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Unveiling a New Perspective on Cadmium-Induced Hormesis in Soil Enzyme Activity: The Relative Importance of Enzymatic Reaction Kinetics and Microbial Communities

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Abstract: Hormesis in soil enzymes is well-established, yet the underlying mechanism remains elusive. In this novel study, we investigated the effects of low-dose Cd exposure (0, 0.03, 0.3, 3, and 30 mg·kg^{-1}) in farmland soil within a typical constructed wetland environment. We assessed the activities of four soil enzymes (urease (URE), denitrification enzyme (DEA), dehydrogenase (DHA), and alkaline phosphatase (ALP)) at varying exposure durations (0 h, 24 h, and 48 h), evaluating hormetic characteristics across these time intervals. Additionally, we determined kinetic parameters, specifically the Michaelis constant (K_m) and maximum reaction velocity (V_{max}), for these enzymes while examining potential alterations in microbial community structure. Our findings revealed hormesis in all four soil enzymes at 24 h of exposure, with varying stimulus width and maximum hormesis rates. Interestingly, heavy metals did not significantly influence the diversity of soil microbial communities, but they did inhibit the ability of soil microbial communities to secrete extracellular enzymes. This resulted in a reduction in the soil enzyme pool and a consequential shift in overall soil enzyme activities. The conclusion of this study is that low-dose Cd primarily reduced extracellular enzyme secretion by soil microorganisms, leading to a reduction in the size of the soil enzyme pool and thereby inducing hormesis in soil enzyme activities.

Keywords: hormesis; cadmium; dose–response relationship; soil enzyme; bacterial community composition

Citation: Wu, J.; Wu, Z.; Agathokleous, E.; Zhu, Y.; Fan, D.; Han, J. Unveiling a New Perspective on Cadmium-Induced Hormesis in Soil Enzyme Activity: The Relative Importance of Enzymatic Reaction Kinetics and Microbial Communities. *Agriculture* **2024**, *14*, 904. https://doi.org/10.3390/agriculture14060904

Academic Editor: Anna Piotrowska-Długosz

Received: 13 May 2024
Revised: 3 June 2024
Accepted: 5 June 2024
Published: 7 June 2024

1. Introduction

Hormesis was initially observed by German biologists Hugo Arndt and Rudolph Schultz in 1888. In 1943, this stimulatory effect on organisms resulting from exposure to low-dose chemicals was officially termed "Hormesis". Following a prolonged period of silence, Calabrese, Kaiser, Renner et al. published articles in prestigious journals, such as *Nature*, *Science*, and *E&ST*, in 2003 to comprehensively review the phenomenon of hormesis and its associated effects [1–3]. This groundbreaking concept not only challenges the conventional linear dose–response theory but also compels us to reevaluate the fundamental problem of environmental management and risk control: how clean is clean [4]? In other words, when the "low dose" surpasses the limit or critical threshold, this stress-inducing dose

not only does not necessitate repair or control measures but may even confer benefits when compared against established environmental quality benchmarks or standards [5–8]. Undoubtedly, these findings hold significant implications for assessing environmental risks.

The prevailing perspective regarding the mechanism of hormesis is Overcorrection/Overcompensation [9]. While a substantial body of experimental data supports this theory, further experimental results are required to quantify this process for environmental risk assessment across diverse subjects and stressors [10–12]. Notably, several studies have demonstrated that low-dose Cd in soil can enhance eggplant and rice yield while also significantly increasing intracellular enzyme activity in earthworms [13,14]. In fact, due to the traditional research paradigm of hormesis, the majority of previous studies have been limited to investigating individual organisms. However, a longstanding inquiry remains: what are the implications of a "low dose" that exhibit "beneficial" effects on soil, animals, or plants for the soil itself [15,16]? Specifically, how can individual-level traits (organisms) be extrapolated to encompass community or ecosystem levels and establish associations between low-dose pollution and ecosystem characteristics [5]?

In contrast to the rapid advancements in the models of individual organisms, research on soil as an experimental model remains significantly limited. Recent studies utilizing soil as an experimental model have revealed the sensitivity of soil enzymes to stress. These enzymes play a pivotal role in all biochemical processes within the soil [17,18]. Consequently, soil enzymes play an important role in all soil biochemical processes, and changes in soil enzyme activities can indicate the direction and degree of soil biochemical reactions [19–21]. Fan et al. discovered a wide range of stimulation in soil alkaline phosphatase (ALP) activity induced by Cd, with the maximum stimulation varying from 8% to 66% and the stimulating dose ranging from 0.1 to 3.0 mg/kg [22]. This observation highlights the substantial variability in soil enzyme-related hormesis, which can be attributed to both the complexity of the subject system and the inherent biological characteristics of soil enzymes. Previous studies have primarily focused on investigating the impact of Cd on various aspects of soil enzymes, including their activity, structure, ecotoxicity, kinetics, and thermodynamic processes under single or combined stress [23,24]. Additionally, researchers have explored the potential role of soil enzymes as indicators for assessing Cd pollution. The mechanism underlying the effects of Cd on soil enzyme activities is believed to involve its interaction with substrates, enzyme structures, or enzyme–substrate complexes [25]. Moreover, it is also suggested that Cd indirectly influences enzyme activities through its influence on associated microbial communities [26]. Obviously, previous studies have made significant progress and achievements in both the effects of activity and discussions on mechanisms. However, it is worth noting that most of these studies primarily focus on the inhibitory effect of high-dose Cd, leaving a considerable gap in research regarding the stimulatory effect of low doses. The responses exhibited by soil enzymes to Cd stimulation can manifest in various forms, including enzyme deformation or deconstruction, as well as Cd binding to substrates or enzyme–substrate complexes [27–29]. These alterations' impact on enzyme activity can be effectively elucidated through the application of enzyme kinetics theory and methodologies.

There are frequent reports of hormesis in microbial populations, but it is crucial to elucidate the relationship between these changes and alterations in enzyme activities. For instance, the hormetic response observed in soil ALP under Cd stress cannot solely be attributed to ALP-associated microbial strains [30]. In other words, this response reflects a collective modification and interaction among all microorganisms in response to stressors, which can be characterized by ALP as well as other soil enzymes. Therefore, the response of microbial quorum sensing may serve as a crucial mechanism driving soil enzyme hormesis [31,32]. Notably, recent studies on microbial responses to heavy metal stress have increasingly focused on analyzing changes in Gram-positive (G+) and Gram-negative (G-minus) bacteria [7,33,34]. The ecological significance of rare microorganisms (relative abundances < 0.1%) has also been highlighted more recently, thereby expanding our emphasis on soil microbial ecology [35–37]. Clearly, classifying microorganisms under

Cd stimulation based on the responses of G+ and G− bacteria as well as abundant and rare microorganisms is expected to provide a clearer understanding of the relationship between microbial quorum sensing and the expression of soil enzyme hormesis.

Previous studies on the response of soil enzymes to heavy metal stress have focused on the response of single soil enzymes. Some studies have also suggested that the community response of soil microorganisms may be an important mechanism of hormesis of soil enzymes. In this paper, we wanted to know how the swarm response of microbes relates to changes in soil enzyme hormesis. Is soil enzyme hormesis dominated by changes in the affinity of enzymes to substrates due to stress, or is it due to microbial responses that alter the total amount of soil enzymes? In order to study the relative importance of enzymatic reactions and microbial activity in the process of soil enzyme hormesis, this experiment was conducted using soil as the subject and Cd as the stressor. We sought to evaluate the activities of four typical soil enzymes (urease, dehydrogenase, denitrification enzyme, and alkaline phosphatase) under low doses of Cd (0, 0.03, 0.3, 3, and 30 $mg \cdot kg^{-1}$) at different exposure times (0, 24, and 48 h) in order to determine enzyme-specific hormesis characteristics as a function of time. Here, we hypothesize that the hormesis of enzymes is mainly caused by two aspects, enzyme inactivation and enzyme accumulation, which coincides with the kinetic parameters of enzymatic reaction. Based on this assumption, we also evaluated kinetic parameters of the four enzymes, including the Michaelis constant (Km) and the maximum reaction velocity (Vmax), as well as the microbial community structure in an integrated manner. This study provides a plausible enzymatic and microbiological explanation for the phenomenon of soil enzyme hormesis.

2. Materials and Methods

2.1. Soil Sample Collection and Soil Properties

Five sampling areas were selected in the study area. The distance between each of two sampling areas was at least 50 m, and $0.5 \times 0.5 \times 0.2$ m^3 samples were collected in each area. Before sampling, stones, litter, roots, and other large debris were removed, and soil from the 0–20 cm layer was collected with a spade and stored separately. One part was air-dried in sterile plastic bags and sieved through a 2 mm nylon sieve for chemical and soil enzyme assays, and the other part was placed in a sterile frozen incubator for microbiological analysis. The basic physical and chemical properties of farmland soil are shown in Table 1.

Table 1. Physical and chemical properties of farmland soil (SOC: soil organic carbon, DOC: dissolved organic carbon, TN: total soil nitrogen, NH_4^+-N: soil ammonia nitrogen, NO_3^--N: soil nitrate).

pH	EC $ms \cdot cm^{-1}$	SOC $g \cdot kg^{-1}$	DOC $mg \cdot L^{-1}$	TN $g \cdot kg^{-1}$	NH_4^+-N $mg \cdot kg^{-1}$	NO_3^--N $mg \cdot kg^{-1}$
7.95 ± 0.05	245 ± 18	13.62 ± 1.11	14.75 ± 1.05	1.31 ± 0.05	6.99 ± 1.15	3.44 ± 0.84

Soil pH was measured with a pH meter (PB-10, Sartorius, Göttingen, Germany) at a soil to water ratio of 1:2.5, while soil EC was measured with an EC meter (DS-11A, Leici, China) in soil/water suspension (1:1).

Soil organic carbon (SOC) was measured with a TOC analyzer (multi N/C 3100, Analytik Jena, Jena, Germany), and fresh soil was drip-added with 10% hydrochloric acid to eliminate inorganic carbon until bubbles were no longer generated, which was then determined using the external solid module of the TOC instrument. Dissolved organic carbon (DOC) was measured with a TOC analyzer (multi N/C 3100, Analytik Jena, Jena, Germany) at a soil to water ratio of 1:5.

Total soil nitrogen (TN) was determined using the Kjeldahl method. Samples were digested using an induction cooker and then distilled and titrated using a semi-automatic nitrogen analyzer (NKB-3200, Yihong Instrument, Guangdong, China). Soil nitrate (NO_3^-N) was measured with an ultraviolet spectrophotometer (UV-2550, Elemental Analysis Instrument, Suzhou, China). After extraction with KCl, the color was analyzed at 220 nm and

275 nm wavelengths to identify the corresponding nitrate content on the working curve. Soil ammonia nitrogen (NH_4^+-N) was analyzed using an ultraviolet spectrophotometer (UV-2550 type, Chromatography Instrument, Hefei, China). After extraction with KCl, phenol solution and alkaline sodium hypochlorite solution were added and maintained at room temperature for 1 h. Subsequently, colorimetry was performed at a wavelength of 625 nm to identify the corresponding ammonia nitrogen content on the working curve.

