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A large-scale field investigation revealing the distribution characteristics of arsenic in earthworm tissues

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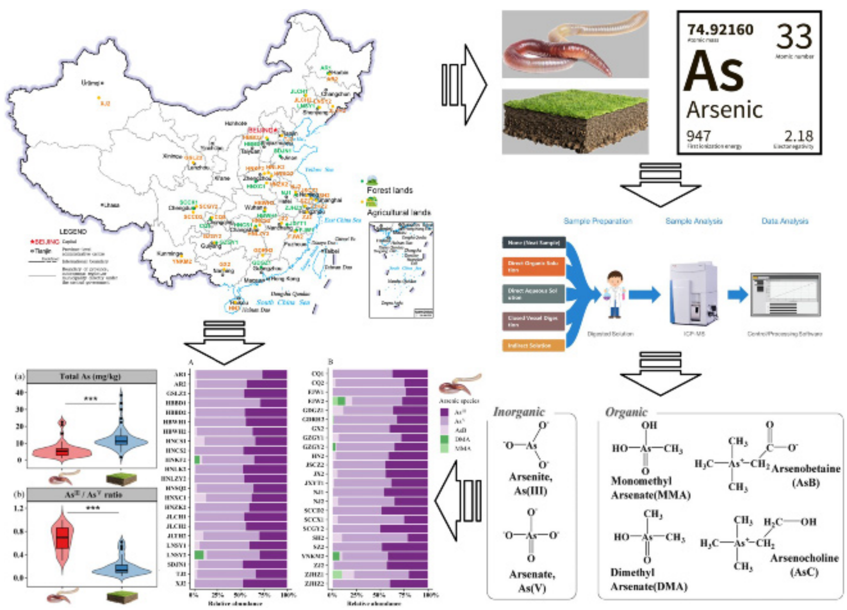
ABSTRACT



• Arsenic characteristics in earthworms and soils across 47 sites in China were studied.
• Earthworm tissues showed lower arsenic levels than surrounding soils.
• Higher arsenite to arsenate ratio was observed in earthworm tissues.
• Positive correlation of arsenic levels in earthworm tissues with soil nitrate.

The total arsenic (As) and As species of earthworm body tissues and surrounding soils were investigated in 47 locations (16 forested lands and 31 agricultural lands) at a national scale across China using inductively coupled plasma-mass spectrometer (ICP-MS) and high-performance liquid chromatography-inductively coupled plasma-mass spectrometer (HPLC-ICP-MS). Earthworm body tissues had an average total As concentration of 6.21 mg kg^{-1}, significantly lower than the soil As concentration of 12.99 mg kg^{-1}. The ratio of arsenite to arsenate (As^{III}/As^{V} ratio) in earthworm body tissues (67%) was significantly higher compared to that in surrounding soils (19%). HPLC-ICP-MS analysis detected small amounts of organic As forms, such as arsenobetaine (2.9%), dimethylarsinic acid (1%), and monomethylarsonic acid (0.3%), mainly in earthworm tissues from certain locations. The total As content and As^{III}/As^{V} ratio in earthworm tissues exhibited a strong positive correlation with soil NO_3^- content. This field study enhances our understanding of As concentration and speciation in earthworm body tissues across China, contributing valuable insights into the biogeochemical cycle of As and its biological risks in diverse soil ecosystems. These findings provide crucial evidence for policymakers to formulate strategies addressing and mitigating soil As pollution and associated health risks.

Keywords arsenic characteristics, earthworm body tissues, surrounding soils, HPLC-ICP-MS, China

1 Introduction

Arsenic (As) is a pervasive toxic metalloid element commonly present in the natural environment, it generally does not pose an environmental risk when it occurs naturally and becomes concentrated in soil through geological processes (Campbell and Nordstrom, 2014). Unfortunately, there has been an observed increase in the spread of As in recent years, primarily stemming from human activities, including mineral resource extraction, the application of As-based pesticides, and the irrigation of As-contaminated wastewater, resulting in pollution in both water bodies and soil (Hartley et al., 2013; Podgorski and Berg, 2020;

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Shi et al., 2023). Once introduced into the environment, As continues to accumulate in the soil, exerting adverse impacts not only on the growth and development of plants and animals but also posing a significant threat to human survival and health by entering the human body through the food chain (Zhu et al., 2014; Wang et al., 2024). The toxicity of As is recognized to be contingent on both its total concentration and chemical species (Zhu et al., 2017). Generally, inorganic As species exhibit greater toxicity than their organic counterparts, with arsenite (As^{III}) being considered more toxic than arsenate (As^{V}) among inorganic forms. Methylated organic species, including arsenobetaine (AsB), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), are relatively less toxic compared to other forms (Xue et al., 2021). AsB was typically regarded as non-toxic (Popowich et al., 2016). Hence, it is imperative to monitor both the total concentration of As and its speciation in environmental and biological samples. The chemical forms of As in the environment play a decisive role in shaping its behavior and fate and are intricately linked to its toxicity (Xue et al., 2021).

