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RESEARCH ARTICLE

Eisenia fetida impact on cadmium availability and distribution in specific components of the earthworm drilosphere

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Abstract

Although the potential of vermiremediation for restoring metal-contaminated soils is promising, the effects of earthworms on the availability of soil metals are still debatable. Most previous studies considered the soil as a "whole black box." Mobilization or immobilization of metals are affected by earthworm activities within drilosphere hotspots under different soil conditions, which has not been specifically studied. Therefore, an improved 2D terrarium was designed to study the impact of earthworm activities on cadmium (Cd) fate in the drilosphere hotspots (burrow wall soils, burrow casts, and surface casts) of different artificially spiked Cd treatments (CK: 0 mg kg^{-1}; LM: 1 mg kg^{-1}; and HM: 5 mg kg^{-1}) with different organic amendments (2% and 10%). The results revealed that Cd increased earthworm activities with the highest cast production in HM and the highest burrow length in LM. Earthworms exhibited a stronger tendency to reduce total Cd concentration by 4.48–13.58% in casts of LM soils, while 3.37–5.22% in burrow walls under HM treatments. Overall, earthworms could increase the availability of Cd in casts under all conditions (55.46–121.01%). The organic amendments decreased the total Cd concentration and increased the availability of Cd in the disturbed soil. A higher amount of organic amendment significantly decreased total Cd concentration of the drilosphere by 1.16–5.83% in LM and HM treatments, while increasing DTPA-Cd concentrations in all components by 23.13–55.20 %, 14.63–35.11%, and 3.30–11.41% in CK, LM, and HM treatments, respectively, except for earthworm non-disturbed soil and no-earthworm soil in HM treatments. Redundancy analysis (RDA) revealed that the moisture, pH, and total carbon contents in soil are the main factors affecting Cd bioavailability. In this study, we decoded the "black box" of soil by making it relatively simple to better understand the effects and mechanisms of earthworm activities on soil metal availability and consequently provided comprehensive insights for using earthworms in soil vermiremediation.

Keywords Improved 2D terraria · Drilosphere · Cadmium availability · Soil organic matter · Redundancy analysis

Highlights • The 2D terraria were used to sample different components of the drilosphere.

• Earthworms redistributed Cd in soil through their habit activities.

• Cd bioavailability in casts was higher than that of other drilosphere components.

• Higher organic amendment increased Cd bioavailability of the drilosphere hotspot.

• Soil Cd availability was influenced by moisture, pH, and total Cd and carbon contents of soil.

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Introduction

Soil contamination by metals is a problem of wide concern, especially cadmium (Cd) pollution (Li et al. 2019). Therefore, various remediation processes have been developed, especially biological remediation, which is considered to be a promising approach associated with environment friendly advantages (Gong et al. 2018; Li et al. 2019). Earthworms are regarded as "soil ecological engineers" that can influence soil physical, chemical, and biological properties (Bertrand et al. 2015; Ferlian et al. 2020). What is more, earthworms are known to alter the fate of metals in the soil ecosystem (Sizmur and Richardson 2020; Zhang et al. 2022). Therefore, many studies suggested that vermiremediation, defined as removing or degrading soil contaminants via earthworms, could be an alternative way to facilitate the restoration of Cd-polluted soils (Milleret et al. 2008; Macci et al. 2012; Kaur et al. 2018; Liu et al. 2020b; Zeb et al. 2020; Xiao et al. 2022).

Even though there are many benefits, vermiremediation has not yet been widely applied because the actual effects of earthworms on the availability of Cd (one of the most toxic inorganic pollutants) have not been consistently documented (Sizmur and Hodson 2009; Sizmur and Richardson 2020). Most previous studies considered the soil as a "whole black box." Several studies suggested that earthworm could increase plant absorption of Cd (Wu et al. 2019; Wu et al. 2020), while some findings showed that earthworm had no impacts on $CaCl_2$-extractable Cd of the bulk soil (Lemtiri et al. 2016) or decreased water-soluble Cd (Gomez-Eyles et al. 2011). The meta-analysis conducted by (Sizmur and Richardson 2020) also showed that earthworms have no impact on the Cd availability of bulk soil.

The drilosphere was defined as the internal gut of earthworm and external worm-soil interfaces, including the surface and below-ground casts, and burrow wall soils (Brown et al. 2000; Kuzyakov and Blagodatskaya 2015). The hotspots of the drilosphere (surface casts, burrow casts, and burrow wall soils) are rich in nutrients and microbiomes and consequently exhibits faster element turnover rates (Brown et al. 2000) and closely interact with other biopores, such as the rhizosphere, porosphere, litter system, and aggregatusphere (Brown et al. 2000; Kuzyakov and Blagodatskaya 2015). Earthworm activities could change the distribution of metals in soil by altering the total concentration and bioavailability and ultimately influence the efficiency of soil metal remediation (Xiao et al. 2022) because the drilosphere was one of the key interfaces that control the fate of soil metals (Zhang et al. 2018; Gong et al. 2021; Zhao et al. 2022). However, in situ experiments were rarely conducted to demonstrate how earthworms influence Cd fate in the soil, mainly in the context of drilosphere formed by burrowing, feeding, and excretion activities of earthworms. Therefore, elucidating the distribution of Cd availability in specific components of the drilosphere and the bulk soil is very important to explain the contradictory effects of earthworms on Cd availability.

Additionally, most studies suggested that these varying results are mainly due to the variability in soil properties, such as contamination status, pH, and especially soil organic matter (SOM) content (Sizmur and Richardson 2020; Xiao et al. 2022). The findings of (Sizmur and Richardson 2020) revealed that the metal concentrations in plants decreased with the addition of earthworms in the soil with < 2% SOM contents while it increased in 5–10% and > 10% SOM-amended soil. Most studies proposed that SOM content played an important role on earthworm activities (Zhang et al. 2016a), and earthworms could also accelerate SOM decomposition (Santos et al. 2021). Therefore, it is important to clarify how the interaction between SOM and earthworms will affect Cd availability.

