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Effects of Wood Vinegar Application and Planting Density on Cadmium Uptake and Accumulation in a Maize–Wheat Rotation System Under Straw Removal Management

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Highlights

- Integrated management enhanced maize Cd phytoextraction potential while reducing Cd accumulation in subsequent wheat.
- ZT100 significantly reduced total and residual Cd in soil.
- The Cd content in wheat grains was reduced under the comprehensive measures of straw removal.
- Integrated management was associated with shifts in soil bacterial communities; PLS-PM indicated a strong negative association between maize straw Cd accumulation and wheat grain Cd.

Abstract

Cadmium (Cd) contamination in agricultural soils threatens food safety and human health. To address this issue, wood vinegar (WV) and high-density planting were combined in our field study. The results revealed that the combined application significantly increased maize (*Zea mays*) grain and straw biomass while enhancing Cd accumulation in aboveground tissues. Notably, grain Cd concentrations remained relatively low, ranging from 0.032 to 0.047 $mg \cdot kg^{-1}$, whereas the Cd enrichment coefficients in stems and leaves rose by 70.03% and 67.42%, respectively. Additionally, WV application altered soil Cd speciation and reduced the exchangeable Cd fraction. The ZT2 treatment reduced the exchangeable Cd fraction by 24.56%, while the ZT3 treatment reduced total soil Cd by 6.91%, indicating its potential to enhance Cd removal. In the subsequent wheat (*Triticum aestivum*) season, maize straw removal combined with high-density planting considerably decreased Cd concentrations across wheat tissues, with maximum reductions of 25.59% in grain and 45.63% in straw. However, wheat grain Cd concentrations remained above the applicable food safety limit. These management practices were also associated with shifts in the soil microbial community, including changes in the relative abundance of Actinobacteria and Acidobacteria. Partial least squares path modeling (PLS-PM) indicated that Cd accumulation in maize straw showed the strongest negative association with Cd concentrations in wheat grain. Overall, integrating WV application with high-density planting represents a promising agronomic strategy for enhancing Cd phytoextraction by maize and mitigating Cd accumulation in subsequent wheat.

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1. Introduction

The quality and safety of farmland are closely associated with food security, which is the fundamental guarantee of human health. Cadmium (Cd) contamination is among the most serious forms of heavy metal pollution in agricultural soils, threatening both food security and human health [1,2]. China suffers annual grain losses of up to 10 million tons because of heavy metal contamination [3,4]. Cd stands out as particularly problematic among the major heavy metal pollutants, exerting toxicity not only by disrupting the metabolic steps of plant physiology, photosynthesis, nutrient absorption, and water utilization but also by reducing the quality and yield of crops [5–7]. It can accumulate in the human body through the food chain and is difficult to eliminate, and long-term accumulation can l bring about severe consequences, such as generalized aches, intestinal diseases, damage to DNA, and even cancer [8,9].

Cd accumulation in soils primarily arises from anthropogenic sources, including agricultural activities (such as the application of pesticides and fertilizers that contain heavy metals), mining operations, irrigation with treated wastewater, and atmospheric deposition resulting from industrial emissions [10–12]. Therefore, an efficient, economical, and environmentally friendly remediation technology is urgently needed. Traditional phytoremediation techniques utilize hyperaccumulator plants to absorb and transfer heavy metals from the soil and offer advantages, such as environmental friendliness and lower costs [13]. Nonetheless, these methods are limited by lengthy remediation cycles, low yields of biomass, and competition with agricultural production for land. Consequently, it is challenging to simultaneously meet the dual demands to protect farmland and ensure food production [14].

Against this backdrop, the "phytoremediation coupled with agro-production" (PCA) model has emerged, enabling the simultaneous safe utilization and purification of contaminated farmland. This model utilizes primary crops (such as maize) as carriers and leverages their high tolerance, high biomass yield, and significant potential to accumulate heavy metals in their non-edible parts. Concurrently, chemical enhancement techniques, including applying organic chelating agents and organic acids, can boost the crop's efficiency in absorbing and transporting heavy metals, such as Cd [15]. Such enhancement measures can significantly activate the heavy metals in soil and promote their accumulation in non-edible plant parts [16–20]. Furthermore, chemical enhancement techniques can further improve the efficiency of remediation [19,21,22]. Among them, WV, which is an environmentally friendly and widely available organic mixture, demonstrates considerable potential for chemically enhancing remediation [23–25]. Additionally, high levels of acetic acid, phenols, alcohols, ketones, and other components are present in this type of vinegar, which has long been employed for soil amelioration and plant growth regulation [26]. Among the various activators, WV is particularly effective in enhancing the uptake of Cd uptake by plants, highlighting its promising application prospects in the remediation of agricultural soils contaminated with Cd [27–30].

However, despite the reported benefits of organic amendments and chemical activators in enhancing heavy metal phytoextraction, studies on the use of WV as a sustainable and low-cost activator to improve Cd remediation efficiency through crop-based phytoremediation remain scarce, especially under field conditions [31,32]. Moreover, most existing phytoremediation strategies focus on remediation efficiency, while insufficient attention has been paid to integrating heavy metal removal with sustainable agricultural production, particularly under field conditions. Therefore, the synergistic effects of chemical activation and agricultural production within the PCA framework require further investigation.

Therefore, this study aimed to address these knowledge gaps by integrating WV-enhanced phytoremediation with agricultural production through the PCA model. Specifically, we investigated (1) how WV application and planting density affect maize growth, Cd uptake, and translocation; (2) whether WV promotes Cd mobilization and transformation in contaminated soils; and (3) whether this remediation strategy can effectively reduce Cd accumulation risks in subsequent crops. We hypothesized that WV application would enhance soil Cd availability and facilitate Cd uptake and accumulation in maize non-edible tissues, thereby improving Cd phytoextraction efficiency, while optimized planting density could regulate maize biomass production, Cd uptake, and transport processes to maximize remediation performance and reduce Cd contamination risks in subsequent crops. This study provides new insights into the feasibility of integrating heavy metal remediation with safe agricultural production and offers a practical approach for the sustainable utilization of Cd-contaminated farmland.

2. Materials and Methods

The field experiment was conducted at a contaminated farm site in China containing Shajiang black soil, whose basic physicochemical properties were as follows: pH, 5.92; available phosphorus (P), 32.74 $mg \cdot kg^{-1}$; available potassium (K), 171.60 $mg \cdot kg^{-1}$; available nitrogen (N), 66.10 $mg \cdot kg^{-1}$; and organic matter, 12.55 $g \cdot kg^{-1}$. Before establishing the field experiment, the spatial variability in soil Cd in the experimental field was evaluated using a grid sampling method. The experimental area was divided into a 4 × 5 grid, and 44 soil samples were collected from evenly distributed sampling locations (two samples from each grid cell and four additional samples from the field boundaries). The initial soil Cd concentrations ranged from 0.698 to 0.753 mg kg^{-1}, indicating relatively low spatial variability across the experimental field. Therefore, the experimental plots had comparable initial Cd contamination levels before treatment application.

The WV used in the study was purchased from Shijiazhuang Hongsen Activated Carbon Co., Ltd. (Shijiazhuang, China) and extracted from the shells of fruit. The main properties were as follows: pH, 4.18; conductivity, 3.50 $mS \cdot cm^{-1}$; organic carbon, 57.75 $g \cdot kg^{-1}$; total N, 2.69 $g \cdot kg^{-1}$; total P, 0.81 $g \cdot kg^{-1}$; and total K, 0.09 $g \cdot kg^{-1}$. In addition, the main organic components of WV were characterized, including organic acids, ketones, esters, and phenolic compounds and derivatives, which accounted for 46.06%, 3.10%, 7.34%, and 32.70%, respectively. The variety of maize used was Zhengyuanyu 432, which was procured from Henan Qiule Seed Industry Technology Co., Ltd. (Zhengzhou, China).