Soil serves as a critical repository for biodiversity, seamlessly integrating geochemical processes across diverse ecosystems and providing a comprehensive reflection of ecosystem metabolism (Thakur et al., 2020). Consequently, alterations in soil quality stand as reliable indicators for the assessment of long-term sustainability and stability in ecosystems (Wagg et al., 2014). Soil fauna constitutes the most active component within this system, standing out as a pivotal group, serving as a significant reservoir of biodiversity, and making substantial contributions to the maintenance of soil functions and resource utilization (Bardgett and Putten, 2014; Zhu et al., 2021a). Among these soil organisms, earthworms are particularly noteworthy, widely distributed across terrestrial ecosystems and ranking as the largest invertebrates inhabiting soil (Blouin et al., 2013). Within ecosystems, earthworms play crucial roles in fundamental processes, including the decomposition of organic matter (Huang et al., 2020) and nutrient cycling in the soil (Sizmur and Richardson, 2020; Xue et al., 2022). Additionally, they significantly improve soil permeability and foster plant growth (Zhao et al., 2024). Moreover, due to their direct interaction with soil pollutants, earthworms exhibit heightened sensitivity to contaminants, making them quintessential bioindicator species for assessing soil pollution (Dhiman and Pant, 2022; Wang et al., 2024; Zhao et al., 2024). Research has demonstrated that in soils containing elevated concentrations of As compounds, these pollutants can accumulate within earthworm tissues through dermal contact or ingestion, resulting in adverse effects on earthworm survival, biomass, and reproductive capabilities (Xing et al., 2023). Additionally, earthworm activities play a significant role in the redistribution and biotransformation of As within the soil environment (Wang et al., 2019c). Therefore, investigating As characterizations in earthworm body tissues could prove invaluable in understanding a comprehensive and systematic evaluation of As ecological risks and biotransformation in terrestrial ecosystems.

Furthermore, terrestrial invertebrates, particularly earthworms, known for their capacity to accumulate elevated levels of As, represent potential vectors for the transfer of As through trophic levels to higher organisms, influencing terrestrial food chains (Button et al., 2012). Despite growing interest in earthworm biomarkers (Calisi et al., 2013; Wang et al., 2018; Wang et al., 2019b; Wang et al., 2019c), most studies have been conducted in controlled laboratory conditions, with limited investigations in natural field settings. Our research in Chinese forested and agricultural lands aims to address this gap, analyzing total As concentrations and species in surrounding soils and earthworm body tissues at a national scale. The goals of this study include characterizing total As concentrations and species, examining differences between surrounding soils and earthworm body tissues, and exploring potential environmental factors influencing changes in total As concentrations and species. This research contributes crucial insights into As dynamics in terrestrial environments, offering valuable implications for environmental health and management.

2 Materials and methods

2.1 Sample collection

Earthworms and surrounding soil samples were collected from the upper 20 cm of soil, corresponding to the cultivated horizon, across 28 provinces in China. This encompassed 16 forested lands and 31 agricultural lands, with sampling conducted between June 15 and August 20, 2017. The geographical details of the sampling sites are provided in Fig. 1. Each collection consisted of three replicates for both earthworms and soil samples, resulting in a total of 141 earthworm samples and 141 surrounding soil samples (31 agricultural lands $\times$ 3 replicates + 16 forested lands $\times$ 3 replicates = 141 samples). To collect earthworm samples, we initially excavated multiple rectangular blocks of soil (measuring 50 cm in length, 30 cm in width, and 20 cm in height) at each location, placing them on clean white fabric (Zhu et al., 2021b). The soil blocks were then broken down, and all adult earthworms with a fully developed clitellum were carefully handpicked. In each replicate, we gathered 3 to 30 individuals per earthworm species found at the sampling site, amounting to 60 to 240 individuals per site, and 20 to 80 individuals per replicate. The dominant earthworm species and soil types at sampling sites are provided in Table S1. Our focus was exclusively on the predominant