Considering the complex activities of earthworms, it was difficult to accurately obtain the samples from the different drilosphere components with traditional pots or a microcosm box (Heinze et al. 2021). To better understand the impact of earthworms on the availability of metals in the specific components of the drilosphere, an improved 2D terrarium based on the previous studies (Barnett et al. 2009; Felten and Emmerling 2009; Du et al. 2014; Huerta Lwanga et al. 2017; Wang et al. 2021) was applied in this study to explore the cadmium footprints in hotspots mediated by earthworm activities. It is hypothesized that 1) the cast production and the burrowing activities were both important pathways of earthworms affecting soil metal availability and showed different effects and 2) SOM content will affect the intensity and extent of earthworm activities and subsequently impacts the whole "black box" soil Cd availability. The specific aims of this study are to 1) assess the heterogeneity of Cd content, bioavailability, and speciation of different parts of the drilosphere in Cd-contaminated soils and 2) investigate the organic amendments and soil factors that influence Cd availability in this earthworm-mediated ecosystem. This study provides insights into a potential method to study how earthworms affect the metal availability of drilosphere hotspots, which will be useful to predict the comprehensive impact of Cd of the whole bulk soil and assess possible benefits of vermiremediation for soil metal contaminants in practice.

Materials and methods

Substrate, experimental device, and earthworms

Surface soil (0–20-cm depth clay loamy typic-hapli-stagnic anthrosol) was collected from an experimental paddy field in Suzhou City (E120°58′, N31°30′), Jiangsu province, China. Experimental soil and well composted cow manure (as organic amendment to simulate soil organic matter) were air-dried and sieved through a 2-mm mesh prepared for experiment and property determination. The chemical properties of the experimental soil and organic amendment are listed in Table 1. The soil metal contents (Cd, Pb, Cu, and Zn) did not exceed the risk screening values according to the Environmental Quality Standard for Soils in China (GB 15618-2018).

To facilitate the sampling of the surface and below-ground casts and burrow wall soils, the size of the experimental 2D terrarium was re-designed with width, height, and thickness of 25 cm, 35 cm, and 1 cm, respectively, based on the previous studies (Barnett et al. 2009; Felten and Emmerling 2009; Du et al. 2014; Huerta Lwanga et al. 2017; Wang et al. 2021). The three-dimensional view of the experimental

Table 1 Chemical properties of the experimental soil and organic amendment

	pH	TC (g kg^{-1})	TN (g kg^{-1})	C/N	CEC (cmol kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Soil	6.84±0.08	15.01±0.36	0.66±0.06	11.71±0.67	9.53±0.31	0.35±0.06	20.05±0.58	22.12±0.47	58.75±8.4
	Threshold for soil metals[a]					0.6	140	100	250
Organic amendment	7.79±0.02	381.03±4.02	17.47±1.04	21.87±1.49	10.56±0.24	0.46±0.01	3.93±0.30	5.98±0.13	21.26±0.52
	Limit for organic fertilizer metals[b]					3	50	No requirement	

CEC cation exchange capacity, *TC* total carbon, *TN* total nitrogen

Values are expressed as mean ± SD (n=3)

[a]Thresholds refer to the risk control standard for soil contamination of agricultural land (GB 15618-2018)

[b]Limits refer to the national quality standard for organic fertilizer of China (NY525-2012)

device is shown in Fig. S1. The side wall was constructed using a transparent acrylic board to observe and record the burrowing track of earthworms.

The model earthworm species *E. fetida* was used in this study (Tang et al. 2016; Wang et al. 2021). Though *E. fetida* is a typical epigeic species, but able to dig down to a depth of 30 cm, when cultivated in a narrow space and homogeneous medium (Li et al. 2016). Earthworms (*Eisenia fetida*) with similar fresh weight (350–450 mg per individual) were bought from Xinyida farm (E113°30′, N37°27′), Hebei Province, China. All adult earthworm acclimated in the no exogenous Cd-contaminated soil for two weeks with approximately 1% composted cow manure as a feed supply under the condition of temperature 22±2 °C and 12 h of light and 12 h of darkness.

Experiment design and exposure process

The experiment adopted a two-factor multi-level design with three levels of Cd concentration (artificially spiked concentrations of 0 mg kg^{-1} (CK), 1 mg kg^{-1} (LM), and 5 mg kg^{-1} (HM)) with 2% and 10% organic amendment (dry weight, *w/w*) respectively (6 treatments in total). The concentrations of Cd were set according to a previous field study in which the low and high soil Cd contaminations was 1.40 and 6.60 in the historically Cd-contaminated field (Huang et al. 2021a). Then, 5 mg/kg Cd did not affect earthworm mortality (Tatsi et al. 2020; Zhou et al. 2020). Then, 0.5 mL and 2.5 mL of Cd^{2+} stock solution (1 mg Cd mL^{-1}, $CdCl_2 \cdot 2.5H_2O$, Sigma, USA) was diluted with deionized water and added to 50 g of substrate to get highly contaminated soil. The substrate was then air-dried and sieved through 1-mm mesh and mixed with 450 g of clean substrate using a grinder to finally obtain the Cd-spiked exposure substance (LM and HM). The prepared soil mixtures were incubated at the room temperature for 2-weeks to achieve equilibrated aged soil (Tang et al. 2006), then air-dried, and re-sieved through 1-mm mesh (Huang et al. 2020). Each treatment had 9 replicates.

The terraria were laid flat and approximately about 100 g of dry soil was spread evenly, and 20 mL of deionized water was sprayed on the surface of the dry soil to obtain a 20% soil moisture content (approximately 60% maximum water holding capacity). This process was repeated five times until the device was completely full. Then ,the side wall was fixed to the main body of the device using a dovetail clip to easily remove it during soil sampling, and finally, earthworms were introduced into the devices (4 adults of *E. fetida* per terrarium) and the experiment lasted for 28 days. To avoid moisture loss, approximately 5 mL of deionized water was dripped on the soil surface of each terraria every 5 days.

Sampling of earthworms and drilosphere soils

After 28-day incubation, earthworm burrows in soils were photographed. Earthworms were collected from each device after removing the side wall, and the number of live earthworms was counted and washed with deionized water. The biomass of earthworms was determined after gut depuration for 24 h to calculate the mean biomass loss rate (Lai et al. 2021). All earthworms were flash-frozen and stored in the −80 °C refrigerator (SANYO, Japan) for further analysis.

After earthworm removal, different components of soil and casts were sampled according to their shape and distribution as shown in Fig. 1. Surface casts (SC) and belowground casts (BC) excreted in the burrow wall were collected. About 2 mm of burrow wall soil was scraped off as BW sample (Hao et al. 2018; Thu Hoang et al. 2020; Heinze et al. 2021). Soil samples collected 3 cm from the burrow wall was considered as non-disturbed soil (ND). Finally, soil without earthworm incubation was randomly sampled as no-earthworm soil (NE). Conclusively, the drilosphere mainly constituted SC, BC, and BW, and non-drilosphere included ND and NE (Xu et al. 2021). All casts and soil samples were mixed well, fully air-dried, and finally sieved through 0.125-mm and 2-mm meshes for subsequent analysis.

