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RESEARCH ARTICLE

Impact of tillage and straw management on cadmium bioavailability and uptake in rice: A long-term field study

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Highlights

- Plow tillage with straw incorporation effectively lowers cadmium (Cd) in rice grains.
- This practice reduces soil Cd availability and enhances iron plaque (IP) on rice roots.
- Available Cd, acid-soluble Cd, and IP directly influence Cd in rice grains.

Abstract

Tillage practices alter the interaction between soil and rice straw, impacting soil quality and cadmium (Cd) dynamics. However, the effects of tillage and straw management strategies on soil Cd accumulation and rice uptake remain unclear. This study investigated how tillage and straw practices influence rice Cd uptake by altering soil Cd mobility and bioavailability. A long-term field experiment was conducted with four treatments: no-tillage with straw return on the soil surface (NTS), rotary tillage with straw incorporation (RTS), plow tillage with straw incorporation (PTS), and plow tillage with straw removed (PT). Results showed that Cd concentrations in rice organs (root, stem, leaf, and rice grain) decreased in the order NTS>RTS>PTS, with only PTS maintaining grain Cd levels below 0.2 mg kg^{-1}. Compared with NTS and RTS, the average Cd concentrations in rice grain under PTS were significantly reduced by 56.76 and 25.88%, respectively. A partial least squares path model indicated that reductions in available Cd (Avail-Cd) and acid-soluble Cd (Aci-Cd), combined with iron plaque (IP) formation on the roots, were key factors in lowering rice Cd levels. PTS reduced Avail-Cd and Aci-Cd by decreasing soil bulk density, increasing soil organic matter, pH, and the abundances of Nitrospirota and Bacteroidota. Moreover, PTS enhanced soil nutrient and Fe^{2+} levels, promoted IP formation on rice roots through improved root morphology and antioxidant activity, and limited Cd uptake. Although PTS increased total and available soil Cd compared to PT, its promotion of IP formation mitigated rice Cd uptake, resulting in comparable grain Cd concentrations between the two. Thus, long-term plow tillage with straw incorporation emerges as a sustainable practice to enhance soil quality and reduce Cd uptake in the rice cropping system.

Keywords: tillage practices, straw return, rice, cadmium, iron plaque

1. Introduction

Heavy metal pollution in farmland, particularly cadmium (Cd), has garnered significant attention due to its severe impacts on agricultural product quality and safety (McLaughlin *et al.* 2021). Surveys indicate that heavy metal pollution in parts of China is severe, with Cd having the highest pollution rate, exceeding standards at approximately 7% of surveyed locations (Shi *et al.* 2022; Zhao *et al.* 2023). As China's most important staple crop, rice has a greater potential for Cd absorption compared to other cereals (Jing *et al.* 2023). Cd can then enter the human body through the food chain, causing serious health risks (Mao *et al.* 2019). Thus, controlling Cd pollution in rice and ensuring its safe production are critical global environmental challenges that

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demand urgent solutions.

Soil tillage practices are vital in rice production, influencing soil physical, chemical, and biological properties, thereby affecting crop growth and yield formation (Cheng *et al.* 2024). Proper tillage management can enhance soil structure, nutrient concentration, microbial properties, and overall crop productivity (Sanchez *et al.* 2016). Common tillage methods include plow tillage, rotary tillage, and no-tillage. Plow tillage, a conventional method, significantly impacts soil moisture, porosity, and aggregate stability, while rotary tillage typically operates at shallower depths. No-tillage, often advocated for reducing costs and preserving soil structure, can slow soil degradation and support biodiversity (Huang *et al.* 2015; Denardin *et al.* 2019). However, prolonged no-tillage can lead to shallow tillage layers, degraded soil structure, and diminished physical and chemical properties, thereby limiting sustainable rice production (Pandey *et al.* 2014).

The impact of tillage on Cd accumulation in crops is inconsistent. For acid-secreting crops like lupins, no-tillage can create acidic soil layers, increasing Cd uptake compared to tilled soils (Zotarelli *et al.* 2012). Conversely, some studies show lower Cd levels in no-tillage rapeseed compared to conventional tillage (Wang *et al.* 2024). For deep-rooted crops, intensive tillage aids root penetration into deeper layers, where Cd concentrations are often lower, potentially reducing Cd uptake (Linh *et al.* 2015).

China produces large amounts of crop straw, and straw returning has replaced burning as a sustainable method for recycling agricultural waste and improving soil quality. Many studies have shown that although straw returning can improve soil fertility, it also brings Cd from the straw into the soil, increasing the Cd accumulation in soil and rice (Shen *et al.* 2019; Yuan *et al.* 2024). However, other studies indicate that organic matter in rice straw can form organic-metal complexes with Cd in the soil, so that Cd^{2+} is wrapped inside the organic matter, reducing its bioavailability and limiting rice Cd uptake (Lu *et al.* 2017; Chen *et al.* 2022). These contradictory findings, often based on potted or short-term field experiments, underscore the need for long-term studies to clarify these effects.

Heavy metal accumulation in soil is a gradual process, and effective prevention and control strategies require long-term investigations. Long-term field experiments provide valuable insights due to their extended timeframes, comprehensive data, and reliability, contributing significantly to agricultural research worldwide (Liu J *et al*. 2021). Currently, few studies address how long-term tillage practices affect soil Cd distribution and availability. Moreover, there is a lack of research on the combined effects of long-term tillage and straw management on soil Cd bioavailability, rice Cd uptake, and underlying mechanisms.

This study aims to address these gaps through a long-term field experiment investigating how tillage practices and straw returning influence soil Cd chemical forms and rice root characteristics. Specifically, we explore how these practices modify soil chemical properties and microbial communities, affecting rice Cd uptake. Additionally, we examine how tillage-straw management enhances iron plaque formation by improving root morphology and enzyme activity, thereby impeding Cd translocation. Finally, we elucidate the mechanisms underlying the impact of tillage and straw management on rice Cd uptake.

