

Intercropping *Pennisetum giganteum* and *Medicago sativa* for Iron-Manganese Mitigation and Control in Coal Gangue: Root System Dynamics and Phytoremediation Potential

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Abstract

Background and aim The diffusion of iron (Fe) and manganese (Mn) pollution caused by coal gangue accumulation has garnered significant attention. While intercropping is recognized as a promising remediation strategy, its efficacy and underlying mechanisms require further investigation.

Methods This study employed a leaching column experiment to evaluate the effects of intercropping *Pennisetum giganteum* Z.X.Lin and *Medicago sativa* L. on Fe/Mn concentrations, chemical fraction in coal gangue-contaminated soil and leachate, and plant root responses.

Results Results demonstrated that: (1) The *P. giganteum*/*M. sativa* intercropping system exerted synergistic regulatory effects on Fe/Mn contents and chemical fraction in coal gangue soils, reducing deep-layer (20–40 cm) soil Fe by 4.55%-5.04% and Mn by 12.01%-14.10%. It increased the residual Fe proportion by 8% and acid-extractable Mn proportion by 7%, while enhancing bioavailable Mn by 14.61%-21.75% at 20–40 cm depth. (2) Intercropping optimized root spatial distribution and Fe/Mn enrichment capacity, significantly increasing specific root length and root mass density. *M. sativa* root Fe and shoot Mn contents rose by 78.96% and 23.51%, respectively, while *P. giganteum* root Fe/Mn increased by 21.99% and 23.81%. Intercropping facilitated the conversion of Fe/Mn into residual fractions within plants, promoting their immobilization. (3) The intercropping system reduced Fe/Mn leaching outputs by 34.80% and 11.75%, respectively. Cropping pattern and root iron plaque Mn content were identified as primary drivers for Fe and Mn leaching, respectively.

Conclusions *P. giganteum*/*M. sativa* intercropping reduces Fe/Mn diffusion via complementary root vertical zonation, providing an effective technical approach for ecological restoration of coal gangue dumps.

Introduction

Coal gangue, a solid waste formed during coal mining and washing processes through the stripping and separation of mineral impurities associated with coal-bearing strata, represents one of the largest-volume industrial solid wastes globally (Liu et al. 2025b; Zheng et al. 2024). Its primary components include clay minerals, aluminosilicate minerals, and Fe-S minerals, alongside trace hazardous constituents (Li and Wang 2019). In the karst regions of southwest China, coal gangue production is substantial, characterized by high sulfide content and complex associated minerals. However, due to unique geographical and geological conditions, its comprehensive utilization rate remains low, leading to pronounced pollution issues (Dong et al. 2023; Zhang et al. 2022). Under precipitation leaching and mine water immersion, Fe and Mn ions originally occluded in coal gangue undergo mobilization and release, resulting in excessive Fe and Mn concentrations in surrounding water and soil environments. Additionally, sulfide oxidation generates sulfate, contaminating groundwater and adjacent soil (Reddy et al. 2025). Long-term stockpiling and weathering processes further release acidic wastewater, accelerating the mobilization of metallic elements (Li et al. 2021). Surveys indicate that Fe and Mn concentrations in 88% and 74% of groundwater samples, respectively, exceed the World Health

Organization (WHO) drinking water standards (Sharma et al. 2021). Chronic excessive intake of Fe and Mn may induce neurological disorders, immune dysfunction, and other health issues (Lv et al. 2022; Singh et al. 2010). Therefore, the mobilization-migration mechanisms, monitoring, and control strategies for Fe and Mn in coal gangue accumulation zones require urgent attention.

Selecting appropriate agronomic management measures is crucial for addressing soil metal contamination through phytoremediation, with remediation efficiency varying significantly under different management strategies (Takim 2012; Zhao et al. 2024). Plant intercropping primarily influences metal bioavailability in soil and plant metal uptake/accumulation indirectly by modifying the rhizosphere environment (Wan et al. 2021). Studies demonstrate that intercropping significantly increases soil exchangeable, reducible, oxidizable, and residual Cd fractions compared to crop rotation and monoculture (El-Tohory et al. 2023; Yang et al. 2025). Additionally, intercropping enhances plant accumulation of Fe and Mn (Senk et al. 2023). For instance, intercropping *Zea mays* L. with *Sedum plumbizincicola* increased maize biomass and yield while reducing Mn content in maize tissues and enhancing Mn uptake by *S. plumbizincicola*, effectively remediating Mn-contaminated soil (Li et al. 2024). Intercropping systems combining legumes and graminoids show high potential for achieving safe production and impeding metal dispersion. *Z. mays* and *Medicago sativa* L. intercropping improved phosphorus uptake and utilization efficiency by optimizing root morphology and distribution, thereby increasing *Z. mays* yield (Ma et al. 2023). *Oryza sativa* L. intercropping systems reduced grain Cd content by elevating soil pH (lowering Cd bioavailability) and promoting iron plaque formation on root surfaces, mitigating heavy metal contamination and enhancing safety (Xu et al. 2021). Furthermore, legume and graminoid intercropping enhances plant Fe uptake: Legumes secrete protons and phenolic compounds to acidify rhizosphere soil, reducing Fe^{3+} to Fe^{2+} and absorbing Fe via transporters (e.g., *AhIRT1*), while graminoids secrete phytosiderophores that bind Fe^{3+} in the rhizosphere, forming soluble complexes to increase available Fe concentrations (Dai et al. 2019). Currently, few remediation studies utilizing legume-graminoid intercropping systems exist for coal gangue stockpiles, and their efficacy in impeding Fe/Mn migration remains unclear.

M. sativa, a common leguminous plant, is characterized by its rich nutritional value, strong stress resistance, and well-developed root system (Guerchi et al. 2024). It effectively absorbs and accumulates heavy metals from contaminated soils and is widely adopted in intercropping systems (Chen et al. 2025). *M. sativa* can enrich heavy metals such as Cd, Cu, and Pb, demonstrating significant remediation potential for multi-metal contamination from coal gangue stockpiles (Song et al. 2025). When intercropped with *Lolium perenne* L., it enhances heavy metal resistance by reducing oxidative damage and boosting antioxidant enzyme activity, thereby alleviating metal-induced growth inhibition (Cui et al. 2018). Gramineous plants have numerous successful applications in ecological restoration (Patra et al. 2021). For instance, *Pennisetum giganteum* Z.X.Lin exhibits strong adaptability to mining areas, high biomass production, and considerable potential for remediating heavy metal-contaminated soils (Tan et al. 2022). Its extensive fibrous root network markedly improves soil texture and particle size distribution, effectively controlling soil and water erosion (Zhang et al. 2024). Following application of poly- γ -glutamic

acid (γ -PGA), *P. giganteum* shows significantly enhanced Hg absorption and translocation efficiency (Zhou et al. 2024). This species effectively accumulates As, Cd, Cu, and Zn, extracting 0.94–1.31 kg/ha of Cd annually from soils containing 8–100 mg/kg Cd while yielding 28–79 t/ha of dry biomass, combining remediation efficiency with economic benefits (Zhang et al. 2010). Although both *M. sativa* and *P. giganteum* show substantial promise in phytoremediation, their combined efficacy in impeding Fe/Mn migration from coal gangue stockpiles through intercropping remains inadequately characterized.

