

Systematic Review

Meta-Analysis of the Impacts of Applying Livestock and Poultry Manure on Cadmium Accumulation in Soil and Crops

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Abstract: The return of livestock and poultry manure (LPM) to fields is necessary for sustainable agricultural development, but it is also a primary source of heavy metal contamination in agricultural land, which potentially threatens soil and crops. Conflicting results have been reported in previous studies on the impacts of returning LPM to fields on cadmium (Cd) accumulation in the soil and crops. Herein, we investigated the impacts of applying such manure on Cd accumulation in the soil and crops through meta-analysis. We also explored the relationships of Cd content in the soil and crops with the soil properties, experimental conditions, and manure properties. Moreover, we evaluated the primary reasons for the differences in the results of different studies and the factors influencing them. Upon applying LPM, the contents of soil total Cd, soil available Cd, and crop grain Cd increased by 30.96%, 86.91%, and 20.43%, respectively, and the crop root Cd content decreased by 16.91%. Random forest analysis and decision tree analysis further quantified the importance of each influencing factor and identified the primary factors influencing soil available Cd and grain Cd contents. From the perspective of safe production, some suggestions were made for returning LPM to fields: (1) applying higher pH LPM or increasing the dosage in soils with low Cd content and pH of <5.7; (2) applying LPM to soils with Cd content of <1.8 mg/kg, pH of >5.7, and soil organic matter content of >19 g/kg, which was associated with a low risk of Cd accumulation in crop grains; and (3) applying LPM while planting low-Cd-accumulating crop varieties. This study provides scientific guidance for the safe use of such manure resources and helps to reduce the risk of cadmium accumulation.

Keywords: LPM; cadmium; meta-analysis; random forest analysis; decision tree analysis



Citation: Tang, T.; Zhou, H.; Yang, Z.; Zeng, P.; Gu, J.-F.; Mu, Y.-S.; Liu, C.-F.; Han, Z.-Y. Meta-Analysis of the Impacts of Applying Livestock and Poultry Manure on Cadmium Accumulation in Soil and Crops. *Agronomy* **2024**, *14*, 2942. <https://doi.org/10.3390/agronomy14122942>

Academic Editor: José L. S. Pereira

Received: 17 October 2024

Revised: 4 December 2024

Accepted: 5 December 2024

Published: 10 December 2024



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1. Introduction

With the rapid development of industry and agriculture, heavy metals enter fields through atmospheric deposition, sewage irrigation, pesticide application, and other processes. Cadmium (Cd) is one of the most prominent heavy metal pollutants in field soil, and a recent survey in China reported that approximately 7% of arable soil is contaminated with Cd [1,2]. In the soil, Cd has toxicity and mobility and can be easily absorbed by food crops. It has serious impacts on the growth of crops, reducing crop yield, and can be transferred to the edible parts for absorption by the human body through the food chain, thereby posing a serious threat to human health [3,4]. The uptake of Cd by crops is affected by various factors, such as environmental factors and crop varieties; thus, its accumulation in soil and crops has been a matter of wide concern for researchers in various countries [5,6]. The

combination of planting and raising is one of the basic concepts of sustainable agricultural development, and the return of LPM to fields is an effective approach for improving soil fertility, increasing crop yields, and mitigating agricultural surface pollution. However, it is also one of the primary sources of heavy metal pollution [7,8].

LPM is a high-quality organic fertilizer resource that can replace chemical fertilizers often applied to fields. It can provide a variety of nutrients for the soil and improve soil quality, among other benefits, and in recent years has attracted extensive attention from researchers [9]. For example, chicken manure and pig manure contain large amounts of organic matter, which has a strong buffering effect on changes in soil acidity and alkalinity, and can adsorb or immobilize heavy metals in the soil through complexation, ion exchange, and other reactions [10,11]. Several studies have shown that LPM can effectively reduce the bioavailability of soil heavy metals [12–14]. However, in other experiments, such manure had no effect on the reduction in bio-availability and even the opposite [15,16]. This may have been due to the different nature of the LPM used and differences between the soil. One study showed that pig manure and chicken manure, despite containing more nutrients, also contain higher levels of heavy metals than cattle manure, so returning them to fields increases the level of soil heavy metals [17]. In addition, a large number of studies have shown that the amount of LPM applied affect the amount of heavy metal in the soil [18–21]. Most previous trials on the return of LPM were conducted at a single soil or crop. Therefore, the different experimental conditions could have produced different results in different studies. To obtain a better understanding of the patterns of Cd content in soils and crops upon the return of LPM to fields, it is necessary to perform quantitative assessment based on peer-reviewed journal articles in order to identify the drivers of differences in the impacts of LPM on Cd accumulation in the soil and crops.

Meta-analysis, an effective approach for performing large-scale analysis of datasets, can bridge the gap between the traditional literature reviews and quantitative analyses by quantitatively aggregating the results of multiple studies to facilitate a comprehensive analysis [22]. In recent years, in the field of environmental ecology, a large number of studies have used meta-analysis to evaluate changes in the soil environment and microbial diversity caused by organic fertilizer application [23,24]. Several meta-analyses have shown that the application of LPM has positive effects on soil organic carbon (SOC), cation exchange capacity (CEC), increase pH, and total phosphorus [25–27]. Furthermore, several studies have explored the impacts of applying LPM on soil properties, quantified the magnitude of responses to LPM application in terms of soil biochemical properties, and revealed significantly increased enzyme activity and soil reactive nitrogen content, reduced NH₃ emissions, accelerated nutrient cycling, and increased crop yields [28,29]. A previous meta-analysis revealed substantial variation in the heavy metal content of LPM, which was higher in south China than in north China [30]. It has also been shown that the long-term application of LPM significantly increases soil Cd, Cu, and Zn contents [31]. Previous meta-analysis on the return of LPM to fields mainly focuses on soil fertility, crop yield, and carbon and nitrogen emissions; however, it lacks systematic analysis and research on the relationship of soil environmental factors, experiments, and other factors with soil and crop Cd contents.

Herein, meta-analysis was used to analyze the effects of the return of LPM on Cd content in the soil and crops (rice, maize, and wheat) through subgroup analyses, random forest (RF) analyses, and decision tree (DT) analysis. The main goals were as follows: (1) to quantify the responses to Cd accumulation in the soil and crops in terms of soil physicochemical properties, application scenarios, and the nature of LPM; (2) to analyze the main factors influencing Cd accumulation in the soil and crops upon the return of livestock manure to fields, such as manure type and dosage, cereal crop type, soil environment, and experimental conditions; and (3) to explore the reasons for the differences in Cd contents and provide guidance for the safe production and efficient and rational use of LPM.

2. Materials and Methods

2.1. Data Collection

To analyze the effects of applying LPM on soil and crop Cd content and the main factors influencing these effects, 56 relevant peer-reviewed articles from CNKI, Elsevier, and Web of Science published between 2004 and 2023 were collected (Figure 1). The search query was performed using the following terms: (“Livestock and poultry manure” OR “Cow dung” OR “Pig manure” OR “Chicken manure”) AND (“Soil”) AND (“Heavy metal”) AND (“Plant” OR “Rice” OR “Corn” OR “Wheat”). To ensure the accuracy of each included study, the following criteria were applied during the data collection process: (1) at least three replicates for each treatment; (2) a control group wherein no LPM was applied and an experimental group wherein LPM was applied; and (3) a main focus on the soil and crop Cd content after the application of LPM.

