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Combination of intercropping maize and soybean with root exudate additions reduces metal mobility in soil-plant system under wastewater irrigation

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ABSTRACT

The effects of root exudates and irrigation with treated wastewater on heavy metal mobility and soil bacterial composition under intercropping remain poorly understood. We conducted a pot experiment with maize and soybean grown in monocultures or intercultures, irrigated with either groundwater or treated wastewater. In addition, the pre-collected root exudates from hydroponic culture with mono- or inter-cropped maize and soybean were applied to the soil at four levels (0 %, 16 %, 32 % and 64 %). The results showed that application of root exudates increased plant growth and soil nutrient content. The analysis of "Technique for Order of Preference by Similarity to Ideal Solution" for higher plant biomass and lower soil Cd and Pb concentrations indicated that the best performance of soybean under treated wastewater irrigation was recorded under intercropping applied with 64 % of exudates, with a performance score of 0.926 and 0.953 for Cd and Pb, respectively. The second-best performance of maize under treated wastewater irrigation was also observed under intercropping applied with 64 % of exudates. Root exudate application reduced heavy metals migration in the soil-plant system, with a greater impact in intercropping than in monocropping. In addition, certain soil microorganisms were also increased with root exudate application, regardless of irrigation water. This study suggests that appropriate application of root exudates could potentially improve plant growth and soil health, and reduce toxic heavy metal concentrations in soils and plants irrigated with treated wastewater.

1. Introduction

Food security is currently at great risk with a projected increase of water scarcity of 40 % by 2030 (WRG, Water Resources Group, 2030, 2030, 2009) as 69 % of the world's water consumption (AQUASTAT, 2010) is used in the agricultural sector (Kama et al., 2023b). Moreover, the continuous population growth is increasing food demand worldwide. As a result, there is a critical need to enhance agricultural water use efficiency and explore alternative resources of water for irrigation. Approximately 380 km^3 of wastewater are produced globally each year, (Natasha et al., 2020), which is equivalent to 15 % of all agricultural water use (Kama et al., 2023b). As urbanization advances, the amount of wastewater will continue to increase. Consequently, treated wastewater (WW) has been proposed as a feasible source of irrigation water (Jaramillo and Restrepo, 2017; Perezvargas y Castor et al., 2023). However, wastewater irrigation in water-limited areas has been identified as contributing to the accumulation of heavy metals in agricultural soils (Kama et al., 2023a; Lyu et al., 2015; Natasha et al., 2020). Long-term heavy metal contamination has been, and remains, a significant agricultural issue (Shah et al., 2022; Zhao et al., 2022). A comprehensive understanding of heavy metal mobility under different cropping practices (e.g., monocropping, intercropping) and wastewater irrigation would provide a guidance for the safe irrigation with WW.

Intercropping is a widely recognized and commonly used method to utilize arable land and other resources efficiently (Baldé et al., 2020). Improving soil health and agricultural production through intercropping has received significant attention over the past two decades (Mousavi and Eskandari, 2011). Intercropping enhances resource-use efficiency

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and restores the ecological and environmental benefits of cultivated soil (Fu et al., 2019; Ma et al., 2022). However, heavy metal mobility in intercropping systems under WW irrigation has not been well examined yet. It is crucial to evaluate the impact of intercropping on the migration of heavy metals under WW irrigation. This assessment will provide safe and effective solutions for the use of WW in agricultural systems.

In an intercropping system, root interactions between different crop species modulate a variety of biotic and abiotic processes, altering soil biophysicochemical properties (Kumawat et al., 2022). Such processes include nutrient acquisition, growth inhibition of neighboring crops, and attraction and repulsion of specific microbial species (Frank and Groffman, 2009). Root exudates contain a wide variety of substances released from different parts of the root system, including low molecular weight organic compounds, macromolecular mucilage, root cell exfoliations and their degradation products, as well as gases, protons and nutrient ions (Vives-Peris et al., 2020; Wen et al., 2022). They play important roles in rhizosphere function and thus in plant-microbe-soil interactions (Song et al., 2022; Wang et al., 2022). Exudates are also an important source of soil organic carbon and can account for up to 11 % of total photosynthetic production (Wen et al., 2022). Root exudates are therefore an important energy source for soil microbes (Bais et al., 2006; Liu et al., 2022), activating microorganisms to release nutrients for plants (Liu et al., 2022).

In addition, it has previously been reported that changes in the composition of root exudates under intercropping can alter the fraction and distribution of heavy metals in soil-plant systems (Wen et al., 2022). Some studies have also reported that root exudation acts as a protection mechanism as it may promote the formation of organo-metal complexes and thus alleviate heavy metal phytotoxicity (Yu et al., 2022; Zhao et al., 2022). Furthermore, organic carbon has been shown to have significant effects on heavy metals mobility (Wang, 2008; Zia-ur-Rehman et al., 2023). However, the amount and composition of root exudates can also be influenced by plants and heavy metals in the soil (Rehman et al., 2020; Zhang et al., 2023).

The altered bioavailability of heavy metals due to root exudates is one mechanism underlying heavy metal accumulation in plant organs (Zhao et al., 2022). However, the effects of WW irrigation and root exudate application on rhizosphere properties and heavy metal mobility in intercropping systems have not been investigated extensively. We hypothesized that application of root exudates could reduce heavy metal mobility in the soil-plant system. To test this hypothesis, a pot experiment was conducted to investigate the effects of root exudate application on crop growth, heavy metal migration and soil bacterial composition under maize/soybean intercropping with WW irrigation. Our findings will provide potential solutions for the safe use of WW for crop irrigation.

2. Materials and methods

2.1. Root exudate collection

Maize (*Zea mays* cv. Zhengdan958) and soybean (*Glycine max* cv. Zhonghuang13) seeds were obtained from Henan Tianzhong Seed Co., Ltd and Jiaxiang Autumn Harvest Seed Industry Co., Ltd, respectively. Maize and soybean were chosen due to their ability to complement each other's yield and improve soil health, as detailed in the work of Fu et al. (2019). Studies suggest that maize is the better intercropping partner for soybean in comparison to other cereals (Chen et al., 2017a; Du et al., 2018). The seeds were sterilized and germinated in sterile Hoagland's nutrient solutions. Twenty-four germinated seedlings were transferred aseptically to individual plastic boxes, each containing 9 L of sterile Hoagland's nutrient solution, in a growth chamber (7000 Lx, 16 h/8 h) at 28 °C. After one month, the plants in one of the boxes were transferred to containers filled with 3 L of deionized water for 24 h to collect root exudates (Zhu et al., 2009). Subsequently, the solution containing root exudates was then filtered through a 0.45-µm filter membrane to eliminate root debris and analyzed for the concentration of total organic carbon (TOC) using a Shimadzu TOC-5050A TOC Analyzer (Zhu et al., 2009). The concentrations of TOC in the solutions collected from mono-maize and mono-soybean were respectively 13.14 mg L^{-1} and 16.06 mg L^{-1}, and 32.42 mg L^{-1} under intercropping. The collected exudates were stored at −80 °C until they were used for the pot experiments.

2.2. Soil cultivation experiments

Pot experiments were conducted between July and September 2022 in the greenhouse of Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences (CAAS) in Xinxiang (35.27°N, 113.93°E), Henan Province, China. Soil properties were as follows: pH, 8.54; electrical conductivity (*EC*), 1.330 mS cm^{-1}; organic matter (*OM*), 1.55 %; concentrations of total Cd, Pb, Zn and Cu, 0.22, 16.08, 61.35 and 24.13 mg kg^{-1}, respectively. The average temperature in the greenhouse during cultivation was 29.58 °C and the relative humidity was 41.36 %.

The maize and soybean seeds used were the same as in the preceding section. Plants were irrigated using either treated livestock wastewater from pig farms or groundwater. Here, the livestock wastewater (the biogas slurry) and the groundwater was collected as mentioned in our previous paper (Kama et al., 2023a). The chemical properties of the groundwater and livestock wastewater are presented in the supplementary material (Table S1) (Kama et al., 2023a). Soil was sieved (2 mm), mixed thoroughly, and used to fill pots. A total of seventy-two pots were each filled with 8 kg of soil that was mixed thoroughly with 10 g of compound fertilizer (N-P_2O_5-K_2O 15:15:15, nutrient ≥45 %). In monocropping systems, either four maize seeds or eight soybean seeds were sown directly into the pot. For intercropping systems, three maize and six soybean seeds were sown in each pot. Two weeks after sowing, the seedlings was thinned to one per pot in the monocrop or intercrop system for maize, and to two for soybean. The seedlings received irrigation every 3–4 days, ensuring that the soil moisture level was maintained between 60 % and 70 % of the water holding capacity using the corresponding waters. Four levels of root exudates (0 %, 16 %, 32 % and 64 %) were applied to the soil of the same planting pattern. As an example, monocultured maize root exudates were applied to the monocultured maize. To prepare the above-stated levels of root exudates, 0, 16, 32 and 64 mL of each (referred as "0", "1", "2", and "3" in the results section) were diluted with deionized water to a final volume of 100 mL. Exudate solutions were applied two times a week immediately after irrigating.

2.3. Sample gathering and analysis

2.3.1. Soils

Soils attached directly to the roots were collected from each pot at a depth of 0–20 cm, and then divided into two sub-samples. The first was used for chemical analysis. Soil pH and *EC* (soil: water at 1:2.5) were determined by a pH meter and a conductivity meter, respectively. Other chemical properties, such as *OM*, total nitrogen (*TN*), total phosphorous (*TP*), water-soluble K^+ and Na^+ were analyzed using standard methods (Liu et al., 2019). The second sub-sample was sent to Shanghai Personal Biotechnology Co., Ltd. in China for bacterial composition analysis (Dai et al., 2023; Guo et al., 2021).