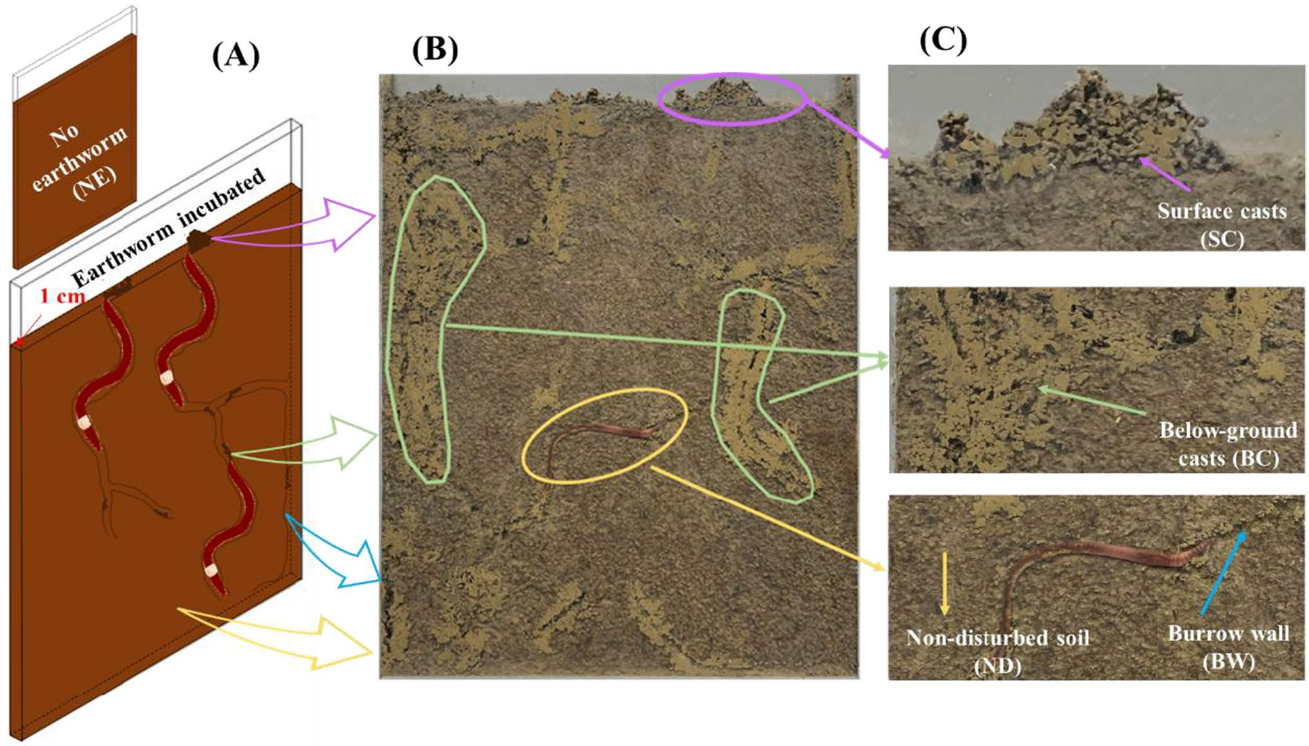

Fig. 1 Schematic representation of earthworm burrow burrows (**A**), different components of soil and casts sampling sites (**B**), and their typical shapes (**C**)

Sample test and analysis

Recording earthworm activity

All BC and SC samples were air-dried and weighed. Pictures of earthworm burrows were taken as described in "Sampling of earthworms and drilosphere soils" (Fig. 1), and the total burrow length was calculated using CAD 2021.

Metal bioaccumulation of earthworms

Freeze-dried earthworms were microwave (MARS-5, CEM Microwave Technology Ltd., USA) digested using a mixture of HNO_3-H_2O_2. The digestion solutions were analyzed for Cd using the ICP-OES (detection limit was 1 ng kg^{-1}). Standard shrimp powder (GBW10050, Cd = 0.039 mg kg^{-1}) was used as the reference sample and measured concentrations were all within 95–100% of certified concentrations, thereby validating the analysis. The values of bioaccumulation factor (BAF) were used to determine the ability of *E. fetida* to accumulate HMs as described previously (Lai et al. 2021):

$$\text{BAF values} = \frac{\text{Cd concentrations in earthworms}}{\text{Cd concentrations in ND}} \quad (1)$$

Metal analysis of different drilosphere soil components

The total Cd concentrations were determined using the ICP-OES after digesting 0.25 g of sieved (0.125 mm) soil sample using the aqua regia extraction (3 M HNO_3 : 2 M HCl (*v/v*) =1 : 3). The blanks and certified reference materials of soil (GSBZ 50014-88, Cd=0.083 mg kg^{-1}) were used to validate the quality of the extraction process (Huang et al. 2021a; Wang et al. 2022a), and the recovery rates for this analysis were between 95 and 105%.

The concentration of DTPA-extractable metal has been widely used to assess the availability of metals to plants and soil organisms (Cheng et al. 2021; Huang et al. 2021b). Briefly, 2.5 g air-dried and ground soil samples (sieved through 2-mm mesh) were digested using 12.5-mL solution of 10 mM $CaCl_2$ and 5 mM diethylenetriaminepentaacetic acid (DTPA) and oscillated at 180 rpm and 25 °C for 2 h. The resulting solution was used to determine Cd concentration using ICP-OES and the blanks were used to validate the extraction quality.

The sequential extraction procedure is generally used in identifying the specific fraction and immobilized progress of HMs. In this work, the three-stage BCR (Community Bureau of Reference) sequential extraction procedure was performed to analyze the fraction of Cd in the contaminated soil. The detailed BCR extraction procedures include four replicates to validate data quality (Huang et al. 2020). Additionally, the residual fraction (F4) was assessed by subtracting the acidic extractable fraction (F1), the reducible fraction (F2), and the oxidizable fraction (F3) from the total concentration.

Chemical properties of different drilosphere components

The total carbon and nitrogen contents of soil were determined using the Vario ELIIICHNSO Elemental analyzer as described previously (Zhan et al. 2021). The moisture content of the soil was determined gravimetrically on a dry weight basis after drying the soil at 105 °C for 24 h. The pH, cation exchange capacity (CEC), and dissolved organic carbon (DOC) concentrations were measured according to the methods described previously (Huang et al. 2020). The concentrations of NH_4^+-N and NO_3^--N in soil were determined using 2 M KCl (1:5 ratio (*w/v*) of soil to KCl) after passing through 0.45-μm filters and analyzed using a continuous flow analyzer (Skalar, Holland). Although these normal factors have been investigated in a limited amount of studies at the drilosphere level, no link has been made to Cd validity (Andriuzzi et al. 2013; Wang et al. 2023b). All eight parameters were analyzed for NE, ND, BW, and BC. Due to the limited production of surface casts, only TC, TN, and moisture content were measured for the components in surface casts.

