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Effects of Nano-zero-valent Iron and Earthworms on Soil Physicochemical Properties and Microecology in Cadmium-Contaminated Soils

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Abstract The capacity of nano-zero-valent iron (nZVI) and soil animals to remediate heavy metal–contaminated soil has been widely studied. However, the synergistic effect of soil animals and nZVI has not been thoroughly investigated. Here, we studied the combined effect of earthworms and nZVI on soil physicochemical properties and microecology during remediation of cadmium (Cd)–contaminated soil. The results showed that although amendment with nZVI reduced earthworm survival and biomass, the combination of nZVI and earthworms was effective at reducing the available Cd (ACd) content of soil and improving its quality. ACd most effectively reduced by 75.3% in the presence of earthworms under the 0.25% nZVI combination. Meanwhile, the combined action of earthworms and nZVI significantly improved soil properties and increased the diversity of soil microorganisms. In the earthworm-free system, nZVI reduced ACd by increasing soil pH and the abundance of Stenotrophobacter in Cd-contaminated soil, in addition to the co-precipitation and adsorption reported in previous studies. Correlation analysis revealed that the combination of nZVI and earthworms synergistically decreased ACd by decreasing soil OM and increasing the relative abundance of *Opitutus* and *Gemmatta*. Overall, our study indicates that the combination of nano-zero-valent iron and earthworms is a potential system for in situ remediation of Cd-contaminated soils and provides a deep understanding of the mechanisms involved in remediation.

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1 Introduction

Anthropogenic activities associated with the rapid development of industry, agriculture, and urbanization have increased cadmium (Cd) contamination of soils worldwide (Mamat et al., 2020). Cd contamination is particularly serious in China, where levels range from 0.003 to 9.57 mg/kg (Sriprachote et al., 2012). In soil, the bioavailability and migration of Cd are significantly higher than for other soil contaminants (Wang et al., 2015; Wang et al., 2020). Cd exposure through the food chain is associated with a variety of toxic and carcinogenic effects, including damage to the kidneys, skeleton, and respiratory system (Fu et al., 2008; Huang et al., 2018). Furthermore, Cd has a negative impact on soil microorganisms and reduces the decomposition rate of litter, leading to reductions in soil quality and crop production (Liu et al., 2020). Therefore, remediation methods have been developed for the treatment of Cd-contaminated soil.

Physicochemical remediation and bioremediation have been employed to reduce Cd toxicity (Kumpiene et al., 2017). For physicochemical remediation, nano-zero-valent iron (nZVI) is a promising material that has undergone intensive study as a remediation agent for heavy metal pollution of water bodies and groundwater (Adeleye et al., 2016; Lei et al., 2018). Previous research has shown that its active chemical properties, large specific surface area, and surface energy confer high heavy metal reduction and adsorption capacities (Vilardi et al., 2017; Xue et al., 2018a). Previous studies have demonstrated that nZVI immobilizes Cd through adsorption and precipitation (Boparai et al., 2013; Guha et al., 2020). However, in the soil environment, the properties of nZVI are affected by the activities of animals and microorganisms, organic matter (OM), moisture content, and pH (Lei et al., 2018). These complex interactions accelerate the aging and agglomeration of nZVI particles, and thus affect remediation efficiency. Meanwhile, recent studies have addressed the biological toxicity of nZVI, which has negative effects on the soil ecosystem. For example, Liang et al. showed that 500 mg/kg nZVI had negative effects on the behavior and physiological responses of earthworms (Liang et al., 2018). Most research to date has not considered the health of soil animals and microorganisms. Therefore, the utility of nZVI as a heavy metal treatment in soil warrants further investigation.

For bioremediation, earthworms have strong potential for improving soil fertility, supporting soil nutrient cycling, modulating soil microflora, and remediating heavy metal-contaminated soil (Gong et al., 2019; Van Groenigen et al., 2019). For example, *Eisenia fetida* can effectively reduce soil available Cd (ACd) (Liu et al., 2020). Little information regarding the impact of nZVI on earthworm activities or cooperative effects of nZVI and earthworms on remediation of Cd-contaminated soil is available. Meanwhile, both nZVI and earthworms are good at remediating heavy metal–contaminated soils, but few studies have focused on their synergy. To fill this gap, we studied the combined effect of earthworms and nZVI on soil physicochemical properties and microecology. Our research provides new insights into the combined use of nZVI and earthworms for remediation of Cd-contaminated soil.

2 Materials and Methods

2.1 Experimental Materials

nZVI (DK-Fe-001; particle size, 30–50 nm, 99.9% (metal basis); true density, 7.87 g/cm^3) (Fig. S2) was purchased from Beijing Dekedaojin Technology Co., Ltd. (Beijing, China). Cadmium chloride ($CdCl_2$, > 99%) was obtained from Fengchuan Chemical Reagent Co., Ltd. (Tianjin, China). Red soil samples were collected in Kunming City, Yunnan Province, Southwestern China (N 24° 49′, E 102°51′; elevation, 1980 m). Humus soil was purchased from a commercial vendor. The red and humus soil were freed of plant rhizomes, seeds, and stones, dried naturally, and filtered through 2- and 0.25-mm mesh sieves. The red and humus soil were mixed at a mass ratio of 2:1. The background contents (measured in four samples) of ACd (available cadmium), Tfe (total iron), SOM (soil organic matter), total nitrogen (TN), alkali-hydrolyzable nitrogen (AN), total phosphorus (TP), and available phosphorus (AP) were 0 ± 0.00 g/kg, 54.52 ± 4.74 g/kg, 93.34 ± 3.00 g/kg, 2.74 ± 0.31 g/kg, 342.34 ± 47.36 mg/kg, 1.18 ± 0.12 g/kg, and 434.32 ± 32.22 mg/kg, respectively. Earthworms (*Eisenia fetida*, Daping-2) were purchased from a commercial breeder in Kunming City, and were allowed to excrete dirt and clear their intestines for 24 h (Liang et al., 2018). The earthworms were then transferred to soil, with a 2:1 mass ratio of red to humus soil and water content of 45%, for a 2-week habituation period. Healthy and segmented earthworms weighing 0.3 to 0.4g were chosen for the experiment.

2.2 Experimental Design

2.2.1 Experimental Procedures

We used indoor simulation experiments to study the efficiency and mechanism through which earthworms and nZVI act in combination to remediate Cd-contaminated soil. We used *Eisenia fetida* as our study species, with a density of five individuals per 500g of soil (Kostecka et al., 2020) and nZVI (at mass ratios of 0%, 0.05%, 0.25%, and 0.5%) (Yirsaw et al., 2016; Zou et al., 2016) as the research objects. An initial 500 g of soil containing Cd (30 mg/kg) was placed in a PVC tube (about 10 cm in diameter and 12 cm in height), and the moisture content was adjusted daily to 45%. After a stabilization period of 28 days, nZVI of various mass ratios was added to PVC tubes containing five *Eisenia fetida* or no earthworms. The temperature was maintained at 22 ± 3 °C and the day/night ratio was set to 12:12 throughout the experiment. On the 45th day of the test period, earthworms were collected from tubes in each treatment group, and counted and weighed after 24 h of intestinal clearing. The survival rate and biomass of earthworms in each sample were calculated. The soil samples collected on day 45 were analyzed for soil aggregate stability, ACd content, soil chemical properties, and soil microbial diversity.

2.2.2 Soil Agglomerate Stability and Physicochemical Properties

The aggregate size distribution (ASD) and mean weight diameter (MWD) were used as indicators to evaluate the stability of agglomerates (Kemper & Rosenau, 1986). Using the wet sieve method, water-stable soil agglomerates were obtained by immersing air-dried soil samples in distilled water for 5 min. Then, the sieve was manually shaken up and down 50 times within 2 min at an amplitude of 3 cm, and the resulting material was sorted into large agglomerates (> 250 μm), small agglomerates (53–250 μm), and clay (< 53 μm). Distilled water was used to wash soil from the sieve into a container, and the soil was then naturally air-dried and weighed. The proportions of different agglomerate sizes, ASD, and MWD were calculated. Soil chemical properties (pH, OM, TN, TP, AN, and AP) and TFe were determined according to the methods of Du and Gao (2006). Determination of ACd in soil was conducted with a flame atomic absorption spectrophotometer using DTPA (0.005 mol·L^{-1} diethylenetriaminepentaacetic acid, 0.1 mol·L^{-1} triethylamine, 0.01 mol·L^{-1} $CaCl_2$) as the extractant.