2. Materials and methods

2.1. Site description

The pilot field experiment was conducted in Changsha, Hunan Province, southern China (28°07′N, 112°18′E). Established in 2005, the region experiences a subtropical monsoonal humid climate, with an average annual temperature of 16.8°C, a mean sunshine duration of 1,740 h, and average annual precipitation and potential evapotranspiration of 1,360 and 1,353.9 mm, respectively. The frost-free period is approximately 300 days, with 9 months having a mean temperature above 10°C. The soil is silty clay loam, baseline soil properties (0–20 cm layer) prior to the experiment were 0.35 mg kg^{-1} of total Cd (Total-Cd), 1.29 g kg^{-1} of total N, 17.63 g kg^{-1} of total K, 1.23 g kg^{-1} of total P, 224.10 mg kg^{-1} of available N (AN), 97.10 mg kg^{-1} of available K (AK), 4.38 mg kg^{-1} of available P (AP), 1.25 g cm^{-3} of bulk density (BD), 34.90 g kg^{-1} of soil organic matter (SOM), and 6.26 of pH.

2.2. Experimental design and management

The field experiment included four tillage practices: 1) no-tillage with straw return on the soil surface (NTS); 2) rotary tillage with straw incorporation (RTS); 3) plow tillage with straw incorporation (PTS); 4) plow tillage with straw removal (PT).

The treatments were arranged in a randomized block design with three replicates. Each plot measured 64 m^2 (8 m×8 m) and was separated by 35 cm-wide ridges covered with plastic film to prevent cross-irrigation or drainage. After rice harvesting, rice straws were either removed manually (PT) or cut into 5–10 cm lengths for incorporation (NTS, RTS, and PTS), while the roots were kept in the soil.

For NTS plots, no tillage was conducted, and rice straws were left as mulch throughout the cropping period. In RTS and PTS plots, straws were incorporated into the soil, with PTS involving plowing (15–20 cm depth) followed by two rounds of rotovation (8–12 cm depth). RTS plots underwent four rounds of rotovation (8–12 cm depth).

The double rice cropping system used the cultivars Zhongzao 25 (early rice) and Taifengyou 2213 (late rice) in 2022 and 2023. Rice seedlings were manually transplanted in April (early rice) and July (late rice) and harvested in July and October, respectively. Fertilization included 375 kg ha^{-1} of compound fertilizer ($N:P_2O_5:K_2O$=20:12:14) as a basal application for both crops, with additional urea applied at rates of 150 kg ha^{-1} (early rice) and 75 kg ha^{-1} (late rice) during growth stages. Pest control was performed one week prior to transplanting using herbicides and insecticides. Intermittent irrigation was implemented, maintaining a 5 cm water depth except for mid-season drainage (7 days) and final drainage (7–10 days before harvest).

2.3. Soil and plant sampling

Soil samples were collected after the late rice harvest in 2022 and 2023. A standard cutting ring with a volume of

100 cm^3 was used to measure soil BD in the layers of 0–5, 5–10, and 10–20 cm. For the determination of pH, SOM, cation exchange capacity (CEC), available nutrients (AP, AK, and AN), available Cd (Avail-Cd), and Total-Cd, a composite undisturbed soil sample (homogenized soil from three replicate plots in one treatment) was collected from the different soil layers with the use of a flat spade. Soil samples (0–20 cm) were collected from each plot using a five-point sampling method. A portion of the fresh soil was stored at –80°C for microbiological analysis (only in 2023), while the remaining soil samples were thoroughly mixed, air-dried, crushed, and sieved (0.149 mm) for chemical fraction analysis of Cd.

Rice samples were collected at maturity. Roots were washed, scanned for morphological analysis, and stored at −80°C for antioxidant enzyme activity, iron plaque (IP), and then Cd concentration analyses. Aboveground parts (stems, leaves, and grains) were dried and ground for analysis of Cd concentrations.

2.4. Analytical methods

Root IP and antioxidant enzymes Fresh roots (0.5 g) were ground in an acid buffer using a tissue grinder (Tissuelyser-48) and centrifuged (10,000 r min^{-1}, 10–15 min) to obtain crude enzyme extract. Antioxidant enzyme activities were measured using standard methods: superoxide dismutase (SOD, nitroblue tetrazolium photoreduction), peroxidase (POD, UV absorption method), catalase (CAT, guaiacol method), malondialdehyde (MDA, thiobarbituric acid colorimetric method) (Upadhyay *et al.* 2022).

The IP on the roots surface was extracted using the dithionite-citrate-bicarbonate (DCB) method (Fang *et al.* 2024), and the concentrations of Fe and Cd in the extract were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent, Santa Clara, CA, USA). After extraction, the roots were oven-dried at 75°C to a constant weight and subsequently analyzed for Cd concentration.

Soil and plant analysis Soil pH was measured at a 1:2.5 soil-to-water ratio. SOM was determined using potassium dichromate oxidation. AP, AK, and AN were analyzed *via* spectrophotometry, flame photometry, and extraction methods, respectively. Soil CEC was measured using $Co(NH_3)_6Cl_3$ extraction. Fe^{2+} concentration was determined colorimetrically using o-phenanthroline.

Cd fractions in soil were assessed using the BCR sequential extraction procedure, which distinguishes acid-soluble, reducible, oxidizable, and residual fractions. Total and available Cd in soils and plants were analyzed using ICP-MS, with quality control performed using certified reference materials.

Soil microbes analysis Soil DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primers 338F and 806R, followed by sequencing on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Bioinformatic analyses were performed using QIIME 2 software (version 2019.4).

2.5. Data analysis

The Cd bioconcentration factor (BCF) of rice was calculated as the following eq. (1)

$$BCF_A=Cd_A/Cd_{soil} \quad (1)$$

where the Cd_A and Cd_{soil} are the Cd concentrations of plant organs (i.e., root, stem, leaf, and rice grain) and soil, respectively (Yuan *et al.* 2024).

Statistical analyses were conducted using one-way ANOVA with Duncan's multiple comparisons (SPSS 24). Graphs were plotted using Origin 2023, and partial least squares path modeling (PLS-PM) was performed in R (4.0.5). Beta diversity was analyzed using Bray-Curtis distances and nonmetric multidimensional scaling (NMDS). Redundancy analysis was performed with Canoco 5.

3. Results

3.1. Cd uptake in rice plants

We investigated the effects of different tillage practices on Cd concentrations in various rice organs and observed significant variations across treatments (Fig. 1). Cd concentrations in each organ decreased with increasing tillage intensity, following the pattern: NTS>RTS>PTS (P<0.05). For rice grains, the average Cd concentrations in NTS and RTS were 0.414 and 0.243 mg kg^{-1}, respectively, which were significantly higher than 131.28 and 34.92% in PTS. Furthermore, the comparison between PT and PTS showed no significant increase in Cd concentrations in any rice organs, indicating that long-term straw returning under plow tillage conditions had no substantial impact on Cd levels in rice.

