors and then merged to ASVs. After removal of chimera, filtered sequences were taxonomically assigned by comparison with the SILVA database (version 138) and by using the RDP naive Bayesian classifier implemented in the DADA2 Rpackage (min boot 80; Quast et al., 2013). ASVs that remained unclassified at phylum level were disregarded (0.6 % of sequences). A total of 100,905 sequences (mean: 6,727, SD: 1557) passed all quality filters and were assigned to 1614 ASVs.

2.5. Statistical analysis

Venn diagrams were generated to visualize the number of resistance genes shared among sample groups or those unique to a sample group (Hulsen, 2021). All further statistical tests were performed in R v4.4.1 (R Core Team, 2024). A confidence interval of 95 % was applied in all tests. The clr-transformed data was subjected to principal component analysis (prcomp) to visualize the differences in the ARG and MGE profiles between the initial sewage sludge, cast and vermicompost samples. Significance levels were inferred by PERMANOVA (adonis 2) using vegan v2.6–6.1 (Oksanen et al., 2024). The differences in gene copy numbers among samples in regard to specific ARGs and MGEs detected via HT-qPCR were tested using the non-parametric Kruskal-Wallis test (Kruskal.test). Network analysis was performed for predicting the taxonomic origin of ARGs and MGEs. Prior to network inference, ASVs occurring in <4 samples were excluded from the dataset to prevent zero inflation. Network inference was done with Spearman correlations. The significance of the correlation coefficient was corrected for multiple comparisons, and only significant correlations were considered in further analysis. Hence, a positive correlation indicates co-occurrence between an ARG and a taxon, and a strong correlation points towards a higher likelihood of the taxon to be the ARG-host (Nguyen et al., 2021). This produced a very clear picture with all significant correlation coefficients $>|0.9|$. Networks were visualized using igraph (Csardi and Nepusz, 2006).

3. Results

3.1. Gut- and cast-associated processes reduce the potential risk of ARG dissemination during vermicomposting of sewage sludge

In terms of composition, the ARG profile in the raw sludge comprised aminoglycoside resistance genes with an average relative abundance of 16 % (Fig. 1A), followed by multidrug (9 %), MLSB (6 %) and tetracycline genes (4 %). Genes conferring resistance to sulfonamides and beta-lactamases accounted for only 1 % of the ARGs in the sludge (Fig. 1A). Heavy metal resistance genes represented in average 8 % of the sludge mobilome and those related to insertional sequences accounted for 36 % (Fig. 1A). In comparison, genes encoding transposases (7 %), MGEs (7 %) and integrases (4 %) were less abundant in the initial sludge (Fig. 1A).

Marked changes in the ARG copy number were observed after transit of the sewage sludge through the earthworm guts (GAPs), with large decreases in the relative abundance of most targeted ARGs in the fresh casts (Fig. 1A). In this regard, the abundances of the genes encoding resistance to aminoglycosides ($p = 0.007$), MLSB ($p = 0.005$) and tetracyclines ($p = 0.007$) were about 3-, 10-, and 5-times lower in the egested materials than in the initial sludge (Fig. 1A). After gut transit, lower abundances were also registered for the genes conferring resistance to beta-lactamases ($p = 0.04$), glycopeptides ($p = 0.005$), multidrug ($p = 0.024$), phenicols ($p = 0.009$) or rifamycins ($p = 0.015$). Moreover, cast-associated processes (CAPs) led to a significant decrease in the relative abundances of rifamycin ($p = 0.015$) and diaminopyrimidine ($p = 0.032$) resistance genes (Fig. 1A).

Focusing on specific ARGs classified as of high risk to human health by the World Health Organization, we found that potential high-risk