2.2.3 Determination of Soil Microecology

On the 45th day of the soil microbial determination test, three devices were randomly selected from among the six devices of a given treatment, and a high-temperature-sterilized key was used to collect soil samples and place them in a high-temperature-sterilized centrifuge tube; these samples were stored at −20 °C until testing. After extraction of genomic DNA from the sample, the V3–V4 region of 16S rDNA was amplified. The primer sequences used in this study are 338F (5′-ACTCCTACGGGAGGC AGCAG-3′) and 806R (5′-GGACTACHVGGG TWTCTAAT-3′). The length of the amplicon was approximately 460 bp. The amplification product was cut and recovered, and then quantified with a QuantiFluor fluorometer. The purified amplification products were mixed in equal amounts, connected to sequencing adapters to construct a sequencing library, and sequenced on the Hiseq 2500 PE250 platform (Ilumina, San Diego, CA, USA). DNA/RNA/Small RNA/cDNA library sequencing was performed on the Hiseq 2500/4000 platform by Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China). Bioinformatics analysis was performed using Omicsmart, a real-time interactive online platform for data analysis and storage (http://www.omicsmart.com). Redundancy analysis (RDA) was performed using the R package vegan; correlation coefficients were calculated based on species abundance tables and environmental factor tables using the R package psych and presented as heat maps; correlations between environmental factors and ACd were analyzed using the R package corrplot.

2.3 Data Processing and Analysis

One-way analysis of variance (ANOVA) was used to determine the effects of the mass ratio of nZVI on earthworms (including survival and biomass). Two-way ANOVA (followed by Duncan's multiple range post hoc test) was used to determine the effects of nZVI concentration and earthworm activity on soil ACd content and soil physicochemical properties.

All were normally distributed and met the requirements for the chi-square test. The analyses were performed using the SPSS statistical package (version 25.0; IBM Corp, Armonk, NY, USA). $P < 0.05$ was taken to indicate statistical significance. RDA was performed using the R package vegan. Correlations between environmental factors and species were analyzed using the R package psych based on species abundance and environmental factor tables; correlations between environmental factors and ACd were analyzed using the R package corrplot and presented as heat maps, based on Spearman's correlation coefficients. Images were plotted and rendered using Origin 2022 software.

3 Results and Discussion

3.1 Effects of nZVI on Earthworm Survival Rate and Biomass

To determine the effects of nZVI on earthworms, various concentrations of nZVI (0, 0.05, 0.25, and 0.5%) were evaluated. Amendment with nZVI reduced the survival rate and biomass, although not significantly (Table 1). Similar to previous reports, nZVI addition to Cd-contaminated soil affected the biomass, survival rate, and physiology of earthworms (Bai et al., 2020; Liang et al., 2018). Liang et al. showed that 500 and 1000 mg/kg of nZVI significantly inhibited earthworm growth and respiration, and also enhanced the avoidance response (Liang et al., 2017). The highest earthworm biomass was detected under the 0.25% nZVI treatment, resulting in an 80% survival rate. Thus, it can be suggested that the 0.25% nZVI mass ratio has the least detrimental effect on earthworm survival.

3.2 Effects of nZVI and Earthworms on Soil Physicochemical Properties

3.2.1 ACd and TFe in Soils

nZVI and earthworms effectively remediated the Cd-contaminated soil. However, the synergistic effects of ZVI and earthworms remain unclear. Thus, the effects of nZVI, alone and in combination with earthworms, on ACd in soil were evaluated in the remediation system. Amendment with nZVI, alone and in combination with earthworms, significantly reduced Cd availability ($P < 0.01$), as illustrated in Fig. 1a. Compared to treatment with nZVI alone, the combination of nZVI and earthworms more effectively reduced the ACd content of soil. Liu et al. (2022) reported a noteworthy decrease of 10.9% and 9.3% in available cadmium content in low-Cd and high-Cd soils, respectively, subsequent to treatment with 0.1% nZVI. Moreover, Jin et al. (2023) demonstrated a nZVI fixation efficiency of 32.05% in soil samples after 20 days of adding 5 g/kg of nZVI. Prior research has confirmed the significance of earthworms in reducing DTPA-Cd concentrations in cadmium-contaminated soils, ranging from 5 to 20 mg·kg^{-1} (Cheng et al., 2021).In our study, the 5–0.25% system had the lowest ACd content (7.40 mg/kg) and ACd was reduced by 75.3% in that treatment. From Fig. 1b, it is apparent that the total iron content of the soil was significantly increased with the addition of 0.05% nZVI in the absence of earthworms (P<0.05). However, no noteworthy alteration was observed in the total iron content of the soil under 0.25% and 0.5% nZVI treatments despite varying concentrations. Compared with the group without earthworms, the inclusion of earthworms and 0.05% nZVI led to a noteworthy fall in soil total iron content by 10.53%, dropping below the total iron background value of the earthworm and nZVI-free group. This suggests that earthworms

Table 1 Survival rate and biomass of earthworms at different nZVI concentrations. Error bars are mean ± standard error (n=6). Different lowercase letters indicate significant differences within each treatment based on one-way ANOVA ($P < 0.05$)

nZVI concentration	Original weight of earthworms (g)	Survival rate	Dry weight of earthworms (g)
0	1.5272±0.04895a	0.7333±0.08433a	0.7619±0.05372a
0.05%	1.4918±0.04759a	0.8333±0.03333a	0.7275±0.09044a
0.25%	1.5083±0.03515a	0.8000±0.05164a	0.8295±0.06734a
0.5%	1.4682±0.02314a	0.8333±0.03333a	0.7083±0.06288a

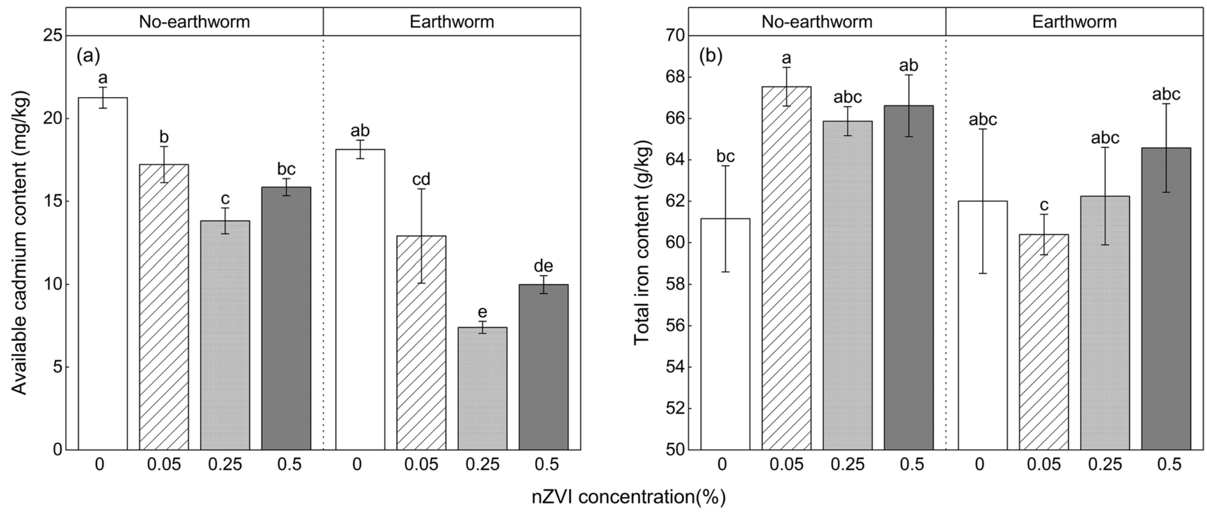

Fig. 1 nZVI and its impact on total iron content in soil in the presence of earthworms (**a**); nZVI and its impact on soil available Cd in soil in the presence of earthworm (**b**). Error bars are mean ± standard error ($n = 6$). Difference lowercase letters indicate significant differences within each treatment based on two-way ANOVA ($P < 0.05$)