2.3.2. Plant harvest

Plants were collected after eight weeks of growth; plant height (*PH*), stem diameter (*STD*), leaf chlorophyll content (*Lchl*), leaf nitrogen level (*LN*), and leaf area (*LA*) were determined prior to harvest. Harvested plant materials were divided into roots and shoots, and then dried in an oven at 50 °C for more than 72 h. The dried shoots were then ground and homogenized for chemical analysis.

2.4. Determination of heavy metal content in soil and plants

2.4.1. Determination of heavy metal concentrations in soil

Briefly, 300 mg of air-dried soil sieved through 0.15 mm was supplied with 9 mL of concentrated nitric acid and 3 mL of concentrated hydrochloric acid. After microwave digestion (Mars CEM 240 / 50), the mixture was shifted to a volumetric bottle and made up to a final volume of 50 mL using deionized water. The total contents of Zn, Cu, Cd and Pb in the solution were determined by atomic absorption spectroscopy (PE900H).

2.4.2. Determination of heavy metal concentrations in plants

The concentrations of heavy metals in plants were determined using standard methods (Franco-Hernández et al., 2010). Approximately 200 mg of plant samples were mixed with 10 mL HNO_3 was and digested in the microwave (Mars CEM 240 / 50). The mixture transfer and heavy metals determination were the same as above.

2.5. Enrichment and translocation factors

An enrichment factor (*EF*) was calculated for Zn, Cu, Pb and Cd to establish heavy metal accumulation in the soil with wastewater irrigation (Cao et al., 2019; Chen et al., 2021).

$$EF = \frac{\text{Concentration of heavy metal in wastewater} - \text{irrigated soil}}{\text{Concentration of heavy metal in groundwater} - \text{irrigated soil}} \quad (1)$$

A translocation factor (*TF*) was also used to determine relative translocation of metals from soil to plant shoots (Branquinho et al., 2007; Galal and Shehata, 2015; Kama et al., 2023a).

$$TF = \frac{\text{Concentration of metal in plant shoots}}{\text{Concentration of metal in soil}} \quad (2)$$

2.6. Determination of optimum root exudates application rates and planting patterns in wastewater-irrigated soil using TOPSIS

The "Technique for Order of Preference by Similarity to Ideal Solution" (TOPSIS) (Lai and Hwang, 1994) was used to assess the optimal root exudates application rates and planting patterns which balanced the negative effects of Pb and Cd accumulated in the soil and the positive benefits of biomass in wastewater-irrigated soil. Pb and Cd were selected based on the high toxicity response coefficients of Pb (5) and Cd (30); and Cu and Zn are plant beneficial transition metals in a certain concentration range (Al-Swadi et al., 2022). The steps are as follows:

1. Evaluation indices (soil Pb and Cd, plant biomass) are represented by the contribution matrix using the following equation (Eq. 3).

$$X = (X_{ij})_{n \times m} \quad (3)$$

where n is 2 (monocropping and intercropping); m is 4 (the number of root exudates treatment); X_{ij} is the contribution of the ith root exudate concentration to the jth evaluation parameter.

2. The matrix is standardized by Eq. 4:

$$\overline{X}_{ij} = \frac{X_{ij}}{\sqrt{\sum_{i=0}^{n} X_{ij}^2}} \quad (4)$$

where $i = 1, 2, \ldots, n; j = 1, 2, \ldots, m$.

3. The weighted normalized matrix is calculated by Eq. 5:

$$V_{ij} = \overline{X}_{ij} \times W_j \quad (5)$$

where positive V_{ij} is the perfect best case and negative V_{ij} is the worst instance. W_j is the weight of the jth criterion and the $\sum_{j=1}^{n} W_j$ is 1; for this work, the weight of Pb and Cd was set to be 0.5, and biomass was set to be 0.5 to equilibrize their respective dedications (Hamani et al., 2023).

4. The Euclidean distances are calculated by (Eqs. 6 and 7):

$$S_i^+ = \sqrt{\sum_{j=1}^{m} \left(V_{ij} - V_j^+\right)^2} \quad (6)$$

$$S_i^- = \sqrt{\sum_{j=1}^{m} \left(V_{ij} - V_j^-\right)^2} \quad (7)$$

5. The performance score of the treatments are calculated by (Eq. 8):

$$P_i = \frac{S_i^-}{S_i^+ + S_i^-} \quad (8)$$

then the treatments are ranked according to the scores.

2.7. Data analysis

Plant growth characteristics, soil chemical properties and concentration of heavy metals in soil and plants were analyzed by one-factor analysis of variance; Tukey's test was employed for multiple comparisons to test the significance of the differences between treatments ($p < 0.05$) using SPSS version 22.0 (SPSS Inc., IL, USA). Data were displayed as means with standard error (SE); figures were prepared with Origin Pro 2021b.

3. Results

3.1. Soil basic properties

Table 1 shows the soil chemical characteristics developed under different application rates of root exudates and planting patterns under groundwater and WW irrigation. WW irrigation had significant effects on soil chemical properties compared with groundwater irrigation (Table 1). Significant differences were observed in soil TP and TN across all treatments with an increase with root exudates application except for TN under monocultured maize irrigated with WW.

Concentrations of soil TP and TN ranged respectively between 2.02 and 3.38 mg g^{-1} and 1.14–1.69 mg g^{-1} and were higher under monocrop soybean in groundwater-irrigated soil without root exudate addition. However, a different situation was observed with root exudate application: in this case, *TP* and *TN* were higher in WW-irrigated soil and root exudate application treatments (Table 1). The results showed that water type, planting pattern and root exudates application rate all had significant positive effects on soil basic chemical properties except for *OM* and pH.

3.2. Crop growth traits

Irrigation with treated wastewater in intercrop systems increased the height of both maize and soybean plants compared to groundwater irrigation. Under monocrop conditions, maize height decreased significantly when root exudates were applied to WW-irrigated soil, whereas it did not decrease in groundwater-irrigated soil when root exudates were applied. Root exudates enhanced the growth of soybean in both mono- and intercrop systems, regardless of whether the soil was irrigated with groundwater or WW (Table 2a and b). Contrarily, in monocrop maize, applying root exudates had no positive effect when soil was irrigated with WW. This contrasts with the positive response observed in both mono- and intercrop systems when root exudates were applied to groundwater-irrigated soil (Table 2a). Despite this, maize showed a positive response when grown in WW-irrigated soil using intercrop methods compared to monocropped maize.

Water source and planting patterns significantly affected plant *SDW*,

Table 1
Soil basic chemical properties under different treatments.