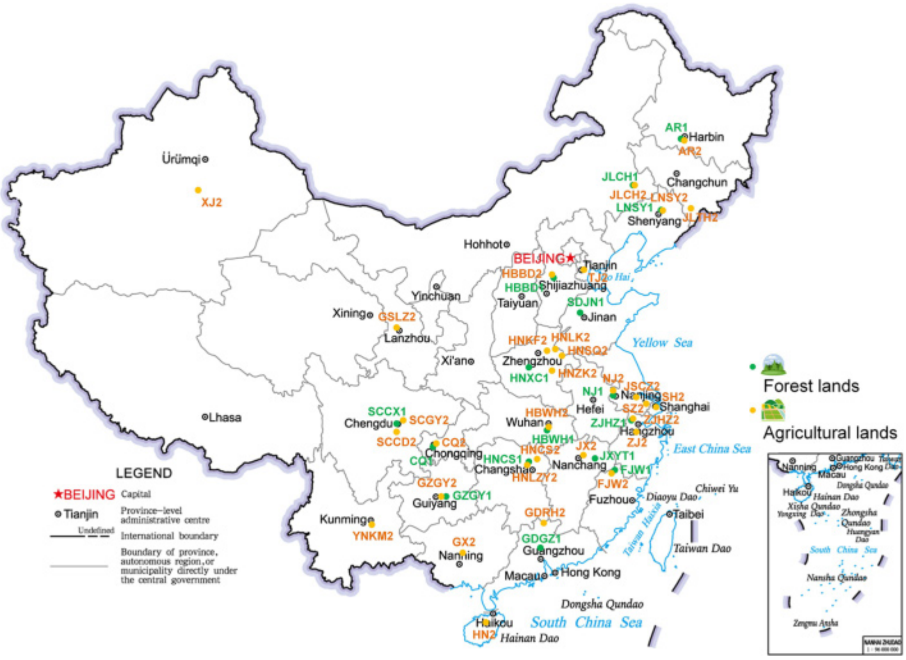

Fig. 1 The distribution of sampling sites across China (A suffix of 1 in the location indicates forest land, while 2 indicates agricultural land).

earthworm species in this study, with species identification conducted through morphological analysis and dominance determined by abundance. Soil samples were collected using a five-point sampling method. Subsequently, soil samples were immediately passed through a 2-mm sieve to remove visible non-soil materials and kept on ice with earthworm samples for less than 3 h before being transferred to the laboratory for a series of physicochemical analyses, total As analysis and As species analysis.

2.2 Determination of soil physicochemical properties

The soil clay content was measured by the wet-sieving method with international and USDA classification systems. Two mol L^{-1} KCl was used to extract soil NO_3^- and NH_4^+ from fresh soil samples (Norman and Stucki, 1981), which were then measured using a complete automatic chemistry analyzer (SmartChem 200, AMS, Italy). Part of the air-dried soil was ground for soil carbon (TC) and nitrogen (TN) content testing, and the dry combustion method with an element analyzer (Solid TOC II, Elementar, Germany) was used to determine the contents (Bao, 2000). The soil pH was measured with a pH meter (Mettler Toledo, Switzerland) in a 2.5:1 water:soil ratio. Electrical conductivity (EC) was determined by employing a conductivity meter (Leici, DDS-307A, China) at a water:soil ratio of 5:1 (weight/volume) after agitating the mixture for 5 min. The concentrations of total chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), cuprum (Cu), zinc (Zn), cadmium (Cd), and plumbum (Pb) in air-dried soils were determined using inductively coupled plasma-Mass Spectrometry (ICP-MS, Agilent 7700, Agilent Technologies, USA).

2.3 Determination of total arsenic concentration and arsenic species

The freeze-dried earthworm body tissues and soil samples were finely powdered by grinding them in an agate mortar with the use of liquid nitrogen before the digestion and extraction of As (Wang et al., 2022). For the analysis of total As concentration, 200 mg of soil (weighed with a precision of 0.1 mg) and 30 mg of earthworm body tissues (weighed with a precision of 0.1 mg) were accurately weighed into 50 mL polypropylene digestion tubes. A mixture of HNO_3 (Merck Millipore, 65%, Darmstadt, Germany) and hydrofluoric acid (HF) (Thermo Fisher Scientific, 49%, USA) in a 5:1 volume-to-volume ratio (6 mL) was added to the soil samples, while a mixture of HNO_3 (Merck Millipore, 30%, Darmstadt, Germany) and H_2O_2 (2:1 volume-to-volume ratio, 6 mL) was added to the earthworm body tissue samples. These mixtures were allowed to stand at room temperature for 2 h before the tubes were sealed with caps and transferred to a microwave-accelerated system (CEM Microwave Technology Ltd., Buckingham, UK). The microwave-assisted digestion for earthworm body tissues in closed tubes was conducted as previously described (Geiszinger et al., 1998). The temperature ramping program for the microwave digestion of soil samples was as follows: 105°C for 20 min, 180°C for 10 min, and 180°C for 30 min. After reaching room temperature, the samples were diluted to 50 mL with Milli-Q water and filtered through 0.45 μm syringe filters (PVDF, Millipore, USA). The As concentrations in the soil and earthworm body tissues were determined using ICP-MS in a collision cell mode to mitigate interference from argon chloride ($^{40}Ar^{35}Cl$) on As (^{75}As). The accuracy of the total As measurements was validated against certified reference materials, specifically GBW07403, GBW07406, and GBW10050, obtained from the National Institute of Metrology of China. These materials had certified As values of 4.4 ± 0.6 mg kg^{-1}, 220 ± 14 mg kg^{-1}, and 2.5 mg kg^{-1} (reference value). Our results showed values of 4.3 ± 0.4 mg kg^{-1}, 216 ± 15 mg kg^{-1}, and 2.4 ± 0.4 mg kg^{-1} (n = 4), respectively. The recovery rates for the CRMs ranged from 90.0% to 108.2%.