Given the geochemical and biological behavioral similarities and synergies between Fe and Mn, their coexistence can induce alterations in plant root architecture and soil microbial community structure, thereby influencing Fe/Mn migration-transformation processes and disrupting their chemical fraction and dynamic equilibrium in soil. Consequently, this study employed dynamic leaching column experiments to investigate the mitigation effects of *M. sativa* and *P. giganteum* intercropping on Fe/Mn diffusion. The research objectives included: (a) characterizing depth-dependent variations in Fe/Mn content and chemical fraction within coal gangue soil; (b) evaluating the impacts of different cropping systems on plant Fe/Mn accumulation and root morphological traits; and (c) identifying temporal patterns and controlling factors of Fe/Mn concentrations in leachate. Clarifying these intercropping-mediated mitigation mechanisms holds significant implications for remediating Fe/Mn-contaminated soils and protecting groundwater quality.

Materials and Methods

Experimental Materials

Coal gangue samples were collected from a coal gangue dump in Mohong Town, Fuyuan County, Yunnan Province, China (104°13'23.86" E, 25°26'40.54" N; elevation: 1,835 m). Four soil layers were sampled vertically: 0–10, 10–20, 20–30, and 30–40 cm. Agricultural soil and mushroom residue were sourced from the experimental greenhouse of Yunnan Agricultural University and Luliang Green Circle Mushroom Co., Ltd. (Qujing City, Yunnan Province, China), respectively, to supply essential nutrients for plant growth. All materials were air-dried, homogenized, and sieved (20-mesh for leaching experiments; 100-mesh for background analysis). *P. giganteum* cuttings were sourced from Luliang County Seedling Base, while *M. sativa* seeds were procured from Beijing Zhengdao Seed Co., Ltd. Background metal concentrations in coal gangue, soil, and mushroom residue are summarized in Table 1. Key heavy metals in coal gangue - As (4.07 mg·kg⁻¹), Cd (0.2 mg·kg⁻¹), Cr (207 mg·kg⁻¹), Hg (0.32 mg·kg⁻¹), Pb (15.1 mg·kg⁻¹), and Cu (175 mg·kg⁻¹) - were below limits of soil environmental quality risk control standard for soil contamination of agricultural land (GB 15618 – 2018).

Table 1
Background value of coal gangue, agricultural soil, and mushroom residue

Parameter	Coal Gangue	Mushroom Residue	Agricultural Soil
pH	7.45 ± 0.15	6.27 ± 0.18	5.24 ± 0.11
Organic Matter (g·kg ⁻¹)	105.26 ± 10.69	128.36 ± 18.34	30.47 ± 3.67
Total N (g·kg ⁻¹)	1.26 ± 0.26	2.82 ± 0.14	0.83 ± 0.08
Total P (g·kg ⁻¹)	0.93 ± 0.16	9.75 ± 0.94	1.11 ± 0.15
Total K (g·kg ⁻¹)	9.73 ± 0.54	10.19 ± 1.34	4.88 ± 0.55
Alkali-hydrolyzable N (mg·kg ⁻¹)	23.10 ± 3.14	168.03 ± 33.14	102.43 ± 12.07
Available P (mg·kg ⁻¹)	1.46 ± 0.05	39.82 ± 3.65	4.81 ± 0.09
Available K (mg·kg ⁻¹)	81.51 ± 10.07	122.93 ± 14.17	31.26 ± 2.33
Fe (mg·kg ⁻¹)	10268.34 ± 120.55	1380.02 ± 38.57	11.69 ± 2.67
Mn (mg·kg ⁻¹)	542.94 ± 52.39	154.87 ± 110.39	5.97 ± 0.74

Experimental Design

The leaching experiment utilized polyvinyl chloride (PVC) columns (20 cm internal diameter, 50 cm height), with the base lined with 300-mesh nylon cloth to prevent coal gangue loss and fitted with a leachate collection valve; sampling ports (3 mm diameter) were installed at 10 cm intervals along the column height to collect leachate from different soil depths. Each column was filled with 10 kg of coal gangue amended with mushroom residue (3% of gangue mass) and soil (10% of gangue mass) as nutrient supplements to enhance plant survival rates (Fig. 1). Four treatments were established: Control (CK), *P. giganteum* and *M. sativa* intercropping (I-PA), *P. giganteum* monoculture (SP), and *M. sativa* monoculture (SA), each replicated four times (16 columns total). Planting followed scaled-down field patterns: *P. giganteum* was propagated by cuttings with 5 cm plant spacing, while *M. sativa* was established by drill sowing with a 5 cm row spacing from *P. giganteum*.

To minimize inter-treatment interference, leaching columns were spaced 10 cm apart in a completely randomized design, with periodic positional randomization. Distilled water (500 mL) was regularly supplied to maintain soil moisture at 60% field capacity, ensuring optimal growth of *P. giganteum* and *M. sativa*. Leaching treatment was carried out every 10 days, for a total of 8 times. During each leaching event, distilled water was added in four separate doses at a rate of 500 mL every 15 minutes, resulting in a total irrigation volume of 2.0 L. This leaching intensity is equivalent to a rainfall rate of 25 mm·d⁻¹, which approximates the average annual precipitation intensity in the study area (Fuyuan County) and is

used to simulate field precipitation conditions. The leachate was collected through sampling ports after each leaching session.

Sample Collection and Analysis

Water sample collection and analysis: A total of eight leachate collections were carried out during the experiment. For sample collection, the soil solution sampler was inserted into the leaching column at stratified depths of 10 cm, 20 cm, and 30 cm. The leachate from a depth of 40 cm was collected through a switch device at the bottom of the column, and the total volume of the leachate was recorded after each leaching event. The collected water samples were immediately placed in polyethylene plastic bottles and brought back to the laboratory for analysis. The pH of the leachate was directly measured using a pH meter, while the contents of Fe and Mn were determined by atomic absorption spectrometry (AAS).

Soil sample collection and analysis: Soil samples were collected in layers from depths of 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm. The contents of Fe and Mn were determined using AAS. The fraction of Fe and Mn was analyzed by the three-stage sequential extraction procedure proposed by the European Communities Bureau of Reference (BCR) (Mossop and Davidson 2003). In this procedure, the acid-extractable fraction (F1) represents the bioavailable components, the reducible fraction (F2) and oxidizable fraction (F3) are considered potentially bioavailable components, while the residual fraction (F4) is the stable components.

Plant sample collection and analysis: After being washed with distilled water, the fresh plant samples were first subjected to blanching in an oven at 105°C for 30 minutes and dried to constant weight at 75°C. High-pressure digestion vessel decomposition method was employed to determine the contents of Fe and Mn in plants. The sequential chemical extraction method was used to analyse the fraction of Fe and Mn in plants. Specifically, 2 g of plant samples were sequentially extracted using 80% ethanol (EtOH), deionized water (d-H₂O), 1 mol/L sodium chloride (NaCl), 2% acetic acid (HAc), 0.6 mol/L hydrochloric acid (HCl), and HNO₃-HClO₄ mixture to measure the EtOH fraction, d-H₂O fraction, NaCl fraction, HAc fraction, HCl fraction, and residual fraction (PRES) (Jahangir et al. 2023; Li et al. 2020). Roots were washed with distilled water and scanned using an Epson Perfection V700 scanner (Seiko Epson, Japan), and then the WinRHIZO root image analysis software (Regent, Canada) was used to quantify root morphological parameters. The contents of Fe and Mn in iron plaque on root surfaces were determined using the sodium dithionite-citrate-bicarbonate (DCB) extraction method.