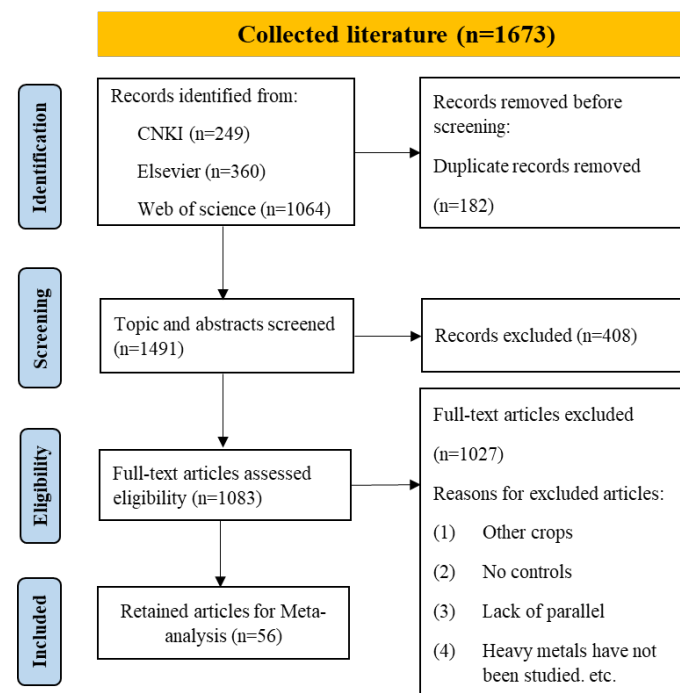


Figure 1. Flow chart of the study.

The impacts of applying LPM on Cd content in the soil and crops varied under different experimental conditions. Therefore, we collected the following information from the articles that we included in this study: experimental location (latitude and longitude), type of experiment (field experiment, pot experiment), type of LPM, soil environmental factors (soil texture, pH, CEC, SOM), Cd content in soil and crops (rice, wheat, and maize), time of application, and dosage. Statistical information on some of the study parameters is shown in Table 1. The main study sites were conducted mainly in Asia and Africa (Figure S1).

Table 1. Statistical description of the mean concentrations of different experimental parameters.

Category	Soil pH	SOM (g/kg)	CEC (cmol/kg)	Initial Soil Cd Content (mg/kg)	LPM pH	LPM OM (g/kg)	LPM Dosages (t/ha)
Minimum	4.4	0.68	3.57	0.1	6.24	15.5	2.2
Maximum	10.5	82.7	45	50.7	8.73	724	390
Median	6.36	28	17.9	0.72	7.54	353	22.5
Mean	6.6	27.9	19	2.86	7.61	307	36.6
Sample size	278	250	117	239	206	200	283

2.2. Data Analysis

Before analyzing the data using MetaWin2.1, it was first necessary to standardize the data. This included the following processing standards: when the soil sampling depth of the study is 0–15 cm, it is considered that the sampling depth is also 0–20 cm; the unit form of LPM dosage was different in pot and field studies, so by converting the mg/kg used in the pot experiment to t/ha used in the field experiment, the basic assumption is that the till layer was 0–15 cm and that the bulk density of 1.5 kg/L (i.e., a soil mass of 2250 tons per hectare), i.e., 1% addition of LPM was used in the pot experiment, then in the field trial this equates to 22.5 t of LPM per hectare [32]. Soil texture was categorized into three main categories, namely, clay, loam, and sand, while soil texture categories that were not specified in the article were based on their percentage of soil particle sizes and the USDA Soil Texture Triangulation Chart.

When an article does not include SOM but does include SOC, its SOM content can be calculated using the following formula:

$$SOM = 1.724 * SOC \quad (1)$$

where 1.724 is the conversion factor between SOC and SOM [33]. For the data where only SE is available in the article, their SD is calculated here using the following formula:

$$SD = SE * \sqrt{n} \quad (2)$$

where n is the number of repetitions. When the estimate of the SE was not available, the coefficient of variation was taken as 10% [34].

2.2.1. Meta Data Analysis

Meta-analysis was used to estimate the magnitude of changes in the target indicators. To assess the magnitude of the response to the application of LPM on the Cd content in the soil and crops, this study used a random-effects model to calculate the effect size of the grouping of different indicators. The magnitude of the effect of livestock manure on an indicator is expressed as the natural logarithmic transformation ratio ($\ln RR$) [35]. The formula for calculating it is as follows:

$$\ln RR = \ln\left(\frac{X_t}{X_c}\right) = \ln(X_t) - \ln(X_c) \quad (3)$$

where RR represents the response ratio, $\ln RR$ represents the effect value, X_t represents the mean value of the variable after manuring treatment, and X_c represents the mean value of the control group. The formula for calculating the variance (v) was as follows:

$$v = \frac{SD_t^2}{n_t X_t^2} + \frac{SD_c^2}{n_c X_c^2} \quad (4)$$

where SD_t and SD_c are the standard deviations of the experimental and control groups, and n_t and n_c are the numbers of replications for the experimental and control groups, respectively [36]. A weighted average of all study data pairs was used to obtain a composite effect value of $\ln RR_{++}$, which was calculated as follows:

$$\ln RR_{++} = \frac{\sum(\ln RR_i * w_i)}{\sum w_i} \quad (5)$$

where $\ln RR_i$ and w_i are the effect values and weights for the i th set of data [37]. The 95% confidence interval (95% CI) is calculated as follows [38]:

$$95\% \text{ CI} = \ln RR_{++} \pm 1.96 S_{\ln RR_{++}} \quad (6)$$

where $S_{lnR_{++}}$ represents the *SD* of the group as a whole, which is calculated as follows:

$$S_{lnR_{++}} = 1.96 \sqrt{1 / \sum w_i} \quad (7)$$

When the *CI* is >0 , it means that the application of LPM has a positive effect on the indicator (i.e., the application of LPM significantly increases the value of the indicator); when the *CI* is <0 , it means that the application of LPM has a negative effect on the indicator (i.e., the application of LPM significantly reduces the value of the indicator). Meanwhile, when the *CI* is 0, it means that the application of LPM does not have a significant effect on the indicator and there is no significant difference compared with the control. To more intuitively reflect the effect of LPM on each index, this study converted the effect value into percentage using the following formula [32]:

$$E = (\exp(\ln RR_{++}) - 1) * 100\% \quad (8)$$

where E represents the rate of change in Cd content after the application of LPM. When E is >0 , this means that the application of LPM has a positive effect on the accumulation of total soil Cd, the content of the effective state of soil Cd, and the content of grain Cd and root Cd of crops (rice, maize, and wheat), while the opposite is true when E is <0 .

2.2.2. DT and RF Analyses

RF analysis (including DT) is a robust and flexible machine learning integration algorithm for a wide variety of practical problems, calculating the relative importance of predictor variables for soil and helps us better understand data characteristics and model performance [39]. It can also explain which of the different attributes contribute the most to the effect of a dependent variable [40]. In this study, a classification and regression algorithm were used to construct a decision tree, referred to as the CART model. As shown in Figure S2, DT applies decision rules to partition input features, forming homogeneous subsets, and identifies key characteristics in the process from root nodes to leaf nodes. The splitting rules in DT define the relationship between input variables and decision outcomes. If Rule 1 is met, then Decision 1 is made; if not, the process continues by moving down to the next layer of nodes within the tree structure. Each leaf node corresponds to a specific decision result [41]. First, the independent variable is divided into k intervals, labeled as $M_1, M_2, \dots, M_k$, and then the expected value for each interval is calculated using the following formula [42]:

$$\bar{Y}_t = \text{ave}(Y_t | X_i \in M_k) \quad (9)$$

where Y_t denotes the response variable for X_i and $\bar{Y}_t$ denotes the mean of the response variable. The optimal split variable “ g ” and the split point “ j ” are calculated using the following formula:

$$MSE_{min} = \min_{\bar{Y}_1} \sum_{X_i \in M_1(g,j)} (Y_i - \bar{Y}_1)^2 + \min_{\bar{Y}_2} \sum_{X_i \in M_2(g,j)} (Y_i - \bar{Y}_2)^2 \quad (10)$$

where D_1 and D_2 denote two subpopulations delineated on the basis of variable “ g ” and segmentation point “ j ”, respectively. The above steps are repeated until the mean square error (*MSE*) within the subgroups is minimized [32].