As species were extracted from soil and freeze-dried earthworm body tissues using specific solvents. Soil samples (200 mg, with a precision of 0.1 mg) were treated with 5 mL of 0.05 M aqueous ammonium sulfate, while earthworm tissues (30 mg, with a precision of 0.1 mg) were combined with 5 mL of a MeOH (HPLC grade, Thermo Fisher Scientific, USA)/H_2O mixture (1:1 v/v) (Geiszinger et al., 1998). These mixtures were agitated on a rotary wheel at 150 rpm overnight and then centrifuged at 4°C (4754 g for 15 min) to separate the supernatant from the pellet. Soil extracts were filtered through a 0.22 μm filter and stored at −80°C. Methanol-containing extracts were evaporated under a nitrogen stream at room temperature and reconstituted in Milli-Q water. As species were analyzed using HPLC (Agilent 1200, Agilent Technologies, USA) coupled with ICP-MS, employing a PRP-X100 (250 × 4.1 mm length, 10 μm particle size) anion column and a mobile phase with 10 mM diammonium hydrogen phosphate and 10 mM ammonium nitrate at pH 9.25 (adjusted with aqueous ammonia). The analysis covered various As species, including As^{III}, As^{V}, AsB, MMA, and DMA. Quantification was carried out based on peak areas compared to external calibration standards (0, 0.1, 0.5, 1, 5, 10, 50, and 100 μg L^{-1}) containing five As species (As^{III}, As^{V}, AsB, MMA, and DMA). A calibration standard (10 ppb) and a blank were analyzed every 10 samples to ensure instrument stability, as established in a previous study (Xu et al., 2012).

2.4 Data analysis

Statistical differences in As characterization were tested using analysis of pairwise t-tests. Ordinary least squares (OLS) linear regression model was used to test the relationships of the total As content between earthworm body tissues and surrounding soils, and relationships between BFs of earthworms and soil total As content. Redundancy analysis (RDA) was performed to determine the relationship between the physicochemical factors and As characterization in both soils and earthworm body tissues, using the 'vegan' package. Prior to the RDA, an assessment of variance inflation factor (VIF) which tested multicollinearity among selected variables was performed using the "vif.cca" function, and variables exhibiting low multicollinearity (VIF < 10) were retained. Subsequently, the "stepAIC" function was utilized to carry out forward selection, resulting in the selection of NO_3^-, TN, and Zn for inclusion in the RDA model. All statistical analyses were executed in R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria), with a significance threshold of P < 0.05. Data normality was assessed using the Shapiro-Wilk test, and non-normally distributed data were analyzed through appropriate standardization. For data visualization, the "ggplot2" package was employed.

3 Results and discussion

3.1 Total arsenic concentration and arsenic species in earthworm body tissues

The total As concentrations in earthworm body tissues collected from 47 locations ranged from 0.89 to 22.69 mg kg^{-1} (Table S2), with an average level of 6.21 mg kg^{-1} (Fig. 2A). The relatively wide distribution of concentrations emphasizes the variability of As contamination across the studied sites. Notably, the absence of a statistically significant difference between forest and agricultural lands (P > 0.05; Fig. 2C) suggests that both environments contribute similarly to the accumulation of As in earthworm tissues. This finding contradicts conventional expectations, as agricultural lands are often associated with higher anthropogenic inputs of contaminants (Tholley et al., 2023). Examining specific inorganic As compounds in earthworm tissues, As^{III} and As^{V} concentrations ranged from 0.03 to 10.04 mg kg^{-1} and 0.09 to 11.59 mg kg^{-1}, respectively, with average levels of 1.17 mg kg^{-1} and 1.61 mg kg^{-1}. The maximum concentrations of As^{III} and As^{V} in the sample from agricultural land in Lanzhou, Gansu Province, draw attention to localized hotspots of contamination (Table S3; Fig. 3). Such hotspots may be influenced by specific anthropogenic activities or geological features contributing to higher As levels (Huang et al., 2019). Moreover, only in a few sampling locations revealed the presence of small amounts of organic As forms, such as AsB, DMA, and MMA, identified in earthworm body tissues. Interestingly, arsenocholine was not detected (Table S3; Fig. 3A and 3B). The presence of organic As compounds implies potential transformation and biotransformation processes occurring in the environment. However, the limited detection of these compounds might suggest their secondary importance in the overall As contamination scenario. In conclusion, the observed variability in total As concentrations, the absence of a significant difference between land types, and the identification of specific inorganic and organic As forms highlight the complexity of As dynamics