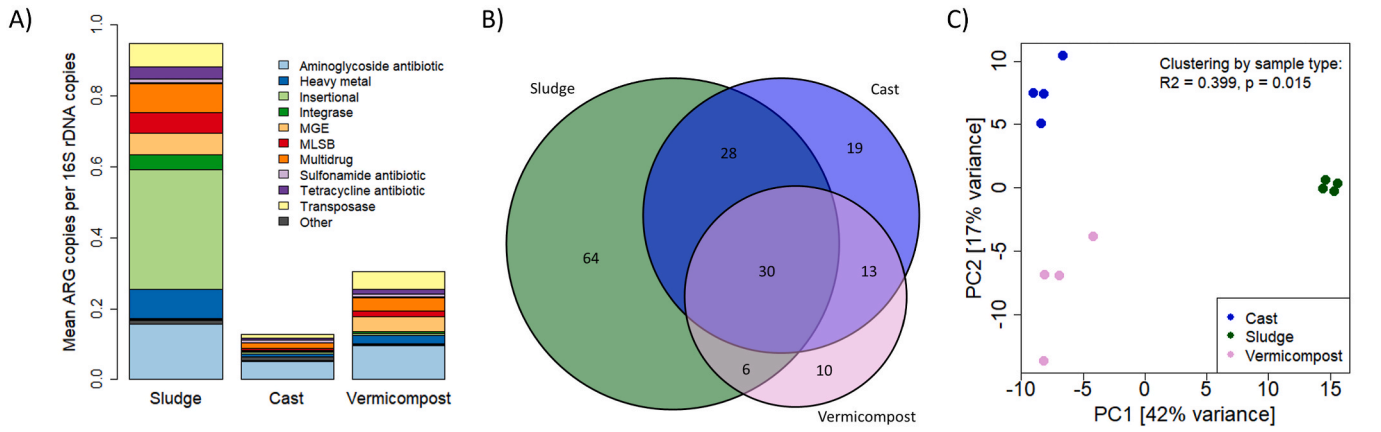

Fig. 1. Changes in antibiotic resistance gene profiles during vermicomposting of sewage sludge. (A) Relative abundance of the ARGs and MGEs detected in the raw sludge, cast and vermicompost samples (3 months old). (B) Venn diagram illustrating the number of resistance genes across sample groups (raw sludge, cast and vermicompost). (C) Changes in beta-diversity composition based on the targeted ARGs and MGEs in the raw sludge, cast and vermicompost samples illustrated by principal component analysis. The targeted genes were classified on the basis of the antibiotic classes to which they confer resistance. MLSB: macrolide-lincosamide-streptogramin; MGE: mobile genetic element.

genes encoding glycopeptide resistance, like *vanYD*, and also the genes *dfrA1* and *dfrA17*, which are involved in diaminopyrimidine resistance (Zhang et al., 2019), were not detected in the egested casts, and they were less abundant in the vermicompost than in the untreated sludge (Table 2). The relative abundance of the *tetM* gene, which confers resistance to tetracyclines, was also significantly lower in the earthworm-processed samples than in the initial sludge (Table 2).

In addition to ARGs, the relative abundances of genes conferring resistance to heavy metals ($p = 0.005$), and those encoding for MGEs ($p = 0.018$) and insertional sequences ($p = 0.045$) were markedly reduced in the earthworm casts (Fig. 1A), with a 9-, 24- and 97-fold decrease when compared to the untreated sludge. Those encoding for integrases ($p = 0.018$) also exhibited an 8-fold reduction in the vermicompost with respect to the initial sludge (Fig. 1A).

In line with the observed differences in terms of abundance, the number of resistance genes also differed among sample types as shown in the Venn diagram (Fig. 1B), with the untreated sludge harbouring greater numbers than the earthworm-processed samples (Fig. 1B). A total of 128 resistance genes were detected in at least two out of the three technical replicates of each sludge biological sample (Fig. 1B). Whilst this number was reduced to 90 and 59 in the fresh casts and the vermicompost samples (Fig. 1B), of these, 21 and 12 referred to distinct MGE subtypes, respectively (Table S2). Among others, they included genes encoding for insertional sequences (i.e., *IS1111*, *IS1133*, *orf 37. IS26*, *orf 39.IS26*, *ISAba3.Acineto*, *ISPps1.pseud*, and *ISSm2.Xanthob;* Table S2); as well as for integrases (i.e., *intl1_337old* and *intl2*; Table S2); and transposases (i.e., *tnpA* genes; Table S2).

Altogether, the abovementioned differences in relative abundance and number led to clustering of the samples based on their ARG and MGE patterns, as reflected by the principal component analyses (Fig. 1C). The first two axes explained 59 % of the total variance, with the raw sludge samples all clustering quite closely together on the positive side of the first component and separately from the cast and vermicompost samples, which were grouped apart from each other along the second axis (Fig. 1C).

Table 2
Variations in the relative abundances of specific genes considered as potentially high risk to human health by World Health Organization (in Zhang et al., 2019) among the initial sewage sludge, cast and vermicompost samples (3 months old). Relative abundances are expressed as the average value of the copy numbers of each gene normalized to the absolute 16S rRNA gene copy number. Standard deviation is given in parenthesis. Significant differences among samples for the targeted genes were tested by the non-parametric Kruskal test. Letters discriminate significantly different sample groups after Bonferroni correction. The relatively high standard deviation is a result of applying detection limits for the genes tested.

Gene target	Antibiotic class	Sludge	Cast	Vermicompost	*p-value*
ermB	MLSB	9.54E-04[a] (9.35E-05)	4.60E-05[a] (1.78E-05)	1.33E-04[a] (2.67E-04)	0.017
dfrA1	Diaminopyrimidines	1.41E-04[b] (3.67E-05)	0[a]	5.43E-05[ab] (1.09E-04)	0.041
dfrA17	Diaminopyrimidines	1.39E-04[b] (4.38E-05)	0[a]	5.09E-05[ab] (1.02E-04)	0.041
tetM	Tetracyclines	1.95E-03[b] (5.11E-04)	9.75E-05[a] (6.93E-05)	1.63E-04[a] (3.27E-04)	0.017
sul 1	Sulfonamides	8.10E-03[a] (9.44E-03)	2.91E-03[a] (7.82E-04)	2.93E-03[a] (5.85E-03)	0.465
vanYD	Glycopeptides	3.20E-04[b] (7.32E-05)	0[a]	1.48E-05[ab] (1.73E-05)	0.010

MLSB: macrolide-lincosamide-streptogramin.