To avoid overfitting and reduce the complexity, ten-fold cross-validation was performed to determine the optimal pruning point based on the smallest X-val relative error (Figure S3) [32,43]. RF prediction model was constructed using soil CEC, SOM, soil pH, soil total Cd, soil type, manure organic matter, manure type, manure pH, crop type, experimental type, application time, and dosage as predictors and changes in soil total Cd, available Cd, and crop root Cd, grain Cd contents as response variables. In the RF model, each decision tree, containing different data samples and feature subsets, was constructed independently based on a random subset of the training data. The 12-dimensional variable test samples in this study were fed into the trained RF model [44]. Assuming that there

are N decision trees in the RF model, each decision tree will output a predicted value Y_i ($i = 1, 2, 3, \dots, N$) for each new variable X entered, and the final prediction is considered as the average of the predicted values of all decision trees (Figure S4) [45]. This stochasticity helps reduce deep model fitting and improve predictive power.

2.3. Data Processing

All data were extracted from the literature included in this study using GetData Graph Digitizer 2.25, processed using Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA, USA), and using OriginPro 2024 (Origin Lab Corp., Northampton, MA, USA). Effect values and 95% CIs were calculated via MetaWin 2.1 [46]. Forest plot results were plotted using GraphPad Prism 9.1. The spatial distribution of the study sites examined in the included literature was plotted using ArcGIS 10.7. RF and DT analysis were performed using the 'randomForest' and 'rpart' packages via R 4.3, respectively, and the factors affecting the cumulative effect of Cd content were ranked. Moreover, the results were used to study the relationship between Cd content and soil environmental factors in soil and crops after the application of LPM.

3. Results and Discussion

3.1. Total Effect of Applying LPM on Soil Properties and Cd Accumulation

The contents of Cd in soil as well as LPM collected in each study varied considerably (Figure 2a). Specifically, the soil total Cd content ranged from 0.1 to 50.7 mg/kg, whereas the Cd content of LPM ranged from 0.001 to 4.11 mg/kg, with mean values of 2.86 and 0.77 mg/kg, respectively. In some cases, the Cd content of LPM was >1 mg/kg; therefore, the increase in soil Cd content may be attributed to the addition of LPM. A study investigating the characteristics of Cd contamination in various types of LPM in China and found that pig manure (0.04–59.7 mg/kg), chicken manure (0.03–4.09 mg/kg), cow manure (0.04–3.40 mg/kg) [47]. Differences in heavy metal content may be related to livestock species, dietary habits and feeding regimes [48]. Reference [49] also proved that the Cd content of LPM was highly variable. If LPM with high Cd content is not effectively treated or is directly applied to fields, it may cause an increase in the soil Cd content, conferring a greater risk of soil and crop contamination [50].

Soil available Cd increased significantly after LPM application (Figure 2b), with the maximum increase reaching 86.9%. Soil available Cd is the key form of heavy metals entering the food chain from the soil and is positively correlated with the Cd content in grain. It was reported that the higher the available Cd content, the higher soil available Cd content accumulated in the crop grains [51]. In this study, soil total Cd and grain Cd increased by 31.0% and 20.4%, respectively, while Cd content in crop roots decreased by 16.9%. The increase in Cd content in grains may have been due to the fact that LPM contains a large amount of organic matter and inorganic ions. After applying LPM, the average SOM content increased from 27.5 to 33.7 g/kg, which improves soil fertility, promotes the growth of the crop, and increases the biomass of all parts of the crop, as well as enhances the extraction of Cd from the crop, which results in an increase in the grains' Cd content [52]. The reduction in root Cd content may be due to the reduction in inter-root soil Cd contents through adsorption, complexation, and other reactions of LPM, which reduces the root Cd content [53].

Differences in soil properties, properties of LPM, and experimental environmental factors between studies increase the uncertainty regarding the cumulative impacts of applying LPM on Cd content in the soil and crops. These effects are thus further discussed in the sections on subgroup analyses below.

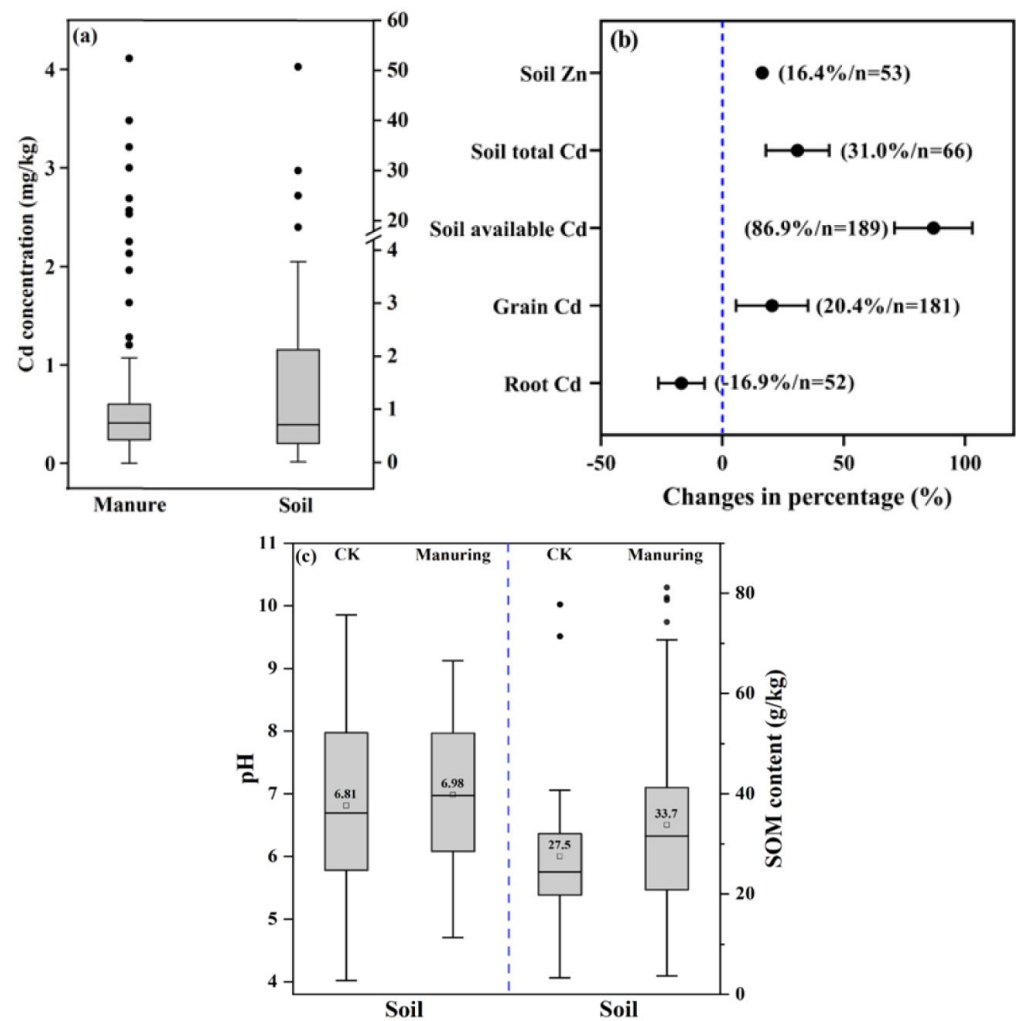


Figure 2. (a) Distribution of Cd contents in soil and LPM used in different studies; (b) overall changes in the impacts of soil and crop on Cd accumulation after the application of LPM, where n indicates the sample size for each category, the horizontal error line represents is the 95% CI; and (c) pH and OM content in soil in the collected dataset (CK represents control treatment, manuring represents LPM application treatment).