Statistical analysis

Root architectural traits: Specific root length (SRL) = Total root length / Root biomass. Specific root surface area (SRSA) = Root surface area / Root biomass. Root tissue density (RTD) = Dry mass per unit root volume (Campos et al. 2017; Sha et al. 2024). Experimental data were processed using Microsoft

Excel 2021, analyzed via ANOVA with SPSS (Version 27.0), Graphical visualizations were generated with Origin 2021, and R (v4.2.4) software was used to establish a random forest model.

Results and Analysis

Output Characteristics of Fe and Mn in Coal Gangue Soil under the *P. giganteum*/*M. sativa* Intercropping System

Variation Characteristics of Fe and Mn Contents in Coal Gangue Soil

The profile distribution of Fe content in coal gangue varied under different cropping patterns (Fig. 2a). At a soil depth of 20–40 cm, the Fe content in coal gangue soil under the *P. giganteum*/*M. sativa* intercropping system significantly decreased by 4.55%-5.04% compared to the control group. At a soil depth of 10–20 cm, the soil Fe content under *M. sativa* monoculture significantly dropped by 4.01%. Across all cropping patterns, the lowest total Mn content in coal gangue was observed in the 10–20 cm soil layer (Fig. 2b). Specifically, under *M. sativa* monoculture, the total Mn content in coal gangue in the 10–20 cm soil layer significantly decreased by 9.97%. In the topsoil (0–20 cm), the Mn content in coal gangue soil under *P. giganteum* intercropping significantly declined by 17.73%-18.73% compared to the control. In the deep soil (20–40 cm), the Mn content in coal gangue soil under *M. sativa* intercropping significantly decreased by 14.10% and 12.01%. Moreover, the Mn content in the deep soil under both *P. giganteum* monoculture and intercropping was significantly higher than that in the topsoil. As shown in Fig. 2c-d, compared to monoculture patterns, *P. giganteum* intercropping significantly reduced the Fe content in coal gangue soil, while *M. sativa* intercropping significantly decreased the soil Mn content. In summary, the intercropping of *P. giganteum* and *M. sativa* reduced the Fe and Mn contents in deep soil, demonstrating a complementary effect. *P. giganteum* intercropping reduced the Fe and Mn content in topsoil, while *M. sativa* intercropping reduced the Mn content in deep soil.

Variation Characteristics of the Chemical Fractions of Fe and Mn in Coal Gangue Soil

The proportion of occurrence fraction of Fe in coal gangue soil under different cropping patterns is shown in Fig. 3a. In all patterns, the residual fraction of Fe was the predominant fraction, accounting for 47%-55% of the total Fe content in the coal gangue soil, while the acid-extractable fraction with relatively high reactivity had a low proportion. Compared to the *M. sativa* monoculture pattern, *M. sativa* intercropping increased the proportion of residual Fe and decreased the proportions of available and potentially available Fe. Compared to the *P. giganteum* monoculture pattern, intercropping increased the proportion of reducible Fe, reduced the proportion of residual Fe, and had a relatively minor impact on the acid-extractable and oxidizable Fe. Mn in coal gangue soil was mainly in the acid-extractable fraction (Fig. 3b), accounting for 45%-52%, while the residual fraction with low reactivity had a low proportion. Under the *M. sativa* intercropping pattern, the proportion of reducible Mn slightly decreased, and the proportion of oxidizable Mn increased. Under the *P. giganteum* intercropping pattern, the proportions of acid-extractable, oxidizable, and reducible Mn all increased, while the proportion of residual Mn decreased. In summary, the intercropping patterns altered the occurrence fraction of Fe and Mn,

affecting their bioavailability. *M. sativa* intercropping passivated Fe in the coal gangue soil. *P. giganteum* intercropping increased the potentially bioavailable components of Fe and Mn, with a greater increase in the proportion of bioavailable Mn components, thereby enhancing the reactivity of Mn in the coal gangue soil.

The vertical distribution characteristics of Fe fraction in coal gangue soil under different cropping patterns are illustrated in Fig. 3c-e. Under the *M. sativa* intercropping pattern, the content of bioavailable Fe component in the 10–40 cm soil layer increased by 34.22%. In the *P. giganteum* monoculture pattern, the content of bioavailable component significantly increased with soil depth. Under both *M. sativa* monoculture and intercropping patterns, the potentially bioavailable components exhibited substantial variations with increasing soil depth. In contrast, under the *P. giganteum* intercropping pattern, the potentially bioavailable component decreased as soil depth increased. In all cropping patterns, the stable Fe component accounted for a relatively high proportion in the topsoil (0–20 cm). As soil depth increased, the content of stable Fe component under the *M. sativa* intercropping pattern decreased by 11.12%.

The variation trend of Mn fractions with soil depth differed from that of Fe (Fig. 3f-h). In the topsoil (0–20 cm) under different cropping patterns, the content of bioavailable Mn component gradually declined. Under the *M. sativa* and *P. giganteum* intercropping patterns, the content of bioavailable Mn component in the 20–40 cm soil layer increased by 21.75% and 14.61%, respectively. This indicates that intercropping enhanced the bioavailability of Mn in the deep coal gangue soil. Moreover, in the deep soil layer, bioavailable Mn component under all patterns was higher than control group. The potentially bioavailable Mn component in the topsoil gradually decreased, while in the 20–40 cm soil layer, they showed an upward trend under the *P. giganteum* and *M. sativa* intercropping patterns. Stable Mn component in the topsoil was low under the intercropping patterns, and under the *P. giganteum* intercropping pattern, residual Mn component in the 10–40 cm soil layer increased by 267%.

Root Characteristics and Fe/Mn Content in *P. giganteum* and *M. sativa* under Intercropping System

Root Morphology and Architecture of *P. giganteum* and *M. sativa* under Different Cropping Patterns

Significant differences in root length, root surface area, and root volume of *P. giganteum* and *M. sativa* were observed across different soil layers under both monoculture and intercropping systems, as shown in Fig. 4a-b. With the increase in soil depth, root length, root surface area, and root volume gradually decline. Under the *M. sativa* monoculture pattern, the root length in the topsoil (0–20 cm) is significantly greater than that in deeper soil layers. Compared to the monoculture pattern, the root length in the 10–20 cm soil layer under the *M. sativa* intercropping pattern exhibits a significant increase of 77.31%. At soil depths of 0–10 cm and 20–30 cm, there are notable differences in root surface area and root volume between the *M. sativa* monoculture and intercropping patterns. The root morphological parameters of *P. giganteum* are higher than those of *M. sativa*. Moreover, these parameters also demonstrate an increasing trend with soil depth. Under the *P. giganteum* intercropping pattern, the root length in the 30–40 cm soil layer shows a significant increase of 40.61% compared to the monoculture

pattern, and the root surface area in the 0–10 cm soil layer increases significantly by 33.50% relative to the monoculture pattern. At a soil depth of 20–40 cm, there are no significant differences in root surface area and root volume of *P. giganteum* between the intercropping and monoculture patterns.