possess the ability to decrease the overall iron content in soil. Consistent with these results, the 5–0.25% restoration system had little impact on earthworm survival. NZVI was oxidized to produce Fe^{2+} (Eqs. 1, 2) and Fe^{3+} (Eq. 3), and can co-precipitate with Cd^{2+} and anions (Eqs. 4–7) in the soil to form precipitates (Boparai et al., 2013; Li et al., 2020), and can adsorb Cd^{2+} via an oxide film on the outer layer of nZVI (Fajardo et al., 2020; Tasharrofi et al., 2020; Zhang et al., 2014). Thus, nZVI effectively reduces the ACd content of soil. Treatment with nZVI alone significantly reduced ACd content and alleviated its toxic effects on the earthworms. Moreover, previous studies showed that nZVI reduces the toxicity of Cd in soil (Xue et al., 2018b), thereby promoting the survival of soil organisms (Guha et al., 2020; Habish et al., 2017). Meanwhile, earthworm activity promoted the formation of aggregates and enhanced the remediation efficiency of nZVI (Al-Maliki & Scullion, 2013 ; Bottinelli et al., 2017). Additionally, a high concentration of nZVI (0.5%) negatively impacts earthworm biomass, movement, and life quality (Liang et al., 2017; Liang et al., 2018; Liu et al., 2015). Accordingly, the combination of a moderate nZVI concentration and earthworms (5–0.25%) yielded the highest remediation efficiency for Cd-contaminated soil. Overall, these results indicate that the combination of nZVI and earthworms is a promising strategy for remediation of Cd-contaminated soil. The main reaction equation is as follows:

$$2Fe^0 + O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4OH^- \quad (1)$$

$$2Fe^0 + 2H_2O \rightarrow 2Fe^{2+} + H_2 + 2OH^- \quad (2)$$

$$2Fe^{2+} + 2H_2O \rightarrow 2Fe^{3+}\ H_2 + 2OH^- \quad (3)$$

$$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3 \quad (4)$$

$$Fe(OH)_3 \rightarrow FeO(OH) + H_2O \quad (5)$$

$$Cd^{2+} + OH^- \rightarrow Cd(OH)^- \quad (6)$$

$$FeOOH + [Cd(OH)]^+ \rightarrow FeOOCd(OH) + H^+ \quad (7)$$

3.2.2 Structure of Agglomerates in Soils

The ASD and MWD are important indicators of aggregate stability (Kemper & Rosenau, 1986). The nZVI-only and earthworm plus nZVI remediation systems in this study showed a significant difference in ASD ($P < 0.05$). Specifically, the proportion of large aggregates (diameter > 250 μm) was highest in the 0–0% (no earthworms and 0% nZVI) system (46.83%), while the proportion of clay (diameter < 53 μm) was lowest (12.34%) among the systems (Fig. 2a). The proportion of large aggregates in the nZVI-only remediation systems decreased with increasing nZVI concentration. After adding 0.05% nZVI, there was a significant increase in the clay content.

As shown in Fig. 2b, the addition of various contents of nZVI to the soil significantly reduced the MWD of aggregates, but this effect leveled off after amendment with earthworms. In the system with earthworms, no significant difference was found in the MWD of soil aggregates with the addition of different low mass ratios (0%, 0.05%, 0.25%) of nZVI. Previous studies showed that reduction of toxic heavy metal content combined with increased soil animal movement was conducive to aggregate formation in soil (Aghababaei et al., 2014; Al-Maliki & Scullion, 2013; Mamat et al., 2020). Furthermore, the burrowing activity of earthworms affects soil structure and quality (Bacher et al., 2020). In summary, both nZVI and earthworm activity during the remediation of Cd-contaminated soil improve soil structure.

3.2.3 Soil Chemical Properties

The nZVI plus earthworm system altered the chemical composition of the soil, including soil pH, OM, TP, TN, AN, and AP, as shown in Fig. 3. Moreover, nZVI significantly influenced soil pH (Fig. 3a). Soil pH (6.34) was highest in the 5–0.25% remediation system. The system with low-concentration nZVI plus earthworms did not impact soil pH, while systems with moderate to high nZVI concentrations increased pH. This difference is likely attributable to alkaline substances released during the remediation process (Yang et al., 2019), and is consistent with previous studies showing that soil pH is an important factor affecting the remediation of heavy metal-contaminated soil with nZVI (Latif et al., 2020; Li et al., 2020). Increasing pH promotes the formation of Cd precipitates and thereby reduces ACd.

The OM content was significantly higher in the combined treatment compared to the nZVI-only remediation systems (Fig. 3b). The OM content was 120.47 g/kg higher in the 5–0% (five earthworms and 0% nZVI) remediation system than other treatment groups. The addition of earthworms significantly increased the OM content of the soil ($P < 0.05$), while an increase in the nZVI mass ratio reduced the OM content, although not significantly ($P >$

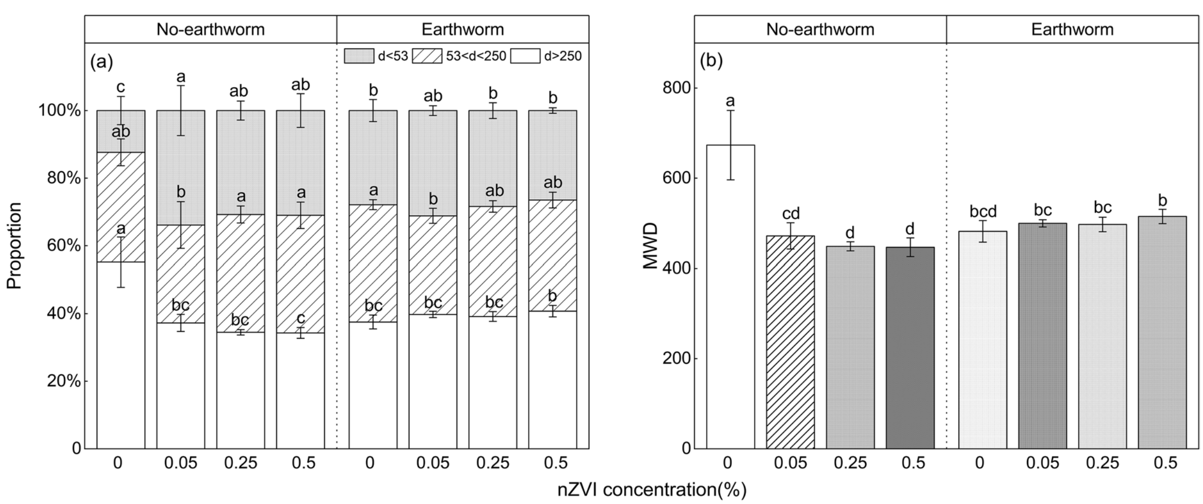

Fig. 2 Effects of nZVI and earthworm activities on aggregate size distribution (**a**) and mean weight diameter (**b**). Error bars are mean ± standard error ($n = 6$). Difference lowercase letters indicate significant differences within each treatment based on two-way ANOVA ($P < 0.05$). d>250 μm represents large soil agglomerates, 53 μm<d<250 μm represents small agglomerates, d<53 μm represents clay, and MWD represents the mean equivalent diameter of the agglomerates. Difference lowercase letters indicate significant differences within each treatment based on two-way ANOVA ($P < 0.05$)