A randomized complete block design (RCBD) was employed in this field experiment. One conventional agricultural practice control (H) and eight remediation treatments were established to evaluate the effects of WV application and increased planting density on Cd phytoextraction in a maize–wheat rotation system. Each treatment was conducted with three independent replicates, resulting in a total of 27 experimental plots (3 m × 9 m), with each plot considered an independent experimental unit for statistical analysis.

The control treatment (H) represented the conventional agricultural practice in the study region, consisting of clear water application, conventional planting density, and straw returning. The other treatments were established by modifying the management practices based on H, including WV application and/or increased planting density (PD). Under conventional planting density (75,000 $plants \cdot hm^{-2}$), treatments included L, T1, T2, and T3. L received clear water application, while T1, T2, and T3 received WV applications at concentrations of 0.25%, 0.5%, and 1%, respectively. Under increased planting density (90,000 plants·hm^{-2}), treatments included ZL, ZT1, ZT2, and ZT3 (Table 1).

Table 1. Test treatment.

Maize Season				Wheat Season	
Treatment	**Wood Vinegar Concentration**	**Plant Density (Plant·hm^{-2})**	**Straw Processing Methods**	**Plant Density (kg·hm^{-2})**	**Straw Processing Methods**
H	clear water	75,000	straw incorporation	225	straw incorporation
L	clear water	75,000	straw removal from fields	225	straw removal from fields
T1	0.25%	75,000	straw removal from fields	225	straw removal from fields
T2	0.5%	75,000	straw removal from fields	225	straw removal from fields
T3	1%	75,000	straw removal from fields	225	straw removal from fields
ZL	clear water	90,000	straw removal from fields	285	straw removal from fields
ZT1	0.25%	90,000	straw removal from fields	285	straw removal from fields
ZT2	0.5%	90,000	straw removal from fields	285	straw removal from fields
ZT3	1%	90,000	straw removal from fields	285	straw removal from fields

In Cd-contaminated farmland, straw removal was adopted in remediation treatments as a necessary supporting management practice to prevent the Cd accumulated in crop straw from re-entering the soil system. Therefore, straw removal was not considered an independent experimental factor, but rather a prerequisite practice for the remediation treatments.

All treatments were randomly allocated among the experimental plots. For plant analysis, three subsamples were collected from each of the three replicate plots within each treatment, and these samples were used to evaluate plant biomass and Cd uptake. For microbial community analysis, soil samples were collected independently from each replicate plot using an S-shaped sampling method. Several soil cores collected from the same plot were pooled and homogenized to obtain one composite soil sample. Thus, each treatment consisted of three biological replicates for microbial community analysis, with each replicate representing one independent field plot.

The fertilizer was applied following local farming standards, and the amount of NPK compound fertilizer (15-15-15) was 750 kg·hm^{-2}. During the maize jointing stage, WV solutions with different concentrations (0.25%, 0.5%, and 1.0%) were applied to the soil surface between maize rows at five-day intervals for a total of three applications. The same WV solution volume was applied in each event, with a total application volume of 6.75 L·m^{-2} (equivalent to 67,500 L·hm^{-2} of diluted WV solution). Based on the WV concentration, the corresponding amounts of undiluted WV applied were 168.75, 337.50, and 675.00 L·hm^{-2} for the 0.25%, 0.5%, and 1.0% WV treatments, respectively. The amount of undiluted WV applied was calculated as follows:

$$\text{Undiluted WV amount}\left(\text{L}\cdot\text{hm}^{-2}\right) = \text{WV solution volume}\left(\text{L}\cdot\text{hm}^{-2}\right) \times \text{WV concentration}$$

The WV solutions were prepared by diluting the original WV with water according to the designed concentrations before application.

In the mature period, the straw was crushed and removed after the maize was harvested. In the wheat season, two planting densities of 225 kg·hm^{-2} (normal density group) and 285 kg·hm^{-2} (conventional planting density increased by 25%) were used. The fertilizer was applied according to the recommendations, and 115 kg·hm^{-2} of NPK compound fertilizer (15-15-15) was applied. Furthermore, standardized field management practices were implemented throughout the cultivation of wheat and maize, involving loosening the soil, weeding, and irrigating the fields.

At the maize maturity stage, three replicate samples of soil (0–20 cm) and whole maize plants were collected from each plot by the "S"-shaped five-point sampling method. Soil samples were air-dried at room temperature (25 ± 5 °C) in the dark, cleared of debris (such as stones and plant residues), ground with a mortar, passed through a 0.15 mm nylon sieve, and stored in sealed plastic bags. Harvested maize plants were washed with deionized water and dissected into five parts: roots, stems, leaves, cobs, and grains. Each part was first heat-deactivated at 105 °C for 30 min and then oven-dried at 65 °C to a constant weight, and the dry mass was recorded.

The collected samples of maize shoots and roots were pulverized, sieved, and subsequently digested with nitric acid: perchloric acid (HNO_3:$HClO_4$) (1:3, v/v) [33]. The digestion was analyzed by atomic absorption spectrophotometry (PerkinElmer AA600, Shelton, CT, USA) to determine the concentrations of Cd in each part of the plant. The Cd accumulation amount (BCA), bioconcentration factor (BCF), and translocation factor (TF) were calculated to evaluate the Cd accumulation and transport capacity of maize tissues. The equations were as follows:

$$\text{BCA}\left(\text{g}\cdot\text{hm}^{-2}\right) = C_\text{i} \times B_i \times 10^{-3}$$

$$\text{BCF} = \frac{C_i}{S_i}$$

$$\text{TF}_{\text{a}-\text{b}} = \frac{C_a}{C_b}$$

where C_i represents the Cd concentration in different plant tissues (mg kg^{-1}), B_i represents the dry biomass of the corresponding plant tissue per hectare (kg hm^{-2}), and S_i represents the Cd concentration in soil (mg kg^{-1}). BCA represents the amount of Cd accumulated in each plant tissue per unit area. BCF indicates the ability of plants to accumulate Cd from contaminated soil, and TF represents the Cd translocation efficiency between different plant tissues [34]. For example, the root-to-shoot translocation factor was calculated as

$$\text{TF}_{\text{root}-\text{shoot}} = \frac{C_\text{shoot}}{C_\text{root}}$$

The plants were sampled, and maize soil samples were collected, ground, and passed through a 100-mesh sieve for storage and subsequent analysis. The soil samples were digested using hydrogen fluoride–hydrochloric acid–nitric acid (HF:HCl:HNO_3) (5:1:0.5, $v/v/v$) [35], and the concentrations of available Cd in the soil were extracted by diethylenetriaminepentaacetic acid/triethanolamine/calcium chloride (DTPA/TEA/$CaCl_2$) [36]. Cd speciation in the soil was analyzed by the Tessier sequential extraction method, which partitions Cd into five fractions: exchangeable, residual, carbonate-bound, iron–manganese oxide-bound, and organic matter-bound [37]. Cd concentrations in the digested or extracted samples described above were determined by the atomic absorption spectrophotometer. Soil pH was measured using a Leici PHS-3E pH meter (INESA Scientific Instrument Co., Ltd., Shanghai, China) after the soil was processed at a soil: liquid ratio of 1:2.5.

The soil microbial community structure was analyzed by high-throughput sequencing at Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The purified PCR products were quantified and subjected to paired-end sequencing on an Illumina NextSeq 2000 platform.