2.2. Soil Incubation Experiment under Cd Stress

2.2.1. Preincubation

A 20 mL brown glass bottle was used to fill 5.0 g of air-dried soil samples, and 2.0 mL of deionized water was added. After sealing with a breathable membrane, the bottle was placed in an incubator at 25 °C for 48 h.

2.2.2. Soil Enzyme Activity Test

Different doses of $CdCl_2 \cdot 2.5H_2O$ solution (1.0 mL) were added to the preincubated soil samples in order to create 8 treatments in addition to the control. Because the hormesis of four soil enzymes was to be investigated simultaneously, doses were selected considering previous studies reporting stimulation in enzymes (see Table S1). The reasons for the reduction in the Cd treatment in the part of the soil enzyme kinetics experiment and the microbial experiment are mainly the following. Firstly, the selected dose was selected according to the definition of hormesis to ensure that the hormetic characteristics of soil enzymes could be accurately reflected. Second, we described the hormetic characteristics of soil enzymes at higher doses (0, 0.006, 0.03, 0.06, 0.3, 0.6, 3.0, 6.0, and 30 $mg \cdot kg^{-1}$ of Cd). Choices included 0 (control), maximum stimulating dose, zero equivalent dose point, and absolute inhibitory dose. Finally, because the response doses of the four soil enzymes were different, the doses selected were as broad as possible and covered the whole "stimulation–inhibition" process. Therefore, the following Cd^{2+} doses were selected for inclusion in this study: 0 (control), 0.006, 0.03, 0.06, 0.3, 0.6, 3.0, 6.0, and 30 $mg \cdot kg^{-1}$.

After sealing the membrane again, the soil was placed in an incubator at 25 °C for 0 (in this study, 0 h refers to a Cd^{2+} exposure time of 0.5 h), 24, and 48 h, respectively. The activities of soil hydrolases (URE, ALP) and oxidoreductases (DEA, DHA) were measured, and the measurement methods are detailed in Section 2.3.

2.2.3. Soil Enzyme Kinetics Experiment

Different concentrations of substrate were added to the precultured soil samples. Urea (0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14 $mol \cdot L^{-1}$), PNPP (5.0, 10, 15, 25, 50 $mmol \cdot L^{-1}$), potassium nitrate (25, 50, 100, 200, 400 $mg \cdot L^{-1}$), and TTC (0.125%, 0.25%, 0.5%, 0.75%, 1.0%, 1.5%, 2.0%) solutions were used to measure the activities of four soil enzymes, as detailed in the succeeding section. For all of the sets of analyses, five sets of replicates were set for each treatment.

2.3. Determination of Soil Enzyme Activity

2.3.1. Urease

Soil URE activity was determined according to the method of Solomon et al. [38]. After preincubation, 2.5 mL of 0.08 $mol \cdot L^{-1}$ urea solution was added to the tested samples, and then the samples were sealed and incubated at 37 °C for 2 h (the control group was not incubated). At the end of incubation, 2.5 mL of distilled water was added. The soil sample in the vial was washed into a 100 mL covered container with 50 mL of 2.0 $mol \cdot L^{-1}$ KCl extract and shaken at 180 $r \cdot min^{-1}$ for 30 min. The shaken soil suspension was quickly filtered. Then, 1.0 mL of the filtrate was put into a 50 mL centrifuge tube, and 9.0 mL of ultrapure water, 5.0 mL of sodium salicylate, and 2.0 mL of sodium dichloroisocyanurate solution were added successively. After 30 min at room temperature, the absorbance was measured at 690 nm with an ultraviolet-visible spectrophotometer using distilled water as a reference.

2.3.2. Dehydrogenase

Soil DHA activity was determined according to the method of Januszek et al. [39]. After preincubation, 5.0 mL of 0.5% 2,3,5-Triphenyltetrazolium chloride (TTC solution, 0.5 mol·L^{-1} Tris-HCl buffer solution, pH = 7.6) was added to the tested samples, and the samples were cultured in a dark incubator with constant temperature (37 °C) for 1 day (the lid was tightly closed). After incubation, 10 mL of methanol was added, and the samples were incubated at 25 °C in the dark at 200 r·min^{-1} for 1 h. Subsequently, it was vortexed for 1 min and filtered (quantitative filter paper), and the absorbance was measured at 485 nm using a spectrophotometer (UV-2550, Elemental Analysis Instrument, China).

2.3.3. Denitrifying Enzyme

Soil DEA activity was determined according to the method of Knowles [40]. In each preincubated sample, 5.0 mL of 10 g·L^{-1} glucose solution and 5.0 mL of 100 mol·L^{-1} potassium nitrate solution were added, and then the sealed samples were incubated for 48 h at 37 °C. The soil samples in the vials were washed into a 100 mL covered container with 25 mL of 1.0 mol·L^{-1} KCl extract and then shaken at 200 r·min^{-1} for 1 h. Subsequently, the liquid was transferred into a 50 mL centrifuge tube and centrifuged at 3000 r·min^{-1} for 10 min. Colorimetry was performed at wavelengths of 220 nm and 275 nm. The no-soil treatment served as the control, and, in order to eliminate the influence of soil-adsorbed nitrate on the experiment, samples with sterilized soil and no substrate were set.

2.3.4. Alkaline Phosphatase

Soil ALP activity was determined according to the method of Liu et al. [41]. Tris-HCl buffer 3.0 mL (pH = 8.4) was added to the preincubated samples. Then, 1.0 mL of 5.0 mmol·L^{-1} p-nitrophenyl phosphate disodium (PNPP) and 1.0 mL of 0.5 mol·L^{-1} $CaCl_2$ solution were added, and the samples were shaken. After a water bath at a constant temperature of 37 °C for 1 h (the control group did not undergo a water bath), the glass bottle was removed, 4.0 mL of 0.5 mol·L^{-1} NaOH was immediately added to terminate the reaction, and the reaction was filtered. Then, 5 mL of filtrate was extracted with a 25 mL plug cuvette, and 1.0 mL of 2.0 mol·L^{-1} Tris solution was added. Ultrapure water was then added to a constant volume. The absorbance was determined with an ultraviolet spectrophotometer (UV-2550 type, Chromatography Instrument, China) at a wavelength of 400 nm, while ultrapure water was used as a reference.

2.4. Extraction of DNA and 16s rDNA Sequencing

The CTAB method was employed to extract the total DNA of microbiome samples from various sources, and the quality of DNA extraction was detected through agarose gel electrophoresis. The V3-V4 fragments were amplified through PCR using primers 341F(5′-CCTACGGGNGGCWGCAG-3′) and 805R(5′-GACTACHVGGGTATCTAATCC-3′). The PCR products were confirmed through electrophoresis on a 2% agarose gel. PCR products were purified using AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified through Qubit (Invitrogen, Waltham, MA, USA). PCR amplification products were examined through electrophoresis on a 2% agarose gel; the AMPure XT beads recovery kit was used. The purified PCR products were evaluated using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and a library quantification kit from Illumina (Kapa Biosciences, Woburn, MA, USA); qualified library concentrations should be above 2 nmol·L^{-1}. For the double-end data obtained through sequencing, it is first necessary to data-split the samples according to barcode information and remove the linker and barcode sequences. Length filtering and denoising were performed by calling dada2 via DADA2 denoise-paired. ASV feature sequences and ASV abundance tables were obtained, and singleton ASVs were removed.

2.5. Fitting of Kinetic Parameters of Enzymatic Reaction

The preincubated soil was incubated under four concentration treatments (0, 0.03, 0.3, 3.0, and 30 mg·kg^{-1} of Cd) for 0, 24, and 48 h. The corresponding substrates (urea, PPNP, potassium nitrate, and TTC) were used to measure the enzyme activities of the preincubated soil. The initial speed of enzymatic reaction V_0 and the known substrate concentration S were obtained. The Michaelis constant (K_m) and the maximum reaction velocity (V_{max}) were calculated using a nonlinear regression of the Michaelis equation:

$$V_0 = \frac{V_{max} \times S}{K_m + S}$$

where V_0 is the initial enzymatic reaction velocity, μg·g^{-1}·h^{-1}; S represents the substrate concentration, μg·mL^{-1}; V_{max} is the maximum reaction rate, μg·g^{-1}·h^{-1}; and K_m indicates the Michaelis constant, μg·mL^{-1}.

2.6. Dose–Response Curve Fitting

Soil enzyme activities were obtained using the method described above, and dose–response curves were calculated. The percentage of stimulation or inhibition (%) of soil enzyme activity was calculated as

$$R = \frac{(E_i - E_0)}{E_0} \times 100\% \quad (1)$$

where R is the inhibition or stimulation rate (negative is inhibition), %, and E_i and E_0 are the soil enzyme activities in the test sample and the control (without added Cd), respectively.

The hormesis dose–response curve has two characteristic indexes: the magnitude of stimulation and the width of the hormetic zone within which stimulatory responses occur [42]. These indexes provide valuable insights to characterize hormesis. The parameters M_{max} (maximum hormesis rates), ZEP (zero equivalent dose point, x_1 and x_2), and Q_i (stimulus width of the hormetic zone) are derived from the fitted curves. In addition, Hor_{zone} (stimulation area of the hormetic zone) was used to integrate the stimulus width and maximum hormesis rate into a unified parameter describing the hormesis-related characteristics of organisms (Figure S1).

In this study, LogNormal Model (peak type, 4 parameters) was employed to fit the dose–response curve using Sigmaplot14.0. In addition, OriginLab 2021 was used to integrate and calculate the area of the inverted U-shaped curve.

2.7. Statistical Analysis

The data related to enzyme activity are presented as mean ± standard deviation, $n = 5$. Analysis of variance (ANOVA) was used to test for differences in soil enzyme activities among nine Cd concentrations, and Dunnett's test was employed. An a priori statistical significance level of $\alpha = 0.05$ ($p < 0.05$) was used for all statistics. Venn plots were used to analyze differences in the number of common and unique soil bacteria OUT between the control (without Cd addition) and four different Cd exposure doses at each culture time. Relative abundances (RAs) were calculated by dividing the number of sequences affiliated with each OUT by the total number of sequences in each sample (%). Gram-positive bacteria (G+) and Gram-negative bacteria (G-) are the two major groups of bacteria identified by the Glanz stain. Abundant taxa (AT) were defined as genera with relative abundance $\geq 1\%$ in the sample, and rare taxa (RT) were defined as genera with relative abundance $\leq 0.1\%$ [43,44]. Subsequently, the Brey–Curtis dissimilarity matrix was generated on the basis of the standardized matrix. Non-metric multidimensional scales (NMDS) were employed to determine the β-diversity of bacterial community structure and implemented using the metaMDS function in R's vegan package (R Core Team, 2020).