3.2. Potential bacterial hosts of ARGs and MGEs during vermicomposting of sewage sludge

The potential ARG hosts were inferred from the correlation between the relative abundance of the ARGs (based on HT-qPCR) and of the bacterial taxa (obtained using 16S rRNA sequencing). Following this rationale, a positive correlation indicates co-occurrence between an ARG and a taxon, and a strong correlation indicates a higher likelihood of the taxon to be the ARG-host (Nguyen et al., 2021). In the present study, Firmicutes were strongly and positively correlated with the *aadA* aminoglycoside-resistance gene and the insertional gene IS21-ISAs29 (Fig. 2; Table S3). Members of other bacterial phyla such as Proteobacteria, Bacteroidota, and Campilobacterota were also strongly and positively correlated with other MGE marker genes (i.e. *cro* and *IncN_korA* genes, Table S3; Fig. 2). In addition, ASVs assigned to Proteobacteria and Bacteroidota were positively associated with genes

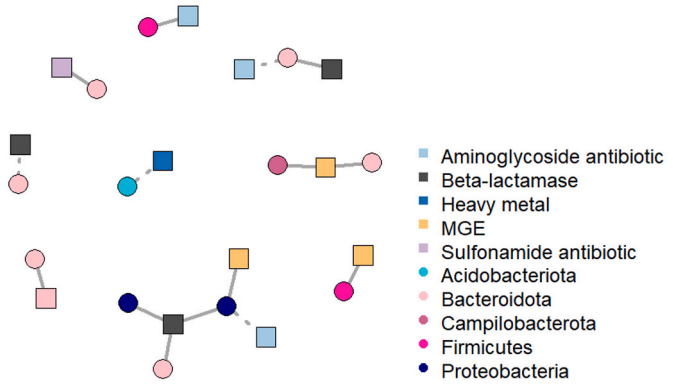

Fig. 2. Network analysis illustrating the positive and negative associations between the bacterial community and the relative abundance of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), respectively. Each circle represents an ASV referring to the bacterial community composition determined by Illumina amplicon sequencing; each square represents a gene (ARGs and MGEs) quantified by qPCR. The colours of the symbols represent the phylum annotation and drug class, respectively. Positive and negative Spearman correlation coefficients are indicated as lines and dashed lines, respectively. The square symbol represents the absolute counts of Bacteroidota measured by qPCR. The correlation between total counts and Bacteroidota ASV supports the validity of the inference method.

conferring resistance to beta-lactamases (i.e. *blaSFO* and *IMP beta--lactamase* genes; Table S3, Fig. 2). The Bacteroidota were negatively correlated with *FOX beta-lactamase* and *AAC(3)-XA* genes (Table S3). Likewise, ASVs belonging to Proteobacteria and Actinobacteriota were negatively correlated with the aminoglycoside- and heavy metal-resistance genes known as *ACC(6′)-IIa* and *arsA* genes (Table S3), respectively.

4. Discussion

The present study broadens our view of variations in the resistome during vermicomposting of sewage sludge by simultaneously considering 384 genes directly or indirectly associated with antibiotic resistance. In this context, we detected a lower relative abundance and smaller number of antibiotic resistance genes in the fresh egested materials from *E. andrei* than in the raw sludge, including some specific genes classified as of potentially high risk to human health. Among them, the t*etM* gene is known to confer resistance to tetracyclines by encoding a ribosomal protection mechanism and, previous quantitative PCR-based studies have shown the effectiveness of vermicomposting in the rapid removal of sludge-borne tetracycline-resistance genes in the presence of *E. fetida* (Huang et al., 2018; Xia et al., 2019; Cui et al., 2022; Guo et al., 2024; Xing et al., 2024). For instance, Cui et al. (2022) reported a remarkable decrease in the relative abundance of *tetM* on day 10th of vermicomposting and, in line with our findings, they primarily ascribed such a reduction to the earthworms' gut associated processes (GAPs) and the concomitant shifts in microbial community composition of sludge. Following this, we also reported other potential high-risk genes such as *dfrA1* and *dfrA17* that encode resistance to diaminopyrimidines (Manna et al., 2021), to be absent in the egested casts further underlining the potential of GAPs as a major shaper of the sludge resistome. This is consistent with previous works on saprophagous insect larvae (i.e., *Protaetia brevitarsis* in Du et al., 2022; and *Pachnoda sinuata* in Gómez-Brandón et al., 2024) underpinning the importance of gut structure, microenvironment, and microbial communities in mitigating the dispersal of this type of ARGs.

More specifically, this could be the result of several processes taking place during gut associated processes that may influence the fate of human pathogenic bacteria, considered as the major carriers and disseminators of ARGs. On the one hand, the reduction of some bacterial taxa due to competition and digestion in earthworm guts can be accompanied by a significant shift in the composition of pathogenic bacteria and the attenuation in their abundance as demonstrated by Aira et al. (2022, 2024) in a vermicomposting trial with *E. andrei*. Other plausible mechanisms are related to the direct elimination of human pathogenic bacteria through the production of bacteriostatic, bactericidal, and fungicidal compounds in the earthworm gut (Fang et al., 2023). On the other hand, an increase in the earthworm guts' microbiota diversity and richness might act as a biological barrier helping to decrease the risk of the dissemination of ARGs (Du et al., 2022). To this end, the fitness cost of carrying ARGs are likely to be more constrained to the host taxa when microbial communities are highly diverse (Reding-Roman et al., 2017). Taken together, the abovementioned plausible mechanisms might have contributed to decrease the resistance selective pressure on ARGs and their dissemination potential during the active phase of vermicomposting in the presence of *E. andrei*.