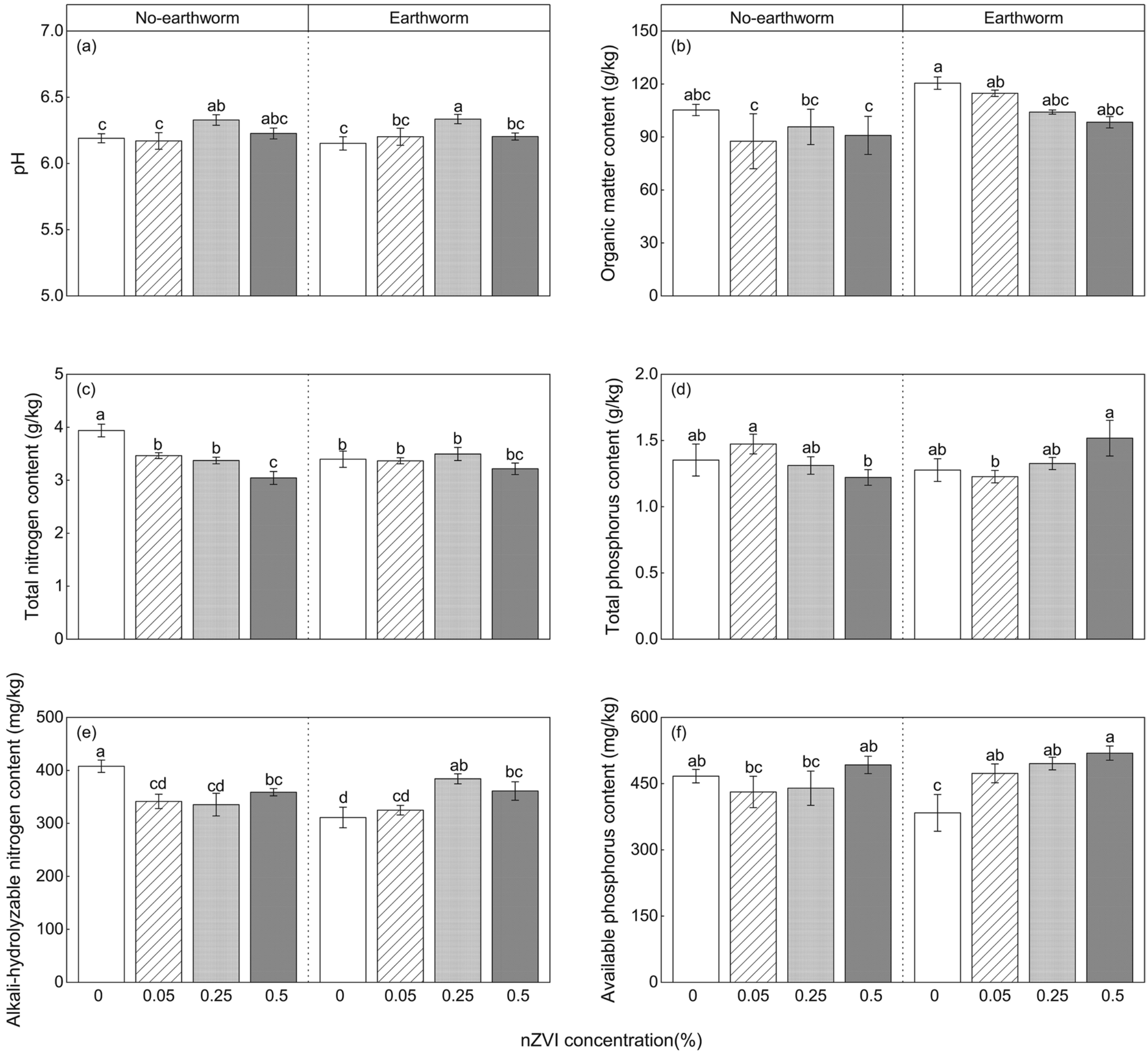

Fig. 3 Effects of nZVI and earthworm activities on soil pH (**a**), organic matter content (**b**), total nitrogen (**c**), total phosphorus (**d**), alkali-hydrolyzable nitrogen (**e**), and available phosphorus (**f**). Error bars are mean ± standard error ($n = 6$). Difference lowercase letters indicate significant differences within each treatment based on two-way ANOVA ($p < 0.05$)

0.05). Similarly, the combination of earthworms and nZVI significantly affected the TN content of soil ($P < 0.01$) (Fig. 3c), consistent with the observation that TN in soil is related to OM content (Zhu et al., 2017). As shown in Fig. 3d, in the treatments without earthworms, TP content was higher with 0.05% and 0.25% mass ratios of nZVI (1.47 g/kg and 1.31 g/kg, respectively) than with a 0% mass ratio. Meanwhile, the TP content was significantly lower with a 0.5% than 0% mass ratio of nZVI. As illustrated in Fig. 3e, an increase in the mass ratio of nZVI decreases the AN content of soil, but this effect was minimized with the addition of earthworms, and the combined nZVI-earthworm remediation system significantly affected the AN content of soil ($P < 0.01$). As shown in Fig. 3f, nZVI significantly altered the available P content of soil ($P < 0.05$), and the available P content of soil was positively correlated with the nZVI treatment level. The AP content of soil was highest in the 5–0.5% remediation system (519.06 mg/kg).

Figure 1b shows that earthworms combined with nZVI significantly reduced the ACd content. Yu et al. showed that a reduction in soil ACd was associated with increases in the OM, TN, and TP contents of soil (Yu et al., 2020). Thus, reducing ACd in the soil reduces its negative impact on soil quality (Boparai et al., 2013; Chen et al., 2019). Earthworms improve soil quality in both chemical and physical terms by maintaining soil structure and recycling nutrients (Bacher et al., 2020). An increase in earthworm abundance promotes the production of water-stable aggregates, which are rich in OM (Bedano et al., 2019). Based on the results of two-factor ANOVA shown in Table 2, the combined effect of earthworms and nZVI significantly affected the proportions of large aggregates, clay, MWD, TN, TP, and AN in the soil ($P < 0.001$), thereby significantly improving the quality of Cd-contaminated soil. Therefore, the combined effect of earthworms and nZVI remediation of Cd-contaminated soil reduces the availability and negative effects of Cd, while earthworm activities prevent the loss of C, N, and P from the soil during the restoration process.

3.3 Effects of nZVI and Earthworms on Soil Microorganisms

3.3.1 Analysis of Bacterial Community Structure and Diversity

Microorganisms are an important component of the soil ecosystem, and alteration of microbial abundances can affect the circulation and flow of substances in the soil ecosystem (Fierer, 2017). The bacterial community from day 45 soil samples was analyzed through high-throughput sequencing of the 16S rRNA V3–V4 region. The results showed that the Sobs index value tended to plateau with increasing depth of sequencing (Fig. S3), indicating that the data obtained from the samples were reasonable. Earthworms and nZVI significantly impacted indices of microbial diversity and species richness ($P < 0.05$) (Table 3). In the 0–0.05%, 5–0.25%, and 5–0.5% remediation systems, the diversity and abundance indices for soil microorganisms did not differ from the control values (0–0%). This similarity indicated that low- and moderate-concentration nZVI plus earthworms did not reduce the diversity or richness of soil microorganisms. Non-metric multidimensional scaling (NMDS) analysis revealed similarity in species composition among treatments, with a significant degree of separation associated with increasing nZVI concentrations. However, the effect of nZVl on the species distribution was dampened by the addition of earthworms (Fig. S4), indicating that amendment with earthworms reduced the impact of nZVI on the soil bacterial community.

Planctomycetes, *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* were the dominant bacterial phyla (Fig. 4a). The composition of microbes was similar among treatments, but the proportions and abundances of specific microbial taxa differed significantly. In the 5–0% remediation system, *Planctomycetes* was dominant (32.2%). The proportion of *Planctomycetes* was higher in the earthworms plus nZVI combined treatment than nZVI-only remediation system. *Verrucomicrobia* and *Firmicutes* were dominant in the 0–0.05% remediation

Table 2 Effects of nZVI (N) and earthworm (E) soil physical and chemical properties, and the stability of aggregates (two-way repeated measures ANOVA, *F* values, and significances). *ns*, not significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Variable	Earthworms (E)	nZVI (N)	E × N
Large aggregates	1.10^{ns}	62.022***	100.238***
Small aggregates	0.082^{ns}	4.308*	1.479^{ns}
Clay	1.413^{ns}	48.099***	40.766***
Mean weight diameter	1.297^{ns}	70.307***	102.167***
pH	0.168^{ns}	5.023**	0.284^{ns}
Organic matter	6.818*	1.909^{ns}	0.666^{ns}
Total nitrogen	1.320^{ns}	13.089***	10.399**
Total phosphorus	0.132^{ns}	0.177^{ns}	3.575*
Alkali-hydrolyzable nitrogen	11.462^{ns}	0.455^{ns}	18.501***
Available phosphorus	0.86^{ns}	4.903*	7.687^{ns}
Available cadmium	15.963***	39.795***	1.236^{ns}

Table 3 Effects of nZVI (mass ratios of 0%, 0.05%, 0.25%. and 0.5%) and earthworms (none or five per tube) on the diversity and abundance indices of soil microorganisms (one-way repeated measures ANOVA). Values are mean ± standard error (n = 3). Different letters indicate a significant difference among treatments at the 5% level according to Duncan's test

Earthworm	nZVI concentration	Shannon	Simpson	Chao	Ace
Without	0	9.88±0.04ab	0.9913±0.0001ab	3862.55±82.79ab	3884.94±88.87ab
Without	0.05%	9.96±0.03a	0.9914±0.0001a	3943.22±71.92a	3961.73±81.49a
Without	0.25%	9.67±0.09c	0.9907±0.0001c	3408.96±195.69c	3396.43±201.2c
Without	0.5%	9.68±0.15c	0.9908±0.0002bc	3511.74±341.27bc	3510.8±353.02bc
With	0	9.72±0.18bc	0.9907±0.0006bc	3524.53±302.38bc	3512.57±325.97bc
With	0.05%	9.75±0.1bc	0.9909±0.0004c	3722.61±160.35abc	3733.11±171.62abc
With	0.25%	9.78±0.07abc	0.9908±0.0003bc	3825.57±68.85ab	3840.46±68.82ab
With	0.5%	9.8±0.02 abc	0.991±0.0002abc	3828.45±160.79ab	3841.63±173.86ab