Variance decomposition analysis (VPA) was used to analyze the contribution of microbial diversity (α-diversity + characteristic microbial distribution) and enzyme kinetics

(affinity K_m and enzyme library V_{max}) to the changes in enzyme activity with Cd dose. VPA is an extension of redundancy analysis (RDA), which aims to analyze the contribution of multiple variables to the variation of a single variable. VPA was conducted using the varpart function in R's vegan package (R Core Team, 2020).

3. Results

3.1. Responses of Four Soil Enzymes to Exogenous Cd Stress

The activities of the four soil enzymes at different times of exposure (0, 24, and 48 h) to exogenous Cd are shown in Figure 1. The maximum stimulations of URE, DHA, DEA, and ALP were 11.62% (0 h, 0.006 mg·kg^{-1} of Cd), 86.96% (0 h, 0.03 mg·kg^{-1}), 2.80% (24 h, 0.6 mg·kg^{-1} of Cd), and 29.62% (24 h, 0.06 mg·kg^{-1} of Cd), respectively. The enzyme activities of URE and ALP did not change much after exposure to all concentrations of exogenous Cd for 48 h. All four soil enzymes produced a phenomenon similar to "hormesis" when exposed to Cd for 24 h.

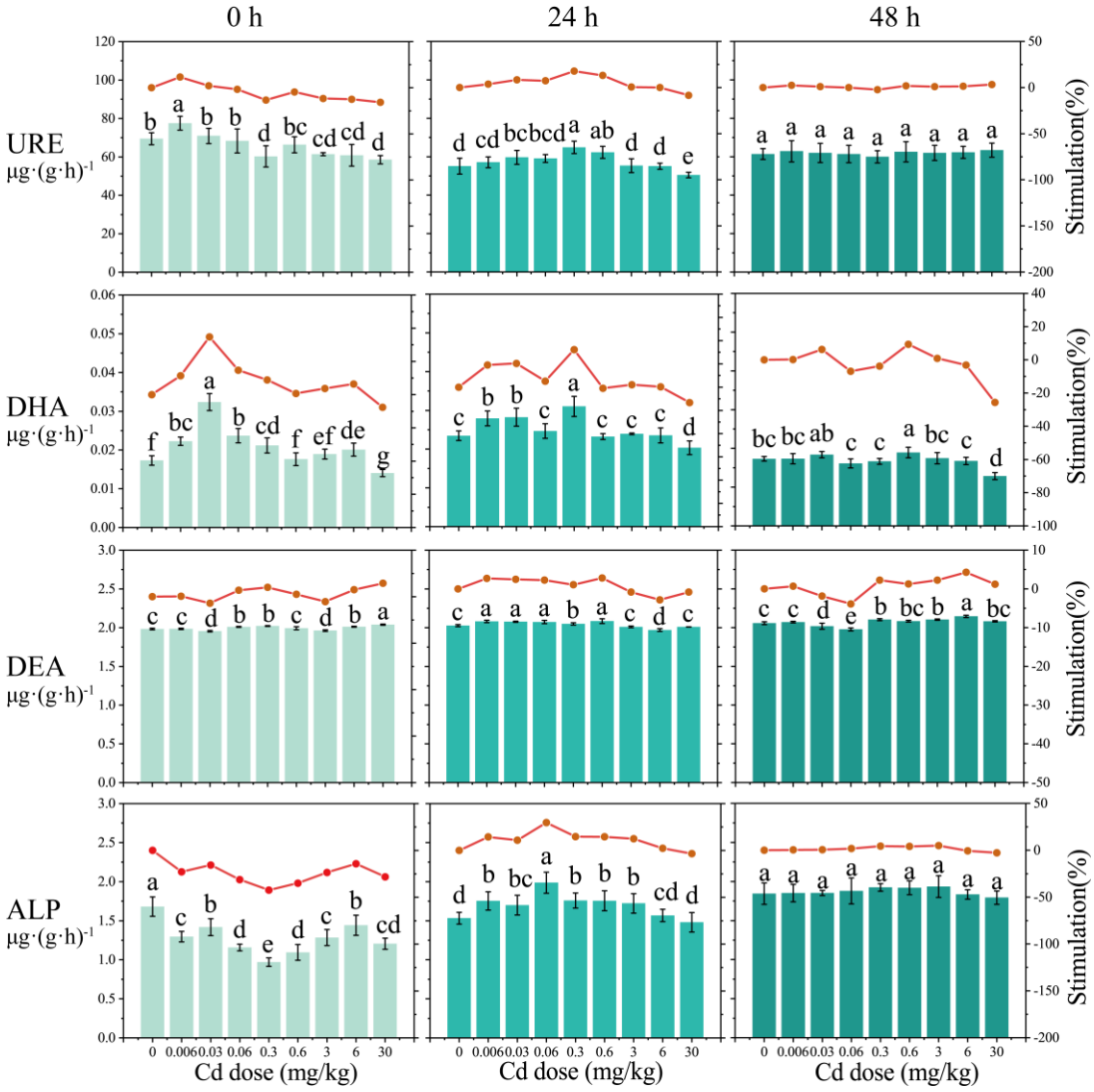

Figure 1. Effect of Cd addition (0, 0.006, 0.03, 0.06, 0.3, 0.6, 3.0, 6.0, and 30 mg·kg^{-1} of Cd) and exposure time (0, 12, and 24 h) on activity of four soil enzymes (URE (urase), DHA (dehydrogenase), DEA (denitrifying enzyme), and ALP (alkaline phosphatase)). Values are presented as means ± SE (n = 5). The red line in the figure indicates the stimulation rate of enzyme activity. Different letters above bars indicate statistically significant differences between treatments ($p < 0.05$, Duncan's test).

3.2. Fitting of Soil Hormesis Parameters

In order to accurately describe the hormesis dose–response relationship and clarify its application to ecological risk assessment, it is necessary to fit the data to obtain some important parameters, such as M_{max}, Q_i, and Hor_{zone} (Figure S1). These parameters are obtained from the fitted model (lognormal model). As shown in Figure 2, M_{max} ranged from 2.86% (DEA, 24 h) to 20.11% (ALP, 24 h). Q_i varied from 634.95 (URE, 24 h) to 91,968.93 (ALP, 24 h), while the Hor_{zone} of the soil enzymes ranged from 0.0130 (DEA, 24 h) to 0.7963 (ALP, 24 h).

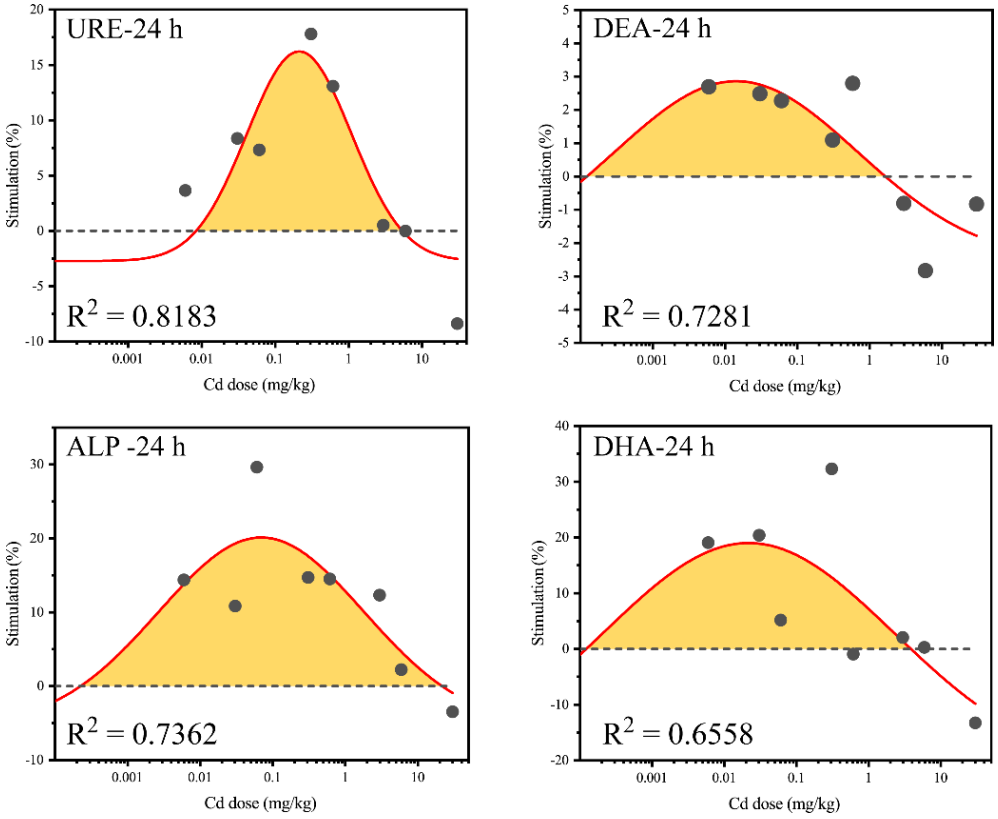

Figure 2. Dose–response relationships of different soil enzymes based on Lognormal distribution model. In the figure, the red line is the fitted curve, and the yellow area represents the integrated area of Hor_{zone} (URE: urease, DEA: denitrification enzyme, DHA: dehydrogenase, and ALP: alkaline phosphatase).

3.3. Kinetic Parameters of Enzymatic Reaction of Soil Enzymes

The kinetic parameters of the enzymatic reaction of four soil enzymes at 0, 24, and 48 h were evaluated (Table 2). Regarding urease, the maximum V_{max} was 125 μg·g^{-1}·h^{-1} and the maximum K_m was 0.05 μg·mL^{-1} at 24 h, and the inducing Cd dose was 0.3 mg·kg^{-1}. For DHA, the maximum values of V_{max} and K_m were 0.0344 μg g^{-1} h^{-1} and 0.00145 μg·mL^{-1}, induced by 0.3 mg·kg^{-1} at 48 h. As for DEA, the maximum V_{max} and K_m values were 11.4 μg·g^{-1}·h^{-1} and 631 μg·mL^{-1} in the control (no Cd supplementation) at 0 h. The maximum V_{max} of ALP was 333 μg·g^{-1}·h^{-1}, while the maximum K_m was 2883 μg·mL^{-1}, induced by 0.3 mg·kg^{-1} at 0 h. It emerges that the maximum $V_{\max}$ and K_m were more determined by the Cd dose (0.3 mg kg^{-1}) than the duration of exposure.

Table 2. Kinetic parameters of enzymatic reaction of four soil enzymes (URE: urease, DEA: denitrification enzyme, DHA: dehydrogenase, and ALP: alkaline phosphatase).