As reflected in the fresh egested casts, the fact that such a reduction in the number and abundance of ARGs was already evident right after the gut transit of *E. andrei*, suggests that the earthworms' density could have played an important role in leading to a quick ARG removal at the active stage of vermicomposting. In line with this, previous trials with *E. fetida* have shown a more pronounced decrease in quinolone resistance genes at higher (150 individuals/kg wet sludge) than lower densities (50 individuals/kg) after a 30-day vermicomposting trial (Cui et al., 2018). The copy numbers of the 16S rRNA gene (a measure of microbial abundance) may directly influence the prevalence of ARGs (Huang et al., 2020). In our study, the 16S rRNA gene was about 1.8 and 2.7 times more abundant in the cast samples (3.72E +05 gene copy number) than in the initial sludge (2.07E +05 gene copy number) and vermicompost (1.36E +05 gene copy number), suggesting that a greater abundance of bacteria appears not to favour the presence of ARGs in the material that the earthworms eject.

At the phylum level, in the present study we found that members of Proteobacteria and Bacteroidota were positively correlated with certain MGE marker genes. Domínguez et al. (2021) reported that these two bacterial phyla were among the most abundant in the fresh casts from *E. andrei*, which might have increased their likelihood to act as potential ARG hosts and be related to MGEs. In the case of Proteobacteria they are also known to carry multiple ARGs and encompass important human pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa* among others (Jiang et al., 2017). In fact, these authors proposed a plausible 'carry-back' mechanism that might facilitate the HGT from actinobacterial ARGs, which make up a large portion of the environmental resistome, to proteobacterial pathogens using conjugative plasmids as the carrier sequence.

During waste conversion, high levels of heavy metals, xenobiotics and other emerging contaminants in the sludge could induce the overexpression of antibiotic resistance functions, and potentially aggravate the spread of ARGs through co-resistance, cross-resistance and co-regulation processes (Zhou et al., 2022). The potential coexistence of ARGs and metal resistance genes could be related to their occurrence within shared DNA segments or the same bacterial host (Wang et al., 2023); and, they can be easily incorporated into MGEs and transferred across microorganisms through bacterial colonization and horizontal gene transfer (Zhang et al., 2020). Hence, the control of heavy metals and other waste-borne bio-hazardous contaminants is also key to help mitigate antibiotic resistance issues in vermicomposting systems (Fang et al., 2023). Our results demonstrated that vermicomposting with *E. andrei* was effective in reducing the abundance of heavy metal resistance genes through the GAPs which might lead to a lower selective pressure on ARGs over the course of the process, and hence in the vermicompost compared to the untreated sludge. The ability of certain metal resistance genes to persist in vermicomposts can be related to a trade-off between the fitness disadvantage imposed by ARG-carrying bacteria and the escalating selection pressure exerted by bioavailable heavy metals as decomposition progresses (Zhang et al., 2020; Zhou and

Li, 2024). In this regard, the transfer of heavy metals to earthworm body could offer a dual advantage, that is to reduce the resistance selective pressure throughout the process and, to decrease the dissemination potential when the vermicompost is applied as a soil amendment in the long term (as reviewed by Fang et al., 2023). However, the underpinning mechanisms regulating the fate of heavy metal resistance genes via earthworms' GAPs and CAPs processes need further exploration and cannot be fully disentangled within the context of our study.

Horizontal gene transfer is a major route of ARG proliferation, and MGEs such as plasmids, integrases, transposons and integrons frequently act as vehicles facilitating the efficient spread of ARGs in different organisms, including clinically important human pathogens (Haudiquet et al., 2022). As shown for ARGs, the genes coding for distinct MGEs were also less abundant (in relative and absolute terms) in the egested casts than in the initial sludge within the context of our study. Such a reduction was observed for genes encoding for insertional sequences like for instance the targeted genes *ISPps1.pseud* and *ISSm2.Xanthob* that displayed the highest abundance in the studied sewage sludge; as well as for class I and II integrase genes (i.e., *intl1_337old* and *intl2*). Due to their properties and mobility, integrons can capture, convert and adapt one or more resistance gene cassettes between a wide variety of pathogenic and nonpathogenic bacterial species (Gillings et al., 2015). Besides, there is also the possibility for MGEs to cluster with ARGs and form mobile multidrug-resistant units that are capable of spreading among commensals, pathogens and even environmental bacteria (Jiang et al., 2017). This is more likely to occur under increased selective pressure caused by the presence of environmental contaminants (Gillings, 2014).

Once earthworms have digested the waste, the egested materials start aging via the cast-associated processes (CAPs; Aira et al., 2019; Muñiz-González et al., 2023), with lower rates of microbial activity being registered during the maturation stage of vermicomposting (Gómez-Brandón et al., 2021). This reduced activity at the end of the process might hamper bacterial cell clustering and mobility, likely diminishing the possibility of horizontal gene transfer in the vermicompost matrix. Considering this together with the fact that the final vermicompost consists of aged casts, we would expect a similar or lower ARG/MGE load in the vermicompost than in the egested casts. Our results are consistent with this rationale since we did not find significant differences between the cast and the vermicompost samples from *E. andrei* in terms of abundance for most of ARG categories, as well as for MGEs. Major differences were indeed recorded when compared these earthworm-processed materials with the untreated sludge.