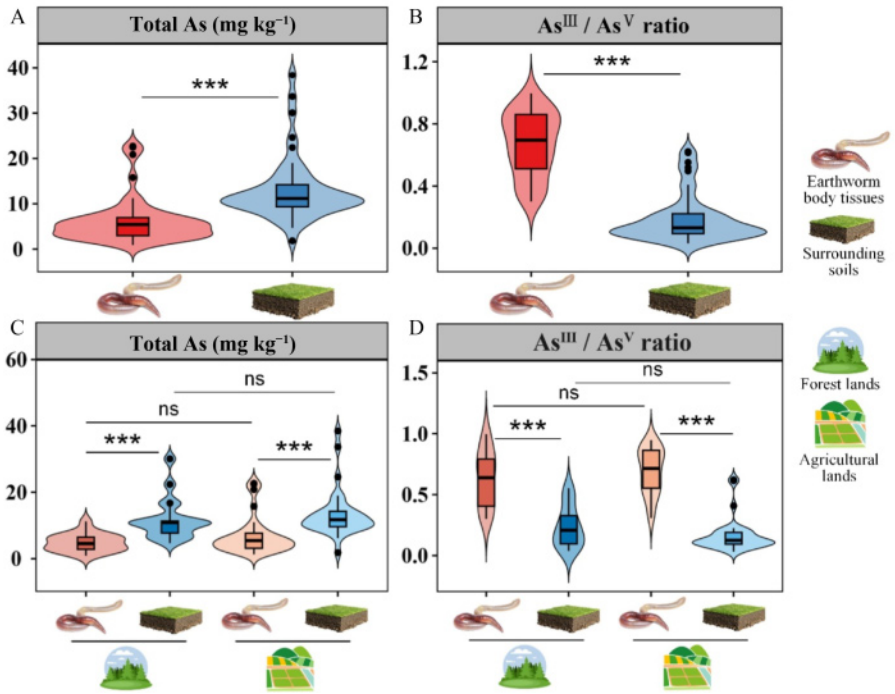

Fig. 2 The abundance of total arsenic content (total As) and the ratio of arsenite content to arsenate content (As^{III}/As^{V} ratio) with an average value between the earthworm and soil across China (A and B), and the comparison of arsenic dynamics between forest land and agricultural land (C and D). Boxplots denote the median value (horizontal line) with hinges representing the 25th and 75th percentiles. Numeric values that using scientific notation above boxplots indicate the significant *P*-values (*, $P < 0.05$; **; $P < 0.01$; ***, $P < 0.001$) based on the two-sided *t*-tests.

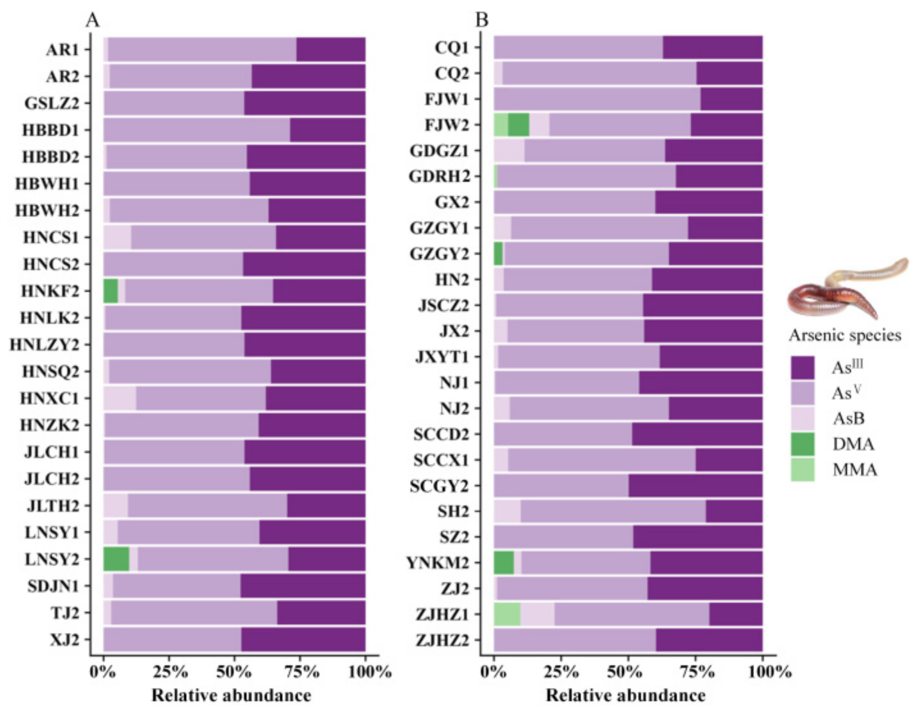

Fig. 3 The relative abundance of arsenic species within earthworm body tissues in the sampling sites across China. As^{III}: arsenite; As^{V}: arsenate; AsB: arsenobetaine; DMA: dimethylarsinic acid; MMA: monomethylarsonic acid.