Raw sequences were quality-filtered using fastp (version 0.23.4) and merged using FLASH (version 1.2.11), and the high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using USEARCH (version 11); chimeric sequences were removed. Taxonomic classification was performed against the SILVA 138/16S_bacteria database. After quality filtering and merging, a total of 3,545,222 high-quality sequences were obtained from 27 samples, ranging from 58,319 to 154,296 sequences per sample, with an average of 131,305 ± 19,560 sequences per sample (Table S1). To minimize biases associated with differences in sequencing depth, the OTU table was rarefied to 47,359 sequences per sample prior to alpha- and beta-diversity analyses.

Alpha- and beta-diversity analyses were conducted using Mothur (version 1.30.2). Beta diversity was evaluated based on Bray–Curtis distance, and PCoA combined with ANOSIM/Adonis analysis was performed to assess differences in microbial community composition among treatments. Functional profiles were predicted using PICRUSt2 (version 2.2.0). All analyses were conducted using the Majorbio Cloud platform.

The data were processed using Microsoft Excel 2021, and statistical analysis was performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) software. The field plot was considered the experimental unit, and all statistical analyses were conducted based on three independent replicates per treatment ($n = 3$). Data normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. WV, PD, and WV × PD denote wood vinegar concentration, planting density, and their interaction, respectively. ** signifies a highly significant difference ($p < 0.01$), * refers to a significant difference ($p < 0.05$), and NS indicates no significant difference. Values are presented as means ± standard errors (SE) of three independent field replicates ($n = 3$). Detailed F- and *p*-values from two-way ANOVA are provided in the Supplementary Tables. Since straw removal was applied as an accompanying remediation practice rather than an independent experimental factor, the factorial analysis focused on the effects of WV application and planting density among the corresponding treatments.

Pearson correlation analysis was conducted to assess relationships among key variables, and partial least squares structural path modeling (PLS-PM) was performed using the "plspm" package in R (R version 4.5.3). Figures were generated using Origin 2022.

3. Results

3.1. Maize Biomass and Cd Distribution in Plant Organs

Significant main effects of WV treatment and planting density were detected for both maize grain yield and straw biomass. (Figure 1A,B). Compared with the H treatment, grain yield and straw biomass in conventional planting increased by 9.11–16.13% and 4.37–7.94%, respectively. Among the treatments, T2 showed the highest grain yield (6662.92 kg·hm^{-2}) (Figure 1A). In the high-density planting, the ZT3 treatment achieved the highest grain yield (6251.09 kg·hm^{-2}), which was 8.95% higher than that of the H treatment. Meanwhile, straw biomass increased by 36.41–42.31% under high-density planting, with the maximum value observed in ZT2 (6762.08 kg·hm^{-2}) (Figure 1B).

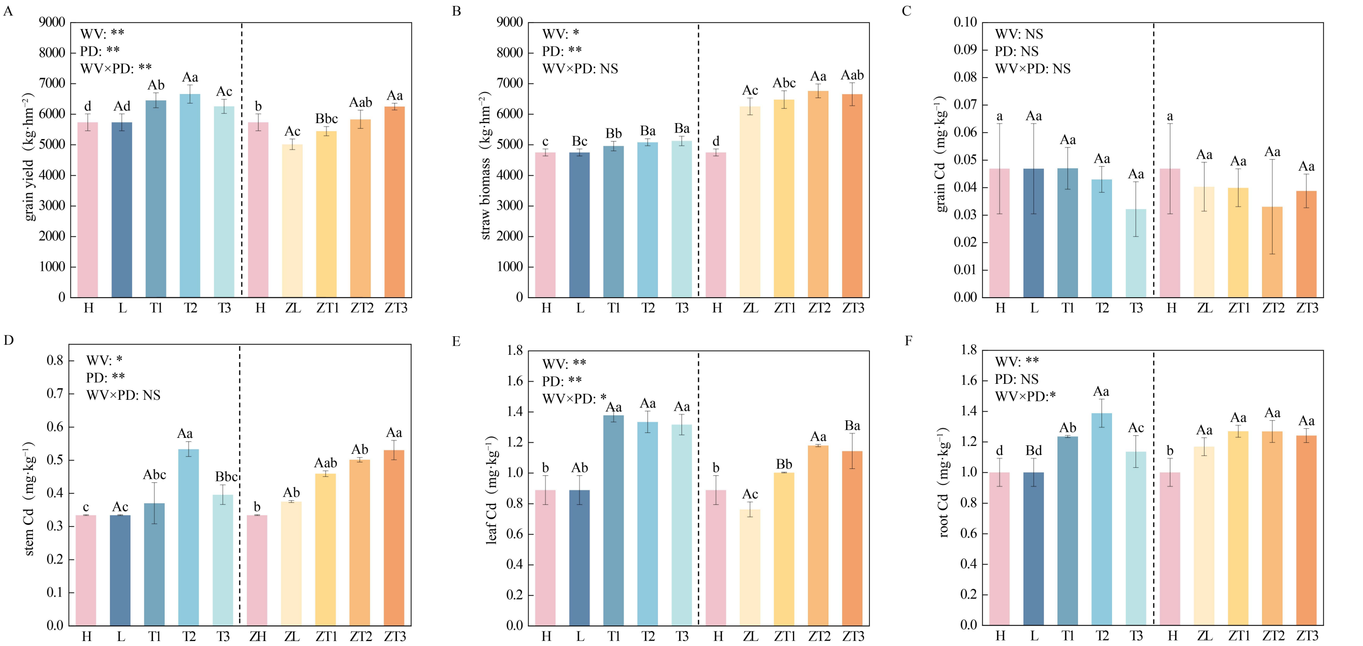

Figure 1. Effects of wood vinegar on maize biomass and cadmium concentrations in different plant parts: (**A**) grain yield; (**B**) straw biomass; (**C**) Cd concentration in grain; (**D**) Cd concentration in stem; (**E**) Cd concentration in leaf; (**F**) Cd concentration in root. Note: Different lowercase letters indicate significant differences among treatments within the same planting density, whereas different uppercase letters indicate significant differences between planting densities under the same WV treatment (Same for other figures). WV, PD, and WV × PD denote wood vinegar concentration, planting density, and their interaction, respectively. ** signifies a highly significant difference ($p < 0.01$), * refers to a significant difference ($p < 0.05$), and NS indicates no significant difference. Values are presented as means ± standard errors (SE) of three independent field replicates ($n = 3$). The same applies below. Detailed F-values and p-values from two-way ANOVA are provided in the Supplementary Tables.

Planting density, WV application, and their interaction affected Cd concentrations in different maize organs (Figure 1). Across all treatments, grain Cd concentrations ranged from 0.032 to 0.047 mg·kg^{-1}, remaining below the maximum limit specified in the Chinese national food safety standard (0.1 mg·kg^{-1}, GB 2762-2025 [38]) (Figure 1C). Under both planting densities, Cd concentrations in stems, leaves, and roots increased relative to the H treatment. Stem Cd concentrations, leaf Cd concentrations, and root Cd concentrations increased by 10.77–59.67%, 12.89–55.02%, and 13.58–38.64%, respectively (Figure 1D–F).