As a matter of fact, as the composition of sewage sludge is highly variable owing to the different methods used in WWTPs, future work considering the performance and magnitude of vermicomposting for ARG removal from different types of sludge is warranted. From a methodological viewpoint, further isolation of bacterial strains with selective culture media, along with their characterization from a phenotypic and genotypic perspective also remains a subject of interest to widen our knowledge about the antibiotic resistance of keystone bacterial groups at the different stages of vermicomposting (Wang et al., 2023). By combining culture-based and culture-independent methods we could achieve a more holistic view of the ARG patterns during the process and, in turn, in the final vermicompost prior to its use as a soil amendment. In addition, integrating shot-gun DNA sequencing in combination with databases like the Comprehensive Antibiotic Resistance Database (CARD2023; Alcock et al., 2023) in future studies could help to further understand the underlying molecular mechanisms involved in the associated pathways of selected genes related to the antibiotic resistome during vermicomposting. The above-mentioned database hosts over 5000 resistance determinants, an untenable number for PCR assays, and its use could pave the way towards overcoming limitations inherent to the amplification-dependent methods. For instance, because of the specificity of PCR, there is little chance of detecting distantly related genes as a single nucleotide substitution may eliminate any signal from the PCR assay (Nguyen et al., 2021; Wang et al., 2023).

Another future research direction requires more detailed studies about the potential host range and the genetic transferability of the various ARGs in order to confirm the link between ARGs and the respective hosts (Wang et al., 2023). This is partly due to the inability to identify the natural bacterial hosts of ARGs and the mobile genetic elements that mediate this spread, such as plasmids and integrons. To this end, metagenomic assembly and binning approaches could help to unveil the genetic location of ARGs and to reconstruct partial or complete genome of ARGs hosts (Zhao et al., 2020).

Furthermore, it has been reported that fungi exhibited a significantly higher frequency of genomic traits associated with both antibiotic tolerance, antibiotic production, and competitive abilities (Egidi et al., 2019). The fungal community, like the bacterial community, was found to be an important driver of ARG patterns in the gut of collembolans and isopods (Wang et al., 2021; Xiang et al., 2022). Nevertheless, up to now the influence of fungal communities on the antibiotic resistome has been unexplored when compared to bacteria, which are considered as the direct ARG hosts. This aspect should be addressed in future vermicomposting studies considering that fungi may affect bacterial communities in both direct and indirect ways (Zhang et al., 2014; Shirakawa et al., 2019) by, for instance, providing nutrients to enrich specific bacteria that can act as ARG hosts; by producing secondary metabolites with antibacterial activity; by competing with bacteria for nutrient resources which could lead to changes in the community composition; or by taking up freely available DNA, such as the plasmids that contained ARGs.

5. Conclusion and outlook

Our findings provide key insights into the feasibility of vermicomposting as an environment-friendly approach for the biological stabilization of sewage sludge in a pilot-scale system. The earthworm species *E. andrei* was capable of lowering the relative abundance and absolute number of a broad spectrum of genes encoding major antibiotic classes, heavy metals, and mobile genetic elements via gut- and cast-associated processes. These attenuation effects on ARGs and MGEs in the presence of *E. andrei* point towards vermicompost to be less prone to horizontal gene transfer than the raw sludge. From a soil perspective, this can be translated into a lower risk of ARG propagation within the soil-plant system upon application of sludge vermicompost. In sum, these findings provide solid evidence of the potential *of E. andrei* for bioremediation applications to redeem value to organic wastes that are chemically contaminated like sewage sludge; as well as, for biomanufacturing purposes as a rapid means of converting large quantities of waste into bio-based, high-value products (i.e., improved fertiliser) for agricultural and horticultural systems. It is therefore advisable to consider the return of nutrients through recycled material via vermicomposting for the transition of extensive to sustainable agriculture. In light of this, future field-scale studies focused on mid-to long-term effects of sludge vermicompost on the soil resistome and how this might influence crop health would be necessary to propel the vermicomposting technology forward and achieve the transition toward a net zero-waste process.

CRediT authorship contribution statement

María Gómez-Brandón: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis. **Manuel Aira:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Maraike Probst:** Writing – review & editing, Visualization, Validation, Data curation. **Ning Liu:** Writing – review & editing, Methodology. **ZhiJian Zhang:** Writing – review & editing. **Yong-Guan Zhu:** Writing – review & editing, Methodology. **Jorge Domínguez:** Writing – review & editing,

Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research has received funding from the Spanish Ministerio de Ciencia e Innovación (PID 2021-124265OB-100 and TED 2021-129437BeI00) and the Xunta de Galicia (ED431C 2022/07).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.125562.

Data availability

Data will be made available on request.

References

Aira, M., Pérez-Losada, M., Domínguez, J., 2019. Microbiome dynamics during cast ageing in the earthworm *Aporrectodea caliginosa*. Appl. Soil Ecol. 139, 56–63. https://doi.org/10.1016/j.apsoil.2019.03.019.

Aira, M., Pérez-Losada, M., Crandall, K.A., Domínguez, J., 2022. Host taxonomy determines the composition, structure, and diversity of the earthworm cast microbiome under homogenous feeding conditions. FEMS Microbiol. Ecol. 98, 1–10. https://doi.org/10.1093/femsec/fiac093.