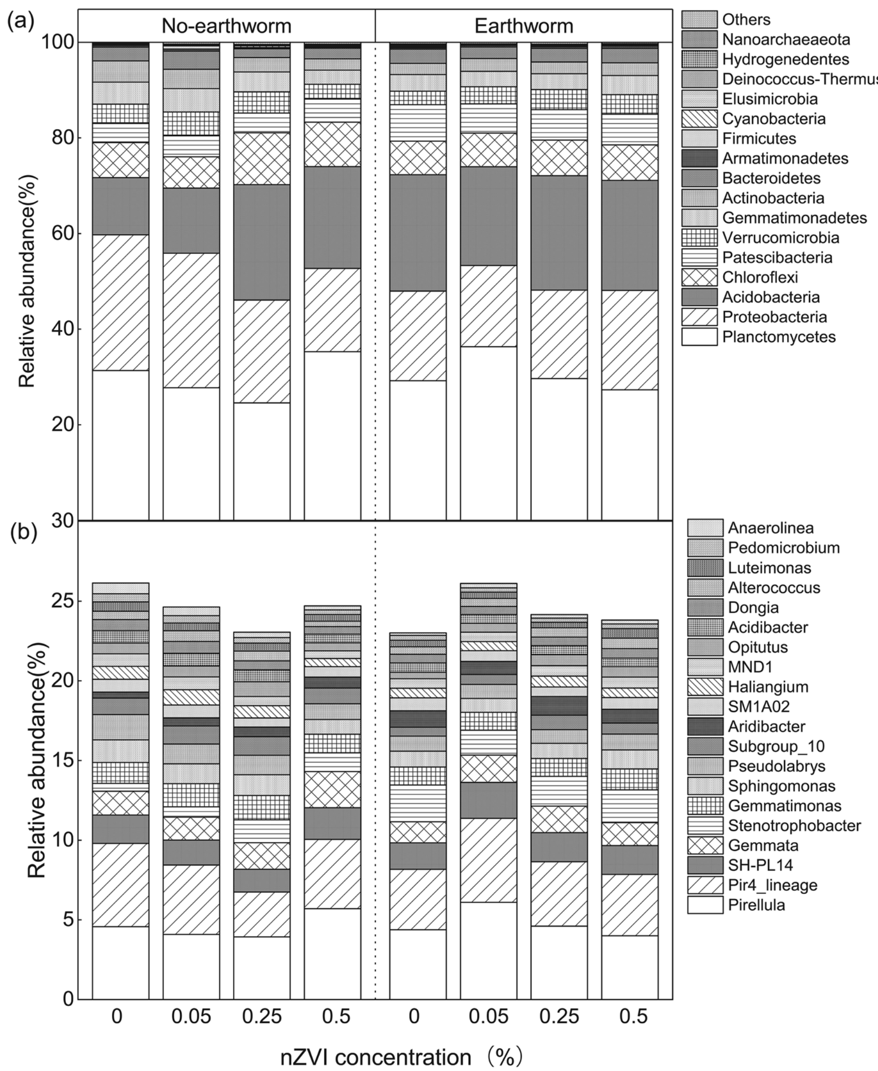

Fig. 4 Average relative abundances of phylum (**a**) and genus (**b**) level of soil bacterial community under different treatments (at the phylum level, the relative abundance < 0.1% was classified as others; top 20 genera based on mean relative abundance rankings at the genus level)

system (5.04% and 1.1%, respectively). In the 5–0% remediation system, *Patescibacteria* was the most abundant taxon (8.98%). The 5–0%, 0–0.25%, and 5–0.25% remediation systems had larger proportions of *Acidobacteria* than other systems (18.95%, 18.93%, and 18.87%, respectively). At the genus level, as shown in Fig. 4b, the three genera with the highest relative abundances, *Pirellula*, *Pir4_lineage*, and *SH-PL14*, all had higher relative abundances in the 5–0.05% system, indicating that the combined effect of earthworms and low to moderate concentrations of nZVI significantly enhanced the abundance of these dominant microbial groups.

3.3.2 Linking the Variations of Soil Physicochemical Properties and Microbial Communities with ACd

To explore the potential mechanisms underlying the reduction of ACd associated with nZVI and earthworms, the correlations of ACd and soil physicochemical properties with species were evaluated using Spearman's rank correlation coefficient (Fig. 5). In the earthworm-free system, ACd was negatively correlated with pH ($P < 0.05$), while *Stenotrophobacter* was negatively correlated ($P < 0.05$) with soil agglomerates (MWD and MA), TN, and ACd. These results indicate that nZVI reduced ACd by increasing pH and the abundance of *Stenotrophobacter*. Soil pH is an important factor affecting the remediation of heavy metal–contaminated soil by nZVI, and has been associated with decreased ACd due to the formation of Cd precipitates (Li et al., 2020). *Stenotrophobacter* belongs to the *Acidobacteria*, which are often enriched in heavy metal–contaminated environments and have the capacity for metal transformation (Xu et al., 2022), indicating adaptability to metal-rich environments. Therefore, we speculated that *Stenotrophobacter* might be involved in Cd immobilization.

In the earthworm system, ACd was significantly positively correlated with soil organic matter ($P < 0.05$). Previous research has indicated that earthworms improve soil quality and stability by altering the proportion of soil macroaggregates, as well as accelerating the decomposition and transformation of OM (Jia et al., 2015). Additionally, *Opitutus* and *Gemmatta* were significantly negatively correlated ($P < 0.05$) with ACd. Thus, increasing AP may decrease soil ACd. These results indicate that the combination of nZVI and earthworms reduced ACd by decreasing soil OM and increasing the relative abundance of *Opitutus* and *Gemmatta*.

3.4 Mechanism Involving the Remediation of Cadmium-Contaminated Soil by the Combination nZVI and Earthworms

In our study, we found the treatment amended with nZVI or the combination of nZVI and earthworms could effectively reduce the effective Cd content in Cd-contaminated soil. Specially, the addition of nZVI decreased the effective Cd content by increasing the proportion of clay, the soil pH, abundance of *Stenotrophobacter*. It is well established that aggregation phenomenon of nZVI within soil matrices exerts a significant influence upon the structural configuration of the soil matrix. Upon the micron-scale aggregation of nZVI, discernible outcomes encompass the attenuation of soil hydraulic conductivity and chemical reactivity (He et al., 2007; Schrick et al., 2004). Consequently, this culminates in an observable elevation in the relative proportion of clay constituents.

The experiments conducted by Xu et al., (2019) have demonstrated that both nZVI and clay are capable of inducing the transformation of Cd within soil matrices into states of diminished biological availability. This finding corroborates with the outcomes of our own research. Similarly, the soil pH value constitutes a pivotal determinant governing the removal of positively charged metal ions by nZVI. An elevation in soil pH engenders the generation of augmented negatively charged adsorption sites on soil colloids and organic matter surfaces, consequently culminating in the diminished bioavailability of metals (Otunola & Ololade, 2020; Zhang et al., 2014). Concurrently, the experiments conducted also reveal a pronounced negative correlation between *Stenotrophobacter* and Cd by Cui et al., (2022).