3.1.1. Impact of Different Soil Properties

Compared with the control, the effect of applying LPM on root Cd contents at different CEC did not differ significantly (95% CI overlapped with the zero axis, Figure 3a). Applying LPM on soil at CEC contents of >20, 10–20, and <10 cmol/kg increased the available Cd content by 230%, 125%, and 178%, respectively. Meanwhile, the grain Cd content increased by 92.8%, 112%, and 26.3%, respectively. It has been demonstrated that SOM is a key factor affecting CEC, and LPM is rich in organic matter, which can increase CEC and enhance the negative charge on the surface of soil colloids, thus affecting the effective fraction of Cd [54,55]. However, the present study showed a substantial increase in the effective Cd content of the soil to which LPM were applied at high CEC levels. This may be because the two studies involved long-term application over a 10-year period or exogenous Cd was added to the soil [56,57]. This suggests that the increase in negative soil colloidal charge caused by the LPM is insufficient to adsorb Cd^{2+} and may be affected by SOM and the application time.

The application of LPM exerted significant effects on soil total Cd, available Cd, and grain Cd contents, depending on the initial content of Cd in the soil (Figure 3b, $p < 0.05$). When the Cd content in the soil was ≤ 1 mg/kg, the soil total Cd and soil available Cd contents increased by 37.9% and 67.9%, while grain and root Cd contents decreased by

20.1% and 31.0%, respectively. The application of LPM increased the soil pH and SOM and decreased the solubility of Cd or changed it to a more stable metal form. The content of Cd in the soil directly affects its accumulation in grains, and it has been shown that the application of such manure (Cd content <0.5 mg/kg) to soil with Cd content of <1 mg/kg reduces the accumulation of Cd in grains [12]. At Cd content of ≥ 1 mg/kg, the soil available Cd and grain Cd contents increased by 105% and 52.1%, respectively. Moreover, at high Cd contents, the bioavailability of Cd in soils was higher. The elevations in soil pH and SOM caused by LPM was insufficient to adsorb and immobilize a large amount of Cd, and the inter-root soil available Cd was maintained at a high content, which enhanced the transport of Cd from the roots to grains [58].

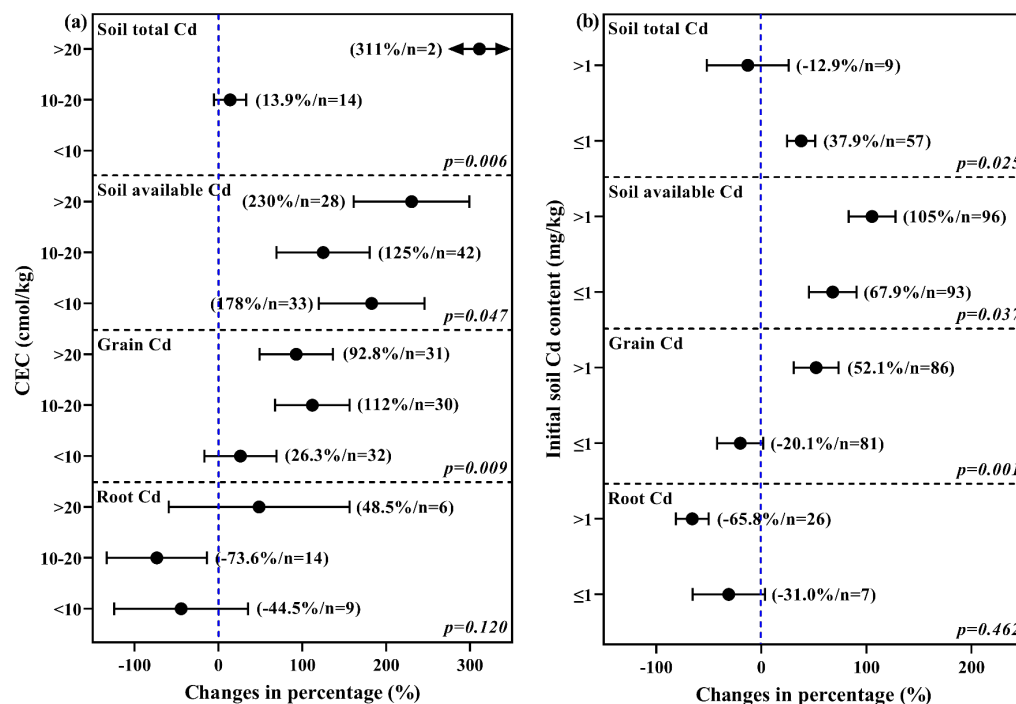


Figure 3. (a,b) show the effects of soil CEC (cmol/kg) and initial soil Cd (mg/kg) content on the changes in soil and crop Cd contents, respectively, with the horizontal error bars indicating 95% CI (horizontal error bars exceeding the axes are indicated by arrows), and the average rate of change in Cd content and sample sizes are shown next to the error bars.

The soil SOM content did not significantly affect the soil total Cd or grain and root Cd contents after the application of LPM (Figure 4a, $p > 0.05$), whereas it showed a significant effect on soil available Cd content ($p < 0.05$). When the SOM content increased from <15 to 15–30 and >30 g/kg, the soil available Cd content changed from a decrease of 86.4% to increases of 128% and 73.7%, respectively. The results indicated that high SOM content in the soil increases soil available Cd content, and a previous study reported that soil SOM enhanced dissolved organic matter (DOM) via root secretion, which lowered the inter-root soil pH and increased the mobility of heavy metals [59]. Another study reported a low correlation between SOM content and Cd content, affecting the variation in soil available Cd contents, which was possibly an effect caused in combination with pH [58]. The soil available Cd may also be affected by interactions between other mobile heavy metals of the soil, such as the relationship between Zn and Pb. Thus, there is a need for further investigation of Cd, especially in field experiments in soils with different SOM contents [60,61].

The application of LPM showed significant effects on soil Cd and grain Cd contents at different pH (Figure 4b, $p < 0.05$). Soil pH plays an important role in controlling Cd bioavailability, and After applying LPM, the average pH increased from 6.81 to 6.98 (Figure 2c).

At pH of > 7.5, the soil total Cd, soil available Cd, and grain Cd contents increased by 63.7%, 119%, and 5.38%, respectively. Meanwhile, at pH 6.5–7.5, the soil available Cd and grain Cd contents increased significantly by 167% and 65.8%, respectively. LPM application in soils with high pH had limited effect on pH improvement, and even lowered pH and increased soil Cd content. This may be due to the fact that the decrease in pH weakens the electrostatic adsorption of Cd ions by soil particles and destroys the structure of inert components in the soil matrix, facilitating the conversion of Cd from stable to unstable states [12,62]. The available Cd and grain Cd contents are the lowest at pH < 6.5 because LPM contain carbonate and phosphate alkaline substances at this pH, which can neutralize soil acidity, increase pH and the negative charge on the surface of the soil colloid, enhance the adsorption of Cd [12], and reduce the flow of Cd in the soil as well as its accumulation in crops [63,64]. The application of LPM to acidic soils (pH < 6.5) thus poses a low cumulative risk to soil and crop Cd contents.