Time (h)	Cd (mg·kg^{-1})	URE		DHA		DEA		ALP	
		V_{max} μg·g^{-1}·h^{-1}	K_m μg·mL^{-1}	V_{max} μg·g^{-1}·h^{-1}	K_m μg·mL^{-1}	V_{max} μg·g^{-1}·h^{-1}	K_m μg·mL^{-1}	V_{max} μg·g^{-1}·h^{-1}	K_m μg·mL^{-1}
0	0	73 ± 6	0.006 ± 0.0002	0.0191 ± 0.0012	0.000038 ± 0.000002	11.4 ± 0.8	631 ± 29	250 ± 8	2225 ± 105
	0.03	105 ± 10	0.032 ± 0.0013	0.0205 ± 0.0017	0.000062 ± 0.000003	6.5 ± 0.5	387 ± 9	167 ± 3	1415 ± 45
	0.3	104 ± 10	0.01 ± 0.0002	0.0212 ± 0.0012	0.00017 ± 0.000003	4.9 ± 0.3	336 ± 8	333 ± 3	2883 ± 70
	3	60 ± 3	0.006 ± 0.0018	0.0199 ± 0.0018	0.00016 ± 0.000005	3.6 ± 0.3	276 ± 12	50 ± 4	436 ± 15
	30	79 ± 3	0.024 ± 0.0011	0.0182 ± 0.0011	0.00027 ± 0.000005	5.2 ± 0.4	345 ± 11	16 ± 1	140 ± 6
24	0	67 ± 6	0.0026 ± 0.0016	0.0313 ± 0.0016	0.00066 ± 0.000012	4.7 ± 0.3	334 ± 8	7.3 ± 0.4	39 ± 1
	0.03	71 ± 5	0.0035 ± 0.0002	0.0303 ± 0.0022	0.00018 ± 0.000003	5.5 ± 0.5	375 ± 12	10.4 ± 0.8	63 ± 3
	0.3	125 ± 7	0.05 ± 0.0024	0.03 ± 0.0024	0.00027 ± 0.000012	6.8 ± 0.5	435 ± 17	12.8 ± 0.9	82 ± 2
	3	91 ± 7	0.0182 ± 0.0022	0.0282 ± 0.0022	0.00017 ± 0.000007	6 ± 0.5	386 ± 12	10.5 ± 0.8	66 ± 2
	30	71 ± 5	0.0143 ± 0.0025	0.028 ± 0.0025	0.00025 ± 0.000003	4.4 ± 0.4	324 ± 11	6.6 ± 0.4	41 ± 1
48	0	114 ± 7	0.011 ± 0.0020	0.0276 ± 0.0019	0.00077 ± 0.000012	4.7 ± 0.3	213 ± 4	12.7 ± 0.7	90 ± 2
	0.03	104 ± 6	0.01 ± 0.0003	0.031 ± 0.0030	0.00108 ± 0.000025	5.5 ± 0.4	233 ± 11	22.2 ± 1.7	166 ± 3
	0.3	94 ± 6	0.003 ± 0.0018	0.0344 ± 0.0018	0.00145 ± 0.000042	6.8 ± 0.5	417 ± 12	55.6 ± 5.5	446 ± 21
	3	98 ± 10	0.008 ± 0.0018	0.0311 ± 0.0018	0.00112 ± 0.000024	6 ± 0.4	182 ± 9	66.7 ± 6.5	559 ± 27
	30	75 ± 4	0.002 ± 0.0026	0.0274 ± 0.0026	0.00142 ± 0.000032	4.4 ± 0.3	204 ± 8	22.2 ± 2.0	183 ± 9

3.4. Soil Bacterial Communities

3.4.1. Abundance Distribution, α-Diversity, and β-Diversity

At 0 h after exposure, the number of ASVs obtained from the soil cultured with different doses (0, 0.03, 0.3, 3.0, and 30 mg·kg^{-1}) of heavy metal Cd was 48,233, 53,943, 54,219, 55,792, and 55,757, respectively. When exposed to Cd for 24 h, the number of ASVs isolated from the soil was 38,182, 26,087, 64,402, 59,604, and 52,413, respectively. When the exposure time was 48 h, the number of ASVs isolated from the soil was 64,071, 61,095, 57,797, 57,682, and 61,596, respectively. Moreover, the total number of ASVs extracted from all of the soil samples was 32 313, and the distributions are shown in the Venn diagrams in Figure 3a. At 0 h, the highest number of ASVs (2586 ± 431) was observed in soil species exposed to 30 mg·kg^{-1} of Cd. When the exposure time was 24 h, the highest number of ASVs was observed at 3 mg·kg^{-1} of Cd (2901 ± 278). After 48 h of exposure, the highest number of ASVs was observed at 0.03 mg·kg^{-1} of Cd (2849 ± 514). It can be inferred that the maximum stimulatory dose decreased gradually with the increase in exposure time.

Based on genus-level taxonomic groups, a total of 1470 soil microorganisms were detected in all soil samples. The relative abundance of the top 30 taxa is shown as a bar chart (Figure 3d). *Bacillus* was the most dominant microbial group in all samples, with a relative abundance ranging from 14.86% to 16.78%. At the phylum level, these 30 genera belonged to *Firmicutes* (25.63% to 40.66%), *Proteobacteria* (23.81% to 32.33%), *Planctomycetota* (4.74% to 15.05%), *Acidobacteria* (7.31% to 10.41%), and *Chloroflexi* (2.68% to 4.26%).

There were significant differences in the observed OTUs of soil bacteria under different Cd exposure times (0, 24, and 48 h) and stress doses (0–30 mg·kg^{-1} of Cd) ($p < 0.05$), but no significant difference in the Shannon index (Figure 3b,c). When Cd was applied for 0 h, the observed OTU index ranged from 1608 to 2366, and the Shannon index ranged from 8.64 to 9.31. After 24 h, the observed OTU index and the Shannon index ranged from 1949 to 2800 and 9.39 to 9.73, respectively. At 48 h, the ranges of the observed OTU index and the Shannon index were 2423 to 2985 and 9.59 to 10.08.

According to the NMDS based on the Bray–Curtis distance difference (Figure 3e), soil the microbial community structure showed an evolving change trend with the increase in Cd exposure time, and the community structure was significantly different between 0 h and 48 h of exposure time. According to the Cd dose classification, the soil microbial community structure was more convergent at higher Cd concentrations but more dispersed at low Cd concentrations. In general, the diversity of the soil microbial community did not change much, but the community structure changed significantly with the increase in exposure time.

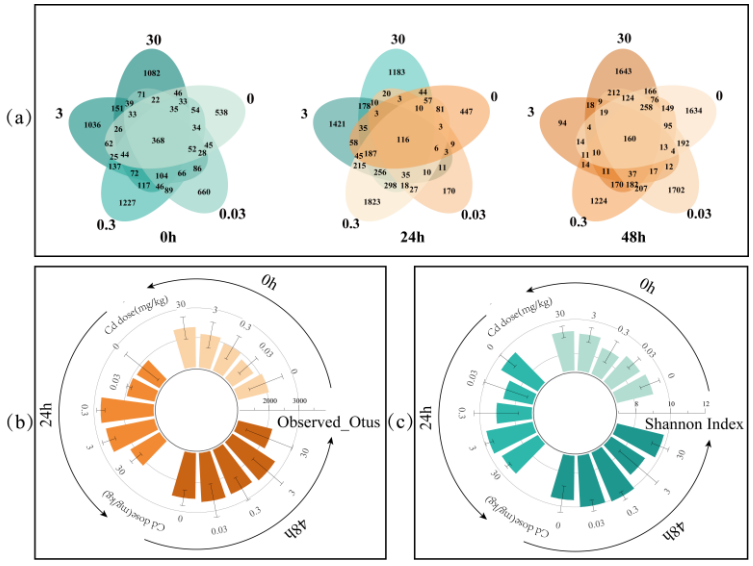

Figure 3. *Cont.*

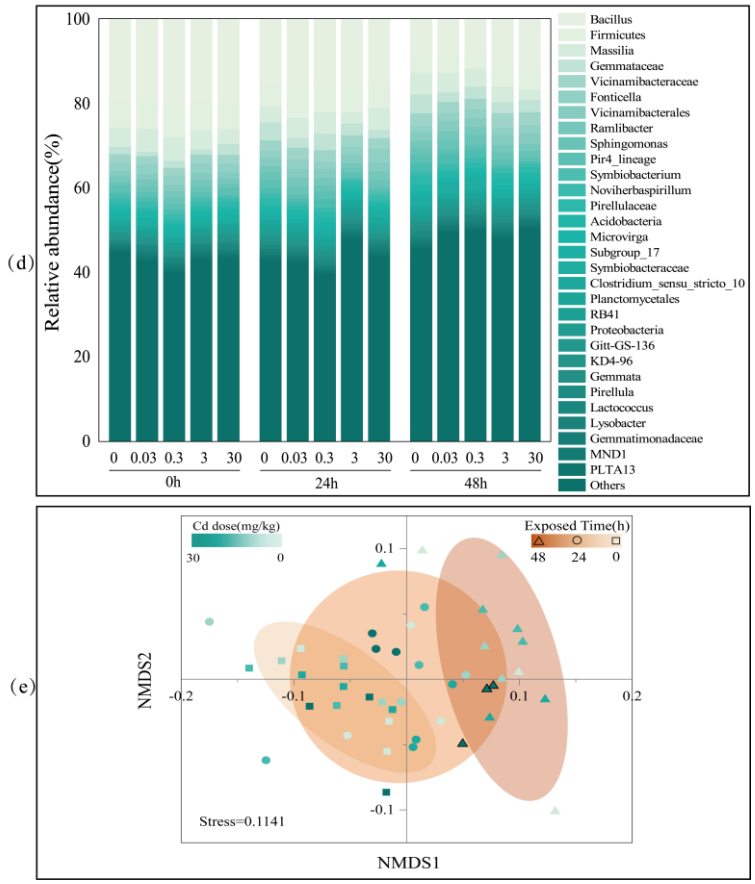

Figure 3. Bacterial community diversity under five Cd doses for three exposure times. (**a**) Venn diagrams. (**b**) Mean (n = 3) observed OTUs (operational taxonomic units) of soil bacterial communities. (**c**) Mean (n = 3) Shannon index of soil bacterial communities. (**d**) Relative abundance of bacterial-abundant genus in soils under five Cd doses and three exposure times. Bars represent mean of the 3 replicate samples per soil. (**e**) Beta-diversity of the bacterial communities presented in a non-metric multidimensional scaling (NMDS) ordination. Dots' color (blue) from light to dark represents elevated Cd doses, and 95% confidence ellipses (orange) represent exposure times.