Within the 0–40 cm soil profile (Fig. 4c-d), the specific root length (SRL) under the *M. sativa* intercropping pattern was significantly higher than that under the monoculture pattern ($p < 0.05$). The SRL in the intercropping treatment reached its maximum value in the 10–20 cm soil layer, while no significant differences in SRL were observed across different soil layers for monoculture *M. sativa* ($p > 0.05$). There were no significant differences in the specific root surface area (SRSA) of plants between the *M. sativa* monoculture and intercropping patterns, and the SRSA generally exhibited a decreasing trend with increasing soil depth. The root tissue density (RTD) under the *M. sativa* intercropping pattern was significantly higher than that under the monoculture pattern at all soil depths, and the RTD in the topsoil was notably higher than that in the deeper soil layers. Compared with the monoculture pattern, the SRL, SRSA, and RTD under the *P. giganteum* intercropping pattern all showed significant increases. In the monoculture system, the SRL of *P. giganteum* in the 0–10 cm soil layer was significantly higher than that in the 10–40 cm soil layer, while no significant differences in SRSA were found across different soil layers. Additionally, the RTD in the 0–10 cm soil layer was 25.85%–29.07% lower than that in the 10–40 cm soil layer. In the intercropping system, the SRSA and RTD in the 0–10 cm soil layer were significantly higher than those in the 10–40 cm soil layer.

2.2.2 Variation characteristics of Fe and Mn contents in *P. giganteum* and *M. sativa*

The spatial distribution characteristics of Fe and Mn in *M. sativa* and *P. giganteum* under four cropping patterns are depicted in Fig. 5a-b. One-way analysis of variance (ANOVA) revealed that the contents of Fe and Mn were significantly correlated with the cropping patterns ($p < 0.05$). There were no significant differences in the shoot Fe concentrations between *M. sativa* monoculture and intercropping, but both were significantly higher than those in *P. giganteum* monoculture and intercropping. Conversely, an opposite trend was observed for root Fe accumulation: the *M. sativa* monoculture pattern exhibited significantly lower root Fe content compared to the other three patterns, and there were also significant differences among the *M. sativa* intercropping, *P. giganteum* monoculture, and *P. giganteum* intercropping patterns. The root Fe content in the *M. sativa* intercropping pattern increased significantly by 78.96% compared to the monoculture pattern, indicating that intercropping significantly promoted root Fe enrichment in *M. sativa*. Similarly, the root Fe content in the *P. giganteum* intercropping pattern increased significantly by 21.99% compared to the monoculture pattern. The shoot Mn content in the *M. sativa* intercropping pattern was significantly higher than that in the *M. sativa* monoculture pattern, while there were no significant differences in shoot Mn content between the *P. giganteum* monoculture and intercropping patterns. The root Mn content in the *P. giganteum* intercropping pattern was significantly higher than that in the *P. giganteum* monoculture pattern. Intercropping increased the Mn content in the shoots of *M. sativa* and the roots of *P. giganteum* by 23.51% and 23.81%, respectively. These findings suggest that intercropping enhanced the root Fe enrichment capacity and shoot Mn enrichment capacity of *M. sativa*, as well as significantly improving the root Fe and Mn enrichment capacities of *P. giganteum*.

As shown in Table 2, significant differences were observed in the Fe content of iron plaque on root surfaces. For *M. sativa* and *P. giganteum* monoculture patterns, the Fe content in the iron plaque of the 20–30 cm soil layer was significantly higher than that in the 0–10 cm soil layer. In contrast, for *M. sativa* intercropping, the Fe content in the iron plaque of the 30–40 cm soil layer was significantly higher than that in the 0–30 cm soil layer, while for *P. giganteum* intercropping, the Fe content in the iron plaque of the 20–30 cm soil layer was significantly higher than that in other soil layers. Intercropping significantly increased the Fe content in the iron plaque on the root surfaces of both *P. giganteum* and *M. sativa* in the topsoil (0–10 cm) and deep soil (30–40 cm) layers, but had a relatively weak effect on the middle soil layer (10–30 cm). The Mn distribution exhibited an opposite trend. *M. sativa* intercropping inhibited Mn accumulation in the iron plaque on root surfaces in the deep soil layer (30–40 cm, with a 44% decrease compared to monoculture), but promoted an increase in Mn content in the iron plaque on root surfaces in the 0–30 cm soil layer. Mn in the iron plaque on *P. giganteum* root surfaces was mainly enriched in the 0–30 cm soil layer, with no significant differences between monoculture and intercropping ($p > 0.05$). These results indicate that intercropping selectively enhances Fe immobilization in the iron plaque on root surfaces in the topsoil and deep soil layers while optimizing Mn retention in the 0–30 cm soil layer by regulating rhizosphere processes.

Table 2
Contents of Fe and Mn in the iron plaques on plant root surfaces

Element	Cropping pattern	0-10cm	10-20cm	20-30cm	30-40cm
Fe(mg·kg ⁻¹)	SA	0.56 ± 0.02Cd	6.32 ± 0.65Bbc	14.88 ± 2.64Ab	0.87 ± 0.10Cc
	IA	4.28 ± 0.82Bc	2.93 ± 0.90Cd	3.74 ± 0.47BCc	8.75 ± 0.27Aab
	SP	9.70 ± 0.54Bb	8.79 ± 0.86Bb	20.41 ± 2.34Aa	7.90 ± 0.25Bb
	IP	15.57 ± 1.59Aa	17.97 ± 4.03Aa	12.55 ± 0.86Ab	12.16 ± 4.21Aa
Mn(mg·kg ⁻¹)	SA	3.07 ± 0.65Bb	4.05 ± 0.92ABb	3.45 ± 0.98Bb	5.07 ± 0.16Aa
	IA	4.92 ± 0.50Aa	5.36 ± 0.25Aa	5.47 ± 0.30Aa	2.84 ± 0.10Bb
	SP	5.62 ± 0.31Aa	5.36 ± 0.32Aa	4.96 ± 0.94ABa	3.70 ± 1.15Bb
	IP	5.38 ± 0.04Aa	5.23 ± 1.50Aa	5.59 ± 0.55Aa	3.36 ± 0.20Bb

Note: Uppercase letters (A, B, C, D) denote significant differences ($p < 0.05$) among different soil depths within the same cropping pattern; lowercase letters (a, b, c, d) indicate significant differences ($p < 0.05$) among different cropping patterns at the same soil depth. The same letter labels signify that there is no statistically significant difference between the compared groups ($p > 0.05$).