Statistic analysis

The experimental data are expressed as mean ± standard deviation (mean ± SD). SPSS 26.0 was used for one-way analysis of variance (ANOVA) and three-way ANOVA, and the Tukey HSD method was used to compare the differences among treatments ($P<0.05$). The relationships between Cd availability (DTPA extractable concentrations and Cd speciation percentages and concentrations) and environmental variables (soil properties and total Cd concentration) were determined by multivariate analysis with the CANOCO software package (version 5.0, Biometris). Redundancy analysis (RDA) was chosen and used according to the results of detrended canonical correspondence analysis (DCCA). The angles and projection positions between the arrows of environmental variables and Cd availability variables reveal their correlation coefficients (Shi et al. 2019). All graphs and figures were prepared using Origin 2021 and Excel 2019.

Results

Growth and activities of earthworms

The mortality and mean biomass loss rates of earthworms are shown in Fig. 2A, B. During the experiment, none of the earthworms died in the treatment CK with 2% and 10% organic-amended soils. The mortality rate of LM and HM treatments was 8.33–12.50%. The mean biomass losses of treatment LM were 46.96% and 35.25% with 2% and 10% organic amendment, respectively, which was significantly higher than treatment CK ($P<0.05$).

Cast production (dry weight) and the total burrow length were shown in Fig. 2C, D. The casts in treatment LM were significantly less (47.36% and 30.90%) compared with the control treatment ($P<0.05$), while the casts in HM were significantly more (11.02% and 44.04%) with 2% and 10% organic-amendment, respectively ($P<0.05$). The burrow lengths of LM and HM were 1.57–1.75 and 1.39–1.46 times greater than CK in 2% and 10% organic-amended soils, respectively. Additionally, 10% organic amendment significantly increased cast production but lowered the burrow lengths compared with those in 2% organic amendment ($P<0.05$).

Cd bioaccumulation in earthworms

The mean concentrations of Cd in *E. fetida* and their BAF values after 28 days of incubation are shown in Fig. 3. The uptake of Cd by earthworms was 1.55–2.36, 20.05–24.28, and 150.21–163.00 mg kg^{-1}, and BAF values were 3.60–4.15, 11.96–15.86, and 27.29–28.94 in CK, LM, and HM, respectively. In LM treatments with 10% organic amendment, Cd concentration and BAF value of earthworms treated were 1.21 and 1.33 times higher than those treated with 2% organic amendment ($P<0.05$), while in HM treatments, they were not significantly different between 2 and 10% organic amendment treatments.

Total Cd concentration of drilosphere and non-drilosphere components

The total Cd concentrations in different components of drilosphere and non-drilosphere are shown in Fig. 4. The total Cd concentrations of BC and SC were 0.33–0.36 mg kg^{-1} and 1.27–1.38 mg kg^{-1}, which were significantly lower (18.26–32.10% and 4.48–13.58 %) than that of NE, ND, and BW (0.42–0.48 mg kg^{-1} and 1.37–1.47 mg kg^{-1}) ($P<0.05$) in CK and LM treatments, respectively. In HM treatments, the total Cd concentrations of BW and SC were significantly lower (3.37–5.22% and 4.59–7.49%, respectively) than that of NE and ND ($P<0.05$). Furthermore, the total Cd concentrations of BW in LM and BW, BC, and SC in HM treatments were 1.16–5.83% lower with 10% organic amendment than that with 2% organic amendment ($P<0.05$).