The combination of nZVI and earthworms enhances by 30% than nZVI alone (35%) (0.25% nZVI) by decreasing soil organic matter and increasing the abundance of *Gemmatta* and *Opitutus*. Soil organic matter (SOM) serves as a carbon source that stimulates microbial proliferation, leading to increased participation of bacterial taxa in heavy metal remediation. Notably, the abundances of *Gemmatta* and *Opitutus* exhibit a significant

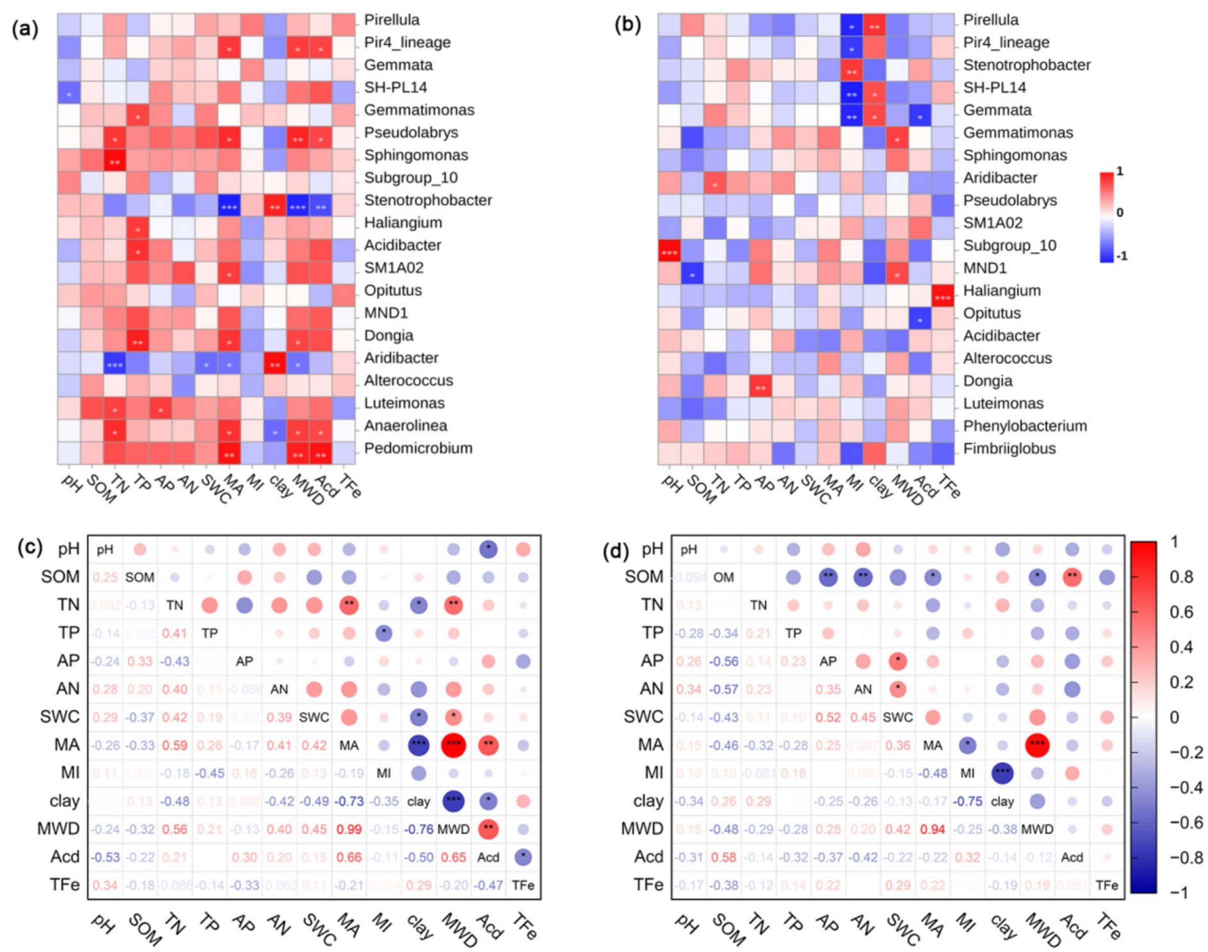

Fig. 5 Effects of various environmental factors on microbial community structure at the genus level [top 20 genera based on mean abundance rankings without earthworms (**a**) and with earthworms (**b**)]; and effects of soil physiochemical properties of ACd without earthworms (**c**) and with earthworms (**d**). Horizontal axis indicates environmental factors; vertical axis indicates species; color indicates the strength of the correlation; asterisks within the square indicate significant correlation. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

negative correlation with the soil-available cadmium content. This observation aligns with the findings of (Li et al., 2021; Wang et al., 2021). Additionally, research has indicated the significant role of *Gemmatta* in cadmium-contaminated soils (Bian et al., 2023; Pei et al., 2021). The combined remediation of cadmium-contaminated soil using nZVI and earthworms demonstrates the potential for sustainable soil remediation practices. This benefits local ecosystems by improving soil quality through cadmium remediation, and the successful application of this remediation approach may have global implications.

4 Conclusions

In this study, we investigated the combined effect of earthworms and nZVI on soil physicochemical properties and microecology during the remediation of Cd-contaminated soil. The results showed that although amendment with nZVI reduced earthworm survival and biomass, the combination of nZVI and earthworms effectively reduced the ACd content of soil. Meanwhile, the combined action of earthworms and nZVI significantly improved soil properties and increased the diversity of soil microorganisms. In the earthworm-free system, nZVI reduced ACd by increasing soil pH, the proportion of clay, and the abundance of *Stenotrophobacter* in Cd-contaminated soil, in addition to the co-precipitation and adsorption reported in previous studies. Correlation analysis revealed that the combination of nZVI and earthworms enhanced the decrease in ACd by decreasing soil OM and increasing the relative abundance of *Opitutus* and *Gemmatta*. Overall, our study indicates that the combination of nano-zero-valent iron and earthworms is a potential system for in situ remediation of Cd-contaminated soils and provides a deep understanding of the mechanisms involved in remediation. In the future, it is worth conducting a thorough investigation into the effect of nZVI and earthworms on soil nutrient cycling and other ecosystem functions. This will facilitate a comprehensive evaluation of the remediation process' impact on soil ecosystems. Furthermore, it is essential to apply this methodology to genuine contaminated sites to assess its practicability and sustainability.

Author Contribution BT: methodology, data curation, formal analysis, roles/writing—original draft, visualization. YZ: conceptualization, formal analysis. WZ: data curation, conceptualization, software. YZ: conceptualization, formal analysis, investigation. MY: conceptualization, formal analysis, investigation. JC: supervision, validation, writing—review and editing. CL: project administration, supervision, validation. CD: funding acquisition, project administration.

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Data Availability The data are given in the paper.

Declarations

Ethical Approval All applicable institutional and/or national guidelines for the care and use of animals were followed. Please feel free to contact me if you have any questions regarding this submission.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Agree.

Conflict of Interest The authors declare no competing interests.

References

Adeleye, A. S., Stevenson, L. M., Su, Y., Nisbet, R. M., Zhang, Y., & Keller, A. A. (2016). Influence of phytoplankton on fate and effects of modified zerovalent iron nanoparticles. *Environmental Science & Technology, 50*(11), 5597–5605. https://doi.org/10.1021/acs.est.5b06251

Aghababaei, F., Raiesi, F., & Hosseinpur, A. (2014). The combined effects of earthworms and arbuscular mycorrhizal fungi on microbial biomass and enzyme activities in a calcareous soil spiked with cadmium. *Applied Soil Ecology, 75*, 33–42. https://doi.org/10.1016/j.apsoil.2013.10.006

Al-Maliki, S., & Scullion, J. (2013). Interactions between earthworms and residues of differing quality affecting aggregate stability and microbial dynamics. *Applied Soil Ecology, 64*, 56–62. https://doi.org/10.1016/j.apsoil.2012.10.008

Bacher, M. G., Schmidt, O., Bondi, G., & Fenton, O. (2020). Influence of dung pats on soil physical quality mediated by earthworms: From dung deposition to decay and beyond. *Soil Research, 58*(5), 421–429. https://doi.org/10.1071/SR19319

Bai, H., Luo, M., Wei, S., Jiang, Z., & He, M. (2020). The vital function of humic acid with different molecular weight in controlling Cd and Pb bioavailability and toxicity to earthworm (Eisenia fetida) in soil. *Environmental Pollution, 261*, 114222. https://doi.org/10.1016/j.envpol.2020.114222

Bedano, J. C., Vaquero, F., Domínguez, A., Rodríguez, M. P., Wall, L., & Lavelle, P. (2019). Earthworms contribute to ecosystem process in no-till systems with high crop rotation intensity in Argentina. *Acta Oecologica, 98*, 14–24. https://doi.org/10.1016/j.actao.2019.05.003

Bian, F., Zhong, Z., Zhang, X., Li, Q., & Huang, Z. (2023). Bamboo-based agroforestry changes phytoremediation efficiency by affecting soil properties in rhizosphere and non-rhizosphere in heavy metal-polluted soil (Cd/Zn/Cu). *Journal of Soils and Sediments, 23*(1), 368–378. https://doi.org/10.1007/s11368-022-03303-y