3.4.2. Relative Abundance of G+ and G− Bacteria

Among the bacterial genera that could be detected, *Bacillus*, *Fonticella*, *Symbiobacterium*, *Microvirga*, *Lactococcus*, and *Clostridium* were G+, whereas *Massilia*, *Pir4_lineage*, *Ramlibacter*, *Sphingomonas*, and *Noviherbaspirillum* were G−. The relative abundance of G+ (Figure 4a) and G− (Figure 4b) varied with Cd dose and exposure time. With the increase in Cd exposure time, the relative abundance of G+ gradually decreased, with overall ranges of 24.01% to 24.84% (0 h), 18.67% to 20.94% (24 h), and 13.64% to 14.96% (48 h). In contrast, the relative abundance of G− did not change much with the increase in Cd exposure time, with overall ranges of 8.53% to 10.11% (0 h), 8.65% to 10.74% (24 h), and 7.87% to 11.02% (48 h). At a given exposure time, the relative abundance of G+ did not change much, and there was no significant difference in the relative abundance of G+ after 0 h and 24 h of Cd exposure. After 48 h of exposure, the relative abundance of G+ was significantly lower (−7.31–7.66%) than that of the control at 3–30 mg·kg^{-1} of Cd. At a given exposure time, the relative abundance of G− significantly changed with the increase in Cd stress dose. After 0 h of exposure, the relative abundance of G− was significantly higher (7.97–26.23%) at 0.03–3.0 mg·kg^{-1} of Cd and significantly lower (11.05%) at 30 mg·kg^{-1} of Cd compared to the control. These results are consistent with the hormesis characteristics of "low-dose stimulation and high-dose inhibition". After 24 h of exposure, the relative abundance of G− was significantly lower (9.62–12.01%) at 0.03 mg·kg^{-1} and 3 mg·kg^{-1} of Cd and significantly higher (9.21%) at 0.3 mg·kg^{-1} of Cd

compared to the control, showing hormesis characteristics. After 48 h of exposure, the relative abundance of G− at 0.03–0.3 mg·kg^{-1} of Cd was significantly higher than that of the control, and the stimulating amplitude ranged from 8.85% to 15.26%. Moreover, the relative abundance of G− at 30 mg·kg^{-1} of Cd was significantly lower (17.69%) than that of the control, which is also in alignment with the hormesis characteristic of "low-dose stimulation and high-dose inhibition".

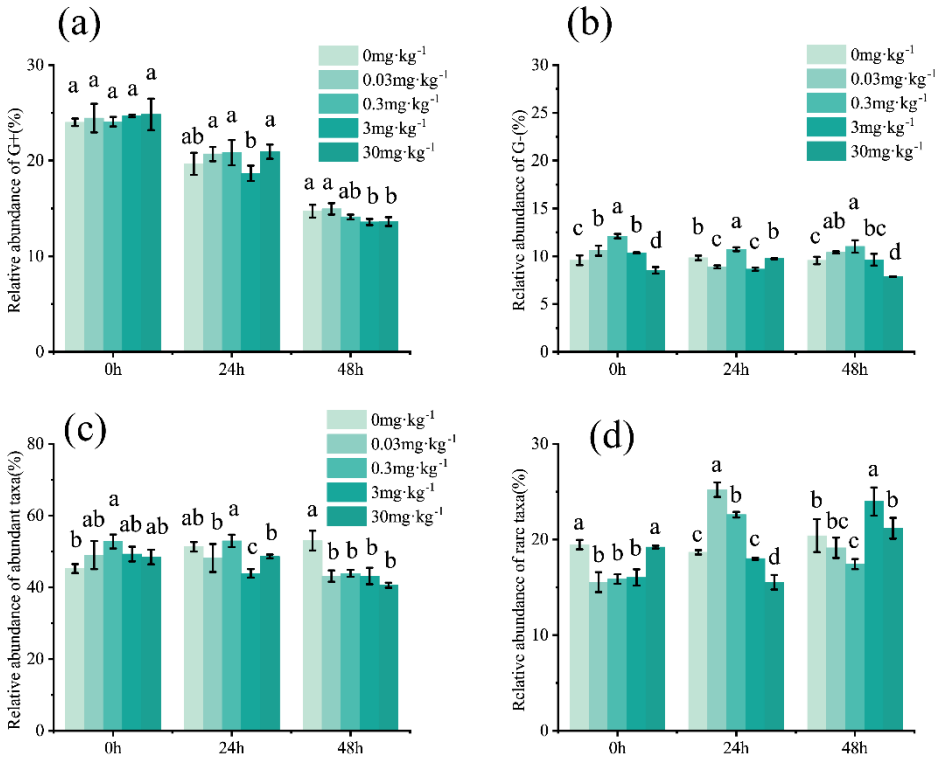

Figure 4. Effect of Cd addition (0, 0.006, 0.03, 0.06, 0.3, 0.6, 3.0, 6.0, and 30 mg·kg^{-1}) and exposure time (0, 12, and 24 h) on the relative abundance of G+ (**a**) and G− (**b**) populations and AT (**c**) and RT (**d**) populations. Gram-positive bacteria (G+) and Gram-negative bacteria (G−) are the two major groups of bacteria identified by the Glanz stain. Abundant taxa (AT) were defined as genera with relative abundance $\geq$ 1% in the sample, and rare taxa (RT) were defined as genera with relative abundance $\leq$ 0.1%. Different letters above bars indicate statistically significant differences between treatments ($p < 0.05$, Duncan's test).

3.4.3. The Relative Abundance of AT and RT Bacteria

According to the bacterial classification method, the relative abundance of two major microbial groups was obtained as a function of the Cd dose and exposure time. At the genus level, the relative abundance of AT ranged from 40.57% to 53.03% (Figure 4c), while the relative abundance of RT ranged from 15.53% to 25.21% under different Cd doses and exposure times (Figure 4d).

AT was significantly higher (16.66%) in the presence of 0.3 mg·kg^{-1} of Cd compared to the control at 0 h, showing a hormesis-like characteristic of low-dose stimulation (Figure 4c). After 24 h of exposure, AT was significantly lower (14.47%) than that of the control, inhibited by 3.0 mg·kg^{-1}. After 48 h of exposure, AT was significantly lower (17.24–23.50%) in 0.03–30 mg·kg^{-1} than in the control. After 24 h of exposure, RT was significantly higher (21.08–35.10%) in the presence of 0.03–0.3 mg·kg^{-1} of Cd but significantly lower (16.81%) in the presence of 30 mg·kg^{-1} of Cd compared to the control (Figure 4d). After 48 h of exposure, RT was significantly lower (14.57%) at 0.3 mg·kg^{-1} of Cd but significantly higher (17.48%) at 3.0 mg·kg^{-1} of Cd compared to the control.

4. Discussion

4.1. Cd-Induced Hormesis of Multi-Enzyme System in Soil

Recent evidence has shown that low doses of stress can induce soil enzymes to exhibit hormesis-like phenomena [11,45–48]. In this study, we evaluated the activity of representative soil oxidases (DHA and DEA) and hydrolases (URE and ALP) under different levels of Cd stress and exposure times to obtain two crucial indexes of the hormesis curve, i.e., the magnitude of stimulation and width of the stimulatory response. The finding that three of the four enzymes exhibited stimulation in the range of 16.21% to 20.12% corroborates the insights of Calabrese and Baldwin, who suggested that the stimulatory response is commonly of modest magnitude and often does not achieve statistical significance in hypothesis testing [49]. An updated evaluation by Calabrese et al., however, suggested that the stimulation magnitude tends to increase with an increasing number of doses included in the study design [50]. While typically the maximum stimulation has a magnitude in the range of 30–60%, it can be increased to 60–90% of the control response when there are at least six doses in the hormetic stimulatory zone [50]. Therefore, new studies should consider including several more doses to confirm whether the response of these enzymes is typically limited in magnitude. Calabrese and Baldwin et al. also reported that the majority (i.e., ~70%) of the observed stimulation ranges are <100-fold of the traditional threshold, with averages of 10- to 20-fold for the traditional experimental model organism. Previous studies on ALP and Cd identified stimulations in doses ranging from 0.004 to 0.2 $mg \cdot kg^{-1}$ (i.e., the stimulus width was 50-fold) based on observed data, with a maximum hormesis rate of 8.0% [51]. Studies on dehydrogenase and Cd found that stimulation based on observed data ranged from 0.6 to 5.0 $mg \cdot kg^{-1}$ (i.e., the stimulus width was 8.3-fold), with a maximum hormesis rate of 45% [52]. However, the stimulus width of the hormetic zone in the experiment was calculated using fitted data, and it was 600- to 10,000-fold. Previous studies mainly calculated the stimulus width based on observed empirical data. Nonetheless, with the development of hormesis research and the proposal of the fitting model, the stimulus width can be estimated for some experiments that have not observed the pre-inhibition effect. The estimates obtained by fitting the curve may be 10–100 times larger than the data obtained through observation. Another possible explanation might be that most of the previous studies focused on biological indicators, such as animal and plant secretions, but soil enzymes have unique properties as biomolecular molecules. As a carrier for many living organisms, the activity and function of soil are affected by many factors, such as physicochemical properties and the survival state of microbial communities [20]. Soil enzymes, as carriers of soil biological functions, are not secreted by a single microorganism. Soil microorganisms have redundant functions, and a single soil enzyme may be secreted and affected by multiple microorganisms [21].

Previous studies using the activity of ALP as the test endpoint have demonstrated that Cd-induced hormesis is time-dependent [53,54]. On this basis, here, we studied additional test endpoints and observed a similar phenomenon, with an inverted U-shaped curve in all four enzymes after 24 h of exposure. Among the four enzymes, the maximum hormesis dose of DEA was the lowest, and the maximum hormesis rate was also the lowest. This may be related to the unique mechanism of resistance of soil-denitrifying communities to heavy metals [55]. Holtan-Hartwig et al. observed that there was a recovery phenomenon in the enzyme activity of a denitrifying community under heavy metal stress [56]. That is, short-term exposure could cause a decrease in the N_2O reduction rate, which returns to the control level with longer-term exposure (2 months) [56]. URE had the highest maximum hormesis dose and a high maximum hormesis rate. This result may indicate that the strains secreted urease, increased the pH of the soil, and immobilized Cd, thus alleviating the toxicity of Cd [57].

4.2. Response of Kinetic Parameters of Enzymatic Reaction

There is a rich theoretical literature on the kinetics and thermodynamics of enzymatic reactions [58,59]. It is assumed that most enzymes follow the Michaelis–Menten kinetic

equation describing the reaction velocity (V). In this study, the kinetic parameters, including the Michaelis constant K_m and the maximum reaction velocity V_{max}, of four soil enzymes were measured under various levels of Cd stress and at different exposure times. For a single enzyme and a corresponding single substrate, K_m can be used to describe the affinity of the enzyme to the substrate [60]. This may be due to the interaction between heavy metals and the protein structure of the enzyme [61]. As a protein, an enzyme requires a certain number of heavy metals as cofactors. Heavy metals can be added to the active center of the enzyme and maintain a certain transition structure, which can change the equilibrium nature of the enzyme reaction and the surface charge of the enzyme protein and increase the activity of the enzyme, that is, generating an activation effect [62]. Conversely, heavy metals occupy the active center of the enzyme or combine with the thiol, amine, and carboxyl groups of the enzyme molecule, resulting in a decrease in the activity of the enzyme, that is, representing an inhibitory effect [27,62].