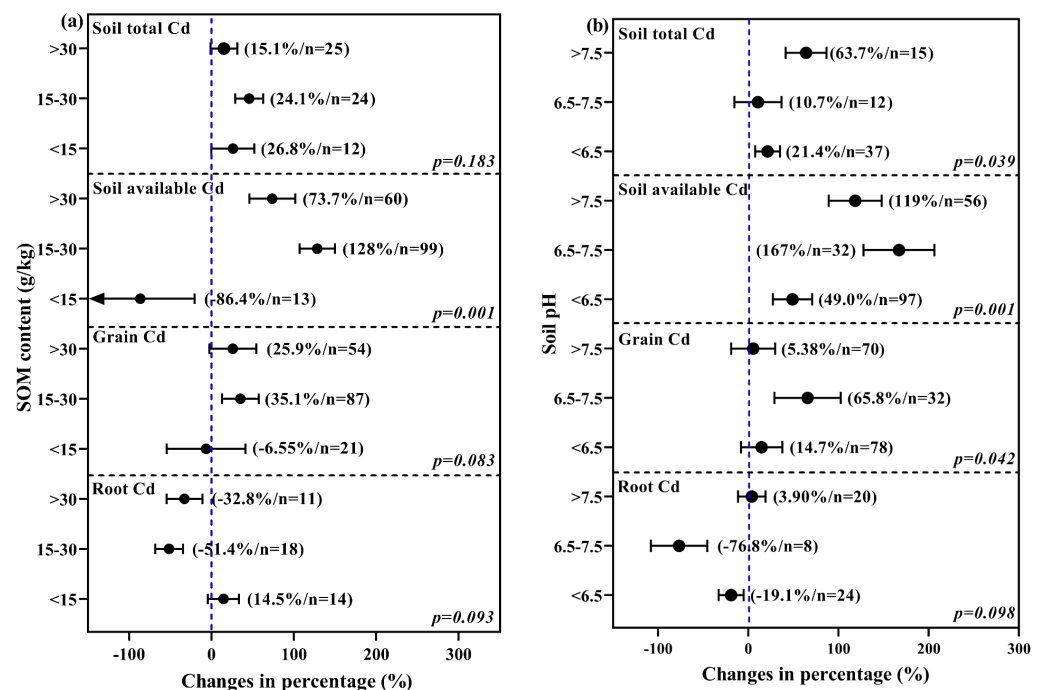


Figure 4. (a,b) represent the effect of different SOM and pH on the variation in soil and crop Cd contents, respectively.

3.1.2. Effect of Different Experimental Conditions

The application of LPM did not significantly affect soil total Cd and root Cd of different crops, but it significantly affected soil available Cd and grain Cd (Figure 5a, $p < 0.05$). It is well known that the biggest difference between the uptake and transfer of Cd in crops is that wheat and maize is aerobic and rice is anaerobic [65]. In this study, soil available Cd and grain Cd content increased by 137% and 45.6% in rice and decreased by 48.1% and 50.0% in wheat, respectively. This might have been due to the application of LPM, which increased the soil Zn content by 16.4% (Figure 2b). It has been reported that OsNramp5 transporter protein is the main pathway for Cd uptake in rice roots, and Zn had no significant inhibitory effect on this channel, whereas wheat mainly absorbs Cd through ZNT1 (a high-affinity transporter for Zn). It was also shown that an increased content of Zn effectively inhibited the uptake of Cd [65,66]. Wheat and maize as dryland crops have better soil aeration, and the amount and activity of fungal microorganisms in the inter-root soil are higher than those in flooded or high-humidity soils, while the secretion of organic acids accelerates the decomposition of SOM and produces more flora involved in the adsorption and immobilization of Cd [67,68]. Therefore, the application of LPM poses a low risk of Cd accumulation in dryland crops such as wheat and corn.

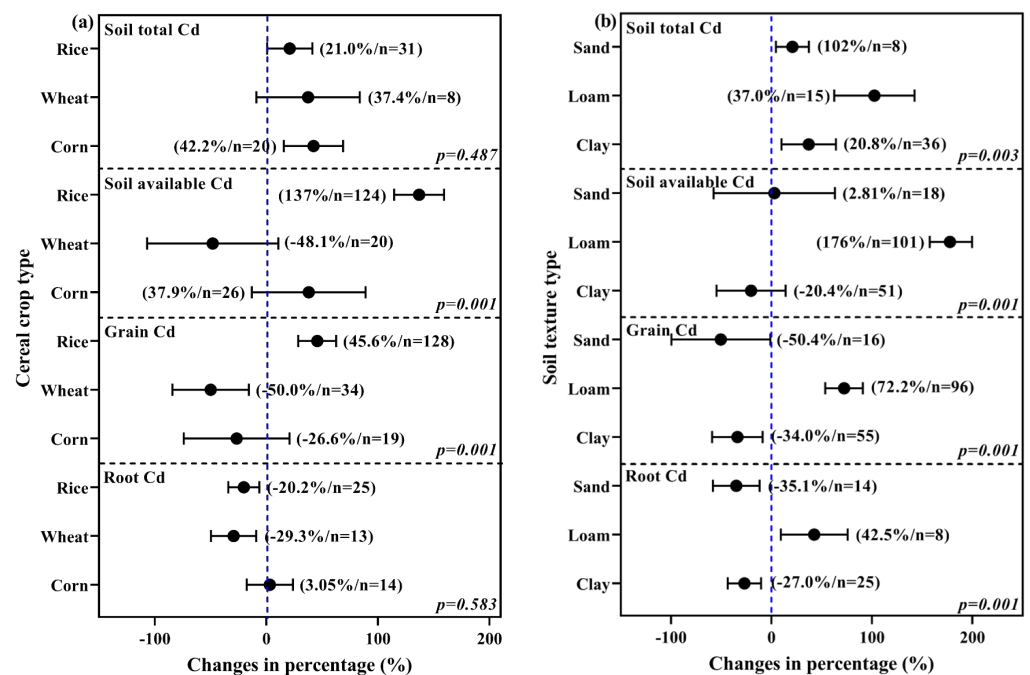


Figure 5. (a,b) show the effects of different cereals and soil textures on the variation in soil and crop Cd contents, respectively.

Soil texture significantly affected the changes in soil and crop Cd contents caused by the application of LPM (Figure 5b, $p < 0.05$). Such application to sandy soils led to approximately 2.5 and 5 times higher soil total Cd content than that to loam and clay soils, respectively. This suggests that the risk of contamination from LPM application in sandy soils is much higher than in loamy and clay soils. This may have been due to the high level of Cd input from some of the LPM [69]. It has been reported that in lower percentage of clay particles Cd has higher mobility and effectiveness and is more readily absorbed by the crop [32,70]. However, contrasting results were observed in our study, the grain and root Cd contents were reduced by 50.4% and 35.1%. This may be due to the higher pH was observed in our study; hence, it may be soil pH rather than soil clay particle content that affects Cd uptake by crops. Meanwhile, soil available Cd, grain Cd, and root Cd accumulation decreased by 20.4%, 34.0%, and 27.0% in clay soil, respectively. In contrast, in loam soil, these values increased by 176%, 72.2%, and 42.5%, respectively. Some studies have reported that clay soils have higher SOM content than sand and loam soils, contain a large amount of clay minerals such as high montmorillonite and kaolinite, which have a high specific surface charge, and are more capable of trapping Cd^{2+} and reducing Cd mobility in the soil [71,72]. The results suggest that, in addition to soil texture, changes in Cd content may be related to pH and SOM.

The application time of LPM had significant effects on the changes in soil and crop Cd contents (Figure 6a, $p < 0.05$). Their long-term (>5 years) application increased the total Cd, soil available Cd, and grain Cd contents of soil by 102%, 111%, and 46.9%, respectively. A study showed that repeated application for 5 years increased the Cd contents in crop grain due to an increase in the bioavailability of heavy metals, which was below the Chinese safety limit but still posed certain risk of Cd contamination [73]. Meanwhile, the soil available Cd and crop grain Cd contents increased by 155% and 49.3%, respectively, upon 1–5 years of application. Overall, long-term application of LPM led to the accumulation of more Cd than short-term application in both soil and crops, which can be attributed to the higher contents of Cd, Cu, and Zn in such manure [74]. It has also been reported that continuous application of LPM for several years improved soil acidification and nutrients, but the soil total Cd and soil available Cd contents also increased significantly [14,75,76]. Therefore, short-term application of LPM is associated with a lower risk, while their long-

term application increases the risk of contamination. Long-term testing and synergistic application with metal-fixing materials are required to ensure safe application.