3.2. Cd Accumulation, Translocation, and Enrichment in Different Maize Organs

WV application altered Cd allocation patterns within maize plants by increasing the relative distribution of Cd in aboveground tissues (Figure 2). In the conventional planting conditions, WV treatments decreased the relative Cd allocation proportions in grains and roots by 20.44–47.95% and 7.84–16.66%, respectively, while increasing Cd allocation to leaves by 7.66–19.80%. Additionally, Cd allocation to stems increased from 12.71% to 13.27% under T2 treatment (Figure 2A). In the high-density planting conditions, WV application further regulated Cd partitioning within maize plants. The relative Cd allocation proportions in grains and roots decreased by 51.70–62.44% and 4.98–14.05%, respectively. Meanwhile, the lowest values appeared under ZT2 treatment (0.78% in grains and 37.94% in roots). Conversely, Cd allocation proportions in stems and leaves increased by 11.11–15.99%

and 2.47–12.87%, respectively (Figure 2A). Moreover, straw Cd accumulation increased by 30.52–114.69% under both planting densities, which demonstrated the highest values at 3.158 and 3.943 g·hm^{-2}, respectively, following the application of T2 (Figure 2B). The enhanced Cd accumulation in maize straw, together with increased straw biomass production, suggests an improved capacity for Cd removal through harvestable aboveground biomass. Subsequent straw removal may facilitate the export of crop-accumulated Cd from contaminated agricultural systems and reduce the potential recycling of Cd back into soils.

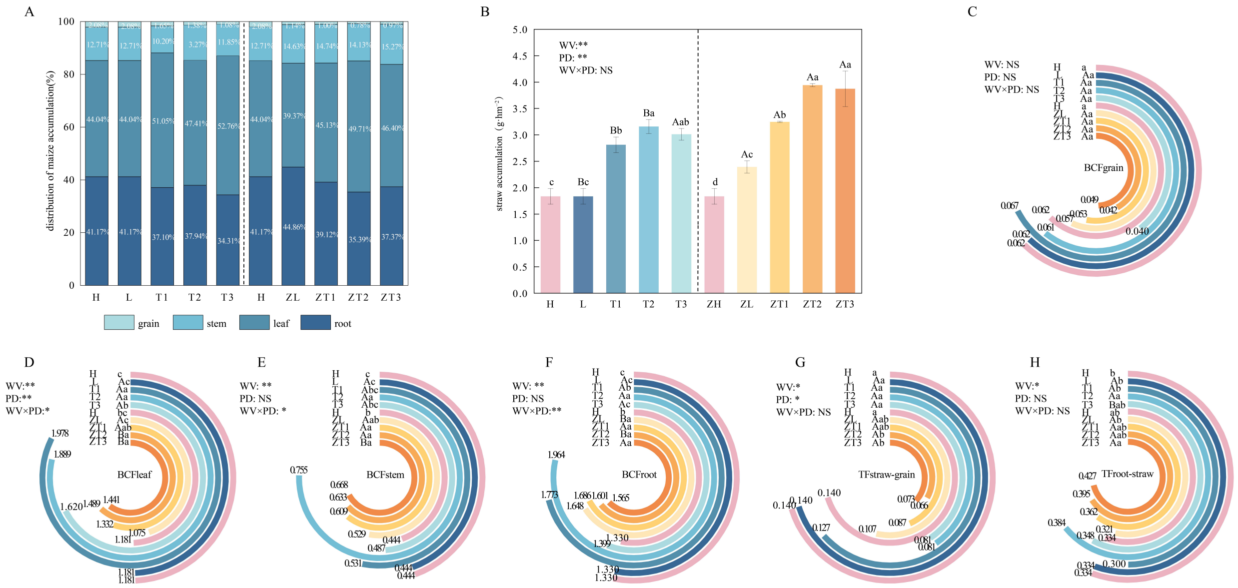

Figure 2. Effects of wood vinegar and high-density group on the accumulation of cadmium and the translocation enrichment coefficient in different parts of maize. The same below. (**A**) distribution of Cd accumulation among different maize organs; (**B**) Cd accumulation in straw; (**C**) BCF of grain; (**D**) BCF of leaf; (**E**) BCF of stem; (**F**) BCF of root; (**G**) TF from straw to grain; (**H**) TF from root to straw. * indicates a significant difference ($p < 0.05$), ** indicates a highly significant difference ($p < 0.01$), and NS indicates no significant difference.

Cd enrichment coefficients reflect the Cd uptake capacity of different maize organs (Figure 2B–E). WV application increased Cd enrichment coefficients in leaves, stems, and roots under both planting density, while altering Cd translocation from roots to aboveground parts and reducing its relative allocation toward grains. The Cd enrichment coefficient in grains showed limited variation, ranging from 0.040 to 0.067. Compared with the H treatment, the Cd enrichment coefficients in leaves, stems, and roots increased by 12.74–67.42%, 9.63–70.03%, and 5.13–47.61%, respectively, across the two planting systems, with the highest values observed in the T1 and ZT2 treatments, respectively. Meanwhile, the translocation factor from straw to grain (TFstraw–grain) decreased by 9.43–54.01%, with the lowest value observed in the ZT2 treatment (0.066). In contrast, the root-to-straw translocation factor (TFroot–straw) reached its maximum under the ZT1 treatment (0.427), representing a 27.96% increase compared with the H treatment (Figure 2G).

3.3. Changes in Soil pH and Cd Speciation

The interaction between the two treatments had no effect on soil pH at maturity (Figure 3A). Exchangeable Cd and organically bound Cd fractions in soil decreased by 12.70–24.56% and 7.12–40.67%, respectively, under both planting density. In contrast, carbonate-bound, iron–manganese oxide-bound Cd, and residual Cd fractions increased by 81.56–118.24%, 31.57–57.15%, and 13.20–127.89%, respectively. To sum up, the combined effect of WV application and maize planting was associated with the transformation of

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Cd from labile fractions to more stable Cd fractions, which was reflected by an increased proportion of residual Cd in soil (Figure 3B).