Planting pattern	Crop	Water type	Root exudate application rate	pH	*EC* (μS cm^{-1})	*OM* (%)	*TN* (mg g^{-1})	*TP* (mg g^{-1})	Water soluble K^{+} (mg g^{-1})	Water soluble Na^{+} (mg g^{-1})
Monocropping	Maize	G	0 %	7.86 ± 0.04c	1785.33 ± 5.75 cd	1.87 ± 0.20a	1.50 ± 0.05bc	2.51 ± 0.11c	0.20 ± 0.05gh	0.08 ± 0.02c
		W		8.07 ± 0.04a	1697.00 ± 9.47d	2.01 ± 0.06a	1.14 ± 0.02e	2.02 ± 0.14d	0.14 ± 0.03j	1.88 ± 0.13a
		G	16 %	7.94 ± 0.03c	1752.00 ± 6.72 cd	1.65 ± 0.15a	1.47 ± 0.06bc	2.55 ± 0.11bc	0.17 ± 0.00ij	0.11 ± 0.01c
		W		7.78 ± 0.02 cd	2113.00 ± 1.62b	1.97 ± 0.04a	1.34 ± 0.07 cd	2.35 ± 0.18c	0.32 ± 0.00c	1.60 ± 0.28ab
		G	32 %	7.94 ± 002bc	1708.00 ± 9.57d	1.70 ± 0.03a	1.46 ± 0.08bc	2.58 ± 0.28bc	0.19 ± 0.01hi	0.16 ± 0.05c
		W		7.70 ± 0.06e	2239.33 ± 2.60a	2.06 ± 0.10a	1.52 ± 0.06ab	2.86 ± 0.09bc	0.38 ± 0.03a	1.74 ± 0.37ab
		G	64 %	7.96 ± 0.06b	1616.67 ± 7.92de	1.81 ± 0.06a	1.47 ± 0.02bc	2.69 ± 0.08bc	0.17 ± 0.01i	0.21 ± 0.04c
		W		7.73 ± 0.04d	2159.33 ± 1.32b	2.07 ± 0.03a	1.51 ± 0.04b	2.98 ± 0.20b	0.33 ± 0.02bc	1.69 ± 0.16ab
	Soybean	G	0 %	7.74 ± 0.01d	2052.33 ± 0.08c	1.90 ± 0.13a	1.69 ± 0.05a	3.38 ± 0.30a	0.34 ± 0.03b	0.39 ± 0.03c
		W		7.69 ± 0.02e	2232.33 ± 3.31a	2.02 ± 0.06a	1.30 ± 0.04 cd	2.75 ± 0.13bc	0.38 ± 0.01a	1.96 ± 0.07a
		G	16 %	7.92 ± 0.06c	1634.33 ± 5.68de	1.82 ± 0.07a	1.44 ± 0.04bc	2.74 ± 0.13bc	0.23 ± 0.04fg	0.23 ± 0.05c
		W		7.73 ± 0.01d	2099.00 ± 6.60bc	2.06 ± 0.04a	1.51 ± 0.01b	3.01 ± 0.05b	0.36 ± 0.01ab	1.54 ± 0.11ab
		G	32 %	7.81 ± 0.04 cd	1965.67 ± 8.69 cd	1.57 ± 0.12a	1.49 ± 0.08bc	2.86 ± 0.07bc	0.32 ± 0.01 cd	0.48 ± 0.10c
		W		7.73 ± 0.01d	2179.33 ± 9.38ab	2.08 ± 0.03a	1.37 ± 0.09 cd	2.72 ± 0.14bc	0.38 ± 0.01a	1.53 ± 0.26ab
		G	64 %	7.99 ± 0.003ab	1522.33 ± 7.91e	1.59 ± 0.05a	1.28 ± 0.03 cd	2.58 ± 0.06bc	0.19 ± 0.01 h	0.30 ± 0.06c
		W		7.79 ± 0.01 cd	1870.00 ± 9.18 cd	1.94 ± 0.15a	1.40 ± 0.02c	2.61 ± 0.09bc	0.33 ± 0.01bc	1.61 ± 0.13ab
Intercropping	Maize+soybean	G	0 %	7.95 ± 0.14bc	1554.80 ± 9.11de	1.84 ± 0.11a	1.23 ± 0.06d	2.29 ± 0.16 cd	0.16 ± 0.06ij	0.42 ± 0.18c
		W		7.77 ± 0.02 cd	2024.00 ± 5.77 cd	2.01 ± 0.11a	1.34 ± 0.04 cd	2.68 ± 0.21bc	0.32 ± 0.01 cd	1.93 ± 0.12a
		G	16 %	7.71 ± 0.03de	1867.67 ± 4.72 cd	1.69 ± 0.16a	1.43 ± 0.08c	3.18 ± 0.29ab	0.28 ± 0.01e	0.46 ± 0.10c
		W		7.80 ± 0.03 cd	1839.33 ± 2.99 cd	2.06 ± 0.06a	1.27 ± 0.10 cd	2.65 ± 0.13bc	0.26 ± 0.01ef	1.93 ± 0.43a
		G	32 %	7.84 ± 0.03 cd	1762.00 ± 4.02 cd	1.86 ± 0.03a	1.21 ± 0.03de	2.34 ± 0.12c	0.21 ± 0.02 g	0.37 ± 0.16c
		W		7.74 ± 0.02d	1980.67 ± 1.01 cd	1.95 ± 0.09a	1.36 ± 0.05 cd	2.63 ± 0.15bc	0.31 ± 0.01d	2.08 ± 0.19a
		G	64 %	7.82 ± 0.03 cd	1870.33 ± 2.96 cd	1.87 ± 0.02a	1.22 ± 0.01d	2.36 ± 0.13c	0.24 ± 0.01 f	0.83 ± 0.06b
		W		7.71 ± 0.04de	1976.00 ± 6.51 cd	1.83 ± 0.03a	1.36 ± 0.03 cd	3.05 ± 0.07b	0.30 ± 0.01de	2.29 ± 0.09a
WT				* ** *	* ** *	ns	* ** *	* ** *	* ** *	* ** *
PP				* ** *	* ** *	ns	* ** *	* ** *	* ** *	* ** *
WT*PP				* ** *	* ** *	ns	* ** *	* ** *	* ** *	* ** *

Notes: Four levels of root exudates (0 %, 16 %, 32 % and 64 %) were applied to the soil of the same planting pattern. As an example, monocultured maize root exudates were applied to the monocultured maize. To prepare the above-stated levels of root exudates, 0, 16, 32 and 64 mL of each were diluted with deionized water to a final volume of 100 mL. Data are listed as means ± standard errors ($n = 3$). Different lowercase letters in the same column indicate significant differences between treatments ($p < 0.05$). G means groundwater, W means treated livestock wastewater, *EC* refers to electrical conductivity, *OM* refers to organic matter, TN refers to total nitrogen, TP refers to total phosphorus, WT refers to water type, PP refers to planting pattern. * ** *$p < 0.0001$.

RDW, *LA*, and leaf area ratio (*LAR*) (Table 2a and b). The effects were intensified when root exudates were applied. For both mono- and intercrop systems receiving groundwater irrigation, maize SDW, RDW, and LA were increased substantially by adding root exudates. Soybean SDW, RDW, LA, and LAR were higher when intercropped and irrigated using WW. Applying root exudates enhanced the positive outcomes on intercropped soybean growth parameters further under both groundwater and WW irrigation. The positive effects of WW irrigation and intercropping on crop growth were augmented by using root exudates, except in the case of monocrop maize receiving WW irrigation.

3.3. Heavy metal concentrations in soil

Except for monocrop maize without root exudate application and with 32 % of root exudates (Fig. 1a), the concentration of Zn in soil irrigated with groundwater was higher than that with WW. Similar trends were observed for Cu in soil irrigated with groundwater and WW for monocrop maize and soybean (Fig. 1b). Compared to soil irrigated with groundwater, soil irrigated with WW in intercrop systems had higher Cu concentrations. For Pb, higher concentration was found in soil irrigated with WW than groundwater for monocrop soybean and the intercropping system, contrary to monocultured maize in which similarities were observed between soil irrigated with WW and soil irrigated with groundwater, except in the treatment without root exudate

Table 2a
Maize growth parameters under different water treatments, planting patterns and root exudate levels.

Planting pattern	Water type	Root exudate application rate	*PH* (cm)	*STD* (mm)	*SDW* (g)	*RDW* (g)	*LA* (cm^2)	*LAR* (cm^2 g^{-1})	*Lchl* (SPAD)	*LN* (mg g^{-1})
Maize in monocropping system	G	0 %	88.67 ± 8.01 cd	8.78 ± 0.71c	37.46 ± 5.86ab	2.05 ± 0.20b	113.56 ± 0.14de	2.94 ± 0.41a	9.10 ± 0.55e	0.97 ± 0.03 f
	W		131.00 ± 6.35a	11.45 ± 0.71b	45.54 ± 5.61a	5.14 ± 0.93a	148.94 ± 0.61 cd	3.07 ± 0.58a	13.73 ± 0.81d	1.50 ± 0.23d
	G	16 %	102.67 ± 2.33bc	10.14 ± 0.34bc	38.69 ± 1.84ab	2.46 ± 0.19b	149.74 ± 0.04 cd	3.66 ± 0.26a	14.07 ± 1.23d	1.43 ± 0.03e
	W		74.67 ± 1.20d	9.40 ± 0.55c	27.35 ± 3.35ab	2.50 ± 0.63b	110.11 ± 0.86e	3.85 ± 0.67a	11.60 ± 0.78	1.47 ± 0.20e
	G	32 %	102.67 ± 2.60bc	11.28 ± 0.44b	43.00 ± 2.65ab	2.52 ± 0.43b	159.51 ± 0.41c	3.54 ± 0.30a	18.43 ± 0.95bc	1.83 ± 0.35b
	W		71.67 ± 9.17d	11.46 ± 0.47b	22.87 ± 7.20b	1.75 ± 0.05b	130.13 ± 2.71 cd	6.10 ± 1.40a	13.87 ± 0.91	1.77 ± 0.13bc
	G	64 %	110.33 ± 5.37b	12.59 ± 0.21ab	47.10 ± 6.18a	3.48 ± 0.70ab	157.85 ± 0.32c	3.24 ± 0.46a	18.30 ± 5.60bc	1.83 ± 038b
	W		89.67 ± 9.87 cd	11.68 ± 0.36b	30.28 ± 4.49ab	2.64 ± 0.69b	123.48 ± 0.46d	3.86 ± 0.39a	14.83 ± 0.24d	1.90 ± 0.06ab
Maize in maize and soybean intercropping system	G	0 %	96.33 ± 2.63c	10.35 ± 0.19bc	28.48 ± 3.44ab	2.40 ± 0.19b	152.13 ± 0.20c	4.89 ± 0.23a	16.23 ± 2.23c	1.60 ± 0.21c
	W		79.00 ± 8.50d	10.70 ± 0.26bc	29.33 ± 3.36ab	2.37 ± 0.37b	168.25 ± 0.13 cd	5.35 ± 0.21a	14.73 ± 0.19d	2.03 ± 0.24ab
	G	16 %	84.33 ± 0.88 cd	11.87 ± 0.35b	29.98 ± 3.24ab	2.16 ± 0.37b	144.06 ± 0.27bc	4.62 ± 0.68a	17.90 ± 1.55bc	1.83 ± 0.12b
	W		72.67 ± 2.73d	11.47 ± 0.10b	31.79 ± 3.01ab	2.26 ± 0.44b	176.26 ± 0.02bc	5.31 ± 0.84a	19.47 ± 1.79b	2.20 ± 0.21ab
	G	32 %	89.67 ± 4.26 cd	12.68 ± 0.26ab	31.15 ± 0.93ab	2.72 ± 0.10b	173.65 ± 0.02bc	5.15 ± 0.34a	19.03 ± 0.73b	1.77 ± 0.24bc
	W		82.00 ± 4.73 cd	12.13 ± 0.15ab	33.39 ± 2.77ab	2.26 ± 0.20b	191.18 ± 0.69ab	5.50 ± 1.09a	21.84 ± 0.93a	2.13 ± 0.28ab
	G	64 %	96.00 ± 2.08c	13.63 ± 0.18a	31.51 ± 2.08ab	2.81 ± 0.10b	183.75 ± 0.74b	5.38 ± 0.25a	20.57 ± 0.88ab	2.23 ± 0.09ab
	W		92.33 ± 8.21c	12.60 ± 0.45ab	34.49 ± 0.73ab	2.51 ± 0.25b	202.46 ± 0.07a	5.47 ± 0.28a	22.00 ± 1.58a	2.70 ± 0.06a
WT			* **	* **	* **	* **	* **	* *	* **	* *
PP			* *	* **	* *	* **	* **	* **	* *	* *
WT*PP			* **	* **	* **	* **	* **	* **	* **	* **