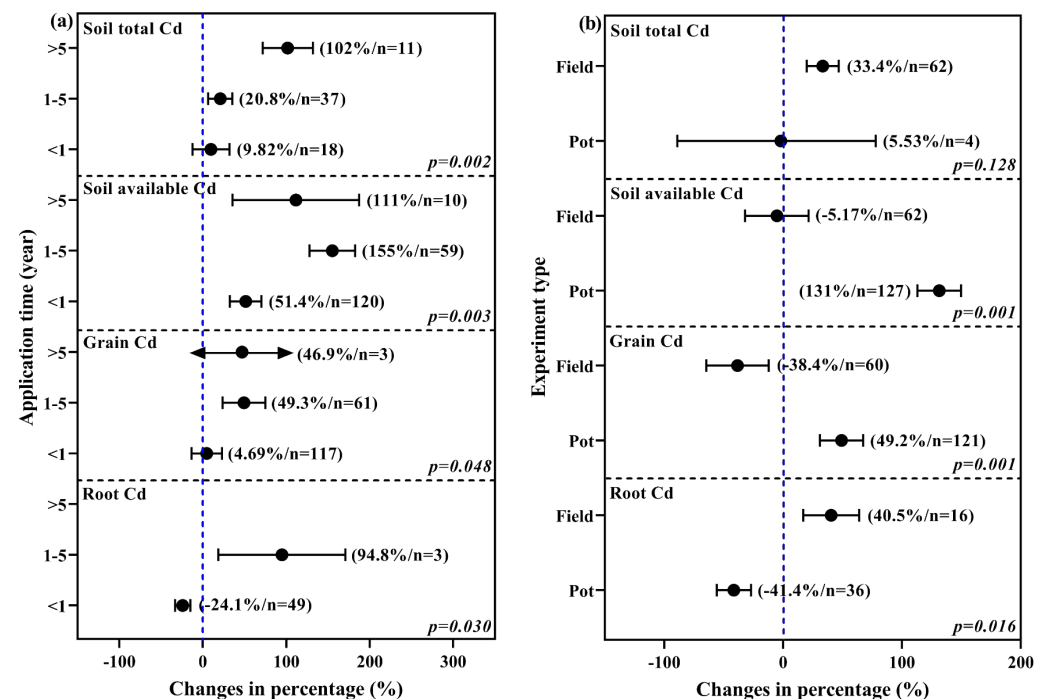


Figure 6. (a,b) present the impacts of duration of LPM application and different types of experiments on the changes in soil and crop Cd contents, respectively.

The application of LPM had a significant effect on the accumulation of soil available Cd and crop Cd in pot and field experiments (Figure 6b, $p < 0.05$). In the field experiment, the soil total Cd and root Cd contents increased by 33.4% and 40.5%, while soil available Cd and grain Cd contents decreased by 5.17% and 38.4%, respectively. However, in the pot experiment, the soil available Cd and grain Cd contents increased by 131% and 49.2%, respectively. The results indicated that the soil available Cd and grain Cd contents in the pot experiment increased significantly compared with those in the field experiment, which may have been due to the relatively high Cd content of the test soil in the former experiment [77,78]. Livestock manure is not effective in reducing Cd in soils with a high Cd content (Figure 5b). Meanwhile, it is possible that drainage occurred in the rice pot experiment at the late stage of growth, which would lower pH, resulting in decreased SOM complexing and chelating ability of Cd and the reconversion of some of the Cd from reducible and oxidized states to the weakly acid-extracted state [79]. Furthermore, Cd-Zn antagonism should be taken into account for under the lower accumulation of Cd in the field; Cd contamination usually occurs in conjunction with Zn contamination, and some studies have proved increased Zn in soils led to decrease accumulation of Cd in grain [80–82]. Therefore, the soil effective Cd and grain Cd contents were higher in the pot experiment than in the field experiment.

The dosage of LPM had significant effects on soil available Cd and grain Cd content, and no significant effect on soil total Cd and root Cd content (Figure 7a). Soil available Cd and grain Cd content increased by 150% and 42.0% at the dosages of 15–45 t/ha, which according to the previous analysis may have been due to the duration of application, soil texture, and other factors. It is possible that, in the long term, the Cd fixed by the organic matter can be released again through the mineralization and decomposition of organic matter to improve the bioavailability of Cd [83]. When the dosage was <15 t/ha, soil available Cd and grain Cd accumulation decreased by 51.1% and 30.9%, respectively. This indicates that application of Low doses of LPM provide nutrients and organic matter binds

Cd reducing its availability. Over fertilization increases the risk of Cd accumulation and other heavy metals [84–86].

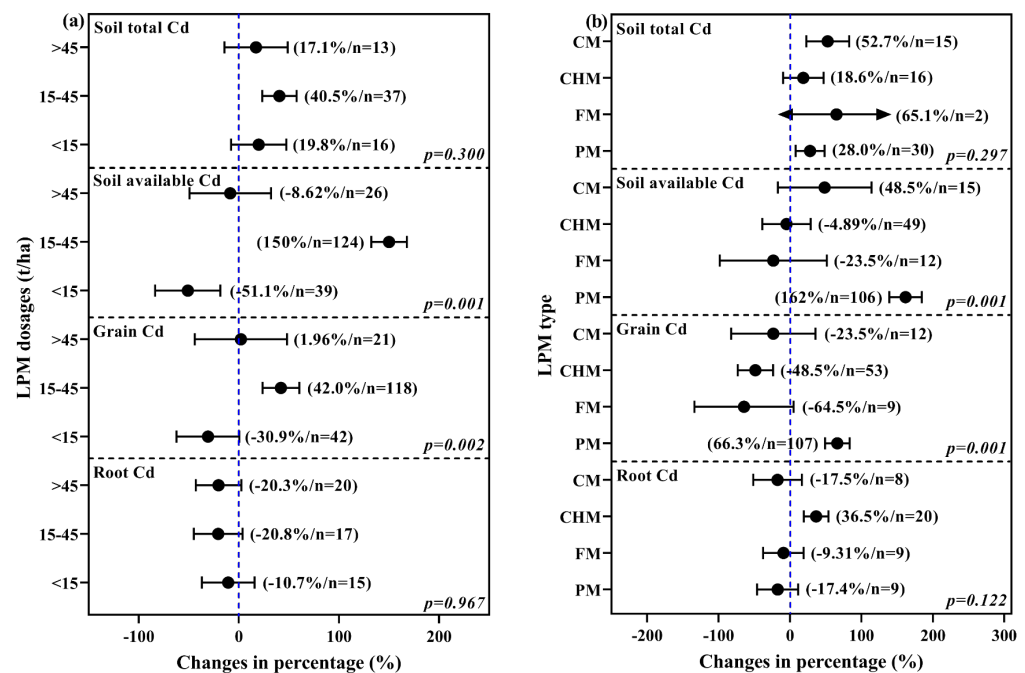


Figure 7. (a,b) represent the effects of different LPM dosages (kg/ha) and LPM types on the changes in soil and crop Cd contents.