Differences in the Speciation of Fe and Mn in *P. giganteum* and *M. sativa*

The distribution of Fe speciation in the shoots and roots of *M. sativa* and *P. giganteum* under different cropping patterns is illustrated in Fig. 6a-b. In the shoots of monoculture *M. sativa*, the residual and d-H₂O fraction of Fe were predominant, accounting for 43% and 29%, respectively. Under the intercropping pattern of *M. sativa*, the proportion of residual Fe in the shoots increased, while that of d-H₂O Fe decreased. A similar trend was observed in the intercropping pattern of *P. giganteum*. In the roots, Fe was mainly present in the residual, NaCl, and d-H₂O fractions. Under intercropping, the proportion of residual Fe in the roots increased, whereas that of HCl and NaCl fractions decreased. The Mn speciation in plants under different cropping patterns is shown in Fig. 6c-d. In the shoots of monoculture *M. sativa*, Mn was primarily in the NaCl, HAc, and d-H₂O fractions. After intercropping, the proportion of residual Mn in *M. sativa* shoots increased significantly, while that of NaCl and d-H₂O Mn decreased. In the shoots of monoculture *P. giganteum*, Mn was mainly in the NaCl, HAc, HCl, and residual fractions. After intercropping, the proportions of HCl and residual Mn increased, while those of NaCl and HAc Mn decreased. In the roots of *M. sativa*, Mn was mainly in the residual and NaCl fractions, whereas in the roots of *P. giganteum*, it was primarily in the NaCl, d-H₂O, and EtOH fractions. After intercropping, the proportion of residual Mn increased, while that of NaCl and d-H₂O Mn decreased, indicating that intercropping promoted the conversion of Mn to the residual fraction in plants.

Output Characteristics of Coal Gangue Leachate and Fe-Mn in the *P. giganteum*/*M. sativa* Intercropping System

Variation Characteristics of Coal Gangue Leachate

The release of elements from coal gangue results from the coupled interaction of minerals with water and gas, generating a large volume of leachate under precipitation leaching. The dynamic trends of the coal gangue leachate volume over leaching days under different cropping patterns are shown in Fig. 7a, with a general gradual decline observed in all leachates. In the control group, the leaching volume was relatively high initially, increased briefly in the middle stage, and then decreased in the later stage. For the *P. giganteum*/*M. sativa* intercropping, *P. giganteum* monoculture, and *M. sativa* monoculture, the leaching volume continuously decreased with increasing leaching days, showing a relatively stable declining trend, with cumulative leachate volumes reduced by 12.41%, 10.18%, and 10.98%, respectively. The variation trends of the pH values of coal gangue leachate over leaching days are depicted in Fig. 7b. The initial pH values of all treatment groups were within the weakly alkaline range. The pH value in the control group remained relatively stable throughout the leaching process, indicating a minor impact of leaching on its pH. In the *P. giganteum*/*M. sativa* intercropping, *P. giganteum* monoculture, and *M. sativa* monoculture, the pH values exhibited a slow decline with increasing leaching days, decreasing by 0.59, 0.51, and 0.49, respectively, before and after leaching.

As shown in Fig. 7c, the Fe content in the coal gangue leachate exhibited a declining trend with the increase in leaching days. In the control group, the Fe content was relatively high in the initial leaching stage, increased briefly in the middle stage, and then tended to decrease in the later stage, which was

consistent with the variation trend of the leaching volume. The decline trend of Fe content in the leachate under the intercropping pattern was more pronounced than that under the *P. giganteum* monoculture and *M. sativa* monoculture patterns, with cumulative Fe outputs decreasing by 34.80%, 24.42%, and 22.79%, respectively. As shown in Fig. 7d, the Mn content in the coal gangue leachate also showed a decreasing trend with the increase in leaching days. The Mn content in the leachate under the *P. giganteum*/*M. sativa* intercropping pattern decreased at the fastest rate, while the decline trends under the *P. giganteum* monoculture and *M. sativa* monoculture patterns were relatively slow, with cumulative Mn outputs decreasing by 11.75%, 9.11%, and 8.43%, respectively, compared to the control group. The minimum leached Fe contents under the four cropping patterns were 3.05, 1.32, 1.75, and 1.90 times the standard limits for centralized surface water sources for drinking water in the Environmental Quality Standards for Surface Water (GB3838-2002), respectively. The minimum leached Mn contents were 18.54, 14.34, 15.01, and 15.18 times the standard limits, respectively. None of them met the standard limits for Fe and Mn specified in Class II and Class III of the Environmental Quality Standards for Surface Water (GB3838-2002).

Analysis of Influencing Factors and Relationships of Fe and Mn in Coal Gangue Leachate

As shown in Fig. 8a, this study employed an enhanced random forest model to evaluate the variable importance of factors influencing the Fe and Mn contents in the leachate, quantifying the impact of each factor on the target variables through %IncMSE (variable importance score). The results revealed that the cropping pattern had an extremely significant effect on the Fe content in the leachate ($p < 0.01$), with the highest variable importance score, indicating that different cropping patterns were the core driving factor regulating the Fe content in the leachate. This effect might be exerted by altering rhizosphere processes, microbial activities, or soil physicochemical properties, thereby influencing the release and migration of Fe. Acid-extractable Fe had a significant impact on the Fe content in the leachate ($p < 0.05$), ranking second in variable importance score, suggesting that as a highly reactive form of Fe, acid-extractable Fe played a crucial role in contributing to the Fe in the leachate. The variable importance scores of the remaining factors were relatively low and their impacts were not significant, indicating that these factors had relatively limited regulatory effects on the Fe content in the leachate.

As shown in Fig. 8b, the Mn content in iron plaque on root surfaces had an extremely significant impact on the Mn content in the leachate ($p < 0.01$), with the highest variable importance score, indicating that the Mn content in iron plaque on root surfaces was a key driving factor influencing Mn release in the leachate. The Mn content in coal gangue soil also had a significant impact on the Mn content in the leachate ($p < 0.05$), ranking second in variable importance score, suggesting that the total Mn content in coal gangue soil played an important role in determining the Mn content in the leachate. The variable importance scores of the remaining factors were relatively low and their impacts were not significant, indicating that these factors made relatively weak contributions to the Mn content in the leachate. Fe and Mn elements share similar physicochemical properties, commonly coexist in nature, and frequently participate together in the physicochemical and biochemical changes in soils and plants. As shown in Table 3, under the four cropping patterns, the contents of Fe and Mn in the leachate exhibited a linear

positive correlation. The *M. sativa*/*P. giganteum* intercropping pattern demonstrated the strongest Fe/Mn linear relationship ($R^2=0.733$), while the linear model explanatory rates were relatively lower under the monoculture patterns of *M. sativa* and *P. giganteum*. The linear relationship was relatively weakest under the control pattern.

Table 3
Linear relationships between Fe and Mn concentrations in leachate under different cropping patterns

Cropping pattern	Fitted equation	R ²	F	Sig.
CK	y = 0.693x + 1.239	0.506	30.74	< 0.001
I-PA	y = 0.901x + 1.118	0.733	82.24	< 0.001
SP	y = 0.738x + 1.198	0.561	38.37	< 0.001
SA	y = 0.879x + 1.091	0.675	62.34	< 0.001

Discussion

Synergistic Mechanisms of Root Morphology and Iron-Manganese Uptake by *P. giganteum* and *M. sativa* under Different Cropping Patterns

Plant root morphology and architectural parameters are key factors determining a plant's capacity to absorb metal elements (Wang et al. 2023). These parameters not only reflect the plant's ability to acquire water and nutrients but also reveal its response strategies and ecological adaptability to metal contamination (Zhang et al. 2023). This study demonstrates significant differences in the spatial distribution characteristics of roots and iron-manganese (Fe-Mn) contents between *P. giganteum* and *M. sativa* under different cropping patterns. In the intercropping system, root length, root surface area, and root architectural parameters of *P. giganteum* increased significantly (Fig. 4). In deeper soil layers (30–40 cm), root length, Fe content in iron plaque on root surfaces (Table 2), and root Fe content increased by 40.61%, 53.92%, and 21.99%, respectively, compared to monoculture patterns. Root Mn content also increased significantly (Fig. 5b), indicating that the intercropping pattern enhanced the root absorption efficiency of Fe and Mn in *P. giganteum*. Conversely, under the intercropping system, *M. sativa* exhibited notable increases in root length, root surface area, and specific root length in shallow soil layers (Fig. 4). Root Fe content increased significantly by 78.96% compared to monoculture patterns (Fig. 5a), and Mn content in iron plaque on root surfaces increased significantly in the 0–30 cm soil layer (Table 2), suggesting that the intercropping pattern also improved the root absorption efficiency of Fe and Mn in *M. sativa*.