V_{max} represents the maximum velocity at which the enzyme reacts with the substrate when the substrate concentration is infinite [58]. Because soil is a complete living structure, the amount of enzyme in the same mass of soil is not the same, so V_{max} can be used as a measure of the size of the enzyme pool in the soil [63]. In this study, the variation of V_{max} showed a lag compared with enzyme activity, and it showed a weak inhibition phenomenon. A possible reason is a lag in the response of microorganisms to heavy metal stress. Another possible reason is that the soil enzyme pool depends on changes in the production velocity of microorganisms and the turnover rate of the enzyme pool. In this study, the variability of V_{max} was greater than that of K_m, indicating that Cd exerted greater control over microbial enzyme production than enzyme–substrate affinity [64].

4.3. Responses of Microbial Communities

Microbial community structure is an important factor affecting enzyme activity [32]. In this study, no significant differences were observed in several α-diversity indices of the community, which may be related to the dose and exposure time employed in this study. For example, Pan et al. (2011) showed that long-term exposure of soil microbial communities to combined Pd and Cd had a greater impact than exposure to single heavy metals, and bacteria were the most sensitive to heavy metals (compared with fungi and actinomycete) [65]. The NMDS results showed that the difference in community structure gradually increased with the increase in exposure time, with a significant difference between 0 h and 48 h; however, a low dose of heavy metal Cd did not change the community structure. Although microorganisms could not physiologically cope well with heavy metal stress, and thus reduced community diversity, natural resistance and dormancy could protect community diversity [66]. Different microbial communities may have similar functions due to functional redundancy, and their microbial activity may be low despite a high microbial diversity [35]. The results showed that the magnitude of change of rare microorganisms was greater than that of abundant microorganisms, and the change of abundance (24 h) of rare microorganisms was similar to that of enzyme activities. This may be attributed to the substitution of resistant species for sensitive species under heavy metal stress, which could preserve community diversity stability while maintaining the same community function and result in a change in community structure [67,68]. Rare taxa play an important role in the mineralization of organic phosphorus in the absence of phosphorus supply in the environment [69]. The changes in relative abundance generated by rare and abundant taxa are affected not only by Cd but also by population dynamics, such as competition or cooperation [35]. Such potential confounding biotic factors cannot be ignored. One possible explanation for why rare taxa exhibit a U-shaped relationship (dose–effect curve) may be due to the higher sensitivity of rare taxa to Cd, and this change can be directly attributed to Cd [44]. Another possible explanation is that abundant taxa are more sensitive to Cd [70]. Such stressors can damage the homeostasis of abundant taxa, change the relationship between competition and cooperation, and thereby affect the

abundance of rare communities. Although a new steady state will eventually be formed, the time of stabilization has not yet been determined.

4.4. A Possible Pathway through Which Low-Dose Cd Affects Enzyme Activities

Soil enzyme activities are affected by the soil's physical and chemical properties and the soil's microbial community [18]. Concurrently, the stress imposed by heavy metals on microorganisms could reduce the release of extracellular enzymes, thereby decreasing the content of enzymes in the soil and indirectly limiting the overall enzyme activity of the soil [56,71].

The results of VPA showed that the contribution rate of microbial factors was 0.03, that of enzymatic reaction kinetics was 0.28, and that of their combined effects was 0.45 (Figure 5a). The single effect of Cd on the enzyme structure was much greater than that on the microbial community structure, and the contribution rate of interaction between Cd and microbial factors to enzyme activity was dominant. One possible explanation is that soil microorganisms have a "resistance" mechanism [72]. The results of α- and β-diversity showed that there was no significant change in the soil microbial community within the observation range, indicating that the changes in enzyme activities might be caused by the direct effect of Cd on the structure of soil enzymes.

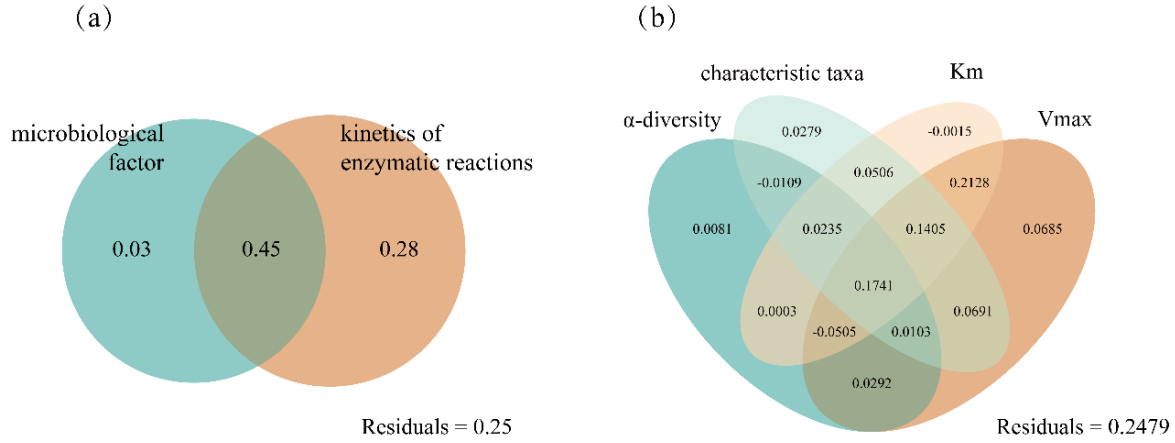

Figure 5. Variance partitioning analysis (VPA) shown the contribution of microbiological factors and enzyme reaction kinetics to the changes in typical soil enzyme activities (**a**), and the contribution of the following subfactors were also shown (**b**). Microbiological factors included α-diversity (Shannon index, Simpson index, chao1, and observed OTUs) and characteristic taxa (G+, G−, AT, and RT). The kinetics of the enzymatic reaction included the K_m and V_{max} of four soil enzymes.

Separating the above two factors of VPA further explained the effect of Cd on enzyme activities in the soil ecosystem (Figure 5b). The contribution rate of V_{max} was the highest (0.6540), followed by K_m (0.5498) and characteristic microbial distribution (0.4851). One possible explanation is that after stress, soil microorganisms allocate much of their energy to "resistance activities" and correspondingly reduce the secretion of extracellular enzymes [72]. The contents of non-inactivated enzymes in soil increased over time, and the overall soil enzyme activities also increased. When the concentration of Cd increased, this balance could be broken, and the direct effect of Cd on enzyme activities was equal to or greater than the effect of Cd on the secretion of extracellular enzymes. At this point, the inhibitory effect of heavy metals could begin to appear, threatening the structural stability of the existing community, until it collapses and gives rise to new distinct communities.

5. Conclusions

Our study revealed a prevalent occurrence of hormesis in various soil enzymes. However, alterations in soil enzyme reaction kinetics and activities exhibited inconsistent patterns. Low-dose heavy metal exposure, as well as exposure time, did not significantly

impact the diversity of soil microorganisms, but exposure time notably influenced the community structure of these microorganisms. Changes in the abundance of rare taxa followed a similar trend to enzyme activities. Importantly, low-dose Cd primarily reduced extracellular enzyme secretion by soil microorganisms, leading to a reduction in the size of the soil enzyme pool and thereby inducing hormesis in soil enzyme activities. This study deepens our comprehension of the mechanisms underlying Cd-induced soil enzyme hormesis, contributing to enhanced insights and applications in the context of soil hormesis.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14060904/s1, Figure S1: Model fitting and parameter setting for a quantitative evaluation of hormesis based on Fan et al. (2021); Table S1: Studies on soil enzyme-related hormesis [22,42,52,73–82].

Author Contributions: J.W.: investigation, formal analysis, resources, writing—original draft. Z.W.: methodology, writing—original draft. E.A.: writing—review and editing. Y.Z.: supervision, funding acquisition. J.H.: supervision, conceptualization, writing—review and editing, project administration. D.F.: conceptualization, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: The National Natural Science Foundation of China (No. 41977354); the Jiangsu Provincial Science and Technology Innovation Special Fund Project of Carbon Emission Peak and Carbon Neutralization (frontier and basis) (Grant Number BK20220016); and The Startup Foundation for Introducing Talent of Nanjing University of Information Science & Technology (NUIST), Nanjing, China (No. 003080).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data analyzed in this study are included in this article and available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Calabrese, E.J.; Baldwin, L.A. Toxicology rethinks its central belief. *Nature* **2003**, *421*, 691–692. [CrossRef]
2. Kaiser, J. Sipping from a poisoned chalice. *Science* **2003**, *302*, 376. [CrossRef]
3. Renner, R. Redrawing the dose—Response curve. *Environ. Sci. Technol.* **2004**, *38*, 90–95. [CrossRef] [PubMed]
4. Agathokleous, E.; Calabrese, E.J. Environmental toxicology and ecotoxicology: How clean is clean? Rethinking dose-response analysis. *Sci. Total Environ.* **2020**, *746*, 138769. [CrossRef]
5. Wang, Q.; Gu, M.; Ma, X.; Zhang, H.; Wang, Y.; Cui, J.; Gao, W.; Gui, J. Model optimization of cadmium and accumulation in switchgrass (*Panicum virgatum* L.): Potential use for ecological phytoremediation in Cd-contaminated soils. *Environ. Sci. Pollut. Res.* **2015**, *22*, 16758–16771. [CrossRef]
6. Shahid, M.; Dumat, C.; Khalid, S.; Niazi, N.K.; Antunes, P.M. Cadmium bioavailability, uptake, toxicity and detoxification in soil-plant system. *Rev. Environ. Contam. Toxicol.* **2017**, *241*, 73–137. [PubMed]
7. Kuppusamy, S.; Thavamani, P.; Megharaj, M.; Venkateswarlu, K.; Lee, Y.B.; Naidu, R. Pyrosequencing analysis of bacterial diversity in soils contaminated long-term with PAHs and heavy metals: Implications to bioremediation. *J. Hazard. Mater.* **2016**, *317*, 169–179. [CrossRef] [PubMed]
8. Chen, X.; Wang, X.; Gu, X.; Jiang, Y.; Ji, R. Oxidative stress responses and insights into the sensitivity of the earthworms *Metaphire guillelmi* and *Eisenia fetida* to soil cadmium. *Sci. Total Environ.* **2017**, *574*, 300–306. [CrossRef] [PubMed]
9. Calabrese, E.J. Hormesis: A revolution in toxicology, risk assessment and medicine: Re-framing the dose–response relationship. *EMBO Rep.* **2004**, *5*, S37–S40. [CrossRef]
10. Milosevic, L.; Osipitan, O.A.; Scott, J.E.; Knezevic, S.Z. Soybean Tolerance to ultra-low doses of dicamba: Hormesis or not. *Crop Prot.* **2023**, *173*, 106356. [CrossRef]
11. Guo, Z.; Chen, G.Q.; Zeng, G.M.; Huang, Z.Z.; Chen, A.W.; Hu, L.; Wang, J.J.; Jiang, L.B. Cysteine-induced hormesis effect of silver nanoparticles. *Toxicol. Res.* **2016**, *5*, 1268–1272. [CrossRef] [PubMed]
12. Gu, Z.; Xie, Y.; Huang, P.; Ding, T.; Tao, M.; Liu, S. Time-dependent hormetic dose responses of skin care product mixtures to *Vibrio qinghaiensis* sp.-Q67: Appearance and quantification. *Sci. Total Environ.* **2023**, *904*, 166651. [CrossRef] [PubMed]
13. Siddhu, G.; Sirohi, D.S.; Kashyap, K.; Khan, I.A.; Khan, M. Toxicity of cadmium on the growth and yield of *Solanum melongena* L. *J. Environ. Biol.* **2008**, *29*, 853–857.
14. Zhang, Y.; Shen, G.; Yu, Y.; Zhu, H. The hormetic effect of cadmium on the activity of antioxidant enzymes in the earthworm *Eisenia fetida*. *Environ. Pollut.* **2009**, *157*, 3064–3068. [CrossRef] [PubMed]