Boparai, H. K., Joseph, M., & O'Carroll, D. M. (2013). Cadmium (Cd2+) removal by nano zerovalent iron: Surface analysis, effects of solution chemistry and surface complexation modeling. *Environmental Science and Pollution Research, 20*(9), 6210–6221. https://doi.org/10.1007/s11356-013-1651-8

Bottinelli, N., Zhou, H., Capowiez, Y., Zhang, Z., Qiu, J., Jouquet, P., & Peng, X. (2017). Earthworm burrowing activity of two non-Lumbricidae earthworm species incubated in soils with contrasting organic carbon content (Vertisol vs. Ultisol). *Biology and fertility of soils, 53*, 951–955. https://doi.org/10.1007/s00374-017-1235-8

Chen, L., Li, F., Wei, Y., Li, G., Shen, K., & He, H.-J. (2019). High cadmium adsorption on nanoscale zero-valent iron coated Eichhornia crassipes biochar. *Environmental Chemistry Letters, 17*, 589–594. https://doi.org/10.1007/s10311-018-0811-y

Cheng, Q., Lu, C., Shen, H., Yang, Y., & Chen, H. (2021). The dual beneficial effects of vermiremediation: Reducing soil bioavailability of cadmium (Cd) and improving soil fertility by earthworm (Eisenia fetida) modified by seasonality. *Science of the Total Environment, 755*, 142631. https://doi.org/10.1016/j.scitotenv.2020.142631

Cui, J., Li, P., Qi, X., Guo, W., & Rahman, S. U. (2022). Assessing the effect of irrigation using different water resources on characteristics of mild cadmium-contaminated soil and tomato quality. *Agronomy, 12*(11), 2721. https://doi.org/10.3390/agronomy12112721

Du, S., & Gao, X. (2006). *Technical specification for soil analysis*. China Agriculture Press.

Fajardo, C., Sánchez-Fortún, S., Costa, G., Nande, M., Botías, P., García-Cantalejo, J., Mengs, G., & Martín, M. (2020). Evaluation of nanoremediation strategy in a Pb, Zn and Cd contaminated soil. *Science of the Total Environment, 706*, 136041. https://doi.org/10.1016/j.scitotenv.2019.136041

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology, 15*(10), 579–590. https://doi.org/10.1038/nrmicro.2017.87

Fu, J., Zhou, Q., Liu, J., Liu, W., Wang, T., Zhang, Q., & Jiang, G. (2008). High levels of heavy metals in rice (Oryzasativa L.) from a typical E-waste recycling area in southeast China and its potential risk to human health. *Chemosphere, 71*(7), 1269–1275. https://doi.org/10.1016/j.chemosphere.2007.11.065

Gong, X., Wang, S., Wang, Z. W., Jiang, Y. J., Hu, Z. K., Zheng, Y., Chen, X. Y., Li, H. X., Hu, F., Liu, M. Q., & Scheu, S. (2019). Earthworms modify soil bacterial and fungal communities through enhancing aggregation and buffering pH. *Geoderma, 347*, 59–69. https://doi.org/10.1016/j.geoderma.2019.03.043

Guha, T., Barman, S., Mukherjee, A., & Kundu, R. (2020). Nanoscale zero valent iron modulates Fe/Cd transporters and immobilizes soil Cd for production of Cd free rice. *Chemosphere, 260*, 127533. https://doi.org/10.1016/j.chemosphere.2020.127533

Habish, A. J., Lazarević, S., Janković-Častvan, I., Jokić, B., Kovač, J., Rogan, J., Janaćković, Đ., & Petrović, R. (2017). Nanoscale zerovalent iron (nZVI) supported by natural and acid-activated sepiolites: The effect of the nZVI/support ratio on the composite properties and Cd 2+ adsorption. *Environmental Science and Pollution Research, 24*, 628–643. https://doi.org/10.1007/s11356-016-7802-y

He, F., Zhao, D., Liu, J., & Roberts, C. B. (2007). Stabilization of Fe− Pd nanoparticles with sodium carboxymethyl cellulose for enhanced transport and dechlorination of trichloroethylene in soil and groundwater. *Industrial & Engineering Chemistry Research, 46*(1), 29–34. https://doi.org/10.1021/ie0610896

Huang, D., Hu, Z., Peng, Z., Zeng, G., Chen, G., Zhang, C., Cheng, M., Wan, J., Wang, X., & Qin, X. (2018). Cadmium immobilization in river sediment using stabilized nanoscale zero-valent iron with enhanced transport by polysaccharide coating. *Journal of Environmental Management, 210*, 191–200. https://doi.org/10.1016/j.jenvman.2018.01.001

Jia, C., Chong, W., Yan, H., Ding-ge, J., & Yi, L. (2015). Effects of earthworm on soil microbes and biological fertility: A review. *Yingyong Shengtai Xuebao, 26*(5). https://doi.org/10.13287/j.1001-9332.20150302.008

Jin, Y., Wang, Y., Li, X., Luo, T., Ma, Y., Wang, B., & Liang, H. (2023). Remediation and its biological responses to Cd (II)-Cr (VI)-Pb (II) multi-contaminated soil by supported nano zero-valent iron composites. *Science of the Total Environment, 867*, 161344. https://doi.org/10.1016/j.scitotenv.2022.161344

Kemper, W., & Rosenau, R. (1986). Aggregate stability and size distribution. *Methods of soil analysis: Part 1. Physical and mineralogical methods, 5*, 425–442. https://doi.org/10.2136/sssabookser5.1.2ed.c17

Kostecka, J., Butt, K. R., Mazur-Pączka, A., Pączka, G., Garczyńska, M., & Podolak, A. (2020). Aspects of the ecology of the earthworm Eisenia lucens (Waga 1857) studied in the field and in laboratory culture. *Environmental Science and Pollution Research, 27*, 33486–33492. https://doi.org/10.1007/s11356-019-06187-7

Kumpiene, J., Nordmark, D., Carabante, I., Sužiedelytė-Visockienė, J., & Aksamitauskas, V. Č. (2017). Remediation of soil contaminated with organic and inorganic wood impregnation chemicals by soil washing. *Chemosphere, 184*, 13–19. https://doi.org/10.1007/s11356-019-06187-7

Latif, A., Sheng, D., Sun, K., Si, Y., Azeem, M., Abbas, A., & Bilal, M. (2020). Remediation of heavy metals polluted environment using Fe-based nanoparticles: Mechanisms, influencing factors, and environmental implications. *Environmental Pollution, 264*, 114728. https://doi.org/10.1016/j.envpol.2020.114728

Lei, C., Sun, Y., Tsang, D. C., & Lin, D. (2018). Environmental transformations and ecological effects of iron-based nanoparticles. *Environmental Pollution, 232*, 10–30. https://doi.org/10.1016/j.envpol.2017.09.052

Li, Y.-J., Fu, H., Zhang, J.-Y., Zhang, Z.-X., & Li, J.-K. (2021). Study of pollutant accumulation characteristics and microbial community impact at three bioretention facilities. *Environmental Science and Pollution Research, 28*, 44389–44407. https://doi.org/10.1007/s11356-021-13801-0

Li, Z., Wang, L., Wu, J., Xu, Y., Wang, F., Tang, X., Xu, J., Ok, Y. S., Meng, J., & Liu, X. (2020). Zeolite-supported nanoscale zero-valent iron for immobilization of cadmium, lead, and arsenic in farmland soils: Encapsulation mechanisms and indigenous microbial responses. *Environmental Pollution, 260*, 114098. https://doi.org/10.1016/j.envpol.2020.114098

Liang, J., Xia, X., Yuan, L., Zhang, W., Lin, K., Zhou, B., & Hu, S. (2018). The reproductive responses of earthworms (Eisenia fetida) exposed to nanoscale zero-valent iron (nZVI) in the presence of decabromodiphenyl ether (BDE209). *Environmental Pollution, 237*, 784–791. https://doi.org/10.1016/j.envpol.2017.10.130

Liang, J., Xia, X., Zhang, W., Zaman, W. Q., Lin, K., Hu, S., & Lin, Z. (2017). The biochemical and toxicological responses of earthworm (Eisenia fetida) following exposure to nanoscale zerovalent iron in a soil system. *Environmental Science and Pollution Research, 24*, 2507–2514. https://doi.org/10.1007/s11356-016-8001-6