Overall, the expansion of *P. giganteum*'s deep root system, coupled with the increased Fe content in iron plaque on its root surfaces, collectively enhanced the fixation of Fe in deeper soil layers. Meanwhile, *M. sativa*'s higher specific root length in surface soil promoted Mn absorption, leading to a reduction in Mn content within the 0–20 cm soil layer. This demonstrates that the spatial complementarity of the root

systems of these two plant species effectively mitigates Fe-Mn pollution caused by coal gangue soil. Such spatial ecological niche complementarity in root systems may facilitate plant root growth and development, thereby expanding the contact area between the rhizosphere and soil (Zu et al. 2024), and enhancing the interception and fixation of active Fe in the soil. Additionally, these two plant species may synergistically activate Fe³⁺ reduction and chelation processes through the combined action of rhizosphere exudates (such as protons, phenolic compounds, and phytochelatin), thereby improving Fe absorption efficiency (Sadeghzadeh et al. 2021).

This experiment demonstrates that the intercropping pattern enhances the Fe and Mn contents in both *M. sativa* and *P. giganteum*, primarily due to the complementary and synergistic rhizosphere functions between leguminous and graminaceous plants (Parks and Friesen 2025). Previous studies have shown that legume/gramineae intercropping can elevate plant Fe content by enhancing jasmonic acid signaling and suppressing ethylene signaling in the root systems of peanut and maize, thereby potentially improving the ecological adaptability of peanut to the intercropping system and promoting Fe uptake during the vegetative stage (Dai et al. 2018). Additionally, intercropping soybean and maize alters the composition of root exudates, leading to significant enrichment of coumarin and oleamide. Coumarin may facilitate Fe absorption, while intercropping also increases rhizosphere microbial diversity. Genera such as *Shinella* and *Rhizobium* could produce Fe chelators or enhance plant Fe uptake (Liu et al. 2025a) findings consistent with our results. As a legume, *M. sativa* secretes organic acids (e.g., citric acid, oxalic acid) and phenolic compounds (e.g., coumarin) from its roots, effectively acidifying the rhizosphere micro-environment and promoting the dissolution of Fe-Mn oxides (e.g., goethite, birnessite) in coal gangue, thereby releasing Fe²⁺ and Mn²⁺ ions (Agnello et al. 2016; Saad et al. 2017). In contrast, *P. giganteum*, a graminaceous plant with a well-developed fibrous root system, exhibits a marked increase in Fe content within root iron plaque under intercropping conditions (Table 2). This enhances its capacity to adsorb and oxidize Fe-Mn ions into stable oxide-bound forms, thereby preventing their downward migration (Wei et al. 2021).

Mitigation Effect and Remediation Potential of the *P. giganteum*/*M. sativa* Intercropping System on Fe-Mn Migration in Coal Gangue

Intercropping enhances remediation efficiency and reduces environmental risks through functional complementarity, rhizosphere regulation, and optimized resource utilization, representing an effective and low-cost ecological strategy for metal-contaminated soil remediation. Leaching experiments demonstrated that the *P. giganteum*/*M. sativa* intercropping system significantly reduced the cumulative output of Fe-Mn in coal gangue leachate, with decreases of 34.80% and 11.75%, respectively (Fig. 7). This effect is closely associated with the functional complementarity of the two plant species and the regulation of rhizosphere micro-environments. For instance, intercropping peanut and maize facilitated the enrichment of *Pseudomonas* around peanut roots, where secreted siderophores promoted plant uptake of iron compounds (Wang et al. 2024). The intercropping system notably enhanced root iron reduction capacity, improving Fe²⁺ absorption efficiency and subsequently reducing Fe content in both leachate and coal gangue soil (Figs. 7 and 2). Additionally, by modulating rhizosphere biochemical

processes, intercropping significantly improved plant nutrient acquisition efficiency for Zn, P, and K (Inal et al. 2007). In the *P. giganteum*/*M. sativa* intercropping treatment, the leachate pH decreased by 0.59. While studies indicate that lower pH promotes mineral dissolution (Luo et al. 2018), the formation of iron plaque significantly suppressed secondary Fe-Mn release from surface soil (Table 2), thereby reducing Fe-Mn concentrations in both leachate and coal gangue soil.

Root iron plaque can reduce the bioavailability of heavy metal elements through adsorption and immobilization, preventing their translocation into plants and mitigating their toxic effects (Deng et al. 2020). In this experiment, intercropping with *M. sativa* significantly increased Fe concentration in root iron plaque on deep roots and raised the proportion of residual Fe to 53% (Fig. 3a), indicating a reduction in bioavailability by promoting Fe transformation into stable fraction. This may be attributed to the oxidation of soil Fe by *Burkholderia* and *Pseudomonas* in the root iron plaque region, leading to the formation of precipitates or stable compounds (Wei et al. 2021). In contrast, intercropping with *P. giganteum* increased the proportion of acid-extractable Mn (Fig. 3b), likely because its roots secrete substantial amounts of low-molecular-weight organic acids (e.g., citric acid, oxalic acid). The carboxyl groups in these acids dissolve manganese oxides through proton exchange and chelation, converting stable manganese into exchangeable Mn^{2+} and increasing the proportion of bioavailable fraction (Flynn and Catalano 2019). Notably, Mn concentrations were higher in deep soil (20–40 cm) than in surface soil (Fig. 2b), with bioavailable Mn content increasing with soil depth, thereby elevating ecological risks. Additionally, Mn content in root iron plaque of *P. giganteum* decreased significantly in deep soil (Table 2), indicating reduced Mn adsorption capacity. Therefore, vertical mitigation measures should be integrated to prevent potential groundwater contamination risks.

This study demonstrates that the *P. giganteum*/*M. sativa* intercropping system reduces the migration risk of iron and manganese from coal gangue through spatial complementarity of root systems. However, the relatively high Mn concentrations in deep soil and leachate indicate that the risk of groundwater contamination still warrants attention. Moreover, leaching column experiments cannot fully replicate dynamic hydrological conditions in the field. Future research should integrate field trials to investigate the interactive mechanisms among root exudates, rhizosphere microorganisms, and speciation transformations of iron and manganese. This will facilitate the optimization of plant intercropping strategies for mitigating metal loss, providing a reference for the large-scale application of this technology in high-sulfur coal gangue stockpiles in southwest China.