in earthworm body tissues. The localized hotspots of elevated concentrations warrant further investigation into the contributing factors, facilitating a more nuanced understanding of As contamination in terrestrial ecosystems.

3.2 Comparative analysis of earthworm body tissues and surrounding soils

The total As concentrations in earthworm body tissues were much lower than those in surrounding soils with a range of 1.82 to 38.48 mg kg^{-1} (Table S2). Notably, the agricultural land samples from Lanzhou in Gansu Province (site GSLZ2), Changzhou in Jiangsu Province (site JSCZ2), and Zhoukou in Henan Province (site HNZK2) were exceptions to this trend (Table S2). The average total As concentration in earthworm body tissues was significantly lower compared to that in surrounding soils with an average level was 12.99 mg kg^{-1} across China ($P < 0.001$; Fig. 2A). Similarly, we have observed this trend in both forest and agricultural lands ($P < 0.001$; Fig. 2C). This phenomenon likely stems from As entering the earthworm's system through dietary intake and subsequently being expelled through various routes (Button et al., 2009). Previous studies suggest that As undergoes a complex series of biotransformation processes within the earthworm, resulting in the production of non-toxic or mildly toxic organic forms, such as AsB. These transformed compounds are then excreted through surface secretions, worm excrement, and urine, among other pathways (Wang et al., 2023). Moreover, our earlier investigations revealed a significant accumulation of arsenic transformation genes (e.g., *arsA*, *arsB*, *arsD*) in the earthworm's digestive tract (Wang et al., 2019c). We have a compelling basis to believe that these genes play a pivotal role in facilitating As conversion within the earthworm, ultimately leading to its elimination. In the surrounding soil samples, only the agricultural land sample from Kunming in Yunnan Province (site YNKM2) exhibited an elevated total As concentration, exceeding the As risk screening threshold specified in China's Soil Environmental Quality Risk Control Standard for Contamination of Agricultural Land (GB 15618-2018) within the pH range of 6.5 to 7.5, which is 30 mg kg^{-1} (Table S2). Fortunately, it did not exceed the As risk controlling threshold for soil contamination of agricultural land (GB 15618-2018). Moreover, we noted a consistent uptrend in As content within earthworm tissues as soil As concentrations escalated, particularly at the GSLZ2, JSCZ2, and HNZK2 sites mentioned earlier (Fig. 4A).

The BFs of the earthworms (the ratio of the earthworm tissue concentration to the soil concentration) from locations across China in this study ranged between 0.08 and 2.69, with an average level of 0.53 (Table S2). In general, our research indicates that as the concentration of As in soils increases, the BFs tended to decrease (Fig. 4B), suggesting that As is predominantly autoregulated and can be sequestered by earthworms. However, it is important to note that BFs reported in other studies vary significantly. Button et al. (2011) reported BFs for As in *Lumbricus terrestris* of 0.37 upon exposure to soils contaminated with 16 mg kg^{-1} As in Nottingham, UK. Similarly, Button et al. (2012) found a BF of 0.31 for *Dendrodrilus rubidus* in soils with a high As concentration of 1400 mg kg^{-1}. Watts et al. (2008) reported BFs for *Lumbricus rubellus* and *Dendrodrilus rubidus*, with values ranging from 0.04 to 0.41 and from 0.05 to 0.44, respectively. Inconsistent with our results, these studies all reported BFs below 1, which indicates a low level of As accumulation in earthworms. However, there are reports of higher BFs in certain conditions. For instance, Lee et al. (2013) found that the BFs of *Eisenia fetida* ranged from 0.26 to 1.97 in soils with As concentrations of 18–2297 mg kg^{-1}. Fu et al. (2011) reported that the BFs of *Pheretima aspergillum* collected from the Xikuangshan antimony mine in China could be as high as 6 in soils with As concentrations below 120 mg kg^{-1}. Similarly, Romero-Freire et al. (2015) found that in *Eisenia andrei*, the BFs ranged between 0.83 and 11.1 in soils with As concentrations ranging from 3.4 mg kg^{-1} to 600 mg kg^{-1}. These results collectively suggest that the extent of As accumulation in earthworms may depend on various factors, including soil properties, As mobility, and the specific earthworm species involved. The variability in BFs reported in different studies underscores