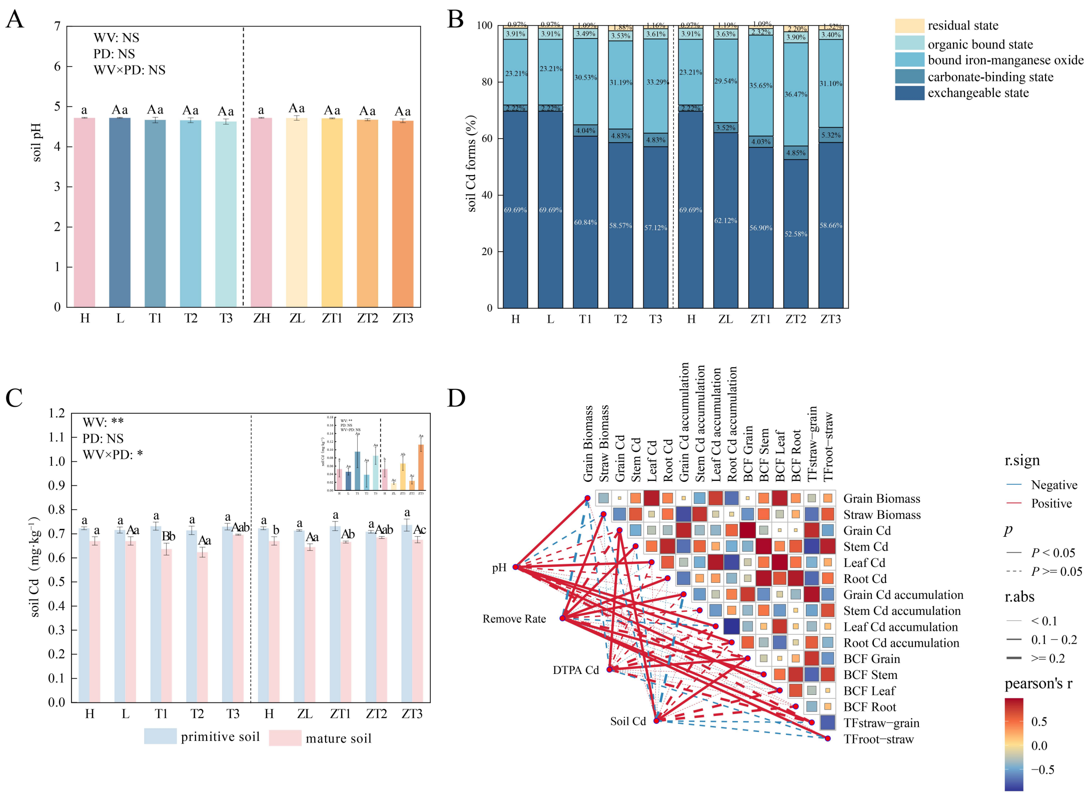

Figure 3. Effects of wood vinegar and high-density group on soil pH, Cd concentrations, and morphology. (**A**) soil pH, (**B**) Cd speciation, (**C**) soil Cd concentrations, (**D**) correlation analysis. * indicates a significant difference ($p < 0.05$), ** indicates a highly significant difference ($p < 0.01$), and NS indicates no significant difference.

3.4. Soil Cd Concentration Changes Before and After Planting

Soil Cd concentrations decreased at maturity. They were significantly influenced by WV application and its interactions with planting density (Figure 3C). Compared with the pre-planting levels, soil Cd concentrations declined by 0.058–0.083 mg·kg^{-1} and 0.065–0.118 mg·kg^{-1} under the two planting densities, respectively. Nonetheless, a proportion of Cd remained retained in maize roots, suggesting that harvesting aboveground biomass alone may not be sufficient for substantial Cd removal from soils. The Cd retained in roots could potentially be released back into the soil during root residue decomposition.

3.5. Correlation Analysis

Correlation analysis revealed that soil pH was negatively correlated with stem Cd concentration, leaf Cd concentration, leaf Cd accumulation, the translocation coefficient, and soil Cd removal efficiency. In contrast, soil Cd concentration was positively correlated with Cd accumulation in stems and leaves as well as soil Cd removal efficiency. Available Cd exhibited positive correlations with Cd concentrations in stems and leaves, as well as leaf Cd accumulation. Additionally, soil Cd removal efficiency was positively correlated with Cd concentrations in stems and roots, stem-leaf Cd accumulation, and the translocation coefficient (Figure 3D). Overall, lower soil pH was associated with greater Cd availability, while higher total and available Cd levels were associated with higher Cd uptake and accumulation in plants. Moreover, Cd removal efficiency showed positive correlations with aboveground Cd accumulation processes.

3.6. Biomass and Cd Concentrations in Different Parts of Wheat Plants at Maturity

In the conventional planting conditions, the straw biomass increased by 4.90–16.56% under the WV treatments and decreased by 14.42% under the LT treatment. The grain yield under the T1 treatment increased by 22.64%, reaching the highest value of 8069.90 kg·ha^{-1}. In the high-density planting conditions, the straw biomass of treatments increased by 6.35–16.51%; the grain yield under the ZT3 treatment increased by 11.15%, reaching the highest value of 7313.92 kg·ha^{-1} (Figure 4A,B).

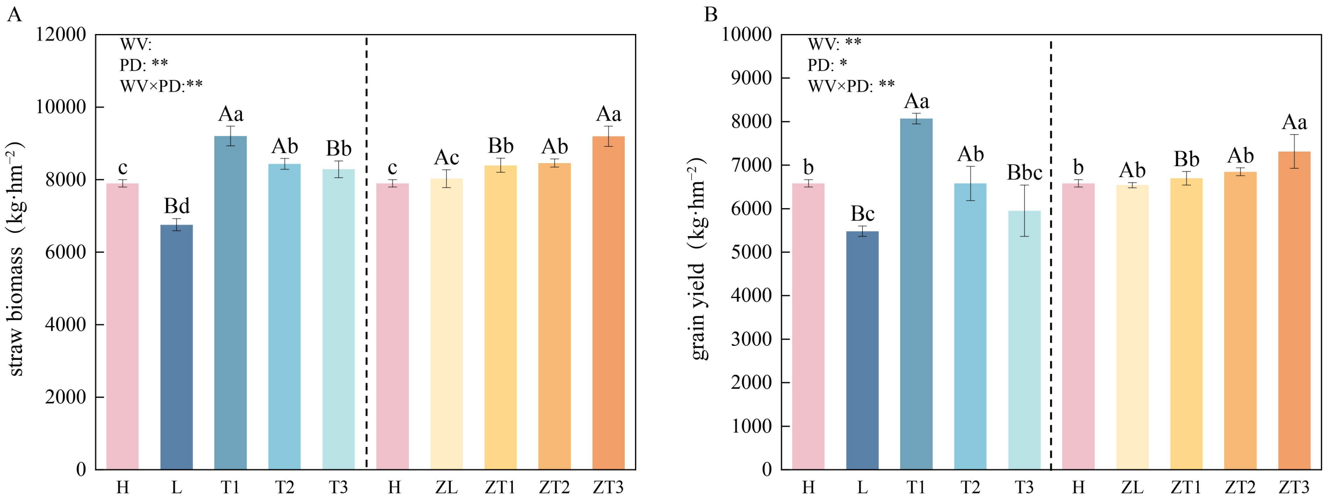

Figure 4. Grain yield and straw biomass of the different treatments during the wheat season. (**A**) straw biomass, (**B**) grain yield. * indicates a significant difference ($p < 0.05$), ** indicates a highly significant difference ($p < 0.01$).

Compared with the H treatment, Cd concentrations in wheat grains, straw, and roots decreased by 8.73–12.83%, 29.53–45.63%, and 13.62–24.26%, respectively, under conventional planting conditions. The corresponding reductions under high-density planting conditions were 16.51–25.59%, 29.77–38.92%, and 24.19–44.78%, respectively. The lowest grain Cd concentration was observed in the ZT3 treatment (0.243 mg·kg^{-1}), which was 0.143 mg·kg^{-1} lower than that in the H treatment. Although the ZT3 treatment reduced Cd accumulation in wheat grain compared with the conventional management practice, the grain Cd concentration (0.243 mg kg^{-1}) remained above the maximum permissible level of 0.1 mg kg^{-1} specified in the Chinese National Food Safety Standard (GB 2762-2025). Therefore, the reduction in grain Cd under ZT3 indicates the potential of the integrated management strategy to mitigate Cd transfer to wheat grain, but it was not sufficient to ensure compliance with the food safety standard. Thus, WV application and planting density optimization during the maize season increased Cd accumulation in maize and were associated with reduced Cd accumulation in subsequent wheat tissues under straw removal conditions (Figure 5).