Aira, M., Garrido-Maestu, A., Prado, M., Domínguez, J., 2024. Earthworm activity reduces bacterial pathogen loads in sewage sludge. Environ. Sci. Pollut. Res. 31, 61959–61966. https://doi.org/10.1007/s11356-024-35358-4.

Alcock, B.P., Huynh, W., Chalil, R., et al., 2023. Card 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Res. 51, 690–699. https://doi.org/10.1093/nar/gkac920.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, M.W., Johnson, A.J.A., Holmes, S.P., 2016. Dada2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research. Inter. J. Comp. Syst. 1695. https://igraph.org.

Cui, G., Li, F., Li, S., Bhat, S.A., Ishiguro, Y., Wei, Y., Yamada, T., Fu, X., Huang, K., 2018. Changes of quinolone resistance genes and their relations with microbial profiles during vermicomposting of municipal excess sludge. Sci. Total Environ. 644, 494–502. https://doi.org/10.1016/j.scitotenv.2018.07.015.

Cui, G., Bhat, S.A., Li, W., Wei, Y., Kui, H., Fu, X., Gui, H., Wei, Ch, Li, F., 2019. Gut digestion of earthworms significantly attenuates cell-free and-associated antibiotic resistance genes in excess activated sludge by affecting bacterial profiles. Sci. Total Environ. 691, 644–653. https://doi.org/10.1016/j.scitotenv.2019.07.177.

Cui, G., Fu, X., Bhat, S.A., Tian, W., Lei, X., Wei, Y., Li, F., 2022. Temperature impacts fate of antibiotic resistance genes during vermicomposting of domestic excess activated sludge. Environ. Res. 207, 112654. https://doi.org/10.1016/j.envres.2021.112654.

Chen, Q., An, X., Li, Hu, Su, J., Ma, Y., Zhu, Y.-G., 2016. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environ. Int. 92–93, 1–10. https://doi.org/10.1016/j.envint.2016.03.026.

Domínguez, J., Ferreiro, A., Velando, A., 2005. Are *Eisenia fetida* (savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Oligohaeta, Lumbricidae) different biological species? Pedobiologia 49, 81–87. https://doi.org/10.1016/j.pedobi.2004.08.005.

Domínguez, J., Aira, M., Crandall, K.A., Pérez-Losada, M., 2021. Earthworms drastically change fungal and bacterial communities during vermicomposting of sewage sludge. Sci. Rep. 11, 15556. https://doi.org/10.1038/s41598-021-95099-z.

Domínguez, J., 2023. State-of-the-art and new perspectives on vermicomposting research: 18 years of progress. In: Mupambwa, H.A., Horn, L.N., Mnkeni, P.N.S. (Eds.), Vermicomposting for Sustainable Food Systems in Africa. Sustainability Sciences in Asia and Africa. Springer, Singapore, pp. 27–44.

Du, S., Zhang, Y., Shen, J.-P., Hu, H.-W., Zhang, J., Shu, C., He, J.-Z., 2022. Alteration of manure antibiotic resistance genes via soil fauna is associated with the intestinal microbiome. mSystems 7, e00529. https://doi.org/10.1128/msystems.00529-22, 22.

Egidi, E., Delgado-Baquerizo, M., Plett, J.M., Wang, J., Eldridge, D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., 2019. A few Ascomycota taxa dominate soil fungal communities worldwide. Nat. Commun. 10, 2369. https://doi.org/10.1038/s41467-019-10373-z.

European Commission, Directorate-General for Communication, 2020. Circular Economy Action Plan – the European Green Deal. Publications Office. https://data.europa.eu/doi/10.2775/458852.

Fang, R., Liu, X., Zheng, Z., Lv, B., Wang, J., Su, Y., Xie, B., Wu, D., 2023. Can antibiotic resistance genes in household food waste be reduced by earthworm vermicomposting? Underpinning mechanisms and strategies. Rev. Environ. Contam. Toxicol. 261, 1. https://doi.org/10.1007/s44169-023-00025-1.

Gillings, M.R., 2014. Integrons: past, present, and future. Microbiol. Mol. Biol. Rev. 78, 257–277. https://doi.org/10.1128/MMBR.00056-13.

Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.G., 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. ISME J. 9, 1269–1279. https://doi.org/10.1038/ismej.2014.226.

Gómez-Brandón, M., Martínez-Cordeiro, H., Domínguez, J., 2021. Changes in the nutrient dynamics and microbiological properties of grape marc in a continuous-feeding vermicomposting system. Waste Manage. (Tucson, Ariz.) 135, 1–10. https://doi.org/10.1016/j.wasman.2021.08.004.

Gómez-Brandón, M., Beesigamukama, D., Probst, M., Klammsteiner, T., Zhou, Y., Zhu, Y. G., Tanga, C., 2024. Garden fruit (*Pachnoda sinuata* L.) accelerates recycling and bioremediation of animal waste. Waste Manage. (Tucson, Ariz.) 173, 131–140. https://doi.org/10.1016/j.wasman.2023.11.019.

Gómez-Roel, A., Aira, M., Domínguez, J., 2024. Vermicomposting enhances microbial detoxification of sewage sludge, enabling potential application of the treated product in agroecosystems. Appl. Sci. 14, 7894. https://doi.org/10.3390/app14177894.