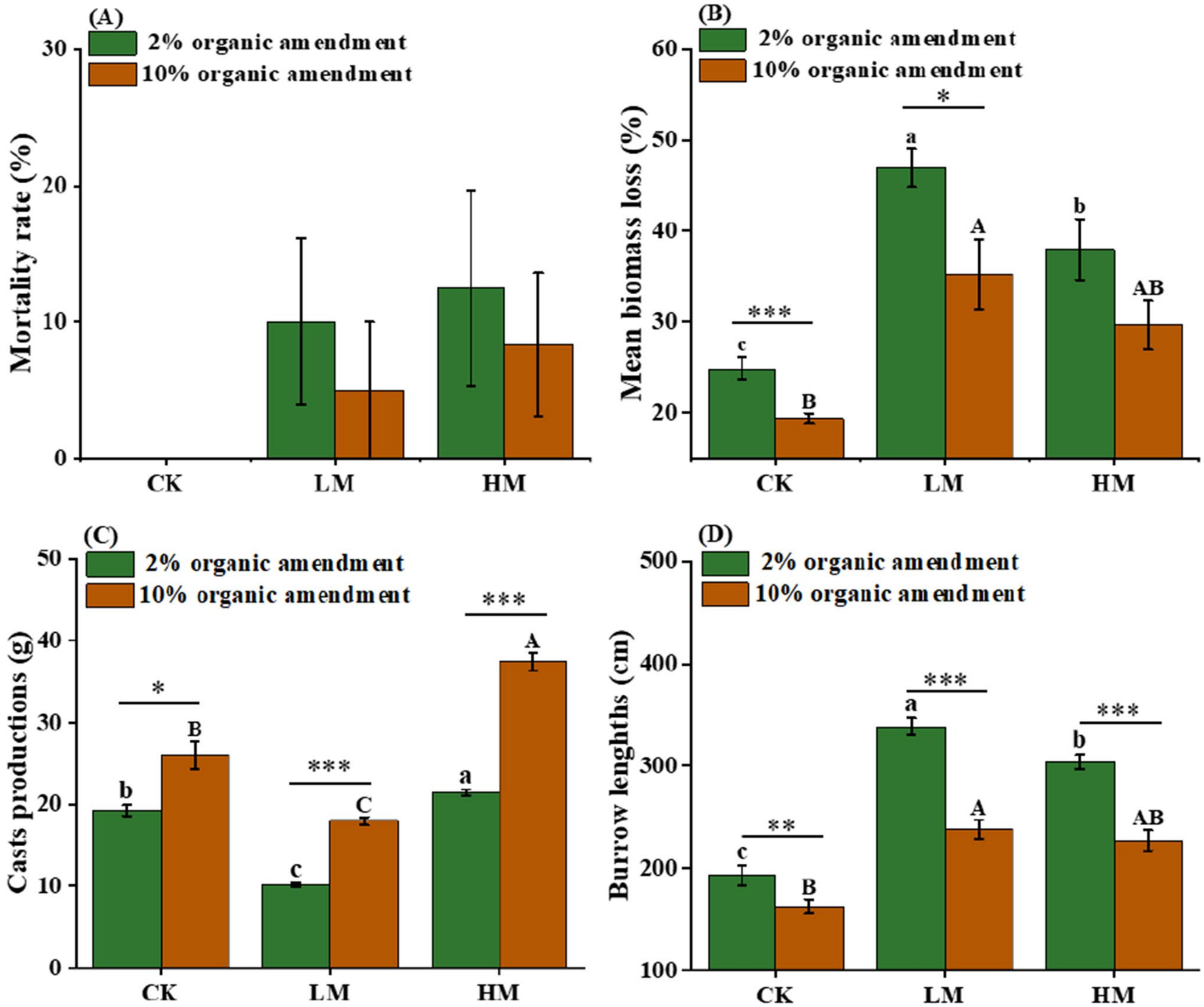

Fig. 2 Growth and activities of earthworms after 28 days of exposure (n=9). CK, LM, and HM represent 0, 1, and 5 mg kg^{-1} Cd addition treatments, respectively. *, **, and *** represent P<0.05, 0.01, and 0.001 for factors effected significantly between 2 and 10% amendment under the same Cd treatment. Different lowercase (a–c) and uppercase letters (A–C) indicate the significant differences (P<0.05) among Cd treatments within the same organic amendment, respectively

Cd availability and chemical fractions of drilosphere and non-drilosphere components

DTPA-extractable Cd contents are used to access Cd availability in different sampling components (Fig. 5). In LM and HM treatments, DTPA-Cd concentrations of BW were the lowest ranging from 0.22 to 1.53 mg kg^{-1} and SC were the highest ranging from 0.57 to 3.19 mg kg^{-1}. All the sampling components exhibited significantly higher DTPA-Cd concentrations ranging from 23.13 to 55.20%, 14.63 to 35.1%, and 3.30 to 11.41% with 10% organic amendment in CK, LM, and HM treatments, respectively, except for NE and ND in HM treatments.

The Cd speciation percentages of different sampling components are shown in Fig. 6. In the control treatments, residual Cd was the main fraction accounting for 28–54%, and in HM treatments, acid-soluble Cd accounted for the largest proportion (46–56%). In LM and HM treatments, the residual Cd content was 12–32% in BW, which was 7–13% higher than that of other components. However, the oxidizable Cd was lowest in BW compared with other components accounting for 4–16%. The percentages of acid-soluble Cd were 13–54% and 15–56% in BC and SC, respectively, which were significantly higher than that of other components (P<0.05).

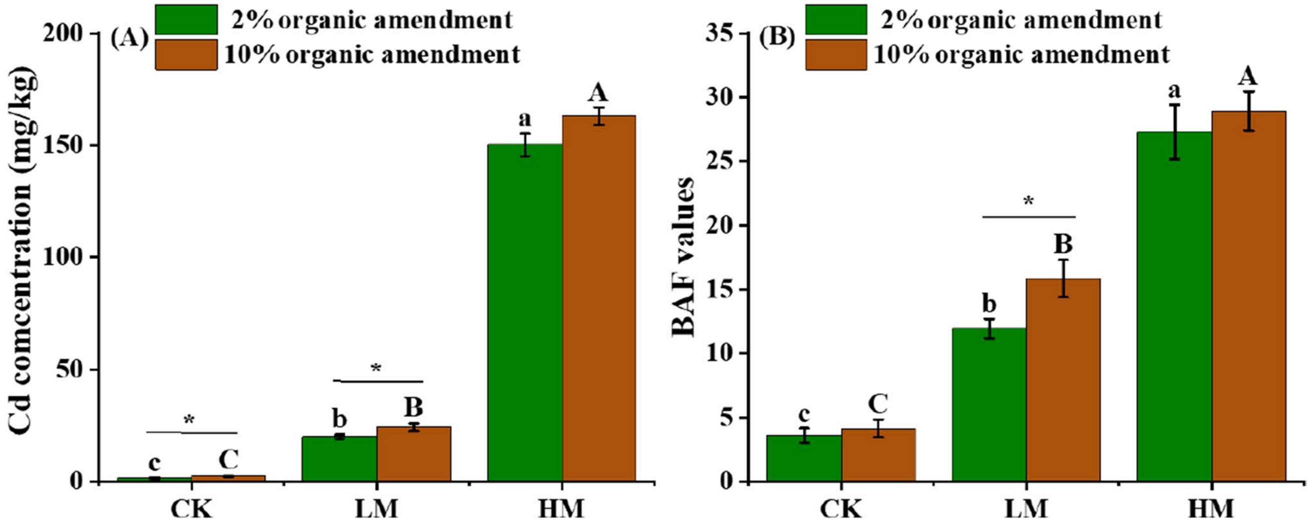

Fig. 3 **A** Cd concentrations in *E. fetida,* and **B** bioaccumulation factors (BAFs) of earthworms after 28 days of exposure (n=9). CK, LM, and HM represent 0, 1, and 5 mg kg^{-1} Cd addition treatments, respectively. * represents P<0.05 for factors effected significantly between 2 and 10% amendment under same Cd treatment. Different lowercase (a–c) and uppercase letters (A–C) indicate the significant differences (P<0.05) among the Cd treatments within the same organic amendment, respectively