Tillage and straw management also significantly influenced the BCF in rice organs (Table 1). For straw return treatments,

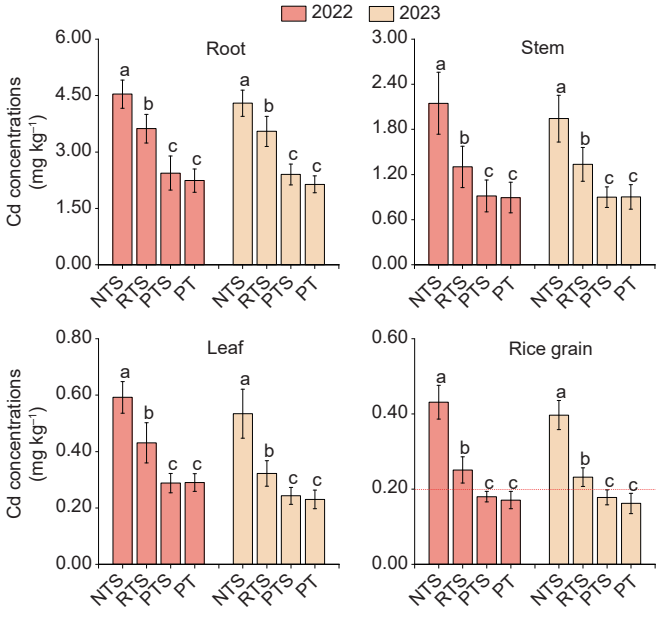


Fig. 1 Cadmium (Cd) concentrations in various rice organs. The dashed horizontal line in the rice grain panel indicates the maximum allowable Cd concentration in rice grain according to the Chinese food safety standard (0.20 mg kg^{-1}). NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed. Bars mean SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

Table 1 Effect of tillage and straw management on cadmium (Cd) bioconcentration factor in rice

Treatment[1)]	2022				2023			
	Root	Stem	Leaf	Rice grain	Root	Stem	Leaf	Rice grain
NTS	9.614±0.845 a	4.550±0.671 a	1.253±0.091 a	0.885±0.093 a	9.265±0.772 a	4.188±0.573 a	1.151±0.105 a	0.826±0.084 a
RTS	6.883±0.672 b	2.475±0.357 b	0.819±0.078 b	0.474±0.052 b	6.695±0.615 b	2.518±0.340 b	0.607±0.066 b	0.411±0.038 b
PTS	4.718±0.302 d	1.768±0.193 c	0.558±0.044 c	0.348±0.027 c	4.663±0.313 d	1.743±0.212 c	0.472±0.043 c	0.346±0.031 c
PT	5.526±0.425 c	2.207±0.212 b	0.715±0.061 b	0.431±0.033 b	5.376±0.347 c	2.265±0.302 b	0.578±0.051 b	0.401±0.023 b

[1)] NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed.
Values are mean±SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

the BCF in each organ followed the same trend as the Cd concentrations, with the order NTS>RTS>PTS (P<0.05). Additionally, the BCF was significantly higher in PT than in PTS (P<0.05), suggesting that long-term straw return under plow tillage conditions significantly reduces Cd uptake in rice.

3.2. Rice root properties

Iron plaque (IP) on the root surface In straw return treatments, the concentration of IP-Fe and IP-Cd significantly varied across treatments in the order: PTS>RTS>NTS over both years (P<0.05) (Fig. 2). The average IP-Fe concentration in PTS was 65.22 g kg^{-1}, which was 23.63 and 238.88% higher than that in RTS and NTS, respectively, indicating that higher tillage intensity promotes greater IP formation. Compared with PT, the IP-Fe concentration in PTS was significantly higher by 55.53 to 62.27% (P<0.05). The average two-year IP-Cd concentration in PTS was 0.885 mg kg^{-1}, which was higher than that in RTS, NTS, and PT by 15.08, 91.56, and 37.00%, respectively.

Morphological and physiological properties of rice roots Significant differences in root morphology were observed across the different tillage treatments (Table 2). Root length, tip number, surface area, volume, and dry weight were the highest under PTS, followed by RTS, and the lowest under NTS (P<0.05) for both years. Straw return (PTS) significantly improved root morphological properties compared with PT. For root physiological properties, antioxidant enzyme activities (SOD, POD, and CAT) and root bleeding-sap levels decreased in the order: PTS>RTS>NTS. Compared with PT, PTS significantly increased root antioxidant enzyme activity and bleeding-sap (P<0.05).

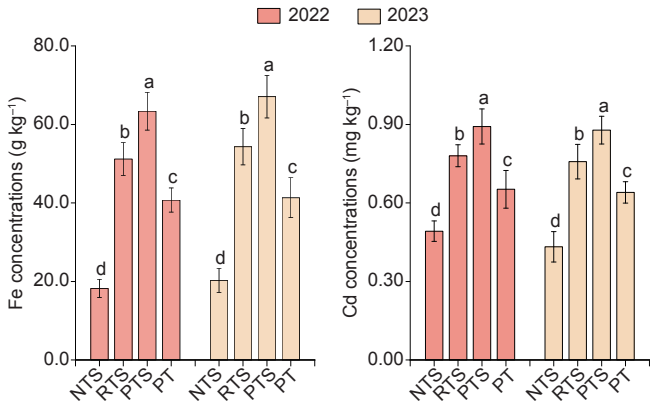


Fig. 2 Iron (Fe) and cadmium (Cd) concentrations in iron plaque (IP). NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed. Bars mean SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

3.3. Soil properties

Cd fractions For straw return treatments, the total Cd concentrations in soil decreased with depth, ranging from 0.379–0.686 mg kg^{-1} in 2022 to 0.381–0.693 mg kg^{-1} in 2023 (Fig. 3). The total Cd concentration at 0–5 cm in NTS was significantly higher than in other treatments, while at deeper layers, NTS showed no significant difference compared with RTS and PTS. At the 5–10 cm depth, the total Cd concentration in RTS was significantly higher than in PTS (P<0.05), but no significant differences were observed at 10–20 or 0–20 cm. Compared with PT, total Cd concentrations in PTS were significantly higher throughout the soil profile (P<0.05).