Conclusions

The *P. giganteum* and *M. sativa* intercropping system significantly suppressed Fe/Mn pollutant migration in coal gangue soil through complementary root spatial distribution. Under intercropping, *M. sativa* developed a high specific root length and root mass density in shallow soil, while *P. giganteum* extended roots into deeper soil layers, collectively expanding the rhizosphere-soil interface. This system reduced Fe/Mn content in deep soil while increasing the residual Fe fraction and enhancing the bioavailable Mn fraction (particularly in the 20–40 cm depth), indicating elevated Mn mobility.

Intercropping enhanced plant metal immobilization capacity. *M. sativa* exhibited significantly increased Mn content in shoots and Fe content in roots, whereas *P. giganteum* showed elevated Fe/Mn accumulation in roots. Concurrently, intercropping raised the proportion of residual Fe/Mn fractions in roots while reducing more labile d-H₂O and NaCl fractions. This demonstrates promoted metal stabilization within plants, minimizing re-release risks.

Intercropping reduced Fe/Mn concentrations in coal gangue leachate, with strong linear correlation between both metals. Cropping pattern and Mn content in root iron plaques were identified as primary factors controlling leachate Fe and Mn outputs, respectively. Thus, the system effectively mitigates secondary release and vertical migration of Fe/Mn through root spatial complementarity, achieving significant pollutant diffusion mitigation in coal gangue stockpiles.

Declarations

Author contributions

Hedian Yan: Conceptualization, Data Curation, Formal Analysis, Writing-Original Draft, Writing-Review and Editing; Tao Zhang: Data Curation, Investigation, Visualization, Writing-Original Draft; Ying Li: Software, Supervision, Validation; Xinran Liang: Investigation, Methodology, Resources; Yongmei He: Project Administration, Funding Acquisition; Fangdong Zhan: Validation, Project Administration; Jianjun Chen (Corresponding author): Investigation, Methodology, Supervision, Writing-Review and Editing; Bo Li (Corresponding author): Conceptualization, Funding Acquisition, Resources, Supervision, Writing-Review and Editing; Yuan Li: Methodology, Supervision, Investigation.

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Data Availability

Data will be made available on request.

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Figures

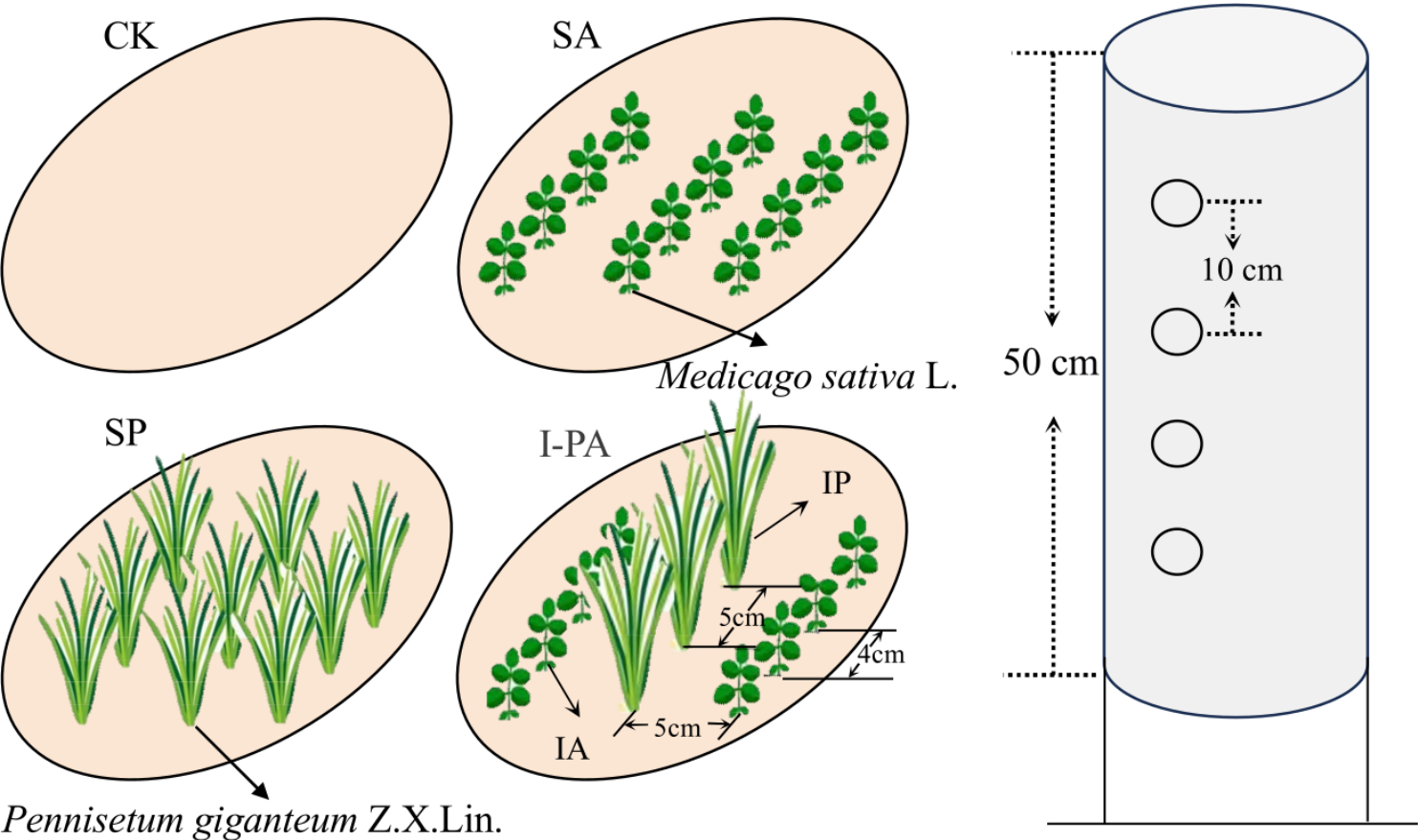


Figure 1

Experimental design diagram. CK: Control group; SA: *M. sativa* monoculture pattern; IA: *M. sativa* intercropping pattern; SP: *P. giganteum* monoculture pattern; IP: *P. giganteum* intercropping pattern; I-PA: *P. giganteum*/M. sativa intercropping. The same applies below.