Guo, H., Li, Z., Sun, X., Xing, M., 2024. Impact of earthworms on suppressing dissemination of antibiotic resistance genes during vermicomposting treatment of excess sludge. Bioresour. Technol. 406, 130991. https://doi.org/10.1016/j.biortech.2024.130991.

Haudiquet, M., de Sousa, J.M., Touchon, M., Rocha, E.P.C., 2022. Selfish, promiscuous and sometimes useful: how mobile genetic elements drive horizontal gene transfer in microbial populations. Philos. Trans. R. Soc. B 377, 20210234. https://doi.org/10.1098/rstb.2021.0234.

Huang, K., Xia, H., Wu, Y., Chen, J., Cui, G., Li, F., Chen, Y., Wu, N., 2018. Effects of earthworms on the fate of tetracycline and fluoroquinolone resistance genes of sewage sludge during vermicomposting. Bioresour. Technol. 259, 32–39. https://doi.org/10.1016/j.biortech.2018.03.021.

Huang, K., Xia, H., Zhang, Y., Li, J., Cui, G., Li, F., Bai, W., Jiang, Y., Wu, N., 2020. Elimination of antibiotic resistance genes and human pathogenic bacteria by earthworms during vermicomposting of dewatered sludge by metagenomic analysis. Bioresour. Technol. 297, 122451. https://doi.org/10.1016/j.biortech.2019.122451.

Hulsen, T., 2021. BioVenn – an R and Python package for the comparison and visualization of biological lists using area-proportional Venn diagrams. Data Sci. 4, 51–61. https://doi.org/10.3233/DS-210032.

Jiang, X., Ellabaan, M.M.H., Charusanti, P., Munck, C., et al., 2017. Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. Nat. Commun. 8, 15784. https://doi.org/10.1038/ncomms15784.

Junior, S.F.S., da Silva, E.O., Araujo, G.F., Soares, L.O.S., Parente, C.E.T., Malm, O., Saggioro, E.M., Correia, F.V., 2022. Antioxidant system alterations and biological health status of earthworms following long-term exposure to antibiotic-contaminated poultry litter. Environ. Sci. Pollut. Res. 29, 23607–23618. https://doi.org/10.1007/s11356-021-17599-9.

Li, W., Bhat, S.A., Wei, Y., Li, F., Tanaka, S., 2023. Occurrence, fate and behavior of antibiotic resistance genes in organic wastes during biological treatments and current elimination strategies. In: Huang, K., Bhat, S.A., Cui, G. (Eds.), Fate of Biological Contaminants during Recycling of Organic Wastes. Elsevier, pp. 1–22.

Manna, M.S., Tamer, Y.T., Gaszek, I., et al., 2021. A trimethoprim derivative impedes antibiotic resistance evolution. Nat. Commun. 12, 2949. https://doi.org/10.1038/s41467-021-23191-z|.

Muñiz-González, A.B., Mendes, L.A., Gómez-Brandón, M., Quintela-Sabarís, C., Rupani, P., Domínguez, J., 2023. Earthworms and microplastics: transport from sewage sludge to soil, antibiotic-resistance genes, and soil remediation. In: Huang, K., Bhat, S.A., Li, F., Kumar, V. (Eds.), Earthworm Technology in Organic Waste Management. Elsevier, pp. 207–220.

Nguyen, A.Q., Vu, H.P., Nguyen, L.N., Wang, Q., Djordjevic, S.P., Donner, E., Yin, H., Nghiem, L.D., 2021. Monitoring antibiotic resistance genes in wastewater treatment: current strategies and future challenges. Sci. Total Environ. 783, 146964. https://doi.org/10.1016/j.scitotenv.2021.146964.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro-Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J., 2024. _vegan: community Ecology package_. R Pack. Version 2.6–6.1. https://CRAN.R-project.org/package=vegan.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

R Core Team, 2024. _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reding-Roman, C., Hewlett, M., Duxbury, S., Gori, F., Gudelj, I., Beardmore, R., 2017. The unconstrained evolution of fast and efficient antibiotic-resistant bacterial genomes. Nat Ecol. Evol. 1 (3), 50. https://doi.org/10.1038/s41559-016-0050.

Seleiman, M.F., Santanen, A., Mäkelä, P.S.A., 2020. Recycling sludge on croplands as fertilizer – advantages and risks. Resour. Conserv. Recycl. 155, 104647. https://doi.org/10.1016/j.resconrec.2019.104647.

Shirakawa, M., Uehara, I., Tanaka, M., 2019. Mycorrhizosphere bacterial communities and their sensitivity to antibacterial activity of ectomycorrhizal fungi. Microb. Environ. 34, 191–198. https://doi.org/10.1264/jsme2.ME18146.

Su, J.Q., Wei, B., Ou-Yang, W.-Y., Huang, F.-Y., et al., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge composting. Environ. Sci. Technol. 49, 7356–7363. https://doi.org/10.1021/acs.est.5b01012.

USEPA, 1996. Method 3050B: Acid Digestion of Sediments, Sludges, Soils. USEPA. Revision 2.