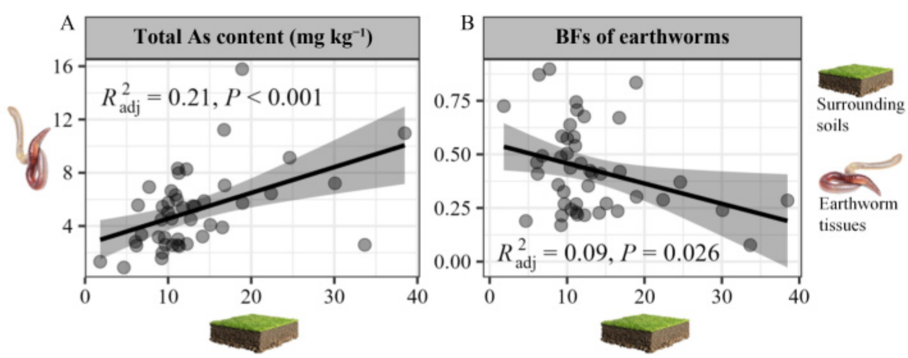

Fig. 4 Relationships of the total arsenic content between earthworm body tissues and surrounding soils (A) and relationships between BFs of earthworms and soil total arsenic content (B).

the need for further research to better understand the complex interactions between earthworms and As in various environmental contexts.

In our research, we exclusively detected inorganic As forms (As^{III} and As^{V}) in the surrounding soils, with average concentrations of 0.22 mg kg^{-1} for As^{III} and 1.46 mg kg^{-1} for As^{V} (Table S3). Notably, the content of As^{V} significantly exceeded that of As^{III}. As is common knowledge, soil represents an aerobic environment with a positive redox potential (pe + pH > 10), which results in the substantial conversion of As^{III} (H_3AsO_3) into As^{V} ($H_2AsO_4^{-}$ and $HAsO_4^{2-}$). Furthermore, the ratio of As^{III} to As^{V} in earthworm body tissues with an average level of 0.67 exhibited a noteworthy increase in comparison to that in the surrounding soils with an average level of 0.19 across China ($P < 0.001$; Fig. 2B). The same results also occurred in both forest land and agricultural land ($P < 0.001$; Fig. 2D). This phenomenon can be attributed to the anaerobic nature of the earthworm gut, characterized by a negative redox potential (Thakuria et al., 2010; Pass et al., 2015; Luo et al., 2024). When earthworms ingest As^{V} from decomposing organic matter, the gut environment, including the gut microbiota, facilitates the reduction of As^{V} to As^{III} during its passage through the earthworm's digestive tract. Previous research on As species in the tissues of the *Eisenia fetida* provides supporting evidence for this process (Lee et al., 2013). Furthermore, studies have indicated that key As biotransformation genes within the earthworm gut are primarily associated with As^{V} reduction and As transport detoxification genes (Zhang et al., 2017; Zhu et al., 2017; Wang et al., 2019c). Consequently, a portion of the more toxic As^{III} becomes assimilated and accumulates in earthworm tissues, resulting in a higher ratio of As^{III} to As^{V} in earthworm body tissues compared to the surrounding soils. In addition, when comparing earthworm body tissues to the surrounding soils, it is noteworthy that the highest As^{III} was 0.96 mg kg^{-1}, observed in an agricultural land sample from Kunming in Yunnan Province (site YNKM2), while the highest As^{V} was 4.92 mg kg^{-1} in the forest land sample from Yingtan in Jiangxi Province (site JXYT1) (Table S3).