Notes: Four levels of root exudates (0 %, 16 %, 32 % and 64 %) were applied to the soil of the same planting pattern. As an example, monocultured maize root exudates were applied to the monocultured maize. To prepare the above-stated levels of root exudates, 0, 16, 32 and 64 mL of each were diluted with deionized water to a final volume of 100 mL. Data are presented as means ± standard errors ($n = 3$). Different lowercase letters in the same column manifest statistically significant differences between treatments at $p < 0.05$. G refers to groundwater, W refers to treated livestock wastewater, *PH* refers to plant height, *STD* refers to stem diameter, *SDW* refers to shoot dry weight, *RDW* refers to root dry weight, *LA* refers to leaf area, *LAR* refers to leaf area ratio, *Lchl* refers to leaf chlorophyll content, *LN* refers to leaf nitrogen level, WT refers to water type, PP refers to planting pattern. * * $p < 0.01$, * ** $p < 0.001$.

application (Fig. 1c). Similar results were observed for Cd but higher Cd concentration was found in in soil irrigated with WW for all treatments, except for monocultured maize with 32 % and 64 % of root exudates application rate (Fig. 1d). These observations suggest that root exudates application was more effective in reducing heavy metal concentrations in WW-irrigated soil under monocrop soybean. The concentrations of heavy metals in soil were generally lower in intercrop systems compared to monocrop maize, except for Pb.

3.4. Heavy metal concentrations in plant shoots

The concentrations of heavy metals in plants varied significantly across the treatments. Heavy metal concentrations were generally lower in soybean plants compared to maize. However, concentrations of Cd were higher in soybean (Fig. 2d). The concentrations of Pb and Cd were lower under intercropping than monocrops (Fig. 2c and d). In monocropping, the concentrations of Zn and Cu were higher in maize shoots than in soybean shoots (Fig. 2a and b). The influence of root exudate treatment on the concentrations of heavy metals in plants was small. This suggests that the concentrations of heavy metals in plants was more influenced by metal species, crop types, planting patterns and water types rather than root exudates treatments.

3.5. Heavy metals mobility in soil-plant system under the supply of root exudates

Fig. 3 shows the capability of plants to transport metals from the soil to the aerial part. Our evidence demonstrated that the translocation factor (*TF*) of heavy metals was influenced by the application of root exudates and planting patterns. The highest rate of Zn translocation occurred under WW irrigation (Fig. 3a). Notably, the *TF* of Pb was higher in intercrop than monocrop systems. In soybean, root exudates resulted in a significant decrease in the *TF*s of both Pb and Cd. In maize, root exudates additions reduced the *TF* of Cd, but that of Pb remained unaffected (Fig. 3c and d). Root exudate additions were not associated with a significant difference in translocated levels of Cu and Zn. However, planting patterns had a stronger impact on the *TF*s of Zn and Cu (Fig. 3a and b).

Pearson correlation coefficients showed that the *TF*s of Cu and Pb were significantly related to plant height and stem diameter. Other growth parameters were not related to the *TF*s of Cu and Pb (Fig. 3e). In this study, the *TF*s of Zn and Cd were not related to plant growth parameters (Fig. 3e).

3.6. Enrichment factors and correlations between translocation factors and growth characteristics

Root exudate application reduced Pb and Cd *EF*s in maize

Table 2b
Soybean growth parameters under different water treatments, planting patterns and root exudate levels.

Planting pattern	Water type	Root exudate application rate	*PH* (cm)	*STD* (mm)	*SDW* (g)	*RDW* (g)	*LA* (cm^2)	*LAR* (cm^2 g^{-1})	*Lchl* (SPAD)	*LN* (mg g^{-1})
Soybean in monocropping system	G	0 %	60.33 ± 3.84bc	2.51 ± 0.23b	2.32 ± 0.17d	0.13 ± 0.03b	7.23 ± 0.27e	3.00 ± 0.34b	9.37 ± 0.77i	1.20 ± 0.06d
	W		73.33 ± 3.93b	2.27 ± 0.09b	3.35 ± 0.23 cd	0.13 ± 0.03b	9.31 ± 0.74d	2.72 ± 0.38b	12.33 ± 1.65 f	2.03 ± 0.13a
	G	16 %	71.33 ± 3.18b	2.96 ± 0.37b	3.47 ± 0.12 cd	0.17 ± 0.02b	7.69 ± 0.36de	2.12 ± 0.04b	13.03 ± 1.59de	1.63 ± 0.12b
	W		73.00 ± 1.15b	2.98 ± 0.46b	3.63 ± 0.13 cd	0.17 ± 0.04b	10.77 ± 0.84 cd	2.83 ± 0.14b	15.10 ± 0.40ef	2.03 ± 0.19a
	G	32 %	72.33 ± 3.48ab	3.01 ± 0.28b	3.33 ± 0.11 cd	0.17 ± 0.01b	8.97 ± 0.46de	2.58 ± 0.19b	14.13 ± 0.82d	1.90 ± 0.12ab
	W		74.33 ± 6.69c	3.46 ± 0.08ab	3.70 ± 0.03 cd	0.18 ± 0.06b	13.19 ± 1.32bc	3.41 ± 0.39a	16.57 ± 1.22e	2.13 ± 0.20a
	G	64 %	74.00 ± 6.11a	3.37 ± 0.12ab	5.58 ± 0.06ab	0.22 ± 0.01b	10.71 ± 2.10 cd	1.85 ± 0.37c	13.63 ± 2.91 cd	2.10 ± 0.40a
	W		82.67 ± 6.74b	3.83 ± 0.09a	5.73 ± 0.46ab	0.22 ± 0.02b	17.43 ± 1.21ab	2.96 ± 0.29b	17.80 ± 0.15ef	2.33 ± 0.38a
Soybean in maize and soybean intercropping system	G	0 %	59.33 ± 8.25c	2.90 ± 0.12b	3.47 ± 0.02 cd	0.30 ± 0.03b	8.66 ± 0.72de	2.30 ± 0.20b	12.67 ± 0.46 f	1.37 ± 0.09c
	W		65.67 ± 7.22bc	3.33 ± 0.12ab	4.31 ± 0.31bc	0.25 ± 0.03b	11.08 ± 1.03c	2.48 ± 0.36b	12.90 ± 1.10ef	1.53 ± 0.27bc
	G	16 %	73.33 ± 1.76b	3.18 ± 0.27ab	4.15 ± 0.33bc	0.55 ± 0.30ab	12.06 ± 0.44bc	2.61 ± 0.20b	12.50 ± 1.82 f	1.43 ± 0.15 cd
	W		76.00 ± 4.58ab	3.40 ± 0.06av	4.23 ± 0.24bc	0.32 ± 0.02b	15.15 ± 1.79b	3.36 ± 0.47ab	12.73 ± 0.88 f	1.83 ± 0.03ab
	G	32 %	76.00 ± 4.93ab	3.31 ± 0.12ab	4.72 ± 0.07c	0.32 ± 0.03b	12.13 ± 0.91bc	2.40 ± 0.16b	18.67 ± 2.33c	1.70 ± 0.32b
	W		75.33 ± 5.36b	3.77 ± 0.09a	5.61 ± 0.37ab	0.31 ± 0.05b	15.22 ± 0.36b	2.59 ± 0.11b	20.47 ± 1.54b	2.17 ± 0.32a
	G	64 %	71.67 ± 3.84b	3.73 ± 0.58a	4.99 ± 0.43b	1.00 ± 0.05a	11.71 ± 1.57c	1.94 ± 0.17bc	18.87 ± 3.98c	1.83 ± 0.18ab
	W		83.00 ± 4.36a	3.80 ± 0.06a	6.27 ± 0.86a	0.40 ± 0.15ab	17.89 ± 1.74a	2.71 ± 0.25b	22.43 ± 1.47a	1.97 ± 0.15ab
WT			* *	ns	* **	* **	* **	* *	ns	ns
PP			* *	* **	* **	* **	* **	* **	* *	*
WT*PP			* **	* **	* **	* **	* **	* **	* **	* *

Notes: Four levels of root exudates (0 %, 16 %, 32 % and 64 %) were applied to the soil of the same planting pattern. As an example, monocultured maize root exudates were applied to the monocultured maize. To prepare the above-stated levels of root exudates, 0, 16, 32 and 64 mL of each were diluted with deionized water to a final volume of 100 mL. Data are displayed as means ± standard errors ($n = 3$). Different lowercase letters in the same column mean statistically significant differences between treatments at $p < 0.05$. G refers to groundwater, W refers to wastewater, *PH* refers to plant height, *STD* refers to stem diameter, *SDW* refers to shoot dry weight, *RDW* refers to root dry weight, *LA* refers to leaf area, *LAR* refers to leaf area ratio, *Lchl* refers to leaf chlorophyll content, *LN* refers to leaf nitrogen level, WT refers to water type, PP refers to planting pattern. ns refers to not significant. * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$.

monoculture (Fig. 4). Furthermore, for intercrop systems root exudate application reduced the *EF* of Cd (Fig. 4). Similar effects were observed in the *EFs* of Zn and Cu with an increase in the root exudate application rate (Fig. 4). Planting patterns exerted no significant impact on heavy metal *EFs*.