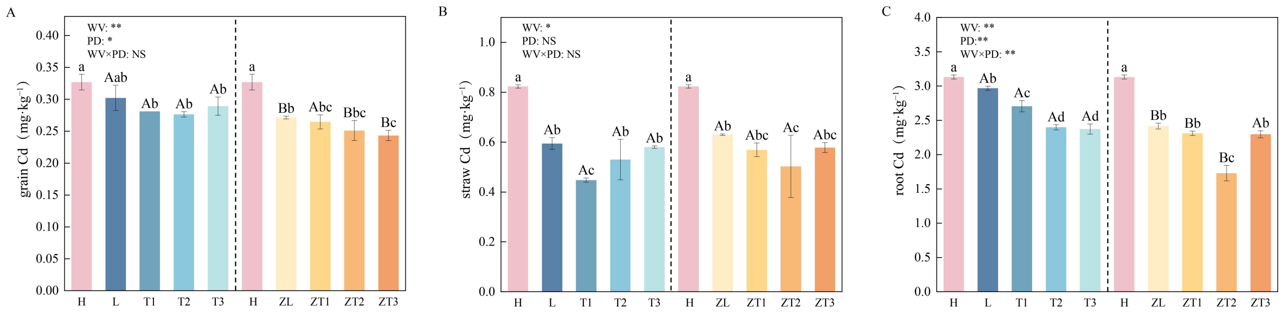

Figure 5. Cadmium (Cd) concentrations in the different parts of wheat under varying treatments. (**A**) grain Cd concentration; (**B**) straw Cd concentration; (**C**) root Cd concentration. * indicates a significant difference ($p < 0.05$), ** indicates a highly significant difference ($p < 0.01$), and NS indicates no significant difference.

3.7. Soil Microorganisms

The sequencing coverage reached 98.87%, indicating sufficient sequencing depth for microbial community analysis (Table 2). Overall, WV application was associated with increased microbial richness and diversity (Chao, Shannon, ACE, and Heip indices) under both planting densities, while the H treatments generally demonstrated lower values. The Simpson index ranged from 0.0061 to 0.0126, indicating differences in community evenness among treatments (Table 2).

Table 2. Effects of different treatments on the alpha-diversity index of the soil microorganisms.

Treatment	Chao	Shannon	ACE	Coverage	Simpson	Heip
H	3432.88 ± 110.5 ab	6.259 ± 0.037 a	3848.18 ± 370.15 a	0.98 ± 0.00 de	0.0061 ± 0.0001 d	0.1761 ± 0.0041 bc
L	3229.10 ± 234.56 bc	6.062 ± 0.056 bc	3304.69 ± 133.41 bc	0.98 ± 0.00 e	0.0068 ± 0.0021 cd	0.1844 ± 0.0051 b
T1	3100.30 ± 97.83 cd	5.976 ± 0.045 bc	2959.99 ± 85.44 d	0.98 ± 0.00 cd	0.0076 ± 0.0007 cd	0.1740 ± 0.0011 c
T2	3351.99 ± 54.99 ab	6.084 ± 0.046 b	3583.38 ± 248.10 ab	0.98 ± 0.00 de	0.0071 ± 0.0004 cd	0.1744 ± 0.0046 c
T3	3473.58 ± 60.09 a	6.301 ± 0.043 a	3554.45 ± 96.44 ab	0.98 ± 0.00 cd	0.0062 ± 0.0010 d	0.1981 ± 0.0106 a
ZL	2900.44 ± 63.89 d	5.650 ± 0.110 f	3138.99 ± 115.98 cd	0.98 ± 0.00 bc	0.0085 ± 0.0015 bcd	0.1431 ± 0.0065 e
ZT1	2593.97 ± 25.07 e	5.825 ± 0.100 e	2858.83 ± 210.10 d	0.99 ± 0.00 ab	0.0104 ± 0.0026 ab	0.1737 ± 0.0063 c
ZT2	3379.93 ± 0.00 ab	5.954 ± 0.027 cd	3304.51 ± 94.79 bc	0.98 ± 0.00 cde	0.0087 ± 0.0001 bc	0.1623 ± 0.0023 d
ZT3	3020.18 ± 197.44 cd	5.846 ± 0.115 de	2288.45 ± 71.49 e	0.99 ± 0.00 a	0.0126 ± 0.0011 a	0.1635 ± 0.0035 d

Note: Different letters in the table denote significant differences between treatments ($p < 0.05$).

OTU analysis identified a total of 1064 OTUs across all treatments, and the H (straw return) treatment exhibited the largest number of unique OTUs (990) (Figure 6A). Compared with the H treatment, OTU richness was lower by 8.14–23.25% in the conventional planting conditions and by 30.42–52.37% in the high-density planting conditions.

PCoA analysis revealed that PC1 and PC2 explained 30.08% and 23.96% of the total variation, respectively, collectively accounting for 54.04% of the variation. Although partial overlaps remained, distinct separation patterns were observed among different treatments under both planting densities. In other words, planting density, remediation practices, and straw management were associated with differences in soil microbial community structure (Figure 6B).

The dominant bacterial phyla included Actinobacteria, Acidobacteria, Proteobacteria, Chloroflexi, and Patescibacteria, with a maximum combined relative abundance of 84.01%. Variations in phylum-level abundance were observed among treatments, with Actinobacteria increased by 27.85% under the T3 treatment, Acidobacteria increased by 11.29% under the ZT2 treatment, and the largest decrease being observed under the ZT3 treatment (31.80%) (Figure 6C).

Further analysis of community composition reflected differences in the dominant genera across treatments, with *Acidobacteriales*, *Occallatibacter*, *Acidothermus*, *WPS-2*, *Candidatus_Solibacte*, and *Sphingomonas* demonstrating distinct distribution patterns among treatments (Figure 7A). Correlation analysis suggested that *Mycobacterium*, *WPS-2*, *Bryobacter*, *Conexibacte*, and *Acetobacteraceae* were negatively correlated with soil pH, while *Acidobacteriales* and *Bacillus* were positively correlated with pH. *Burkholderia-Caballeronia-Paraburkholderia*, *Mycobacterium*, and *Saccharimonadales* were positively correlated with soil available Cd, while *Bradyrhizobium* exhibited a negative correlation. *Mycobacterium* was negatively correlated with soil Cd concentrations, while *Subgroup_2*, *Elsterales*, *Solirubrobacteraceae*, *Acidibacter*, and *Acidimicrobiia*-related taxa presented negative correlations with soil Cd concentrations (Figure 7C).

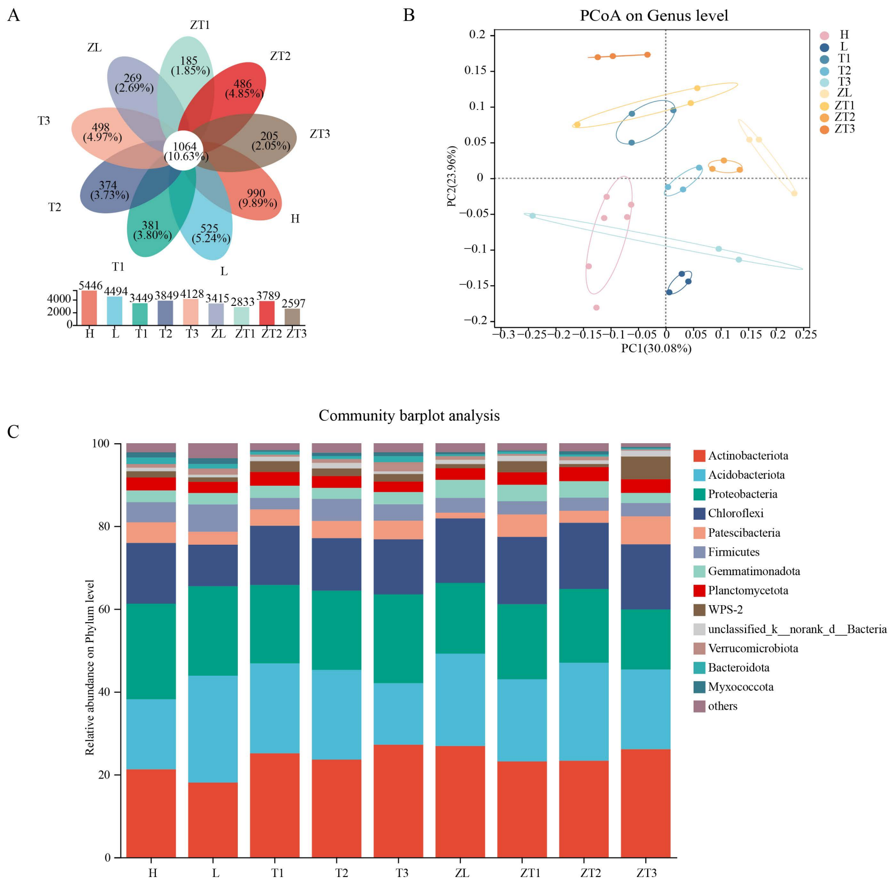

Figure 6. Effects of wood vinegar application and planting density on wheat-season soil bacterial community structure. (**A**): Venn diagram analysis; (**B**): PcoA; (**C**): community composition at the phylum level.