Liu, C., Duan, C., Meng, X., Yue, M., Zhang, H., Wang, P., Xiao, Y., Hou, Z., Wang, Y., & Pan, Y. (2020). Cadmium pollution alters earthworm activity and thus leaf-litter decomposition and soil properties. *Environmental Pollution, 267*, 115410. https://doi.org/10.1016/j.envpol.2020.115410

Liu, J., Xiong, K., Ye, X., Zhang, J., Yang, Y., & Ji, L. (2015). Toxicity and bioaccumulation of bromadiolone to earthworm Eisenia fetida. *Chemosphere, 135*, 250–256. https://doi.org/10.1016/j.chemosphere.2015.04.058

Liu, M., Wang, J., Xu, M., Tang, S., Zhou, J., Pan, W., Ma, Q., & Wu, L. (2022). Nano zero-valent iron-induced changes in soil iron species and soil bacterial communities contribute to the fate of Cd. *Journal of Hazardous Materials, 424*, 127343. https://doi.org/10.1016/j.jhazmat.2021.127343

Liu, X., Xiao, R., Li, R., Amjad, A., & Zhang, Z. (2020). Bioremediation of Cd-contaminated soil by earthworms (Eisenia fetida): Enhancement with EDTA and bean dregs. *Environmental Pollution, 266*, 115191. https://doi.org/10.1016/j.envpol.2020.115191

Mamat, A., Zhang, Z., Mamat, Z., Zhang, F., & Yinguang, C. (2020). Pollution assessment and health risk evaluation of eight (metalloid) heavy metals in farmland soil of 146 cities in China. *Environmental Geochemistry and Health, 42*, 3949–3963. https://doi.org/10.1007/s10653-020-00634-y

Otunola, B. O., & Ololade, O. O. (2020). A review on the application of clay minerals as heavy metal adsorbents for remediation purposes. *Environmental Technology and Innovation, 18*, 100692. https://doi.org/10.1016/j.eti.2020.100692

Pei, P., Sun, Y., Wang, L., Liang, X., & Xu, Y. (2021). In-situ stabilization of Cd by sepiolite co–applied with organic amendments in contaminated soils. *Ecotoxicology and Environmental Safety, 208*, 111600. https://doi.org/10.1016/j.ecoenv.2020.111600

Schrick, B., Hydutsky, B. W., Blough, J. L., & Mallouk, T. E. (2004). Delivery vehicles for zerovalent metal nanoparticles in soil and groundwater. *Chemistry of Materials, 16*(11), 2187–2193. https://doi.org/10.1021/cm0218108

Sriprachote, A., Kanyawongha, P., Ochiai, K., & Matoh, T. (2012). Current situation of cadmium-polluted paddy soil, rice and soybean in the Mae Sot District, Tak Province, Thailand. *Soil Science and Plant Nutrition, 58*(3), 349–359. https://doi.org/10.1080/00380768.2012.686435

Tasharrofi, S., Rouzitalab, Z., Maklavany, D. M., Esmaeili, A., Rabieezadeh, M., Askarieh, M., Rashidi, A., & Taghdisian, H. (2020). Adsorption of cadmium using modified zeolite-supported nanoscale zero-valent iron composites as a reactive material for PRBs. *Science of the Total Environment, 736*, 139570. https://doi.org/10.1016/j.scitotenv.2020.139570

Van Groenigen, J. W., Van Groenigen, K. J., Koopmans, G. F., Stokkermans, L., Vos, H. M., & Lubbers, I. M. (2019). How fertile are earthworm casts? A meta-analysis. *Geoderma, 338*, 525–535. https://doi.org/10.1016/j.geoderma.2018.11.001

Vilardi, G., Verdone, N., & Palma, L. D. (2017). The influence of nitrate on the reduction of hexavalent chromium by zero-valent iron nanoparticles in polluted wastewater. *Desalination and Water Treatment, 86*, 252–258. https://doi.org/10.5004/dwt.2017.20710

Wang, J., Jiang, Y., Sun, J., She, J., Yin, M., Fang, F., Xiao, T., Song, G., & Liu, J. (2020). Geochemical transfer of cadmium in river sediments near a lead-zinc smelter. *Ecotoxicology and Environmental Safety, 196*, 110529. https://doi.org/10.1016/j.ecoenv.2020.110529

Wang, L., Cui, X., Cheng, H., Chen, F., Wang, J., Zhao, X., Lin, C., & Pu, X. (2015). A review of soil cadmium contamination in China including a health risk assessment. *Environmental Science and Pollution Research, 22*, 16441–16452. https://doi.org/10.1007/s11356-015-5273-1

Wang, Y., Yang, R., Hao, J., Sun, M., Wang, H., & Ren, H. (2021). The impact of Pseudomonas monteilii PN1 on enhancing the alfalfa phytoextraction and responses of rhizosphere soil bacterial communities in cadmium-contaminated soil. *Journal of Environmental Chemical Engineering, 9*(6), 106533. https://doi.org/10.1016/j.jece.2021.106533

Xu, C., Qi, J., Yang, W., Chen, Y., Yang, C., He, Y., et al. (2019). Immobilization of heavy metals in vegetable-growing soils using nano zero-valent iron modified attapulgite clay. *Science of the Total Environment, 686*, 476–483. https://doi.org/10.1016/j.scitotenv.2019.05.330

Xu, R., Sun, X., Häggblom, M. M., Dong, Y., Zhang, M., Yang, Z., Xiao, E., Xiao, T., Gao, P., & Li, B. (2022). Metabolic potentials of members of the class Acidobacteriia in metal-contaminated soils revealed by metagenomic analysis. *Environmental Microbiology, 24*(2), 803–818. https://doi.org/10.1111/1462-2920.15612

Xue, W., Huang, D., Zeng, G., Wan, J., Cheng, M., Zhang, C., Hu, C., & Li, J. (2018a). Performance and toxicity assessment of nanoscale zero valent iron particles in the remediation of contaminated soil: A review. *Chemosphere, 210*, 1145–1156. https://doi.org/10.1016/j.chemosphere.2018.07.118

Xue, W., Huang, D., Zeng, G., Wan, J., Zhang, C., Xu, R., Cheng, M., & Deng, R. (2018b). Nanoscale zero-valent iron coated with rhamnolipid as an effective stabilizer for immobilization of Cd and Pb in river sediments. *Journal of Hazardous Materials, 341*, 381–389. https://doi.org/10.1016/j.jhazmat.2017.06.028

Yang, X., Tsibart, A., Nam, H., Hur, J., El-Naggar, A., Tack, F. M., Wang, C.-H., Lee, Y. H., Tsang, D. C., & Ok, Y. S. (2019). Effect of gasification biochar application on soil quality: Trace metal behavior, microbial community, and soil dissolved organic matter. *Journal of Hazardous Materials, 365*, 684–694. https://doi.org/10.1016/j.jhazmat.2018.11.042

Yirsaw, B. D., Mayilswami, S., Megharaj, M., Chen, Z., & Naidu, R. (2016). Effect of zero valent iron nanoparticles to Eisenia fetida in three soil types. *Environmental Science and Pollution Research, 23*, 9822–9831. https://doi.org/10.1007/s11356-016-6193-4

Yu, G., Jiang, P., Fu, X., Liu, J., Sunahara, G. I., Chen, Z., Xiao, H., Lin, F., & Wang, X. (2020). Phytoextraction of cadmium-contaminated soil by Celosia argentea Linn.: A long-term field study. *Environmental Pollution, 266*, 115408. https://doi.org/10.1016/j.envpol.2020.115408

Zhang, Y., Li, Y., Dai, C., Zhou, X., & Zhang, W. (2014). Sequestration of Cd (II) with nanoscale zero-valent iron (nZVI): Characterization and test in a two-stage system. *Chemical Engineering Journal, 244*, 218–226. https://doi.org/10.1016/j.cej.2014.01.061

Zhu, F., Li, L., Ren, W., Deng, X., & Liu, T. (2017). Effect of pH, temperature, humic acid and coexisting anions on reduction of Cr (VI) in the soil leachate by nZVI/Ni bimetal material. *Environmental Pollution, 227*, 444–450. https://doi.org/10.1016/j.envpol.2017.04.074

Zou, Y., Wang, X., Khan, A., Wang, P., Liu, Y., Alsaedi, A., Hayat, T., & Wang, X. (2016). Environmental remediation and application of nanoscale zero-valent iron and its composites for the removal of heavy metal ions: A review. *Environmental Science & Technology, 50*(14), 7290–7304. https://doi.org/10.1021/acs.est.6b01897