3.7. Comprehensive evaluation of root exudates and WW irrigation

The results of the optimization methods based on Euclidian distance, normalized matrices, performance scores, and TOPSIS rank of the WW treatments are shown in Table 3. Monocrop maize without root exudate application ranked first and had a higher biomass with less Cd and Pb accumulation in soil with a best score of 1.000 and 0.807 for Cd and Pb respectively. Intercrop systems receiving 64 % of root exudate application showed the second-best performance (Table 3a). The lowest score of 0.017 and 0.165 (ranked 8th) respectively for Cd and Pb described monocrop systems receiving 32 % of root exudates.

Regarding soybean, the highest score was achieved at a 64 % root application rate in intercrops, with scores of 0.926 and 0.953 respectively for Cd and Pb (ranked first). The poorest soybean performance was observed in monocrops, with root application rate of 16 % for Cd and with no root application for Pb (Table 3b). Application of root exudates was more effective at reducing heavy metal accumulation in soil and improving crop growth in mono- rather than intercrop systems for both crops under WW irrigation.

3.8. Bacterial community compositions

Proteobacteria were the most dominant bacterial phylum in the soils. The composition of bacterial communities was influenced by irrigation water source and the rate at which root exudates were applied, based on the types of crops and planting patterns. The soil bacterial composition was sensitive to WW irrigation at the phylum level (Fig. 5). Furthermore, the application of root exudates increased the relative abundance of Proteobacteria in soil under monocropped maize with groundwater irrigation, relative to WW irrigation (Fig. 5). This suggests that the addition of root exudates did not have a significant impact on the relative abundance of the dominant phylum of soil bacteria in WW-irrigated soil under monocrop maize.

In soil cultivated with monocrop soybean, WW irrigation generally decreased the relative abundance of Proteobacteria and enhanced the relative abundance of Actinobacteria compared to groundwater irrigation, except for 32 % of root exudates (Fig. 5). When applying root exudates, Proteobacteria abundance was reduced under groundwater irrigation compared to no exudate applications, while it remained at a similar level among the three root exudates application rates.

In intercropped soils, the relative abundance of Proteobacteria increased with WW irrigation compared to groundwater irrigation, at root exudates application rates of 0 %, 32 % and 64 %. In groundwater-irrigated soil, the abundance of Proteobacteria was similar between the different root exudates application rates. Compared to other treatments,

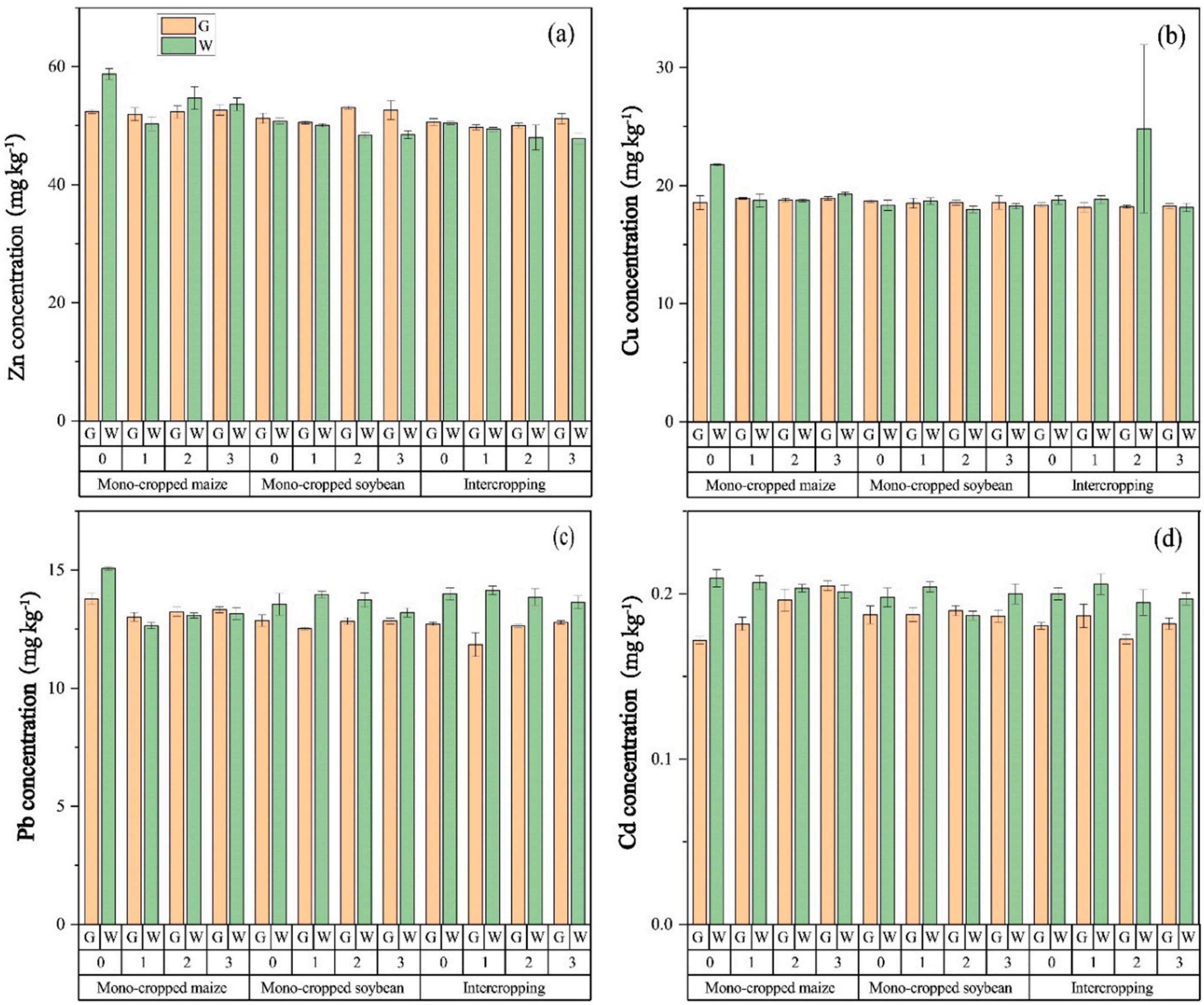

Fig. 1. Concentrations of Zn (a), Cu (b), Pb (c) and Cd (d) in soil at harvest. All data are stated as means with standard errors ($n = 3$). In the abscissa axis, "G" refers to groundwater, "W" refers to wastewater; "0", "1", "2" and "3" refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates, respectively.

the abundance of Cyanobacteria was remarkedly increased in monocrop soybean receiving 16 % of root exudate and groundwater irrigation. In WW-irrigated soil, the abundance of Proteobacteria first decreased and then increased with the root application rate.

Hierarchical clustering indicated that *X67.14* and *subgroup_6*, both from Acidobacteria phylum, were dominant across all treatments followed by *Bacillus* (Firmicutes) and *Solirubrobacter* (Acidobacteria) (Fig. 6). Significant changes in *Nocardioides* and *Streptomyces* abundances were observed based on water source, planting patterns and the root exudate application rates (Fig. 6). Similarities in bacterial community composition were observed between groundwater- and WW-irrigated soils under intercropping at 32 % of root exudates. Also, no significant difference in bacterial taxonomic composition was observed under monocrop and intercrop maize and soybean cultivated soil depending on root exudates application rates. The application rates of root exudates exerted no significant influence on soil bacterial community composition. However, the results revealed that WW irrigation increased the abundance of the main genus in soil and that the bacteria diversity was sensitive to root exudate applications, the type of crops, and planting patterns (Fig. 6).

4. Discussion

4.1. Root exudate applications and wastewater irrigation altered soil properties

Root exudates play a crucial role in plant-soil interactions as they boost soil nutrient content and enhance plant growth, as stated by Zhao et al. (2021). The present study demonstrated that root exudates increased soil nutrient content in soil irrigated with both groundwater and wastewater, particularly under intercrop systems and monocrop soybean. The limited influence of root exudates upon monocrop maize in WW-irrigated soil may arise due to the absence of interactions between maize plants and soil caused by the high nutrient levels in WW, as noted by Shahrivar et al. (2023) and Yerli et al. (2023). Prolonged use of treated wastewater for crop irrigation is known to result in the accumulation of heavy metals in soil (Singh, 2021). Our results indicated that the application of root exudates had a significant impact on soil nutrient content, plant growth and the transportation of heavy metals. Han et al. (2022) found similar results, demonstrating that root exudates increased the microbial population size and soil nutrient content. In addition, root exudates play an essential role in the uptake of several vital metals by plants (Chen et al., 2017b) as well as in protecting them from stress conditions (Liu et al., 2023; Williams and de Vries, 2020). For example,