The available Cd (Avail-Cd) concentration decreased with increasing soil depth in both years, ranging from 0.184–0.395 mg kg^{-1} in 2022 to 0.180–0.408 mg kg^{-1} in 2023 (Fig. 3). In straw return treatments, Avail-Cd concentrations were highest under NTS, followed by RTS and the lowest under PTS in the 0–5 and 5–10 cm layers (P<0.05). PTS significantly increased Avail-Cd concentrations compared to PT. In 2022 and 2023, the Avail-Cd concentration in the 0–20 cm layer under PTS was 15.42 and 19.29% higher, respectively, than under PT.

The Aci-Cd concentrations followed the same trend as Avail-Cd, being the highest under NTS, followed by RTS, and the lowest under PTS (Fig. 4). The average two-year Aci-Cd concentration in NTS was 0.371 mg kg^{-1}, which was higher by 26.41 and 43.24% than in RTS and PTS, respectively. However, the concentrations of Red-Cd, Org-Cd, and Res-Cd were significantly higher in RTS and PTS than in NTS. Moreover, PTS significantly increased concentrations of Aci-Cd, Org-Cd, and Res-Cd compared to PT.

Physicochemical properties Soil physicochemical properties, except for BD, generally decreased with increasing soil depth across all treatments (Fig. 5). There were no significant differences in soil SOM, AN, and Fe^{2+} concentrations in the 0–5 cm layer. However, the soil AP and AK were significantly higher under NTS than under RTS and PTS. In the 5–10 cm layer, significantly lower SOM, AN, AP, AK, and Fe^{2+} were observed under NTS compared with RTS and PTS. In the 10–20 cm layer, SOM, AN, AP, AK, and Fe^{2+} were significantly higher under PTS than under RTS and NTS (P<0.05). In the entire soil profile, soil pH and CEC were highest under PTS, followed by RTS and NTS (P<0.05). Bulk density was

Table 2 Effect of tillage and straw management on root morphological and physiological properties

Year	Treatment[1)]	Root morphological properties per plant					Root physiological properties[2)]				
		Root length ($\times10^3$ cm)	Root tip number ($\times10^4$)	Root surface area ($\times10^3$ cm^2)	Root volume (cm^3)	Root dry weight (g)	Root bleeding-sap (g h^{-1})	SOD (U g^{-1} FW)	POD (U g^{-1} FW)	CAT (U g^{-1} FW)	MDA (nmol g^{-1} FW)
2022	NTS	6.25±0.73 c	4.82±0.21 c	0.70±0.08 c	7.24±0.83 c	2.09±0.17 d	0.73±0.07 c	98.68±10.51 c	10,112.7±989.5 c	589.6±47.6 c	26.07±3.11 a
	RTS	11.68±1.42 b	7.89±0.52 b	1.71±0.19 b	14.02±2.06 b	3.97±0.29 b	1.22±0.08 b	141.61±12.94 b	13,603.8±1,275.6 b	937.4±59.2 b	17.43±2.04 b
	PTS	15.69±1.73 a	10.24±0.84 a	2.42±0.27 a	19.81±2.85 a	4.83±0.35 a	1.51±0.13 a	174.48±17.98 a	17,281.7±1,934.1 a	1,104.8±84.3 a	13.69±1.57 c
	PT	10.92±1.13 b	8.61±0.45 b	1.60±0.16 b	13.32±1.57 b	3.21±0.24 c	1.19±0.10 b	138.53±13.68 b	13,040.9±1,531.2 b	843.1±75.5 b	18.75±1.83 b
2023	NTS	5.80±0.65 c	3.99±0.24 c	0.65±0.07 c	6.68±0.64 c	1.97±0.15 d	0.69±0.05 c	94.70±11.54 c	8,324.8±612.8 c	617.1±41.7 c	25.56±2.74 a
	RTS	11.12±1.55 b	8.83±0.48 b	1.74±0.20 b	15.53±1.35 b	4.06±0.27 b	1.13±0.14 b	147.47±15.13 b	11,719.4±1,519.3 b	884.1±72.1 b	19.20±1.76 b
	PTS	15.47±1.43 a	10.09±0.71 a	2.57±0.22 a	20.97±3.02 a	5.01±0.41 a	1.40±0.11 a	185.56±20.27 a	15,814.7±1,836.3 a	1,085.0±90.6 a	14.41±1.25 c
	PT	10.39±1.20 b	8.57±0.50 b	1.66±0.13 b	14.31±1.44 b	3.35±0.33 c	1.08±0.09 b	151.44±14.39 b	11,024.9±1,425.9 b	869.6±60.3 b	18.59±1.44 b

[1)] NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed.
[2)] SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde.
Values are mean±SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

significantly higher under NTS than under RTS and PTS, with RTS also showing significantly higher BD than PTS at 10–20 cm depth. Compared with PT, PTS significantly improved all soil physicochemical indicators except BD and pH (P<0.05).

Soil microbes The Bray-Curtis distance matrix revealed significant differences in microbial community structure at the phylum level between the different tillage treatments (NTS *vs*. RTS and PTS; P<0.01) (Fig. 6-A). After the 16S rDNA sequencing, we observed that the total number and diversity of microbial

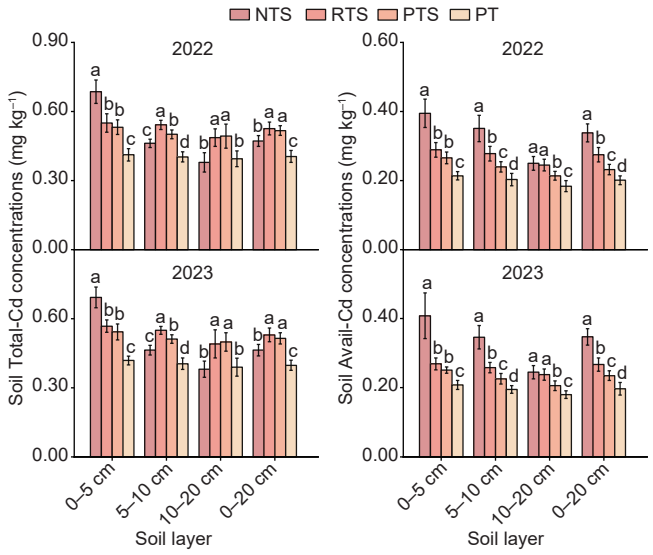


Fig. 3 Total cadmium (Cd) (Total-Cd) and available Cd (Avail-Cd) concentrations in soil. NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed. Bars mean SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