15. Liu, Z.; Chen, W.; He, X.; Jia, L.; Huang, Y.; Zhang, Y.; Yu, S. Cadmium-Induced Physiological Response in *Lonicera japonica* Thunb. *CLEAN–Soil Air Water* **2013**, *41*, 478–484. [CrossRef]
16. Jia, L.; Liu, Z.; Chen, W.; Ye, Y.; Yu, S.; He, X. Hormesis effects induced by cadmium on growth and photosynthetic performance in a Hyperaccumulator, *Lonicera japonica* Thunb. *J. Plant Growth Regul.* **2015**, *34*, 13–21. [CrossRef]
17. Wang, T.J.; Wang, S.L.; Tang, X.C.; Fan, X.P.; Yang, S.; Yao, L.G.; Li, Y.D.; Han, H. Isolation of urease-producing bacteria and their effects on reducing Cd and Pb accumulation in lettuce (*Lactuca sativa* L.). *Environ. Sci. Pollut. Res.* **2020**, *27*, 8707–8718. [CrossRef]
18. Zhang, Y.; Wu, C.; Deng, S.; Zhang, J.; Hou, J.; Wang, C.; Fu, Z. Effect of different washing solutions on soil enzyme activity and microbial community in agricultural soil severely contaminated with cadmium. *Environ. Sci. Pollut. Res.* **2022**, *29*, 54641–54651. [CrossRef]
19. Gianfreda, L.; Rao, M.A. Potential of extra cellular enzymes in remediation of polluted soils: A review. *Enzyme Microb. Technol.* **2004**, *35*, 339–354. [CrossRef]
20. Xu, H.; Qu, Q.; Chen, Y.; Liu, G.; Xue, S. Responses of soil enzyme activity and soil organic carbon stability over time after cropland abandonment in different vegetation zones of the Loess Plateau of China. *Catena* **2021**, *196*, 104812. [CrossRef]
21. Zhu, J.; Peng, H.; Ji, X.; Li, C.; Li, S. Effects of reduced inorganic fertilization and rice straw recovery on soil enzyme activities and bacterial community in double-rice paddy soils. *Eur. J. Soil Biol.* **2019**, *94*, 103–116. [CrossRef]
22. Fan, D.W.; Han, J.G.; Chen, Y.; Zhu, Y.L.; Li, P.P. Hormetic effects of Cd on alkaline phosphatase in soils across particle-size fractions in a typical coastal wetland. *Sci. Total Environ.* **2018**, *613*, 792–797. [CrossRef]
23. Frederick, O.O.; Iroha, A.E.; Oswald, E.C. Evaluation of the concentration of selected heavy metals and the effects on soil enzymatic activities in an abandoned cement factory Nigercem Nkalagu and its environs. *Int. J. Biochem. Res. Rev.* **2014**, *4*, 16–27. [CrossRef]
24. Tian, H.; Kong, L.; Megharaj, M.; He, W. Contribution of attendant anions on cadmium toxicity to soil enzymes. *Chemosphere* **2017**, *187*, 19–26. [CrossRef] [PubMed]
25. Khan, S.; El-Latif Hesham, A.; Qiao, M.; Rehman, S.; He, J.-Z. Effects of Cd and Pb on soil microbial community structure and activities. *Environ. Sci. Pollut. Res.* **2010**, *17*, 288–296. [CrossRef] [PubMed]
26. Lorenz, N.; Hintemann, T.; Kramarewa, T.; Katayama, A.; Yasuta, T.; Marschner, P.; Kandeler, E. Response of microbial activity and microbial community composition in soils to long-term arsenic and cadmium exposure. *Soil Biol. Biochem.* **2006**, *38*, 1430–1437. [CrossRef]
27. Aponte, H.; Meli, P.; Butler, B.; Paolini, J.; Matus, F.; Merino, C.; Cornejo, P.; Kuzyakov, Y. Meta-analysis of heavy metal effects on soil enzyme activities. *Sci. Total Environ.* **2020**, *737*, 139744. [CrossRef]
28. Wang, J.; Shi, J.; Yang, S.T.; Lv, X.L. Effects of applying accumulator straw in soil on nutrient uptake and soil enzyme activity of capsella bursa-pastoris under Cadmium stress. In Proceedings of the 2015 6th International Conference on Manufacturing Science and Engineering, Guangzhou, China, 28–29 November 2015; pp. 719–723.
29. Xian, Y.; Wang, M.; Chen, W. Quantitative assessment on soil enzyme activities of heavy metal contaminated soils with various soil properties. *Chemosphere* **2015**, *139*, 604–608. [CrossRef]
30. Liu, S.; Wang, Z.Y.; Niu, J.F.; Dang, K.K.; Zhang, S.K.; Wang, S.Q.; Wang, Z.Z. Changes in physicochemical properties, enzymatic activities, and the microbial community of soil significantly influence the continuous cropping of *Panax quinquefolius* L. (*American ginseng*). *Plant Soil* **2021**, *463*, 427–446. [CrossRef]
31. Guo, Q.; Majeed, S.; Xu, R.; Zhang, K.; Kakade, A.; Khan, A.; Hafeez, F.Y.; Mao, C.L.; Liu, P.; Li, X.K. Heavy metals interact with the microbial community and affect biogas production in anaerobic digestion: A review. *J. Environ. Manag.* **2019**, *240*, 266–272. [CrossRef]
32. Zuber, S.M.; Villamil, M.B. Meta-analysis approach to assess effect of tillage on microbial biomass and enzyme activities. *Soil Biol. Biochem.* **2016**, *97*, 176–187. [CrossRef]
33. Kaur, G.; Sharma, R.; Singh, K.; Sharma, P.K. Delineating bacterial community structure of polluted soil samples collected from cancer prone belt of Punjab, India. *Biotech* **2015**, *5*, 727–734. [CrossRef] [PubMed]
34. Mounaouer, B.; Nesrine, A.; Abdennaceur, H. Identification and characterization of heavy metal-resistant bacteria selected from different polluted sources. *Desalin. Water Treat.* **2014**, *52*, 7037–7052. [CrossRef]
35. Jousset, A.; Bienhold, C.; Chatzinotas, A.; Gallien, L.; Gobet, A.; Kurm, V.; Kusel, K.; Rillig, M.C.; Rivett, D.W.; Salles, J.F.; et al. Where less may be more: How the rare biosphere pulls ecosystems strings. *ISME J.* **2017**, *11*, 853–862. [CrossRef] [PubMed]
36. Klimenko, A.I.; Matushkin, Y.G.; Kolchanov, N.A.; Lashin, S.A. Spatial heterogeneity promotes antagonistic evolutionary scenarios in microbial community explained by ecological stratification: A simulation study. *Ecol. Model.* **2019**, *399*, 66–76. [CrossRef]
37. Wu, S.J.; Zhou, Z.J.; Zhu, L.; Zhong, L.M.; Dong, Y.X.; Wang, G.J.; Shi, K.X. Cd immobilization mechanisms in a Pseudomonas strain and its application in soil Cd remediation. *J. Hazard. Mater.* **2022**, *425*, 127919. [CrossRef] [PubMed]
38. Solomon, C.M.; Alexander, J.A.; Glibert, P.M. Measuring urease activity in aquatic environmental samples. *Limnol. Oceanogr. Methods* **2007**, *5*, 280–288. [CrossRef]
39. Januszek, K.; Blonska, E.; Dluga, J.; Socha, J. Dehydrogenase activity of forest soils depends on the assay used. *Int. Agrophysics* **2015**, *29*, 47–59. [CrossRef]
40. Knowles, R. Denitrification. *Microbiol. Rev.* **1982**, *46*, 43–70. [CrossRef]
41. Liu, S.; Wang, X.; Guo, G.; Yan, Z. Status and environmental management of soil mercury pollution in China: A review. *J. Environ. Manag.* **2021**, *277*, 111442. [CrossRef]