The H treatment generally enhanced the functional potential of soil microbial communities involved in energy metabolism, substance transport, and genetic information processing. The conventional planting density primarily contributed to the reinforced functions related to amino acid transport and metabolism (10.82–14.23%) and transcription (8.14–11.13%). In the high-density planting conditions, functions associated with carbohydrate transport and metabolism, transcription, and cellular structure were further enhanced by 9.54–9.86%, 7.74–10.70%, and 11.02–12.87%, respectively (Figure 7B).

PLSPM analysis further elucidated the pathways associated with Cd accumulation in maize straw, soil properties, wheat-season microbial factors, and wheat grain Cd concentrations under different planting densities. Straw Cd accumulation, soil pH, soil Cd concentrations, and microbial abundance accounted for 85.9%, 35.6%, 36.6%, and 58.6% of the variation in wheat grain Cd concentrations, respectively (Figure 8A). WV showed a direct negative association with wheat grain Cd concentrations, as well as indirect effects through soil pH and soil Cd. Straw Cd accumulation and microbial abundance were directly associated with wheat grain Cd, while planting density affected grain Cd mainly indirectly through straw Cd accumulation. Standardized total effects suggested that straw Cd accumulation showed the strongest negative association with wheat grain Cd concentrations Figure 8B).

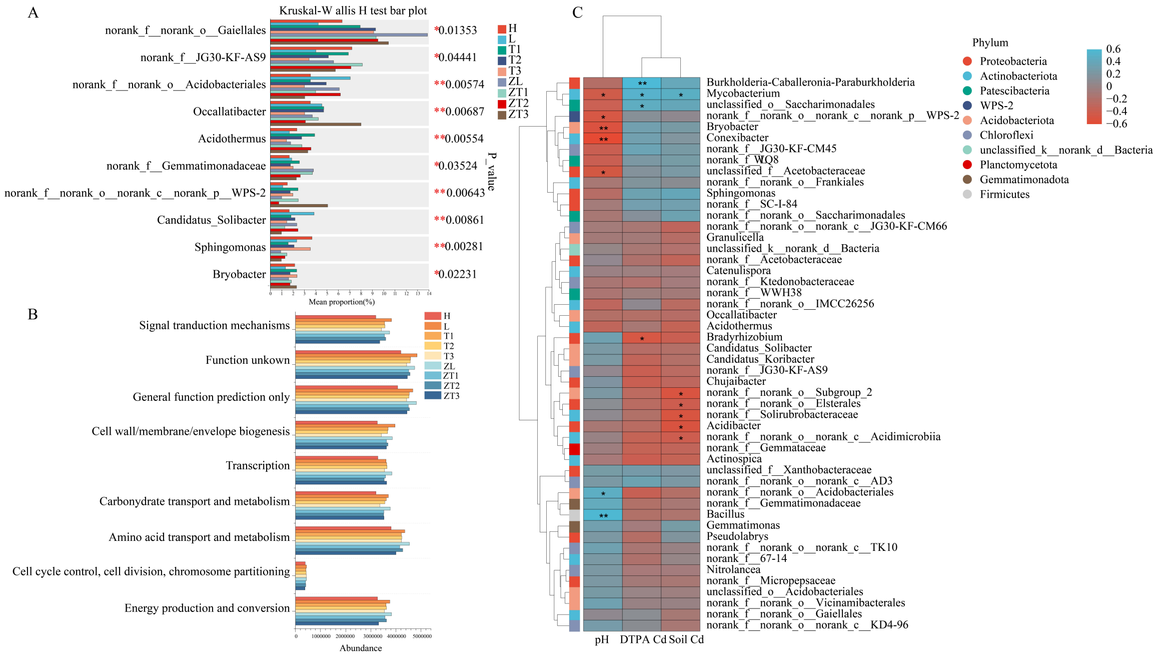

Figure 7. Effects of wood vinegar application and planting density on wheat-season soil bacterial communities. (**A**): differences between groups at the genus level; (**B**): functional prediction analysis; (**C**): correlation analysis between environmental factors and community composition. * indicates a significant difference ($p < 0.05$), ** indicates a highly significant difference ($p < 0.01$).

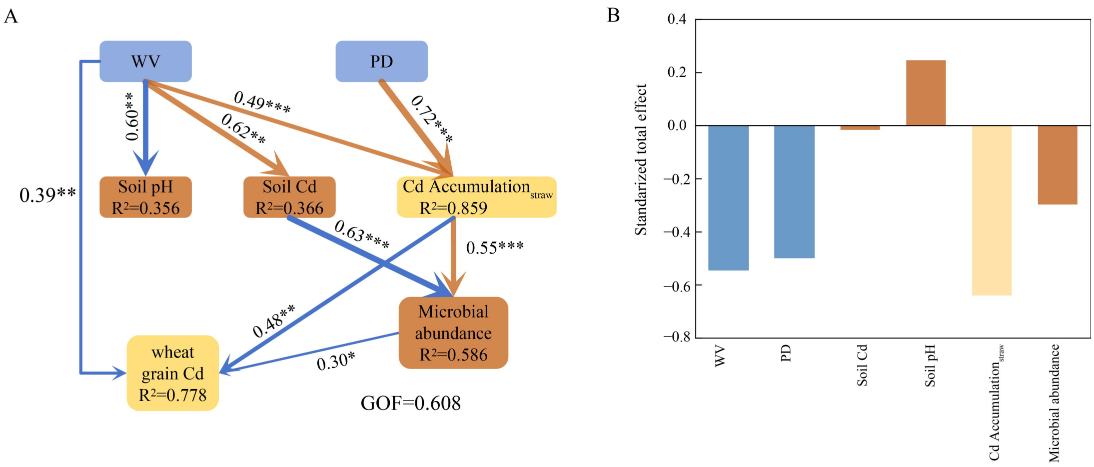

Figure 8. Partial least squares path model of wood vinegar concentration, planting density, soil pH, Cd concentrations, and soil bacterial community. (**A**) Structural equation model showing the relationships among wood vinegar (WV), planting density (PD), soil properties, Cd accumulation in straw, microbial abundance, and grain Cd concentration; (**B**) standardized total effects of the variables included in the structural equation model. R^2 indicates the proportion of variance explained by endogenous variables. * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$. The width of each arrow and the attached values signify standardized path coefficients, indicating the relative strength of the relationships. Orange lines and blue lines indicate positive coefficients and negative coefficients, respectively.