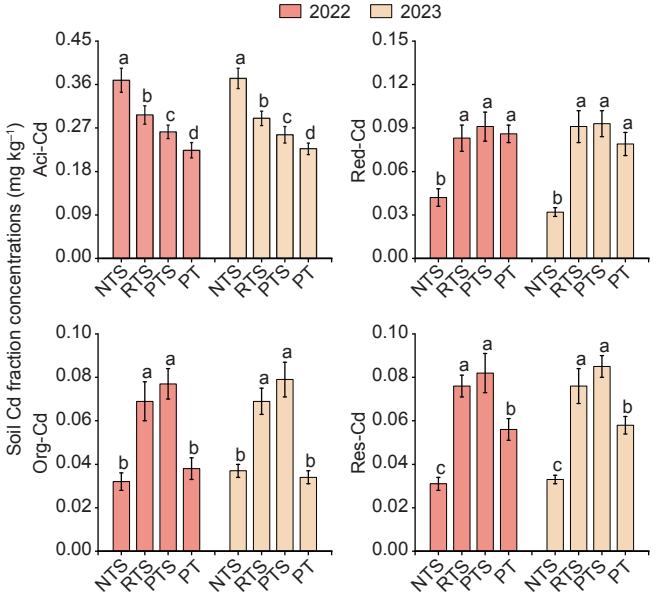


Fig. 4 Chemical forms of cadmium (Cd) in soil (0–20 cm). NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed. Bars mean SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

species (Chao1 and Shannon indices) increased with greater tillage intensity. However, community distribution (Simpson index) became less even as tillage intensity increased (Fig. 6-B). Compared to PT, PTS significantly increased both the Chao1 and Shannon indices (P<0.05), suggesting a more diverse microbial community under plow tillage with straw incorporation.

The dominant bacterial phyla across all soil samples were *Proteobacteria* (21.35–29.41%), *Acidobacteriota* (15.38–17.89%), *Nitrospirota* (5.65–11.47%), *Chloroflexi* (5.52–8.20%), *Desulfobacterota* (7.08–7.77%), *Bacteroidota* (4.20–8.16%), *Gemmatimonadota* (2.08–4.76%), *Myxococcota* (3.70–6.09%), *unclassified_Bacteria* (1.99–3.49%), and *Methylomirabilota* (1.94–2.98%). Significant differences in the relative abundance of these phyla were found between treatments (Fig. 6-C). For straw return treatments, the relative abundance of *Proteobacteria* and *Myxococcota* was significantly higher under NTS than under RTS or PTS, whereas the relative abundance of *Nitrospirota* and *Bacteroidota* was significantly lower under NTS than under RTS or PTS. Furthermore, the relative abundance of *Methylomirabilota* was significantly higher under PTS than under NTS. In contrast, *Chloroflexi* and *Gemmatimonadota* showed significantly higher abundance under PTS compared to PT, while *Proteobacteria* was significantly less abundant under PTS than under PT.

3.4. Analysis of the associations among Cd concentrations in rice, root properties, and soil properties

Redundancy analysis (RDA) was performed to assess the contribution of soil microbial community composition to key soil and rice properties, as well as to identify the relationships between the top 10 phyla and Cd concentrations in rice and soil (Rice grain-Cd, IP-Fe, Aci-Cd, and Avail-Cd) under different treatments (Fig. 7-A). RDA results indicated that Cd concentrations in rice grain (Rice grain-Cd), as well as Aci-Cd and Avail-Cd in soil, were negatively correlated with the relative abundance of *Nitrospirota* and *Bacteroidota*. Conversely, Fe (IP-Fe) and Cd (IP-Cd) concentrations in IP were negatively correlated with the relative abundance of *Proteobacteria*. Fig. 7-B highlights that *Nitrospirota* and *Bacteroidota* contributed the most to the response variables, significantly influencing the Cd concentrations in both rice and soil.

To explore the mechanisms through which soil and root properties influence Cd accumulation in rice, we conducted correlation analyses. The Cd concentrations in various rice organs were highly positively correlated with Aci-Cd and Avail-Cd in the soil (P<0.01), and negatively correlated with IP-Fe and IP-Cd (P<0.05) (Appendix A). A subsequent analysis of the relationships between key soil properties and microbial indices revealed that soil organic matter (SOM), pH, cation exchange capacity (CEC), and Fe^{2+} were significantly negatively correlated with Aci-Cd and Avail-Cd, while they were strongly positively correlated with IP-Fe and IP-Cd (Appendix B). Additionally, rice root properties were significantly positively correlated with iron plaque formation (IP) and several soil properties, except bulk density (BD). These results suggest that changes in soil microbial communities and physicochemical properties resulting from long-term tillage and straw management are closely linked to the speciation of soil Cd and the formation of iron plaques on rice roots, which, in turn, affect Cd uptake by rice plants.

We constructed a PLS-PM to visualize the complex interrelationships among tillage practices, rice, and