3.3 The drivers of total arsenic and arsenic species in earthworm body tissues and surrounding soils

Redundancy analysis (RDA) was performed to explore potential associations between As contents and species in earthworm body tissues, those in surrounding soils and environmental variables (Fig. 5). Significant environmental variables, including soil TN, NO_3^{-}, and Zn, were selected through a forward selection procedure with a variance inflation factor of 999 Monte Carlo arrangement. The first two constrained RDA axes accounted for 37% of the total variance of As contents and species, with the first axis contributing 28.86%. This primary axis showed a positive correlation with soil TN and a negative correlation with soil NO_3^{-} and Zn contents. The second axis exhibited a positive correlation with soil Zn content and a negative correlation with soil TN and NO_3^{-} contents. Notably, total As content and the As^{III}/As^{V} ratio in earthworm body tissues strongly and positively correlated with soil NO_3^{-} content ($P < 0.001$; Fig. 5), highlighting a potential link between nitrogen availability in the soil and As accumulation in earthworms. When earthworms consumed nitrogen-rich organic matter, it furnished abundant carbon and nitrogen sources to the gut microbiota (Zhu et al., 2022). This, in turn, amplified bacterial diversity, fostered growth, and augmented the abundance of As^{V} reduction genes. Consequently, it resulted in the accumulation of As^{III} in both earthworm tissues and the gut (Zhu et al., 2022; Wang et al., 2023). Conversely, total As content and As^{III}/As^{V} ratio in surrounding soils were significantly and positively associated with soil Zn content ($P < 0.01$; Fig. 5). This observation aligns with existing research indicating that the presence of Zn^{2+} enhances the capacity of goethite in soil to adsorb As (Gräfe et al., 2004). Goethite, a common soil mineral, plays a crucial role in controlling As mobility in various climatic regions (Liu et al., 2014). Therefore, our findings emphasize the pronounced correlation between soil zinc content and As levels in surrounding soils. Prior research has revealed a substantial presence of As biotransformation genes in the earthworm gut, with the highest gene abundance associated with the reduction of As^{V} and the transport and efflux of As^{III} (Wang et al., 2023). Simultaneously, previous studies have demonstrated that even at low concentrations, As pollution induces significant alterations in the earthworm gut bacterial community, leading to changes in the abundance of As biotransformation genes within the earthworm. This is particularly evident in the increased abundance of As^{V} reduction genes, resulting in the enrichment of As^{III} in both the earthworm gut and tissues (Wang et al., 2019a; Wang et al., 2019c).

In summary, this study significantly advances our understanding of As concentration and species in earthworms, shedding light on the As biogeochemical cycle in diverse soil ecosystems. The findings are pertinent to soil health assessments, emphasizing the vital role of earthworms in influencing As dynamics. This knowledge informs strategies for managing biological risks associated with As contamination in soils, with broader implications for soil ecology. The insights gained provide a foundation for further investigations into the intricate interplay between soil, biota, and contaminants in terrestrial environments.

4 Conclusions

This study investigated As concentration and speciation in earthworm tissues across China. Earthworm tissues showed

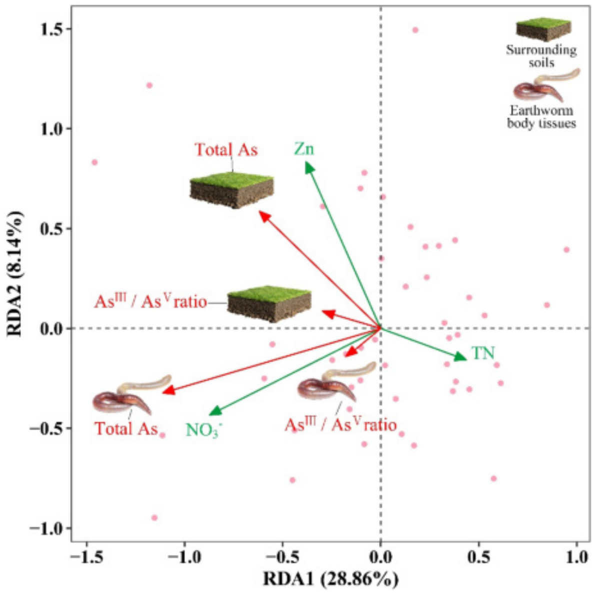

Fig. 5 Redundancy analysis of arsenic characteristics and environmental variables. NO_3^-, soil nitrate nitrogen content; TN, total nitrogen content; Zn, soil total zinc content. Red arrows represented arsenic characteristics in earthworm body tissues and surrounding soils; green arrows represented environmental variables.

an average total As concentration of 6.21 mg kg^{-1}, significantly lower than the soil As concentration of 12.99 mg kg^{-1}. The As^{III}/As^{V} ratio in earthworm tissues was higher than in soils. Only in a few sampling locations revealed the presence of small amounts of organic As forms, such as AsB, DMA, and MMA, identified in earthworm body tissues. Total As content and As^{III}/As^{V} ratio in earthworm tissues correlated positively with soil NO_3^- content. The research contributes to our understanding of As dynamics in earthworm body tissues and surrounding soils, providing insights into the biogeochemical cycle of As and its biological risks in diverse soil ecosystems.

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Conflict of 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.

Compliance with ethics guidelines

The authors affirm that all studies described in the manuscript were carried out in an ethical and responsible manner, adhering fully to all pertinent codes of experimentation and legislation.

Author contributions

S.L. Fu and H.T. Wang conceived the study; D. Zhu collected samples and conducted the arsenic characterization analyses; A. Yang and D. Zhu ran the statistical analyses and wrote the first draft. S.L. Fu, H.T. Wang, D. Zhu, Y.H. Shao, W.X. Zhang, and A. Yang discussed and commented on the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The data, materials and R codes that support the findings of this study are available from the corresponding author upon reasonable request.

Electronic supplementary material

Supplementary material is available in the online version of this article at https://doi.org/10.1007/s42832-024-0235-5 and is accessible for authorized users.

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