The type of LPM had a significant effect on soil total Cd, soil available Cd, and grain Cd content accumulation (Figure 7b, $p < 0.05$). Upon the application of cattle manure, soil total Cd and soil available Cd content increased by 52.7% and 48.5%, respectively. The results of many studies have highlighted that Cu-, Zn-, and even Cd-containing mineral additives are often added to feed to meet the growth requirements of livestock and poultry [75]. The average reductions in grain Cd content upon applying chicken manure and farmyard manure were 48.5% and 64.5%, respectively. Some studies have shown that farmyard manure and chicken manure have higher OM, N, and P content than pig manure, and that the higher the organic matter content, the more binding sites are provided for Cd in the soil. This can in turn immobilize more active Cd and reduce the accumulation of Cd in the grains [12,87]. Meanwhile, the application of pig manure was shown to have a higher risk of accumulation of soil available Cd and grain Cd content with 162% and 66.3% increases in Cd content, respectively. Among the manure types, pig manure is the main contributor of soil Cd in cultivated land, and it has a slightly lower pH, which may reduce soil pH and promote the dissolution and migration of Cd [88]. Cattle manure, chicken manure, and farmyard manure can all reduce grain Cd content, but the choice needs to be based on a combination of factors: nutrient content, impact on soil properties, and impact on the environment.

The organic matter (OM) content of LPM had no significant effect on the soil total Cd and root Cd contents, while it significantly affected the soil available Cd and grain Cd contents (Figure 8a, $p < 0.05$). When the OM content of LPM was <200 g/kg, no significant difference was noted in the soil available Cd and grain Cd contents compared with those in the control, indicating that LPM with OM content of <200 g/kg had a minimal effect on the Cd content of soil and crops. When the OM content was >400 g/kg, the soil available Cd and grain Cd contents decreased by 65.1% and 39.4%, respectively. The effectiveness and mobility of Cd are closely related to SOM, humic acid, and phosphate contents, probably due to the increase in the negative surface charge of soil particles and

functional groups, such as carboxyl and phenolic groups, which physically, chemically, and biologically interact with Cd and reduce its bioavailability [89].

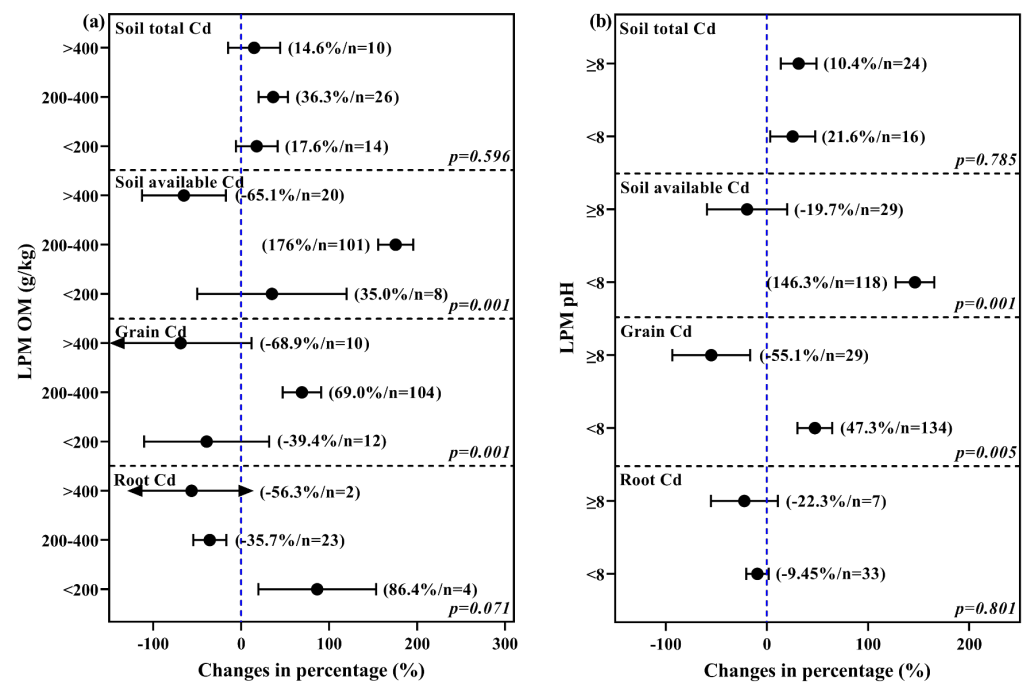


Figure 8. (a,b) show the effects of LPM OM content and pH on the variation in soil and crop Cd contents, respectively.

It has been demonstrated that SOM content is significantly and positively correlated with the effectiveness of Cd, and the higher the SOM content the more the absorption of Cd by the crop [90]. In this study, when the OM content was 200–400 g/kg, the soil available Cd and grain Cd contents exhibited maximum increases of 176% and 69.0%, respectively. This may have been due to the fact that organic acids in manure may reduce the soil pH and increase the solubility of Cd. Alternatively, it may have been due to the fact that the application of manure to the soil elevates soil DOM content, which, when present in large quantities, alters the mineral surface structure, reduces the contact between Cd and clay minerals, and hinders the immobilization of Cd [91,92]. Thus, the OM content of LPM affects the variation in Cd content, possibly via a combination of manure source and component differences, and the soil environment.

The pH of LPM had a significant impact on soil available Cd and grain Cd contents (Figure 8b, $p < 0.05$). Soil available Cd and grain Cd decreased by 19.7% and 55.1%, respectively, when the pH was ≥ 8 . However, soil available Cd and grain Cd contents increased by 146% and 47.3%, respectively, when LPM pH was < 8 . pH is a key factor influencing the soil adsorption of Cd due to the acidification effect caused by the lower pH of LPM. This effect is uncommon in acidic soils but leads to a decrease in pH in alkaline soils, which reduces the negative charge on the soil colloid surface. It also leads to the detachment of Cd from the surface of the particles, which increases the soil available Cd content as well as the accumulation of Cd in roots and grains [8,12].

3.2. Identification of the Main Factors Affecting Soil and Crop Cd Content upon the Application of LPM

The degree of influence of each environmental factor on soil and crop Cd content was quantified by random forest analysis, and the relative importance of each factor in the four models is shown in Figure S5. Their model training errors are shown in Figure S5. The comparison showed that soil texture, soil pH, and duration of application of LPM were the three most influential factors in the model of soil total Cd (Figure S5a),

contributing 11.8%, 11.3%, and 10.7% to the model, respectively. In the model of soil available Cd (Figure S5b), LPM OM, soil initial Cd content, and LPM type were the three most influential factors, contributing 18.2%, 18.1%, and 12.3% to the model, respectively. In the model of crop grain Cd (Figure S5c), SOM, duration of LPM application, and soil pH were the three most influential factors, contributing 9.97%, 9.56%, and 9.53% to the model, respectively. Finally, in the model of crop root Cd (Figure S5d), soil pH, LPM type, and type of experiment were the three most influential factors, contributing 9.38%, 9.22%, and 9.22% to the model, respectively.

Soil pH had a significant effect on grain and root Cd accumulation, which is consistent with previous studies [93]. In addition, pH had a moderate effect on soil available Cd. Soil pH affects Cd solubility, mobility, and consequently crop uptake of Cd by influencing the adsorption and binding of Cd by soil particles [94]. A study has shown that heavy metals accumulate more in soil with a low pH [95].

Soil texture is another key factor influencing the variation in soil total Cd content, and also has a moderate effect on soil available Cd. Soil composition and structure vary among soils with different textures; for example, clay content influences the distribution and total amount of soil Cd, in which clay minerals, oxides, and humus with large specific surface and high adsorption capacity can adsorb and immobilize Cd well [96]. In addition, SOM and LPM OM are among the key factors affecting grain Cd and soil available Cd, respectively. Organic ligands (complexing and chelating functional groups) in SOM can interact with Cd ions through complexation and chelation, and play a key role in Cd's accumulation and morphological distribution in the soil [97].