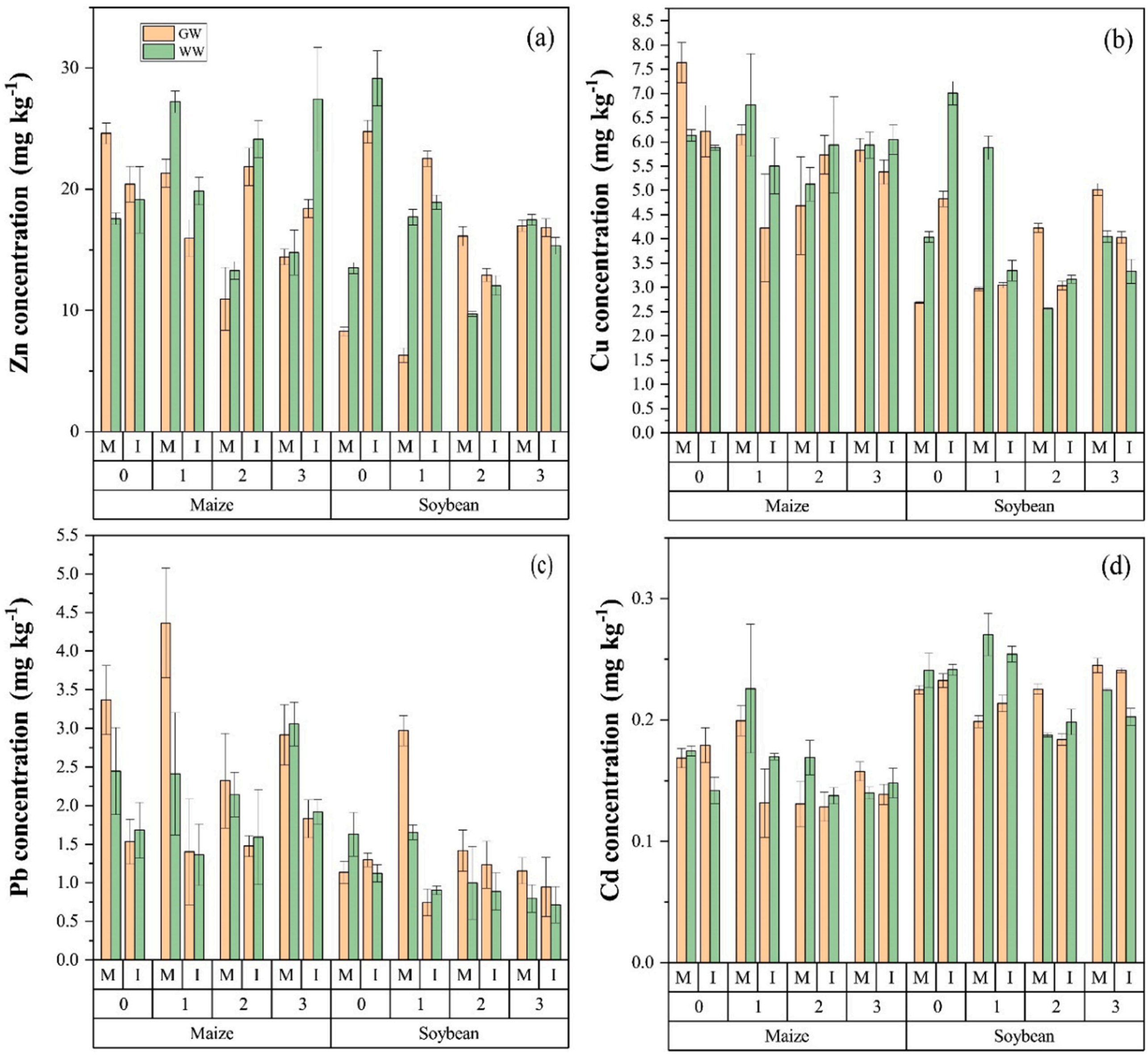

Fig. 2. Concentrations of Zn (a), Cu (b), Pb (c) and Cd (d) in plant shoots at harvest. All data are presented as means with standard errors ($n = 3$). GW and WW in the legend refer to groundwater and wastewater, respectively. In the abscissa axis, the "M" refers to monocropping; the "I" refers to intercropping; "0", "1", "2" and "3" refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates, respectively.

researchers have demonstrated some plant species can tolerate toxic metals in the rhizosphere by exuding citric and malic acids (Podar and Maathuis, 2022). According to this study, which is consistent with Fajardo et al. (2019) findings, plant root exudates enhanced soil nutrient content and promoted plant growth, as well as lowered the concentrations of Cd and Pb in the soil.

4.2. Root exudate addition improved crop growth

Plant root exudates also have supportive roles in plant growth and interactions with physicochemical and biological factors in the rhizosphere (Bais et al., 2006; Chai and Schachtman, 2022). In line with previous studies (Upadhyay et al., 2022), our results showed that root exudate application contributed to plant growth, particularly in soil irrigated with oligotrophic groundwater. Adding root exudates improved plant growth in WW-irrigated soil, except for monocrop maize. The results showed that applying root exudates was more effective in promoting plant growth under intercrops compared with monocrops in WW-irrigated soils. This might be due to the stronger plant-plant and plant-soil interactions in an intercropping system than in a monocropping system (Koskey et al., 2023). Application of root exudates improved plant growth over systems where no exudates were applied, particularly under intercropping and groundwater irrigation, consistent with previous studies (Bais et al., 2006; Podar and Maathuis, 2022; Song et al., 2022). This highlights the importance of plant-soil interaction and its influence on plant growth and soil nutrient content.

4.3. Root exudates influence heavy metal bioavailability

In this study, application of root exudates resulted in a decrease in heavy metal concentrations in soil exclusively monocropped with maize under WW irrigation but not under groundwater irrigation. However, while metal concentrations in plants did not respond consistently to the total concentration of metals in the soil. Previous studies have not commonly reported interactions between root exudate applications and wastewater irrigation. Zia-ur-Rehman et al. (2023) demonstrated the contrasting effect of low molecular weight organic acids (LMWOAs) and high molecular weight organic acids (HMWOAs). Application of

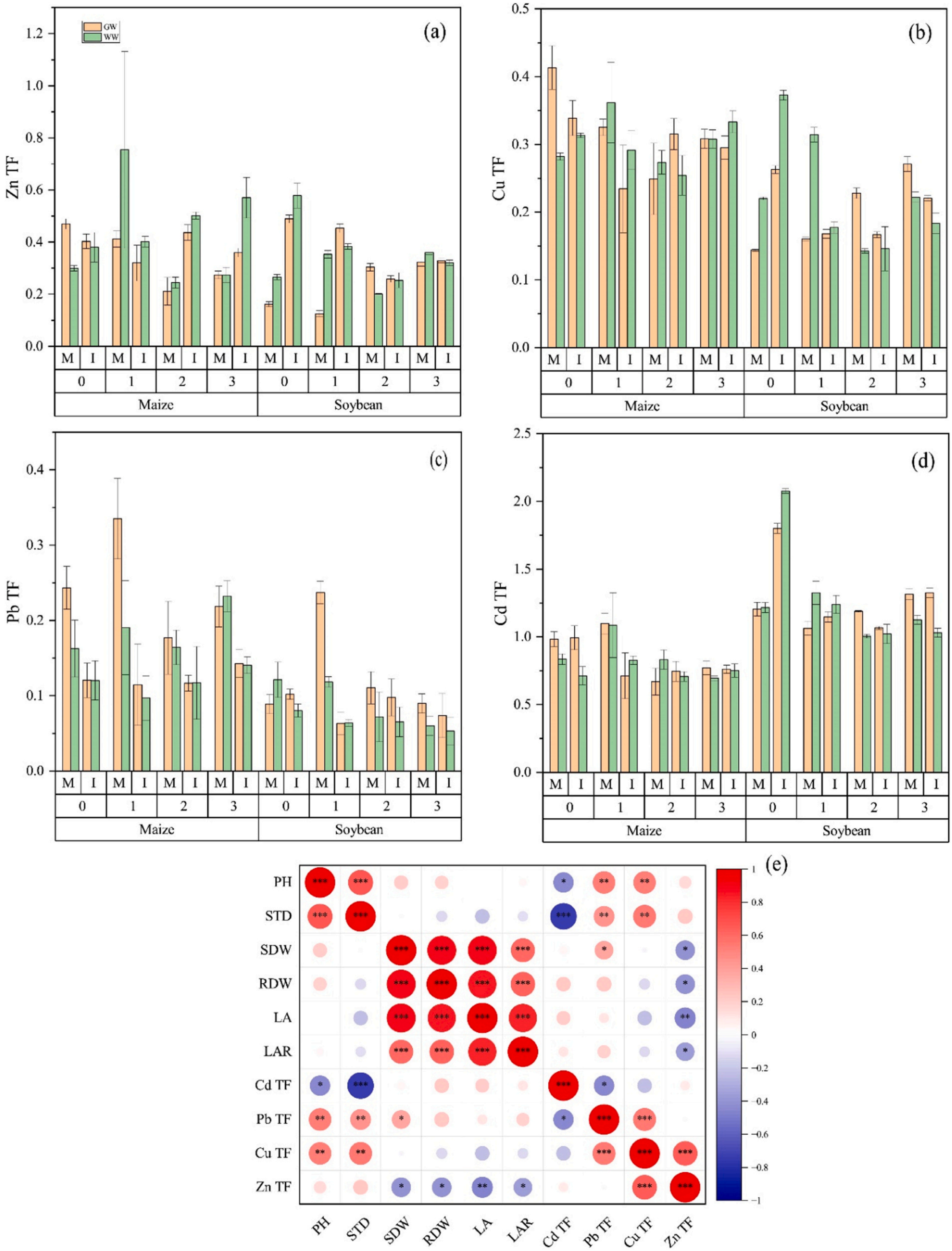

Fig. 3. Translocation factor (TF) of Zn (a), Cu (b), Pb (c) and Cd (d) from soil to plant shoots and their correlations between plant growth characteristics (e). All data are exhibited as means with standard errors ($n = 3$). For Figs. 3a-3d, GW and WW in the legend refer to groundwater and wastewater, respectively. In the abscissa axis, the "M" refers to monocropping; the "I" refers to intercropping; "0", "1", "2" and "3" refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates, respectively. For Fig. 3e, *PH* refers to plant height, *STD* refers to stem diameter, *SDW* refers to shoot dry weight, *RDW* refers to root dry weight, *LA* refers to leaf area, *LAR* refers to leaf area ratio. * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$.