Wang, Y.F., Qiao, M., Duan, G.-L., Li, G., Zhu, D., 2021. Insights into the role of the fungal community in variations of the antibiotic resistome in the soil collembolan gut microbiome. Environ. Sci. Technol. 55, 11784–11794. https://doi.org/10.1021/acs.est.0c08752.

Wang, J., Xu, S., Zhao, K., Song, G., Zhao, S., Liu, R., 2023. Risk control of antibiotics, antibiotic resistance genes (ARGs) and antibiotic resistance bacteria (ARG) during sewage sludge treatment and disposal: a review. Sci. Total Environ. 877, 162772. https://doi.org/10.1016/j.scitotenv.2023.162772.

White, A., Hughes, J.M., 2019. Critical importance of a one health approach to antimicrobial resistance. EcoHealth 16, 404–409. https://doi.org/10.1007/s10393-019-01415-5.

Wolters, B., Fornefeld, E., Jechalke, S., Su, J.-Q., Zhu, Y.-G., Sørensen, S.J., Smalla, K., Jacquiod, S., 2019. Soil amendment with sewage sludge affects soil prokaryotic community composition, mobilome and resistomes. FEMS Microbiol. Ecol. 95, fiy193. https://doi.org/10.1093/femsec/fiy193.

Wu, Y., Deng, S., Wang, X., Thunders, M., Qiu, J., Li, Y., 2023. Discovery and mechanism of action of a novel antimicrobial peptide from an earthworm. Antimicrob. Chemother. 11, e03206. https://doi.org/10.1128/spectrum.03206-22, 22.

Xia, H., Chen, J.Y., Chen, X.M., Huang, K., Wu, Y., 2019. Effects of tetracycline residuals on humification, microbial profile and antibiotic resistance genes during vermicomposting of dewatered sludge. Environ. Pollut. 252, 1068–1077. https://doi.org/10.1016/j.envpol.2019.06.048.

Xiang, Q., Chen, Q.-L., Yang, X.-R., Li, G., Zhu, D., 2022. Microbial multitrophic communities drive the variation of antibiotic resistome in the gut of soil woodlice (Crustacea:Isopoda). Environ. Sci. Technol. 56, 15034–15043. https://doi.org/10.1021/acs.est.2c02471.

Xing, M., Zhao, R., Yang, G., Li, Z., Sun, Y., Xue, Z., 2024. Elimination of antibiotic-resistant bacteria and resistance genes by earthworms during vermifiltration treatment of excess sludge. Environ. Sci. Pollut. Res. 31, 7853–7871. https://doi.org/10.1007/s11356-023-31287-w.

Zhang, M., Silva, M., Maryam, C.D., van Elsas, J.D., 2014. The mycosphere constitutes an arena for horizontal gene transfer with strong evolutionary implications for bacterial-fungal interactions. FEMS Microbiol. Ecol. 89, 516–526. https://doi.org/10.1111/1574-6941.12350.

Zhang, A.-N., Gaston, J.M., Dai, C.L., Zhao, S., Poyet, M., Groussing, M., Yin, X., Li, L.-G., van Loosdrecht, M.C.M., Topp, E., Gillings, M.R., Hanage, W.P., Tiedje, J.M., Moniz, K., Alm, E.J., Zhang, T., 2019. An omics-based framework for assessing the health risk of antimicrobial resistance genes. Nat. Commun. 12, 4765. https://doi.org/10.1038/s41467-021-25096-3.

Zhang, M., He, L.-Y., Liu, Y.-S., Zhao, J.-L., et al., 2020. Variation of antibiotic resistomes during commercial livestock manure composting. Environ. Int. 136, 105458. https://doi.org/10.1016/j.envint.2020.105458.

Zhao, R., Yu, K., Zhang, J., et al., 2020. Deciphering the mobility and bacterial hosts of antibiotic resistance genes under antibiotic selection pressure by metagenomic assembly and binning approaches. Water Res. 186, 116318. https://doi.org/10.1016/j.watres.2020.116318.

Zhou, L., Li, S., Li, F., 2022. Damage and elimination of soil and water antibiotic and heavy metal pollution caused by livestock husbandry. Environ. Res. 215, 114188. https://doi.org/10.1016/j.envres.2022.114188.

Zhou, Y., Li, Q., 2024. Preference and regulation mechanism mediated via mobile genetic elements for antibiotic and metal resistomes during composting amended with nano ZVI loaded on biochar. Environ. Pollut. 358, 124520. https://doi.org/10.1016/j.envpol.2024.124520.

Zhu, Y.-G., Johnson, T.A., Su, J.-Q., Qiao, M., Guo, G.-X., Stedfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. USA 110, 3435–3440. https://doi.org/10.1073/pnas.1222743110.

Zhu, D., Xiang, Q., Yang, X.R., Ke, X., O'Connor, P., Zhu, Y.G., 2019. Trophic transfer of antibiotic resistance genes in a soil detritus food chain. Environ. Sci. Technol. 53, 7770–7781. https://doi.org/10.1021/acs.est.9b00214.

Zhu, D., Ding, J., Yin, Y., Ke, X., O'Connor, P., Zhu, Y.-G., 2020. Effects of earthworms on the microbiomes and antibiotic resistomes of detritus fauna and phyllospheres. Environ. Sci. Technol. 54, 6000–6008. https://doi.org/10.1021/acs.est.9b04500.