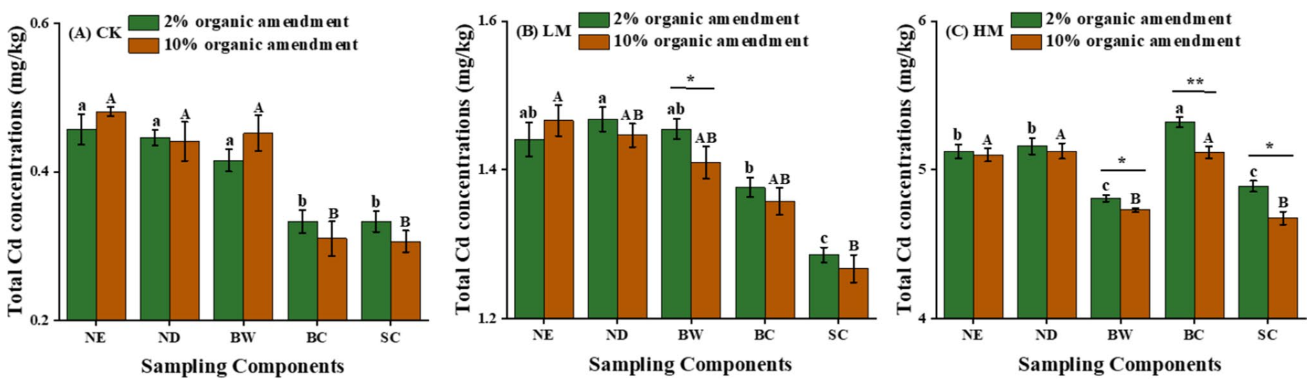

Fig. 4 Total Cd concentrations of different components of soils and casts (n=9). CK, LM, and HM represent 0, 1, and 5 mg kg^{-1} Cd addition treatments, respectively. NE, no-earthworm soils; ND, non-disturbed soils; BW, burrow wall soils; BC, below-ground casts excreted on the burrow wall; SC, surface casts. * and ** represent P<0.05 and P<0.01 for factors effected significantly between the treatments with 2% and 10% organic amendment, respectively. Different lowercase (a–c) and uppercase letters (A, B) indicate the significant differences (P<0.05) among different components within the same organic amendment, respectively

Factors affecting Cd availability

Chemical properties of different components are shown in Table S1. Chemical properties of NE and ND were similar. Among drilosphere components, the chemical properties of SC and BC were significantly different from BW (P<0.05). Specifically, the order of TC concentrations was SC>BC>BW, and for moisture content, it was BC≈BW>SC for all six treatments. The pH values, CEC, DOC, NH_4^+-N, and NO_3^--N of drilosphere components were significantly higher than those of non-drilosphere components (P<0.05), and SC always had the highest level properties among different soil components.

Redundancy analysis (RDA) was performed to determine the potential impacts of soil chemical properties on Cd availability in soil. As shown in Fig. 7, the RDA ranking of soil parameters indicated that the first and second principal components axes contributed 72.8% and 4.6% of the total variance for the factors affecting Cd availability in soil, respectively. The total Cd concentration in soil was the major factor impacting the DTPA-Cd concentration and different chemical fractions of Cd, thereby explaining 70.5% of the variance. Among all soil chemical properties, moisture, pH, and TC were the major factors influencing soil DTPA-Cd concentration and different chemical fractions of Cd, explaining 3.1%, 1.9%, and 1.4% of the

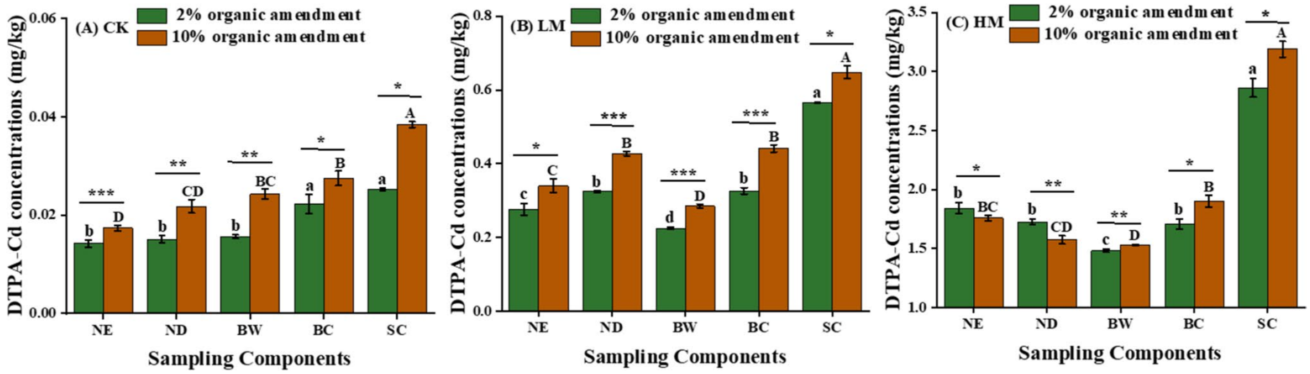

Fig. 5 DTPA extractable Cd concentrations in different components of soil and casts (n=9). CK, LM, and HM represent 0, 1, and 5 mg kg^{-1} Cd addition treatments, respectively. NE, no-earthworm soils; ND, non-disturbed soils; BW, burrow wall soils; BC, below-ground casts excreted on the burrow wall; SC, surface casts. *, **, and *** represent P<0.05, 0.01, and 0.001, respectively, for factors effected significantly between the treatments of 2% and 10% organic amendment at. Different lower case (a–c) and uppercase letters (A, B) indicate the significant differences (P<0.05) among different components within the same organic amendment, respectively