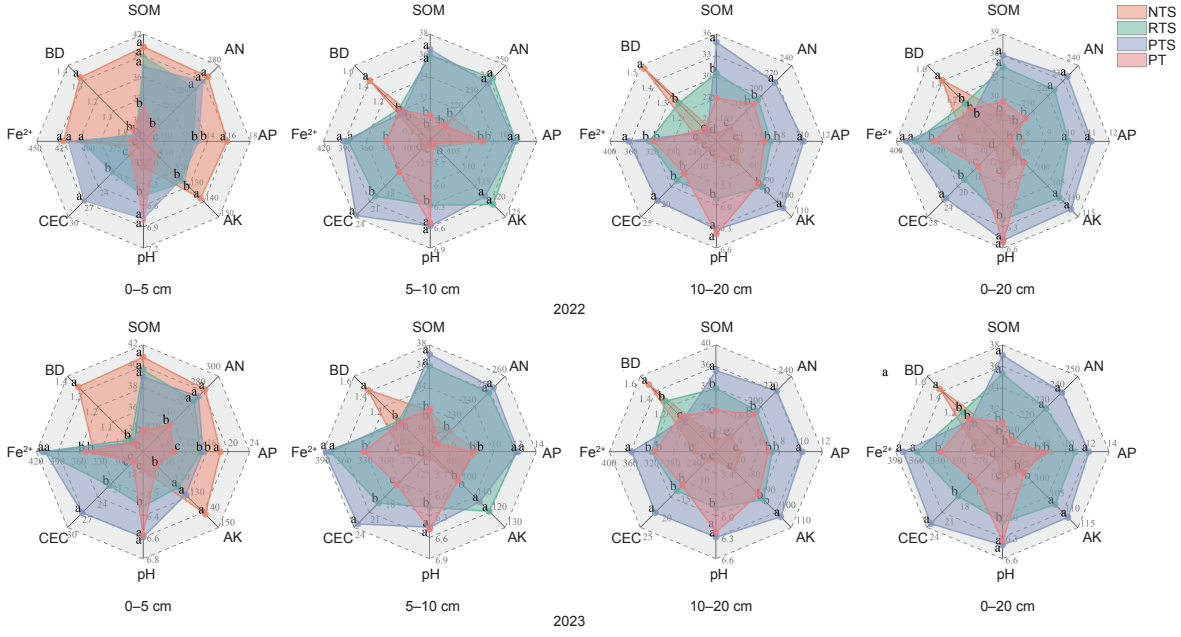


Fig. 5 Effect of tillage and straw management on soil physicochemical properties. NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed. BD, bulk density; SOM, soil organic matter; AN, available N; AP, available P; AK, available K; CEC, cation exchange capacity. Bars mean SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

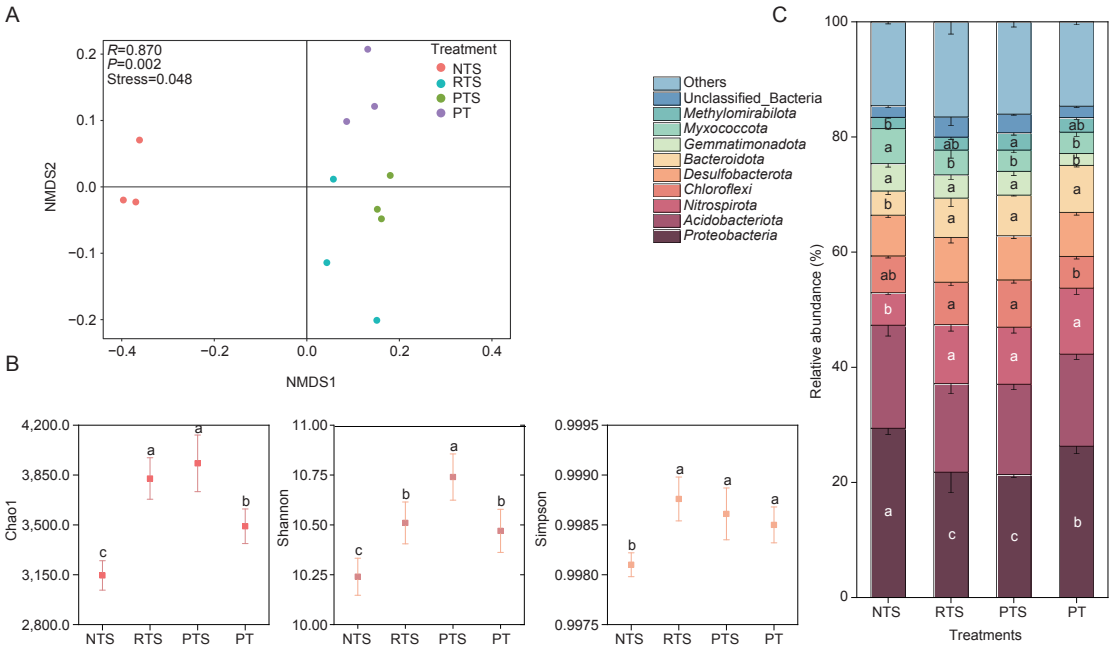


Fig. 6 Soil microbial community at the phylum level. A, nonmetric multidimensional scaling (NMDS) analysis of bacterial community beta diversity. B, alpha diversity at the phylum level. C, relative abundance of dominant bacterial phyla in the bacterial community. NTS, no-tillage with straw return on the soil surface; RTS, rotary tillage with straw incorporation; PTS, plow tillage with straw incorporation; PT, plow tillage with straw removed. Bars mean SD (n=3). Different lowercase letters indicate significant differences among groups under different treatments (P<0.05).

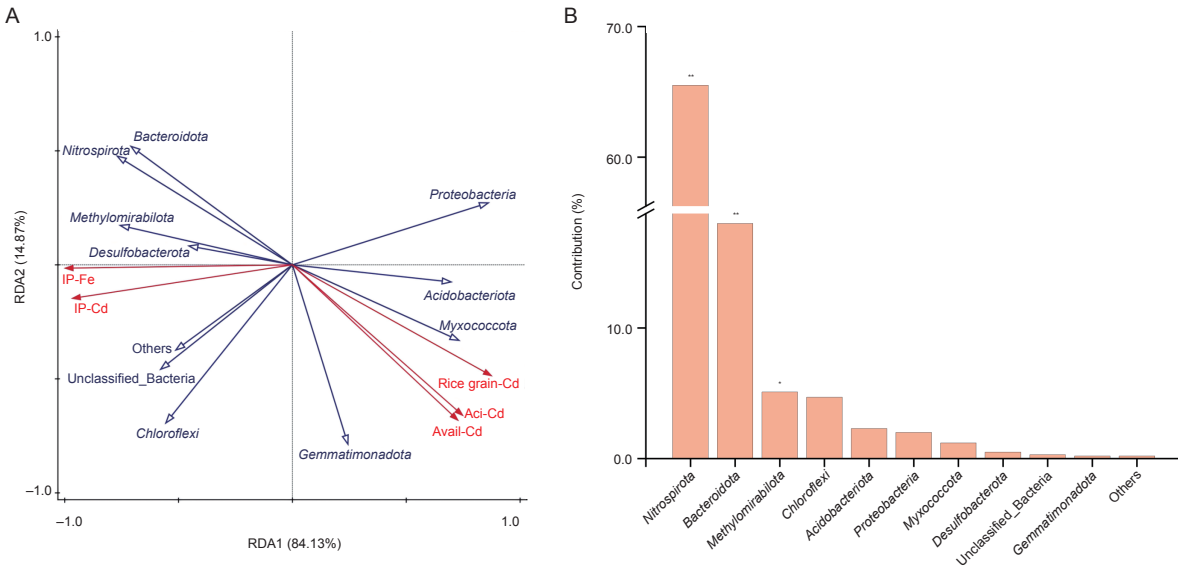


Fig. 7 Relationship between the top 10 bacterial phyla and cadmium (Cd) concentrations in rice and soil. A, redundancy analysis (RDA) of the top 10 bacterial phyla and their relationship with Cd concentrations in rice and soil. B, contribution rates of the top 10 bacterial phyla. *, P<0.05; **, P<0.01.