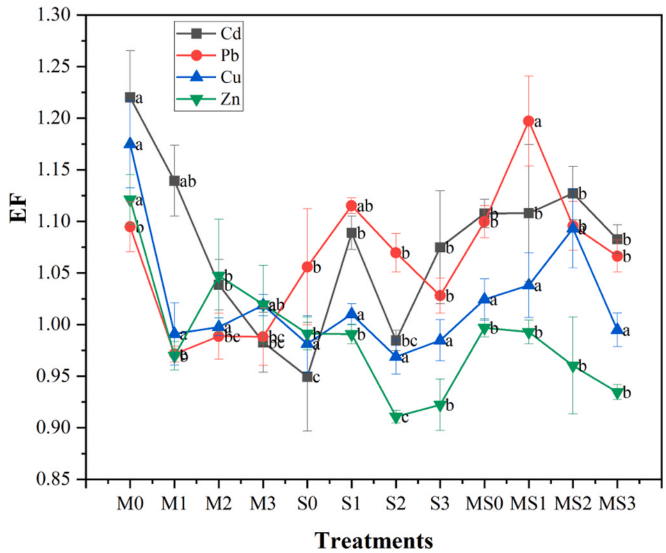

Fig. 4. Enrichment factor (EF) for Zn, Cu, Pb and Cd in wastewater-irrigated soil at harvest relative to groundwater-irrigated soil. "M0″, "M1″, "M2″ and "M3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under mono-cropped maize, respectively; "S0″, "S1″, "S2″ and "S3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under mono-cropped soybean, respectively; "MS0″, "MS1″, "MS2″ and "MS3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under intercropping, respectively. All data are showed as means with standard errors ($n = 3$). Different lowercase letters on the same line mean statistically significant differences between treatments at $p < 0.05$.

LMWOAs (oxalic acid and tartaric acid) increased the bioavailability of metals in soils and the concentration of metals in plant tissues. Application of HMWOAs (citric acid and humic acid) had the opposite effect. Detecting the content of bioavailable metals in the soil is essential to establish more direct associations between soil metal bioavailability and plant uptake of metals in the future. Moreover, the composition of LMWOAs and HMWOAs in root exudates likely changed under the dual action of exogenous exudates and WW irrigation, with their ratio determining the final outcome. Heavy metal bioavailability is also influenced by the organic acid structure as well as the number and type of functional groups that they contain, as heavy metals can form complexes with various organic acids (Vega et al., 2022). Organic acids exhibit not only a variety effect but also a dose effect on heavy metal transport in the soil as reported by Schwab et al. (2008). Appropriate application of root exudates could decrease heavy metals bioavailability in soil and plants as exogenous exudate applications have been reported to reduce heavy metals content in plants (Rehman et al., 2020). Moreover, previous research has indicated that the fate of a particular metal in soil is determined by various factors, which include metal properties and its interaction with plants and soil organic matter (Fouda-Mbanga et al., 2021; Usman et al., 2023; Zhao et al., 2022). While the impact of root exudates on plants and heavy metal content in soil varied across all treatments, this study revealed significant positive effects under intercrop systems. This shows the positive effects of combining root exudate application and intercropping to reduce heavy metal concentrations in soil and plants (Zia-ur-Rehman et al., 2023). In general, the present study has provided evidence that applying root exudates can reduce heavy metals transportation in soil and concentrations in plants under WW irrigation, and that this effect is enhanced under intercropping.

Table 3a
TOPSIS score and rank of wastewater treatments based on balancing maize plant biomass as well as soil Pb and Cd content.

Planting pattern	Root exudate application rate	Normalized matrix		Euclidean distance		Performance Score	TOPSIS rank	Normalized matrix		Euclidean distance		Performance Score	TOPSIS rank
		Biomass	Cd	S-	S+			Biomass	Pb	S-	S+		
Monocropping	0 %	0.254	0.156	0.134	0.000	1.000	1	0.254	0.194	0.131	0.031	0.807	1
	16 %	0.150	0.165	0.034	0.105	0.246	7	0.150	0.163	0.041	0.104	0.281	7
	32 %	0.123	0.185	0.002	0.134	0.017	8	0.123	0.169	0.026	0.131	0.165	8
	64 %	0.165	0.183	0.042	0.093	0.311	5	0.165	0.170	0.048	0.089	0.352	5
Intercropping	0 %	0.159	0.182	0.036	0.098	0.267	6	0.159	0.180	0.038	0.097	0.283	6
	16 %	0.171	0.187	0.047	0.089	0.347	4	0.171	0.182	0.049	0.085	0.363	4
	32 %	0.179	0.177	0.056	0.078	0.418	3	0.179	0.179	0.057	0.077	0.428	3
	64 %	0.185	0.179	0.063	0.072	0.464	2	0.185	0.176	0.065	0.070	0.482	2

Notes: Four levels of root exudates (0 %, 16 %, 32 % and 64 %) were applied to the soil of the same planting pattern. As an example, monocultured maize root exudates were applied to the monocultured maize. To prepare the above-stated levels of root exudates, 0, 16, 32 and 64 mL of each were diluted with deionized water to a final volume of 100 mL. The concentration of Pb and Cd in the soil as well as the plant biomass were given the weight of 0.5 to balance their respective contributions.

Table 3b
TOPSIS score and rank of wastewater treatments based on balancing soybean plant biomass as well as soil Pb and Cd content.

Planting pattern	Root exudate application rate	Normalized matrix		Euclidean distances		Performance Score	TOPSIS rank	Normalized matrix		Euclidean distances		Performance Score	TOPSIS rank
		Biomass	Cd	S-	S+			Biomass	Pb	S-	S+		
Monocropping	0 %	0.124	0.168	0.017	0.113	0.129	7	0.124	0.174	0.008	0.113	0.062	8
	16 %	0.135	0.183	0.011	0.103	0.098	8	0.135	0.179	0.011	0.103	0.100	7
	32 %	0.138	0.168	0.022	0.099	0.183	6	0.138	0.176	0.015	0.099	0.131	6
	64 %	0.211	0.179	0.088	0.028	0.757	3	0.211	0.170	0.088	0.026	0.775	2
Intercropping	0 %	0.162	0.179	0.039	0.076	0.337	4	0.162	0.180	0.038	0.076	0.335	4
	16 %	0.162	0.185	0.038	0.077	0.328	5	0.162	0.182	0.038	0.076	0.331	5
	32 %	0.210	0.175	0.087	0.028	0.758	2	0.210	0.178	0.086	0.028	0.755	3
	64 %	0.237	0.177	0.113	0.009	0.926	1	0.237	0.175	0.113	0.006	0.953	1

Notes: Four levels of root exudates (0 %, 16 %, 32 % and 64 %) were applied to the soil of the same planting pattern. As an example, monocultured soybean root exudates were applied to the monocultured soybean. To prepare the above-stated levels of root exudates, 0, 16, 32 and 64 mL of each were diluted with deionized water to a final volume of 100 mL. The concentration of Pb and Cd in the soil as well as the plant biomass were given the weight of 0.5 to balance their respective contributions.

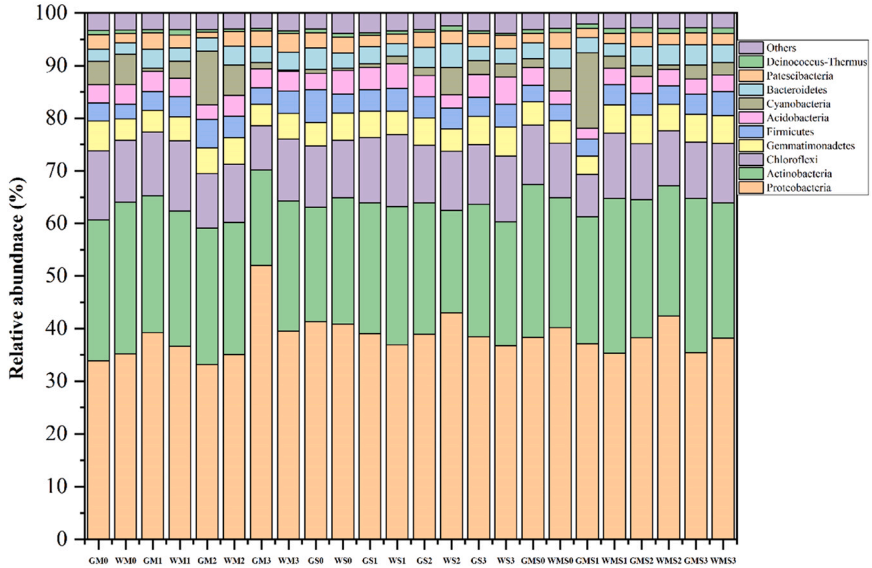

Fig. 5. Soil bacterial community composition in different treatments at harvest. All data are displayed as mean relative abundance ($n = 3$) of soil bacterial communities at phylum level. "G" and "W" refer to groundwater and wastewater, respectively. "M0″, "M1″, "M2″ and "M3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under mono-cropped maize, respectively; "S0″, "S1″, "S2″ and "S3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under mono-cropped soybean, respectively; "MS0″, "MS1″, "MS2″ and "MS3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under intercropping, respectively.