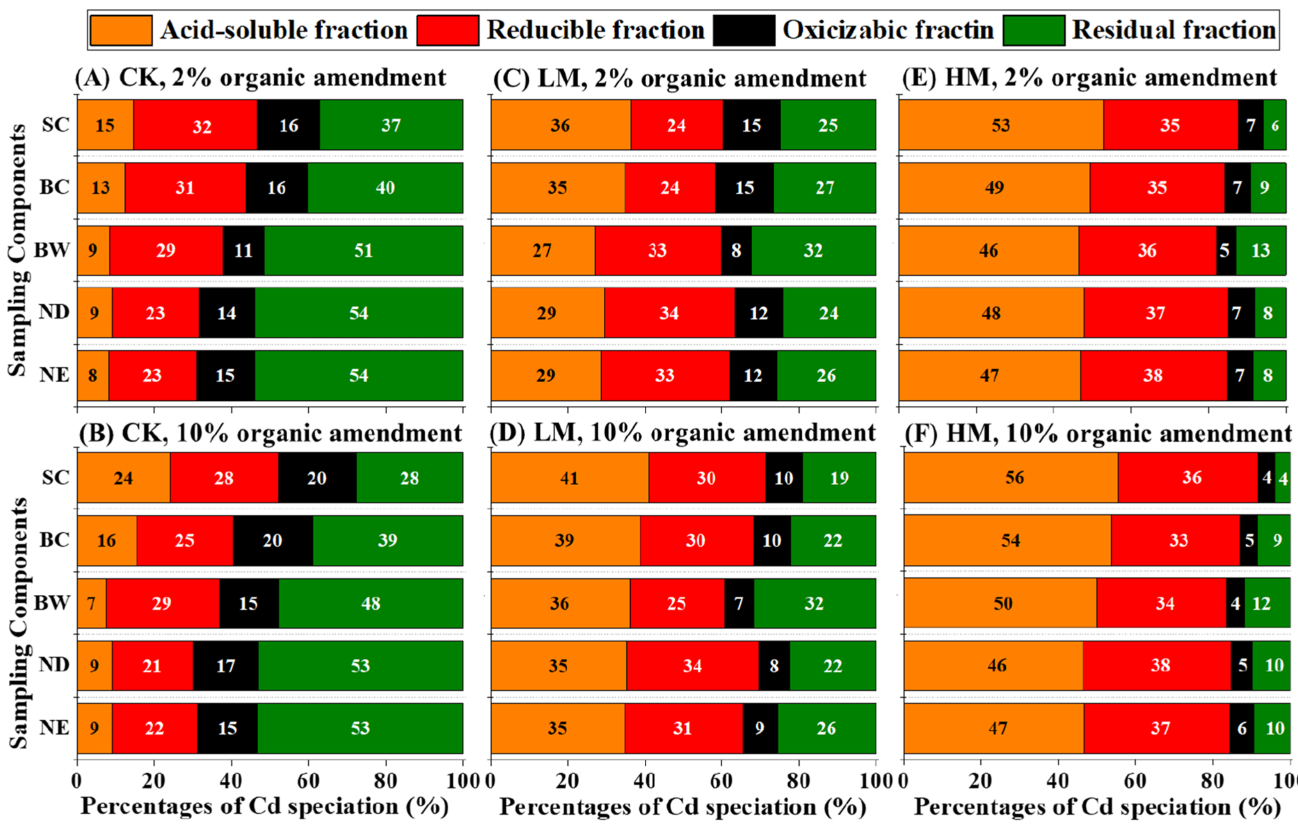

Fig. 6 Percentages of Cd speciation released by BCR sequential extraction. CK, LM, and HM represent 0, 1, and 5 mg kg^{-1} Cd addition treatments, respectively. NE, no-earthworm soils; ND, non-disturbed soils; BW, burrow wall soils; BC, below-ground casts excreted on the burrow wall; SC, surface casts

variance, respectively. Overall, external Cd addition, moisture content, pH, and TC were the main factors influencing Cd availability in soil.

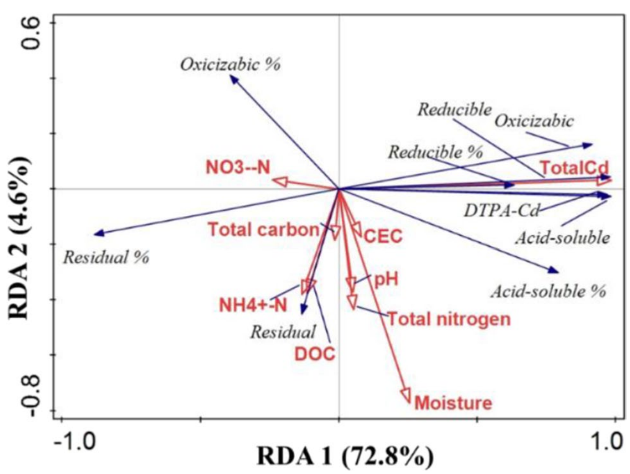

Fig. 7 Redundancy analysis (RDA) between soil Cd availability and chemical parameters of drilosphere and non-drilosphere components. TC, total carbon; TN, total nitrogen; CEC, cation exchange capacity; DOC, dissolve organic carbon

Discussion

Deep into the drilosphere: Cd availability and distribution in different components

As ecosystem engineers, earthworms influence the function of soil ecosystems through their activities in the soil. Soil pollutants can induce behavioral changes in earthworms, which in turn impacts soil function (Wang et al. 2018a; Liu et al. 2020a). To investigate the activities of earthworms and to relatively simply and accurately sample different components of drilosphere in real time under long-term experimental conditions, 2D terraria were utilized in the present experiment improved based on previous studies (Capowiez et al. 2003; Du et al. 2014).

Since the thickness of the device was increased from 2 mm to 1 cm, the burrow trajectories of earthworms were relatively complete, which indicated that the improvement of the device was necessary. Moreover, the maximum sampling amount of casts was up to 3 times higher than the previously conducted pot experiment (Zhang et al. 2016a), which reflected the excretion activities of earthworms more comprehensively. Therefore, the newly designed 2D terraria facilitated the sampling of more casts for further analysis.

With more complex interactions of physicochemical and biological properties both directly and indirectly, the findings of higher Cd bioavailability in SC and BC (Fig. 4) were consistent with most previous studies (Singh et al. 2017; Wang et al. 2018b; Wang et al. 2020), and the gut passage and cast excretion were considered as the main route of increasing metal availability in soil mainly due to the alterations in pH, DOC, or microbial population (Zhang et al. 2016a; Sizmur and Richardson 2020). After being excreted by earthworms in soil along with frequent feeding and excretion activities, native soil may completely turn into wormcast, which could increase the bioavailability and thereby the efficiency of phytoremediation.

In addition to the gut passage, the epidermis is an important mode for earthworms to absorb metals and also influence metal behaviors in soil. In the current study, Cd availability of BW components was decreased (Fig. 5B, C). During burrowing, earthworms secrete mucus to reduce resistance against digging in the soil (Zhang et al. 2016b). The mucus was rich in soluble carbon and nitrogen and could provide energy and a carbon source for microbial growth and the burrowing of earthworms could increase the aeration near the burrow wall, which could together stimulate the growth of Cd resistant microorganisms to immobilize Cd (Cheng et al. 2021). Furthermore, mucus secreted by earthworms could accelerate the humification of substances that could further protect Cd from releasing as soil particles (Sizmur et al. 2010; Sizmur et al. 2011; Huang and Xia 2018; Guhra et al. 2020; Shutenko et al. 2022). Additionally, the decrease of Cd availability in BW under different Cd contamination treatments (LM and HM) might be attributed to high mucus secretion and inhibition of epidermal absorption (Sizmur et al. 2010) under higher metal exposure. However, the mechanisms are unknown and how gut passage and epidermal pathway impact metal bioavailability in the soil is still needed to be studied further from the perspective of SOM molecular characteristics, interaction of mineral-organic matter, and microbial community (Barthod et al. 2020; Ren et al. 2021).

Organic amendment plays important roles in the vermiremediation

Total Cd concentrations of BW in 10% organic-amended soil were significantly lower than that in 2% organic-amended soil for both LM and HM (Fig. 2B, C). From the results of total Cd concentration and burrow length, higher SOM content could lead to a higher burrow reuse rate and further caused stronger epidermal absorption, which in turn resulted in the decrease of Cd concentration in burrow wall soil (Zhang et al. 2018; Wang et al. 2021).