soil properties (Fig. 8). The model revealed that under tillage management, Avail-Cd (0.901), Aci-Cd (0.696), and IP (–0.713) had direct and significant effects on Cd concentration in rice grain. The soil SOM (0.759) and BD (–0.875) significantly influenced Avail-Cd *via* functional microbes (–0.778), while soil pH exerted a substantial negative effect on Aci-Cd and Avail-Cd (–0.956 and –0.863, respectively). Additionally, the soil BD (–0.819) and NPK (0.720) significantly influenced IP formation through root morphological properties (0.867), and NPK (0.848) also positively impacted IP formation through root physiological properties (0.701). Moreover, Fe^{2+} (0.835) directly and positively influenced IP formation.

In contrast, under straw management, soil physicochemical indicators did not significantly impact Aci-Cd, Avail-Cd, or functional microbes. However, NPK (0.891 and 0.912)

significantly influenced IP formation *via* root morphology (0.926) and root physiology (0.839), with Fe^{2+} (0.914) also having a direct positive effect on IP formation. Notably, under straw management, IP, Aci-Cd, and Avail-Cd did not significantly impact Cd concentrations in rice grain.

4. Discussion

4.1. Effects of tillage-straw management on Cd fractions through soil properties

The environmental behavior of Cd in cultivated soils, particularly in paddy soils, is complex due to the considerable influence of tillage practices on the distribution and availability of heavy metals (Weidhuner *et al.* 2021). In this study, the highest total Cd concentration was observed in the 0–5 cm soil layer under NTS conditions, whereas the lowest concentration was found in the 5–20 cm soil layer in the same treatment (Fig. 3). This result may be attributed to the limited soil disturbance under NTS, leading to a higher concentration of Cd at the surface. In contrast, rotary tillage (RTS) and plow tillage with straw return (PTS) resulted in significantly higher total Cd concentrations across the entire 0–20 cm soil profile. Long-term no-tillage practices can lead to surface soil compaction, causing some Cd to migrate downward or be lost, whereas tillage practices like RTS and PTS mix the soil, redistributing Cd to deeper layers.

Although the total Cd concentration in soil is related to rice Cd uptake, Cd bioavailability is considered to have a more substantial impact on Cd uptake in rice (Rao *et al.* 2018). In this study, the soil Avail-Cd and Aci-Cd concentrations were highest under NTS and lowest under PTS (P<0.05), whereas Red-Cd, Org-Cd, and Res-Cd were most concentrated under PTS (Figs. 3 and 4). These findings indicate that increased tillage intensity significantly reduced Cd bioavailability by promoting the transformation of Aci-Cd into a more stable state, such as Org-Cd. The speciation of Cd dictates its mobility and availability, with Aci-Cd being the most bioavailable due to its weak binding to soil particles (Zhao *et al.* 2020). The increase in tillage intensity under straw return conditions reduced Cd bioavailability, although it increased the overall soil Cd concentration. This suggests that the effects of tillage practices on Cd speciation and availability are complex and influenced by various soil physical and chemical factors.

Rice straw return increases Cd concentrations in the soil because rice straw itself contains Cd, which is released into the soil as it decomposes (Huang *et al.* 2024). In our study, the PTS resulted in higher Total-Cd and Avail-Cd concentrations than PT (Figs. 3 and 4). Plow tillage facilitates thorough mixing of the straw with the soil, enhancing the contact between straw and soil, promoting straw decomposition, and subsequently releasing Cd into the soil. As the Total-Cd concentration in the soil increases over time, the concentrations of various Cd fractions also rise under certain conditions.

Tillage practices also influence soil physicochemical properties, which in turn affect Cd bioavailability and its uptake by rice (Bergkvist *et al.* 2003). In this study, NTS resulted in higher BD and lower pH and CEC than RTS and PTS (Fig. 5). Increasing tillage intensity reduced soil BD, improving aeration and water retention, which in turn increased soil pH and CEC. Correlation analysis found that the Avail-Cd and Aci-Cd were positively correlated with BD and negatively correlated with pH and CEC (Appendix B). Previous studies have shown that higher pH reduces Cd bioavailability by increasing soil particle surface charge, thereby enhancing Cd adsorption and precipitation (Yu *et al.* 2016; Li *et al.* 2020). These findings support the conclusion that the higher pH and CEC associated with tillage intensification reduce Cd availability by increasing adsorption and precipitation, particularly under PTS, which promotes the transformation of Avail-Cd into more stable forms.

Rice straw return also enhances soil nutrients, CEC, and Fe^{2+} concentrations (Fig. 5), which can further reduce Cd availability. Although soil nutrients were lower in the 0–5 cm layer under PTS and RTS compared to NTS, they were higher in the 5–20 cm layer, indicating that tillage practices redistribute soil nutrients across layers. This redistribution can help prevent nutrient loss while promoting more uniform nutrient distribution. SOM and Fe^{2+} were negatively correlated with Avail-Cd and Aci-Cd (Appendix B), consistent with the role of SOM in reducing Cd bioavailability through complexation (Chen *et al.* 2020). Fe^{2+} also competes with Cd for adsorption sites, further reducing the availability of Cd in the soil (Liu N *et al.* 2021).