The duration of applying LPM is also one of the most important factors affecting soil total Cd and grain Cd accumulation. Soil Cd, Cu, and Zn contents upon the long-term application of LPM were reported to exceed the soil environmental quality standards, in contrast to the findings in the control; in particular, the cumulative risk of Cd accumulation in grains was significantly higher [98,99]. In addition, the type of LPM is one of the key factors affecting soil available Cd and root Cd content, probably because different types of LPM have different OM, N, P, K, and Cd contents, which lead to variation in the accumulation of heavy metals in the soil. It has also been reported that pig manure in China is more severely contaminated with heavy metals than cattle manure, chicken manure, and other manure types [8].

3.3. DT Analysis of the Factors Influencing the Impacts of LPM on the Cd Content of Crop Grains

The factors influencing the accumulation of Cd in crop grains upon the application of LPM were analyzed by DT analysis (Figure 9). The results showed that soil total Cd content was the main factor affecting Cd accumulation in grains. When the Cd content was <20 mg/kg, soil pH, SOM, and LPM pH had greater effects on the grain Cd content. The application of LPM in soil with pH of <5.7 reduced the grain Cd content by 2.5% to 64%, and the Cd-reducing effect was greater when LPM had a pH of ≥ 7.3 . In addition, the dosage of LPM had an effect on grain Cd accumulation when the soil pH was <5.2. At soil pH of ≥ 6.8 and SOM content of ≥ 19 g/kg, the application of livestock manure was not associated with any risk of Cd accumulation in grains. However, when the soil pH was between 5.7 and 6.8, the application of LPM had a risk of 4.4% to 39% of Cd accumulating on the grains, especially when the soil Cd content was >1.8 mg/kg. Overall, the factors affecting the effectiveness of LPM were as follows, in order of importance: soil total Cd > soil pH > LPM pH and SOM > dosage.

Although both analyses ranked the importance of the influencing factors, the results of RF analysis differed from those of DT analysis in that the duration of manure application had a greater effect on grain Cd content, while the pH and dosage of LPM had smaller effects. Meanwhile, in DT analysis, the effect of the duration of application was weaker, but the pH and dosage of LPM showed greater importance. This may be because the decision tree focuses on determining the optimal feature delineation point, which is influenced by data characteristics, while the random forest avoids overfitting and has better generaliz-

ability and stability than a single decision tree. The combined application of these two approaches would be valuable by exploiting both the intuitive nature of DT analysis and strong predictive power of RF analysis.

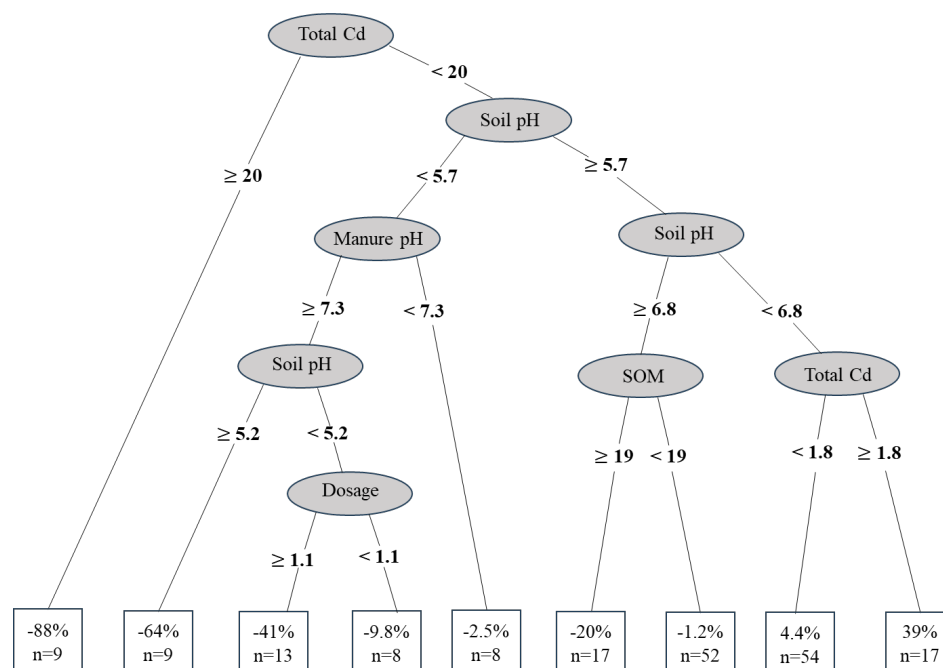


Figure 9. Decision tree analysis of factors affecting the influence of LPM on the Cd content of crop grains.

4. Conclusions

Herein, reports published in the literature were used to quantitatively analyze the effect of LPM returned to fields on the accumulation of Cd in the soil and crops as well as the effect of applying such manure under different experimental conditions on the Cd content of the soil and crops. Meta-analysis revealed that the effect of applying such manure on the Cd content in the soil and crops varied significantly among the different studies, but the average Cd content of grain increased by 20.4%. The passivation effect of LPM was influenced by several factors, with total soil Cd content, low effective Cd content, and high clay content being particularly effective in reducing the risk of Cd accumulation. The risk of Cd accumulation in soil and crops was lower when LPM were applied to acidic soils than to neutral and slightly alkaline soils. In addition, Cd accumulation was more severe upon the application of swine manure. Compared with rice, wheat and maize had lower risks of Cd accumulation. The results of the randomized forest analysis showed that from the perspective of safe production, the main factors influencing the effective soil Cd content were LPM SOM content, initial soil Cd content, and type of LPM. The most important factors influencing grain Cd content included soil SOM, duration of LPM application, and soil pH, which were significantly correlated. The results of decision tree analysis indicated that, against a background of low Cd content, when the soil pH was <5.7 and LPM pH was >7.3, the use of a larger amount of LPM exerted a better passivation effect and reduced the risk of Cd accumulation in grains. These findings are useful to guide the resource utilization of LPM as well as safe production of grains.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14122942/s1>, Figure S1: Distribution of study sites in research articles; Figure S2: Conceptual scheme for decision tree modeling; Figure S3: 10-fold cross-validation estimation error for each pruned node in the decision tree analysis; Figure S4: Predictive modeling schemes for random forest models; Figure S5: Random forest analysis of the relative

importance of the effects of each factor on soil and crop Cd contents. (a): Model of soil total Cd; (b): model of soil available Cd; (c): model of grain Cd; (d): model of root Cd; Figure S6: Random forest error training; Table S1: Details of the experiments used in the collected articles.

Author Contributions: T.T.: Data curation, Investigation, Validation, Visualization, Formal analysis, Writing—original draft. H.Z.: Conceptualization, Writing—review and editing. Z.Y.: Conceptualization, Methodology, Formal analysis. P.Z.: Methodology, Formal analysis. J.-F.G.: Methodology, Formal analysis. Y.-S.M.: Methodology, Formal analysis. C.-F.L.: Methodology, Formal analysis, Funding acquisition, Writing—review and editing. Z.-Y.H.: Conceptualization, Project administration, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Key Research and Development Program of China (2023YFD1701805) and National Social Science Fund “14th Five-Year Plan” 2021 Education General Project: Research on the Connotation, Standards, and Cultivation Strategies of Ecological Civilization Literacy among Primary and Secondary Schools (BHA210123).

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

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

Abbreviations

Cd	Cadmium
LPM	livestock and poultry manure
CEC	Cation exchange capacity
CART	Classification and regression tree
CI	Confidence interval
CM	Cattle manure
CHM	chicken manure
FM	farmyard manure
PM	pig manure
DOM	Dissolved organic matter
OM	Organic matter
SD	Standard deviation
SE	Standard error
SOC	Soil organic carbon
SOM	Soil organic matter
DT	Decision tree
RF	Randon Forest

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