It is well known that organic matter plays an important role in the biogeochemical distribution of metals in soil. In the present study, 2% and 10% organic amendment aimed to simulate low and high organic matter-amended soils and subsequently TC was considered as one of the factors that influence the availability of Cd (Sizmur and Richardson 2020). Additionally, the nitrogen and oxygen atoms in the active functional groups of soil organic matter could form coordination bonds with Cd and for stable complexes to reduce Cd availability (Elyamine et al. 2018; Li et al. 2021; Wang et al. 2023a). Therefore, the changes of TN and DOC,

NH_4^+-N, and NO_3^--N due to the addition of organic matter in the amended soil and decomposition of SOM due to earthworm activities were important factors influencing the availability of Cd. CEC and pH were important factors influencing the adsorption and desorption ability on soil particles of Cd (Sizmur and Richardson 2020; Ribeiro et al. 2022).

In this study, different proportions of organic amendment treatments were performed to simulate soils with different organic carbon and had a positive effect on the availability of metals in soil (Fig. 3), which was contrary to the findings of a previous study (Elyamine et al. 2018). Decomposition and mineralization of organic matter are the dominant processes in soil compared to the humification and adsorption (Bottinelli et al. 2020; Siedt et al. 2021). The higher bioavailability induced by earthworms in soils with higher SOM content might make it more beneficial for combining vermiremediation and phytoremediation of metal pollution in higher SOM-content soils (Sizmur and Richardson 2020; Wang et al. 2022b).

Among soil properties, moisture content was the most important factor identified in the present study (Fig. 7), which was positively correlated with DTPA-Cd and percentages of acid-soluble fraction and negatively correlated with the residual fraction (Table S2). Higher moisture content was observed in the underground cast components which is consistent with previous studies (Wang et al. 2020; Santos et al. 2021). Earthworms can secrete abundant intestinal mucus with high moisture content during feeding (Wang et al. 2020; Santos et al. 2021) which may have contributed to Cd release from the active functional groups of soil organic matter. Although the burrow wall was well aerated, the subsurface of the burrow lining formed by the activity of earthworms still exhibited lower redox potential, and bioavailable metals (Chen and Whalen 2016; Le Mer et al. 2021; Liu et al. 2021).

Implications for vermiremediation

Earthworm activities were affected by Cd contamination of soil as shown in Fig. 2C, D. The production of casts was used as an indicator of behavioral toxicity in this study for the first time as it has previously been used to indirectly reflect the feeding rate of earthworms (Zhang et al. 2016a). Compared to the control treatment, the burrow lengths increased in LM and HM, which was consistent with the results of (Dittbrenner et al. 2010; Duarte et al. 2014). Compared with the burrowing inhibition of the much higher concentration Cd of 45 mg kg^{-1} (Liu et al. 2020a), the increase of burrow length under 1 and 5 mg kg^{-1} Cd concentrations might be due to the hormetic effect, defined as an overcompensated response mechanism of biological homeostasis when exposed to low concentrations of pollutants (Zhang et al. 2009; Velki and Ecimovic 2017). Furthermore, in our study, the moisture content increased in soil with higher Cd levels, which contributed to the increase in the burrow length due to the decreased soil mechanical resistance (Arrázola-Vásquez et al. 2022).

Generally, SOM affected the distribution of earthworms and played an important role in regulating earthworm activities to modulate the stress induced by pollutants (Capowiez et al. 2021). The results of our study indicate that with an increase in the levels of SOM, the capacity of earthworms to produce casts increased, which was in line with previous studies (Zhang et al. 2016a). This result suggests that earthworms exhibit increased ingestion to acquire more energy and nutrients in response to soil toxicant stress (Hobbelen et al., 2006). It is worth noting that, unlike the increase in the production of casts, burrowing was not active under 10% organic-amended soil. Sufficient food for energy around the burrow in higher SOM content soil made earthworms inactive to dig further to get more food, which resulted in less burrow length, higher burrow reuse rate (Wang et al. 2019), and wider burrow diameter (Bottinelli et al. 2017).

Earthworms behave differently in soils with different pollution levels and organic matter content, it is necessary to choose different remediation strategies under different pollution conditions. Under lower Cd contaminated soil, earthworm could build longer burrows with lower Cd availability, this might explain of the decreased availability at the whole "black box" concept, and in this condition, earthworms could be used alone or combined lower organic matter to immobilize soil Cd for remediation by the *in-situ* immobilization strategy. Under higher Cd contaminated soil and 10% organic amendment treatments, earthworms could product more casts with higher Cd availability which was consistent most studies. Therefore, it seems that the combination of vermiremediation and higher organic amendment (crop residuals or compost) with plants (ryegrass and sedum), and even microorganisms will be able to achieve higher remediation efficiency by the extraction strategy (Liu et al. 2021; Xiao et al. 2021; Sun et al. 2022; Wang et al. 2022b).

Except for the selection of remediation strategies, earthworm species also needs to be taken into account. Considering different activities, such as feeding source, burrow type, and the main living soil horizons, different ecotypes of earthworms might impact the element turnover and metal behavior in soil differently, and therefore, it is necessary to conduct further research on earthworms in different ecotypes (Frazão et al. 2019; Hallam and Hodson 2020; Richardson et al. 2020). Based on the environmental behavior of metals at the earthworm-soil micro-interface, it is necessary to investigate the vertical migration and redistribution of metals between different soils using earthworms to assess more comprehensive impacts at the level of ecosystem (Heinze et al. 2021).

Conclusions

In this study, a promising method (2D terraria) to decode the “black box” of soil and assess how earthworms contribute to the metal availability in soil has been described. The present study explained the debate about the effect of earthworms on the availability of soil metals from the concept of drilosphere. The findings showed that earthworm activities increased the availability of Cd in casts and decreased it in the burrow wall soils. Considering there were both the process of immobilization in the burrow wall and the mobilization process in the casts, we can combine earthworms with other remediation methods and regulate earthworm activity according to the remediation needs. What is more, the increase in organic matter led to a decrease in the total Cd concentration and increased the availability of Cd in the disturbed soil which indicated that the organic amendment addition could be combined with earthworm-mediated remediation to improve the efficiency of potential biological extracts, which makes vermiremediation a potential method for the improvement of metal contaminated soils.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Y. G., Z. S., and W. Z. The first draft of the manuscript was written by Y. G., and all authors commented on previous versions of the manuscript. Supervision, project administration, funding acquisition, and writing, review, and editing were performed by Y. Q. All authors read and approved the final manuscript.

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Declarations

Ethics approval This experiment strictly abides by the Animal Management Regulations of China and Laboratory animal-Guideline for ethical review of animal welfare (GB/T 35892-2018).

Consent to participate All authors give consent to participate.

Consent to publish All authors give consent to publication.

Competing interests The authors declare no competing interests.

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