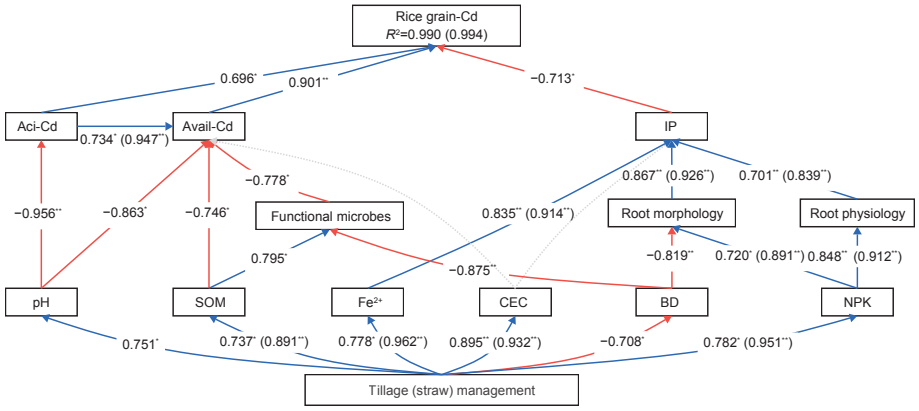


Fig. 8 Partial least squares path model (PLS-PM) explaining cadmium (Cd) concentration in rice grain. Aci-Cd, acid-soluble Cd; Avail-Cd, available Cd; IP, iron plaque; SOM, soil organic matter; CEC, cation exchange capacity; BD, bulk density. *, P<0.05; **, P<0.01.

4.2. Role of IP in Cd uptake by rice under tillage-straw management

Tillage practices can significantly influence rice root growth, which affects Cd uptake from the soil. In our study, the Cd concentration in rice organs decreased in the order NTS>RTS>PTS (P<0.05), and the BCF in rice followed a similar pattern (Table 1). This suggests that the primary factor influencing Cd concentration in rice across different tillage treatments is Cd uptake, which is strongly related to soil and rice root properties. Notably, although PTS increased both Total-Cd and Avail-Cd concentrations in the soil compared with PT, it did not elevate Cd concentrations in rice grain due to a reduction in the Cd bioconcentration factor. This indicates that PTS did not increase Cd uptake in rice, possibly due to enhanced root Cd retention.

The formation of IP on the rice root surface plays a crucial role in controlling Cd uptake. Iron plaques, composed of amorphous iron oxides, can adsorb Cd *via* Cd-Fe precipitation, thereby preventing Cd entry into the plant (Liu *et al.* 2007). In our study, the concentrations of IP-Fe and IP-Cd under PTS were significantly higher than those in other treatments (Fig. 2). Correlation analysis revealed a strong negative correlation between IP-Fe and IP-Cd concentrations and Cd concentrations in rice grain (Appendix A). The iron oxides in iron plaques co-precipitate with mobile Cd in the soil or chelate Cd in the root epidermis, thereby inhibiting Cd uptake by rice (Zandi *et al.* 2021). Additionally, the competitive adsorption of Cd and Fe on rice root surfaces limits Cd entry into the plant (Fu *et al.* 2018).

Root morphology also influences IP formation, as more extensive root systems provide greater surface area for Fe and Cd adsorption. In this study, PTS significantly promoted rice root development, increasing root length, surface area, and volume, thereby enhancing the formation of iron plaques (Table 2). Moreover, the activity of antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) in rice roots also increased with tillage intensity. Antioxidant enzyme activity enhances root oxidative capacity, facilitating the formation of iron plaques through oxidative processes on the root surface (Yang *et al.* 2014).

4.3. Mechanisms for the effects of tillage-straw management on Cd concentration in rice

In this study, the microbial community structure of RTS and PTS differed significantly from that of NTS, and species diversity and richness were significantly higher than in NTS (Fig. 6-B). RDA results showed that *Nitrospirota* and *Bacteroidota* had strongly negative effects with Avail-Cd and Aci-Cd (P<0.01) (Fig. 7-A). Interestingly, the relative abundances of *Nitrospirota* and *Bacteroidota* under RTS and PTS were significantly higher than NTS (Fig. 6-C).

Based on our results, we propose a mechanistic model explaining how long-term tillage practices and rice straw management affect Cd concentration in rice. Tillage practices influence Cd concentration in rice from two main pathways: first, by affecting soil Cd availability, and second, by modifying the formation of iron plaques on rice roots. The increase in pH with higher tillage intensity reduces Aci-Cd and Avail-Cd, while SOM indirectly decreases Avail-Cd by influencing soil microbial communities. In addition, increased BD increases Avail-Cd by reducing the abundance of functional microbes. The second pathway involves the formation of iron plaques, which is promoted by increased NPK fertilizer use under tillage management and by higher Fe^{2+} concentrations, both of which contribute to greater Cd retention in iron plaques and reduced Cd uptake by rice.

In contrast, straw management affected the formation of iron plaques by altering soil properties, but it did not significantly affect rice Cd concentration. Rice straw return increased the total and available Cd concentrations in soil due to the addition of exogenous Cd from the straw. However, it also reduced Cd uptake in rice by promoting the formation of iron plaques, resulting in no significant difference in rice Cd concentration between PT and PTS. Although rice Cd concentrations were not significantly affected by straw management, long-term accumulation of Cd in soil from continuous straw return could pose potential ecological risks, including soil degradation and increased risks to crop production.

5. Conclusion

Our field experiment demonstrated that plow tillage with PTS effectively maintained Cd concentrations in rice grain below the threshold of 0.2 mg kg^{-1} across different tillage practices. This was primarily due to a reduction in the BCF in rice. PTS reduced the mobility and bioavailability of Cd in the soil by decreasing the soil bulk density and increasing soil organic matter, pH, and the abundance of microbial phyla such as *Nitrospirota* and *Bacteroidota*. Furthermore, PTS improved soil nutrient levels (e.g., available nitrogen, phosphorus, potassium) and Fe^{2+} concentrations, promoting their accumulation in the middle and lower soil layers. The increase in soil nutrients enhanced rice root morphology and antioxidant enzyme activity, thereby facilitating the formation of IP on the root surface. The higher Fe^{2+} concentration in the soil directly contributed to increased Fe in the IP. Although PTS resulted in significantly higher soil total Cd and available Cd than PT, it mitigated rice Cd uptake by promoting IP formation, resulting in no significant difference in Cd concentration in rice grain between the two treatments. In conclusion, long-term plow tillage with straw incorporation can not only improve soil quality and reduce soil Cd bioavailability but also reduce Cd uptake in rice by promoting root growth and IP formation.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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