4.4. Planting patterns and root exudate application significantly altered heavy metal mobility in soil-plant system

Root exudate application had a significant effect on soil nutrient content, plant growth and transport of heavy metals. The *TF* is employed widely to assess the mobility of heavy metals in soil-plant systems (Wu et al., 2021). Results of this study demonstrated that under intercropping systems, the *TF* of Zn was higher, and that of Cd for soybean decreased in most cases due to root exudate applications. This may be because root exudates altered the physical and chemical properties, including the form and bioavailability of metals, in the rhizosphere, thus influencing absorption of heavy metals by plants (Agarwal et al., 2023). Application of organic acid can also enhance the antioxidant enzyme activity and mineral uptake in plants (Alshegaihi et al., 2023). Earlier investigations have shown that intercropping improves crop growth and facilitates the transfer of heavy metals from soil to different plant organs (Cao et al., 2021; Wang et al., 2020; Wu et al., 2021). These could be attributed to soil acidification and the related changes in the mobility of heavy metals in the rhizosphere (Kang et al., 2020). Therefore, the application of root exudates could provide an alternative solution for reducing the potential concentration of toxic heavy metals in soil and plants.

4.5. Soil bacterial community composition was sensitive to irrigation water source and root exudate application

Root exudate application significantly altered soil bacterial community structure. This findings is consistent with previous research (Han et al., 2022; Wen et al., 2022), which demonstrated the significant effect of applying root exudates on the composition of soil bacterial communities in soil irrigated with groundwater or wastewater. Furthermore, this study showed that the effects of root exudates on bacterial community were affected by the irrigation waters, crop types and planting patterns. This observation can be explained by the crucial function of root exudates in interactions between plants and soil at the rhizosphere (Kundu and Ganesan, 2023). Additionally, soil bacterial community composition showed a more marked response when exposed to 32 % of root exudates irrespective of irrigation water source. Variations in the community composition of soil bacteria were observed consistently based on irrigation water sources, crop types and planting methods. The results imply that root exudate application plays a role in the composition of soil bacterial community and that an optimum concentration of root exudates could potentially enhance the effectiveness with a specific planting pattern in the rhizosphere. This is significant in intercropping systems, where applying root exudates has been found to be particularly effective in enhancing plant and bacterial growth (Chai and Schachtman, 2022; Shi et al., 2011). This study showed that the application of root exudates can improve soil health, increase plant growth and the relative abundance of certain types of specific soil bacterial taxa, and at the same time, reduce heavy metals migration in soil-plant system.

5. Conclusions

Since there has been little research concerning the interactions between root exudate application and wastewater irrigation in controlling heavy metal mobility, crop performance and soil bacterial communities under intercropping system, we initially harvested root exudates from hydroponic culture. Subsequently, we carried out pot experiments, where maize or soybean was grown in either monoculture or interculture, and they were irrigated using either wastewater or groundwater and treated with root exudates at 0 %, 16 %, 32 %, or 64 % level. We concluded that the combined application of wastewater and root exudates substantially enhanced soil health and stimulated plant growth, particularly under intercropping system. The study found that when maize and soybean were grown together under WW irrigation and amended with a 64 % solution of root exudates, both plants observed high plant biomass and soil accumulated little Cd and Pb. Root exudates had the potential to reduce the migration of heavy metals in the soil-plant system. The use of root exudates had a significant impact on the composition of soil bacterial community. In general, root exudates applied with appropriate rates can effectively reduce the concentration of toxic heavy metals in the soil and improve plant growth, particularly under intercropping system. More research is necessary to investigate the various mechanisms by which root exudates and bacteria may reduce heavy metal accumulation in wastewater-irrigated soil. This knowledge is needed to ensure that wastewater can be safely utilized in agricultural systems.

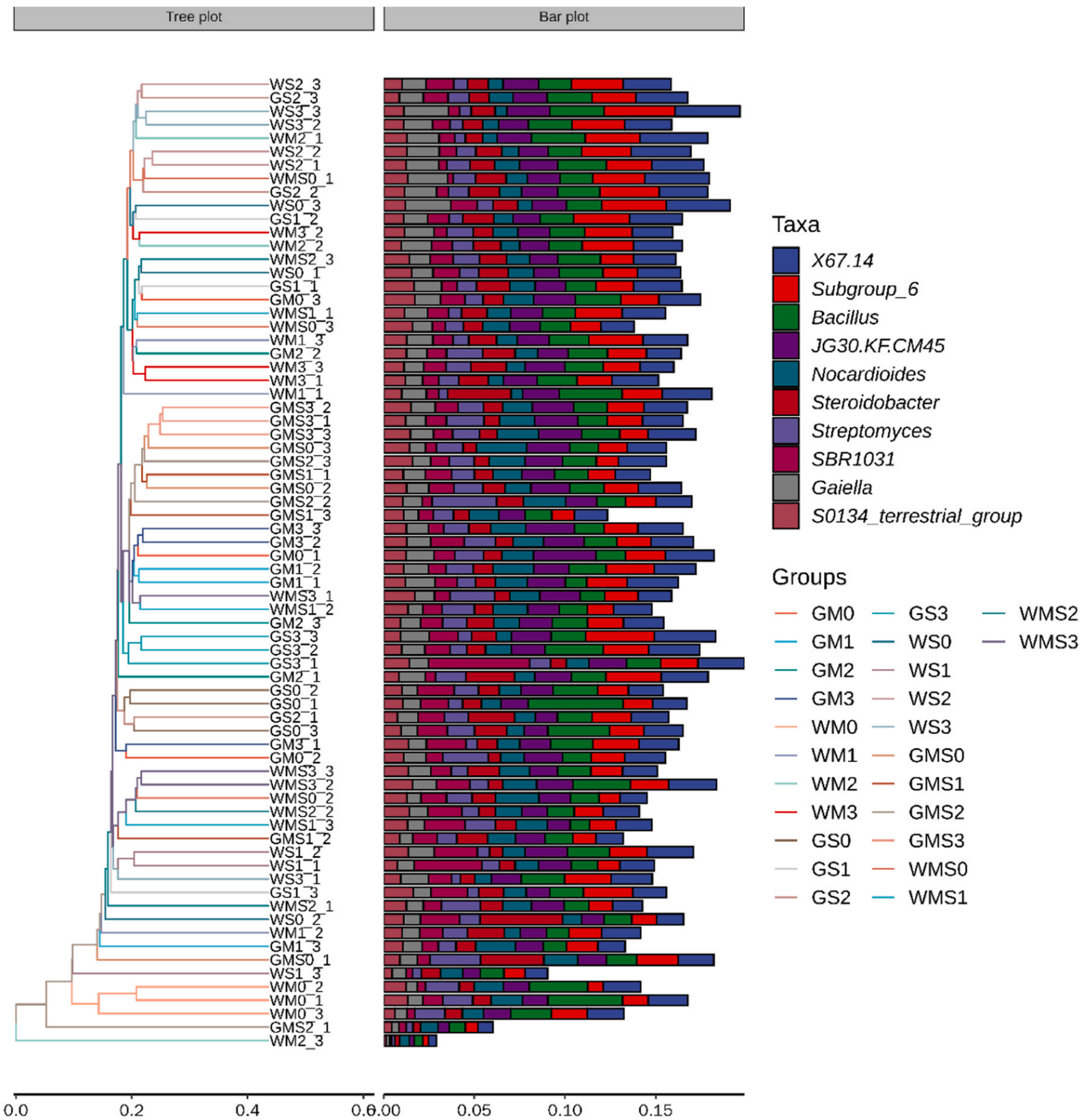

Fig. 6. Hierarchical cluster analysis of the top 10 soil genus in terms of the abundance in different treatments at harvest. The way these treatments clustered in the tree plot is according to their similarity to each other. "G" and "W" refer to groundwater and wastewater, respectively. "M0″, "M1″, "M2″ and "M3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under mono-cropped maize, respectively; "S0″, "S1″, "S2″ and "S3″ refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under mono-cropped soybean, respectively; "MS0″, "MS1", "MS2" and "MS3" refer to 0 %, 16 %, 32 % and 64 % application rate of root exudates under intercropping, respectively. The number "1″, "2″ and "3″ after the short underscore indicate each repetition.

CRediT authorship contribution statement

Conceptualization, Rakhwe Kama, Yuan Liu. and Zhongyang Li; methodology: Rakhwe Kama and Yuan Liu; software: Rakhwe Kama, and Bingjian Cui; validation: Zhongyang Li, and Yuan Liu; formal analysis: Rakhwe Kama; investigation: Rakhwe Kama and Yuan Liu; resources: Zhongyang Li; data curation: Rakhwe Kama, Shouqiang Zhao; writing—original draft preparation: Rakhwe Kama; writing—review and editing: Abdoul Kader Mounaila Hamani, Jibin Song, Maimouna Aidara, Chuncheng Liu, and Yuan Liu; visualization: Yuan Liu.; supervision: Zhongyang Li; project administration: Zhongyang Li; funding acquisition: Zhongyang Li and Yuan Liu. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115549.

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