42. Fan, D.W.; Sun, J.W.; Liu, C.L.; Wang, S.Y.; Han, J.G.; Agathokleous, E.; Zhu, Y.L. Measurement and modeling of hormesis in soil bacteria and fungi under single and combined treatments of Cd and Pb. *Sci. Total Environ.* **2021**, *783*, 147494. [CrossRef]
43. Zhao, Z.; Ma, Y.; Feng, T.; Kong, X.; Wang, Z.; Zheng, W.; Zhai, B. Assembly processes of abundant and rare microbial communities in orchard soil under a cover crop at different periods. *Geoderma* **2022**, *406*, 115543. [CrossRef]
44. Xu, M.; Huang, Q.; Xiong, Z.; Liao, H.; Lv, Z.; Chen, W.; Luo, X.; Hao, X. Distinct Responses of rare and abundant microbial taxa to in situ chemical stabilization of cadmium-contaminated soil. *mSystems* **2021**, *6*, 21. [CrossRef]
45. Zhang, J.Y.; Tang, Z.; Agathokleous, E.; Zheng, G.L.; Xu, L.; Li, P. Hormesis in the heavy metal accumulator plant *Tillandsia ionantha* under Cd exposure: Frequency and function of different biomarkers. *Sci. Total Environ.* **2023**, *889*, 164328. [CrossRef]
46. Tao, M.T.; Liu, S.S.; Gu, Z.W.; Ding, T.T.; Huang, P. Beneficial or harmful: Time-dependent hormesis induced by typical disinfectants and their mixtures with toxicological interaction. *Sci. Total Environ.* **2023**, *904*, 167204. [CrossRef] [PubMed]
47. Cao, X.; Chen, Q.; Xu, L.; Zhao, R.; Li, T.; Ci, L. The intrinsic and extrinsic mechanisms regulated by functional carbon nanodots for the phytoremediation of multi-metal pollution in soils. *J. Hazard. Mater.* **2024**, *462*, 132646. [CrossRef]
48. Zhang, C.; Li, H.; Yang, Y.; Zhou, X.; Zhuang, D.; Liu, W.; Wang, K.; Wang, P.; Zhang, W.; Bai, Y.; et al. Induced mechanism of phosphatase hormesis by Cd ions and rhizosphere metabolites of *Trifolium repens* L. *Chemosphere* **2023**, *344*, 140219. [CrossRef]
49. Calabrese, E.J.; Baldwin, L.A. Defining hormesis. *Hum. Exp. Toxicol.* **2002**, *21*, 91–97. [CrossRef] [PubMed]
50. Calabrese, E.J.; Agathokleous, E.; Kozumbo, W.J.; Stanek, E.J.; Leonard, D. Estimating the range of the maximum hormetic stimulatory response. *Environ. Res.* **2019**, *170*, 337–343. [CrossRef]
51. Zhu, Y.; Liu, C.; You, Y.; Liu, J.; Guo, Y.; Han, J. Magnitude of the mixture hormetic response of soil alkaline phosphatase can be predicted based on single conditions of Cd and Pb. *Ecotoxicology* **2019**, *28*, 790–800.
52. Tan, X.P.; Liu, Y.J.; Yan, K.H.; Wang, Z.Q.; Lu, G.N.; He, Y.K.; He, W.X. Differences in the response of soil dehydrogenase activity to Cd contamination are determined by the different substrates used for its determination. *Chemosphere* **2017**, *169*, 324–332. [CrossRef] [PubMed]
53. Wang, S.Y.; Huang, B.; Fan, D.W.; Agathokleous, E.; Guo, Y.H.; Zhu, Y.L.; Han, J.G. Hormetic responses of soil microbiota to exogenous Cd: A step toward linking community-level hormesis to ecological risk assessment. *J. Hazard. Mater.* **2021**, *416*, 125760. [CrossRef] [PubMed]
54. Guo, Y.H.; Sun, Y.; Fan, D.W.; Wang, S.Y.; Agathokleous, E.; Zhu, Y.L.; Han, J.G. New insights into the role of soil properties in driving cadmium-induced hormesis in soil alkaline phosphatase under vegetation cover change. *Sci. Total Environ.* **2023**, *892*, 164798. [CrossRef] [PubMed]
55. Liu, N.; Liao, P.; Zhang, J.; Zhou, Y.; Luo, L.; Huang, H.; Zhang, L. Characteristics of denitrification genes and relevant enzyme activities in heavy-metal polluted soils remediated by biochar and compost. *Sci. Total Environ.* **2020**, *739*, 139987. [PubMed]
56. Holtan-Hartwig, L.; Bechmann, M.; Hoyas, T.R.; Linjordet, R.; Bakken, L.R. Heavy metals tolerance of soil denitrifying communities: N_2O dynamics. *Soil Biol. Biochem.* **2002**, *34*, 1181–1190. [CrossRef]
57. Wang, Z.; Wang, H.; Wang, H.; Li, Q.; Li, Y. Effect of soil washing on heavy metal removal and soil quality: A two-sided coin. *Ecotoxicol. Environ. Saf.* **2020**, *203*, 110981. [CrossRef]
58. Schnell, S.; Maini, P.K. A century of enzyme kinetics. Should we believe in the Km and vmax estimates? *Comments Theor. Biol.* **2003**, *8*, 169–187.
59. Allison, S.D.; Romero-Olivares, A.L.; Lu, Y.; Taylor, J.W.; Treseder, K.K. Temperature sensitivities of extracellular enzyme Vmax and Km across thermal environments. *Glob. Chang. Biol.* **2018**, *24*, 2884–2897. [CrossRef] [PubMed]
60. Li, W.L.; Fishman, A.; Achal, V. Whole cell evaluation and the enzymatic kinetic study of urease from ureolytic bacteria affected by potentially toxic elements. *Microbiol. Res.* **2022**, *265*, 127208. [CrossRef]
61. Witkowska, D.; Slowik, J.; Chilicka, K. Heavy Metals and Human Health: Possible Exposure Pathways and the Competition for Protein Binding Sites. *Molecules* **2021**, *26*, 6060. [CrossRef]
62. Xia, D.; Lin, L.J.; Li, X.X.; Liao, M.A. Effects of grape seedlings intercropping with bidens species on soil organic matter and soil enzyme activity under cadmium stress. In Proceedings of the Advances in Engineering Research, Shenzhen, China, 25–26 November 2017; pp. 2148–2151.
63. Wang, Z.; Tian, H.; Lei, M.; Megharaj, M.; Tan, X.; Wang, F.; Jia, H.; He, W. Soil enzyme kinetics indicate ecotoxicity of long-term arsenic pollution in the soil at field scale. *Ecotoxicol. Environ. Saf.* **2020**, *191*, 110215. [CrossRef] [PubMed]
64. Tian, P.; Razavi, B.S.; Zhang, X.; Wang, Q.; Blagodatskaya, E. Microbial growth and enzyme kinetics in rhizosphere hotspots are modulated by soil organics and nutrient availability. *Soil Biol. Biochem.* **2020**, *141*, 107662. [CrossRef]
65. Pan, J.; Yu, L. Effects of Cd or/and Pb on soil enzyme activities and microbial community structure. *Ecol. Eng.* **2011**, *37*, 1889–1894. [CrossRef]
66. Sheik, C.S.; Mitchell, T.W.; Rizvi, F.Z.; Rehman, Y.; Faisal, M.; Hasnain, S.; McInerney, M.J.; Krumholz, L.R. Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. *PLoS ONE* **2012**, *7*, e40059. [CrossRef] [PubMed]
67. Tipayno, S.C.; Truu, J.; Samaddar, S.; Truu, M.; Preem, J.K.; Oopkaup, K.; Espenberg, M.; Chatterjee, P.; Kang, Y.; Kim, K. The bacterial community structure and functional profile in the heavy metal contaminated paddy soils, surrounding a nonferrous smelter in South Korea. *Ecol. Evol.* **2018**, *8*, 6157–6168. [CrossRef] [PubMed]
68. Pascoal, F.; Magalhaes, C.; Costa, R. The Link Between the Ecology of the Prokaryotic Rare Biosphere and Its Biotechnological Potential. *Front. Microbiol.* **2020**, *11*, 231. [CrossRef] [PubMed]

69. Wei, X.; Hu, Y.; Razavi, B.S.; Zhou, J.; Shen, J.; Nannipieri, P.; Wu, J.; Ge, T. Rare taxa of alkaline phosphomonoesterase-harboring microorganisms mediate soil phosphorus mineralization. *Soil Biol. Biochem.* **2019**, *131*, 62–70. [CrossRef]
70. Gao, C.X.; Zhu, Y.; Xiao, X.; Liu, R.; Zhu, X.S.; Zhao, Y. Rare soil species impact the rhizosphere bacterial communities and cadmium uptake by *Lolium multiflorum* Lam. *Plant Soil.* **2022**, *473*, 337–350. [CrossRef]
71. Xun, W.; Huang, T.; Zhao, J.; Ran, W.; Wang, B.; Shen, Q.; Zhang, R. Environmental conditions rather than microbial inoculum composition determine the bacterial composition, microbial biomass and enzymatic activity of reconstructed soil microbial communities. *Soil Biol. Biochem.* **2015**, *90*, 10–18. [CrossRef]
72. Li, X.; Meng, D.; Li, J.; Yin, H.; Liu, H.; Liu, X.; Cheng, C.; Xiao, Y.; Liu, Z.; Yan, M. Response of soil microbial communities and microbial interactions to long-term heavy metal contamination. *Environ. Pollut.* **2017**, *231*, 908–917. [CrossRef]
73. Fan, D.W.; Wang, S.Y.; Guo, Y.H.; Liu, J.; Agathokleous, E.; Zhu, Y.L.; Han, J.G. The role of bacterial communities in shaping Cd-induced hormesis in 'living' soil as a function of land-use change. *J. Hazard. Mater.* **2021**, *409*, 124996. [CrossRef] [PubMed]
74. Fan, D.; Wang, S.; Guo, Y.; Zhu, Y.; Han, J. Cd induced biphasic response in soil alkaline phosphatase and changed soil bacterial community composition: The role of background Cd contamination and time as additional factors. *Sci. Total Environ.* **2021**, *757*, 143771. [CrossRef] [PubMed]
75. Han, J.; Wang, S.; Fan, D.; Guo, Y.; Liu, C.; Zhu, Y. Time-dependent hormetic response of soil alkaline phosphatase induced by Cd and the association with bacterial community composition. *Microb. Ecol.* **2019**, *78*, 961–973. [CrossRef] [PubMed]
76. Guo, Y.; Li, X.; Fan, D.; Xue, J.; Han, J.; Zhu, Y. Lysobacter may drive the hormetic effects of Pb on soil alkaline phosphatase. Environ. *Sci. Pollut. Res.* **2020**, *27*, 17779–17788. [CrossRef] [PubMed]
77. Fan, D.; Jing, Y.; Zhu, Y.; Ahmad, S.; Han, J. Toluene induces hormetic response of soil alkaline phosphatase and the potential enzyme kinetic mechanism. *Ecotox. Environ. Saf.* **2020**, *206*, 111123. [CrossRef] [PubMed]
78. Yin, J.; Fan, D.W.; Xu, S.; Han, J.G.; Zhu, Y.L.; Xue, J.H. Hormetic effects of Cr^{3+}, Pb^{2+} and Cd^{2+} on nitrate reductase in soils in Chongming Dongtan wetlands. *J. NJFU. Nat.* **2016**, *59*, 21–26.
79. Fan, D.W.; Tang, Y.F.; Chen, Y.; Dou, L.S.; Zhu, Y.L.; Han, J.G. Hormetic Effects of Cd^{2+} and Pb^{2+} on the Activity of Urease in Soils of Dongtan Wetlands in Chongming Island. *Wetl. Sci.* **2016**, *14*, 421–427.
80. Zhu, S.M.; Dong, Y.Y.; Sha, Z.X.; Fang, Z.; Zou, X.J. Effect of Macrolide Antibiotics on the Urease Activity and Photobacterium phosphoreum. *Asian J. Ecotox.* **2020**, *15*, 116–122.
81. Nweke, C.O.; Ahumibe, N.C.; Orji, J.C. Toxicity of binary mixtures of formulated glyphosate and phenols to Rhizobium species dehydrogenase activity. *J. Microbiol. Res.* **2014**, *4*, 161–169.
82. Zhang, Y.; Yang, S.; Yang, J.; Wu, Z.; Liu, H.; Nie, Z.; Qu, J.; Hu, Y.; Shao, Y.; Liu, J. Temporal hormetic response of soil microbes to cadmium: A metagenomic perspective. *Sci. Total Environ.* **2023**, *891*, 164190. [CrossRef]