4. Discussion

Higher maize grain and straw biomass and greater Cd concentrations in stems and leaves were observed under WV application and high-density planting treatments than under the control. Greater root-to-shoot Cd translocation, particularly to stems and leaves, was observed under these treatments, together with increased total Cd uptake by maize. WV-derived organic acids may interact with soil Cd and potentially facilitate Cd mobilization by forming Cd-organic complexes, as suggested by previous studies [39–41]. Such processes may reduce Cd toxicity and potentially contribute to enhanced Cd uptake by maize. WV application may alleviate Cd-induced physiological stress in maize through multiple pathways, as suggested by previous studies. First, WV-derived low-molecular-weight organic acids may help stabilize cellular processes and alleviate Cd-related stress on mineral uptake, water use, oxidative metabolism, and photosynthesis [42,43]. Second, Previous studies have suggested that WV application may enhance antioxidant defense systems, regulate pigment-related metabolism, and improve photosynthetic performance, which could partially explain the increased maize biomass observed in this study [28,30,44]. Third, WV application may improve soil nutrient availability, potentially by promoting nutrient release, increasing soil fertility, and enhancing urease and sucrase activities, potentially providing a more favorable nutritional environment for maize growth under Cd stress [45,46].

High-density planting was associated with greater straw biomass, which increased the amount of aboveground tissue available for Cd accumulation. Previous studies have suggested that improvements in canopy photosynthesis and resource-use efficiency under appropriate planting densities may contribute to such biomass responses [47,48]. However, because straw management differed between the treatment plots and the control, the magnitude of this response cannot be attributed exclusively to planting density. The combined application of high-density planting and WV was associated with changes in Cd distribution among maize tissues. Specifically, it increased the proportion of Cd allocated to stems and leaves while reducing that in grains and roots, which may have contributed to greater Cd accumulation in aboveground vegetative tissues. Overall, these changes may have enhanced the Cd phytoextraction potential of maize and reduced Cd accumulation in maize grains. In this study, the grain biomass of the ZL treatment did not increase significantly, which may have been related to insufficient N supply. This pattern may indicate the importance of nutrient availability and the supply of nutrients to the growth of maize and its enrichment in Cd. The ability of WV to enhance the concentrations of soil nutrients may partially compensate for this limitation and support plant growth and Cd uptake [49,50].

WV application at different concentrations did not reduce soil pH in farmland soil because the pH of WV was 4.18, and the pH value after dilution was close to the pH value of the soil. Additionally, only a small amount of WV was applied, and the soil was effective at buffering. Hence, no significant changes appeared in the acidity and alkalinity of the soil.

This study suggested that maize absorbed bioavailable Cd from soil through roots and translocated it to aboveground tissues for accumulation, which was associated with reductions in soil Cd concentrations and Cd bioavailability. High-density planting significantly elevated the total biomass of maize straw and its aboveground biomass. The greater biomass may have increased the aboveground capacity for Cd accumulation and may have contributed to greater Cd uptake and the observed decline in soil Cd concentrations. The observed changes in soil Cd fractions likely reflected the combined influences of plant Cd uptake, WV application, planting density, and differences in straw management. WV-derived low-molecular-weight organic acids may contribute to Cd complexation and transformation processes in soil, reducing the interaction between free Cd ions and soil organic matter [51]. Consistent with this possibility, the organic-bound Cd fraction was significantly reduced [52,53]. These organic compounds may influence Cd redistribution among soil fractions, potentially affecting interactions with carbonate minerals and Fe-Mn oxides [54]. In other words, the complexed Cd and free Cd ions may be adsorbed and stabilized by iron and manganese oxides and carbonate minerals in the soil [52,55]. The observed increase in Cd availability and subsequent shift toward more stable Cd fractions may represent different stages of Cd transformation under the integrated management treatments, rather than contradictory processes. Under the integrated management treatments, soil Cd concentrations decreased, and Cd distribution shifted toward relatively more stable fractions.

Cd concentration in grains, which are the edible portion of wheat, is directly associated with the quality and safety of agricultural products, as well as human health. This study revealed a significant reduction in wheat grain Cd concentrations in the remediation group. This reduction may reflect the cumulative effects of the integrated management practices implemented during the preceding maize season, including WV application, planting density, maize Cd uptake, and straw removal. Maize-season early remediation significantly lowered totalsoil and bioavailable Cd concentrations may have reduced Cd availability in the subsequent wheat growth cycle. Simultaneously, the decrease in exchangeable and organic-bound concentrations of Cd in the soil may have contributed to reduced Cd availability for wheat uptake. The increase in the concentrations of stable Cd, such as carbonate-bound and iron–manganese oxide-bound Cd, may have further limited Cd bioavailability. These changes were accompanied by lower Cd accumulation in wheat plants and grains [56]. The lower soil Cd availability observed under the integrated treatments may have contributed to reduced Cd accumulation in wheat grains. However, because straw management differed between the experimental treatments and the control, the respective contributions of WV, planting density, and straw removal to this carryover effect cannot be fully distinguished.

The abundance and distribution of soil microorganisms can directly reflect variation in the characteristics of microbial activity and soil environmental quality [57]. During the wheat-growing season, the Chao, Shannon, and ACE indices of soil microbial communities decreased significantly following straw removal under the treatments. In contrast, straw incorporation may reinforce microbial community diversity by providing sufficient carbon (C) sources for microbial growth and reproduction. Nonetheless, straw removal may alleviate microbial inhibition by limiting oxygen depletion and the formation of toxic substances during straw decomposition, while lessening excessive carbon input into the soil [58–60]. The preceding WV application may also have contributed to the differences in bacterial community composition observed during the wheat season [61,62]. However, because straw management also differed between the experimental treatments and the control, the residual effects of WV cannot be fully separated from those associated with straw removal or incorporation. The low-molecular-weight organic acids contained in the WV, which served as sources of C and energy for microbial growth, were associated with changes in dominant bacterial taxa, including Actinobacteria and Acidobacteria, and inhibited functionally redundant microbial species [63,64]. Moreover, changes in the soil microenvironment may alter the predicted functional potential of microbial communities involved in nutrient metabolism and signal transduction following WV application, and may have been associated with increases in the predicted functional potential related to amino acid transport and metabolism [64].

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5. Conclusions

The combined application of WV and high-density planting during the maize season was associated with increased aboveground biomass and greater Cd accumulation in maize stems and leaves. The ZT3 treatment was associated with a significant reduction in soil total Cd concentrations and increased Cd accumulation in maize aboveground tissues. This strategy was associated with changes in soil Cd speciation and was associated with reduced available Cd fractions while maintaining relatively low Cd concentrations in maize grains. In the subsequent wheat season, lower Cd concentrations were observed in wheat tissues under the straw-removal treatments without significantly affecting biomass production. Meanwhile, the preceding management practices were associated with changes in soil microbial community structure and predicted microbial functional profiles. Overall, the combined application of WV and high-density planting showed the potential to enhance Cd phytoextraction by maize. In the subsequent wheat season, lower Cd concentrations were observed under the treatment regimes that also included maize straw removal. However, because maize straw was removed from the experimental treatment plots but incorporated into the soil in the control after maize harvest, the carryover effects of WV application and planting density on the subsequent wheat season could not be fully separated from the effects associated with the different straw management practices.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy16181851/s1, Table S1: Sequencing statistics for individual samples; Table S2: ANOVA results for maize growth and Cd concentration; Table S3: ANOVA results for maize Cd accumulation; Table S4: ANOVA results for maize soil pH, Cd concentrations, and Cd reduction in soil; Table S5: ANOVA results for wheat growth; Table S6: ANOVA results for wheat Cd concentration.

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Data Availability Statement: The raw microbiome sequencing data generated in this study have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center (NGDC), China National Center for Bioinformation (CNCB), under accession number CRA048419. The other experimental data supporting the conclusions of this article will be made available by the authors upon request.

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