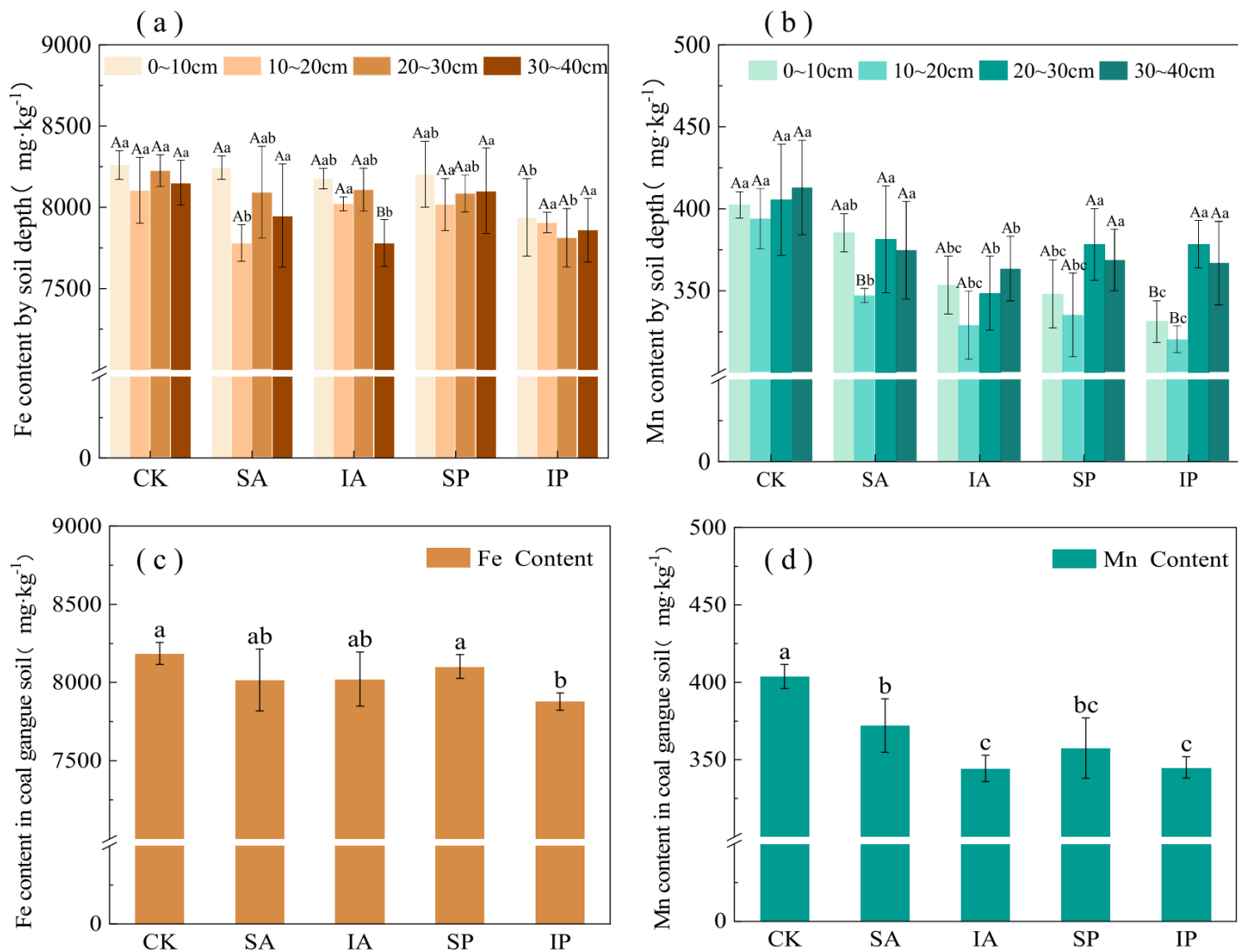


Figure 2

Contents of Fe and Mn in coal gangue soil. Fe (a) and Mn (b) contents in the soil profile of coal gangue under different cropping patterns, as well as Fe (c) and Mn (d) contents in coal gangue under different cropping patterns. Uppercase letters (A, B, C, D) indicate significant differences ($p < 0.05$) among different soil depths under the same cropping pattern; lowercase letters (a, b, c, d) indicate significant differences ($p < 0.05$) among different cropping patterns at the same soil depth. The same letter markings indicate that there is no statistically significant difference between groups ($p > 0.05$).

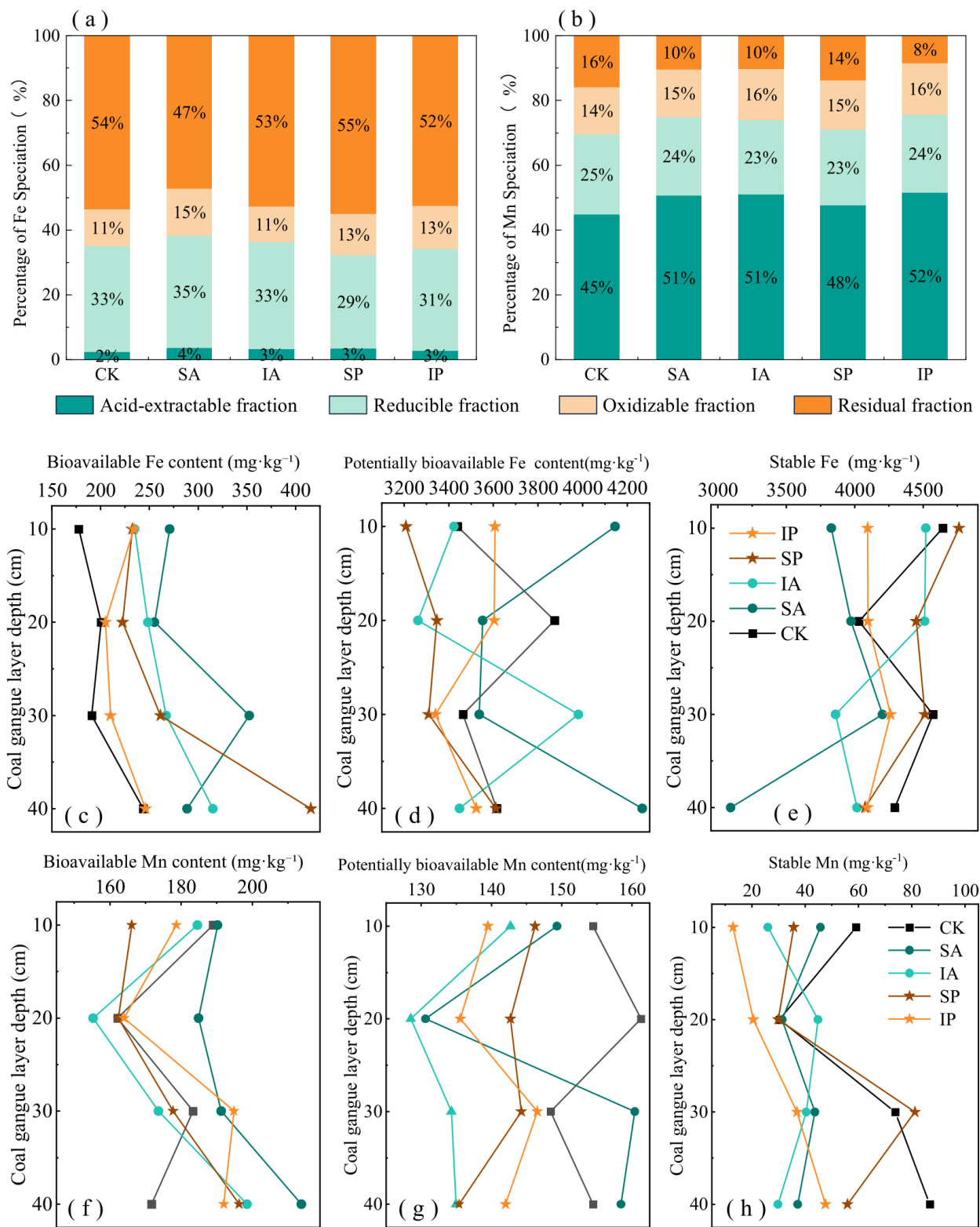


Figure 3

Proportions of Fe and Mn chemical fractions (**a, b**) and vertical distribution characteristics of Fe and Mn chemical fractions in coal gangue under different cropping patterns (**c-h**).

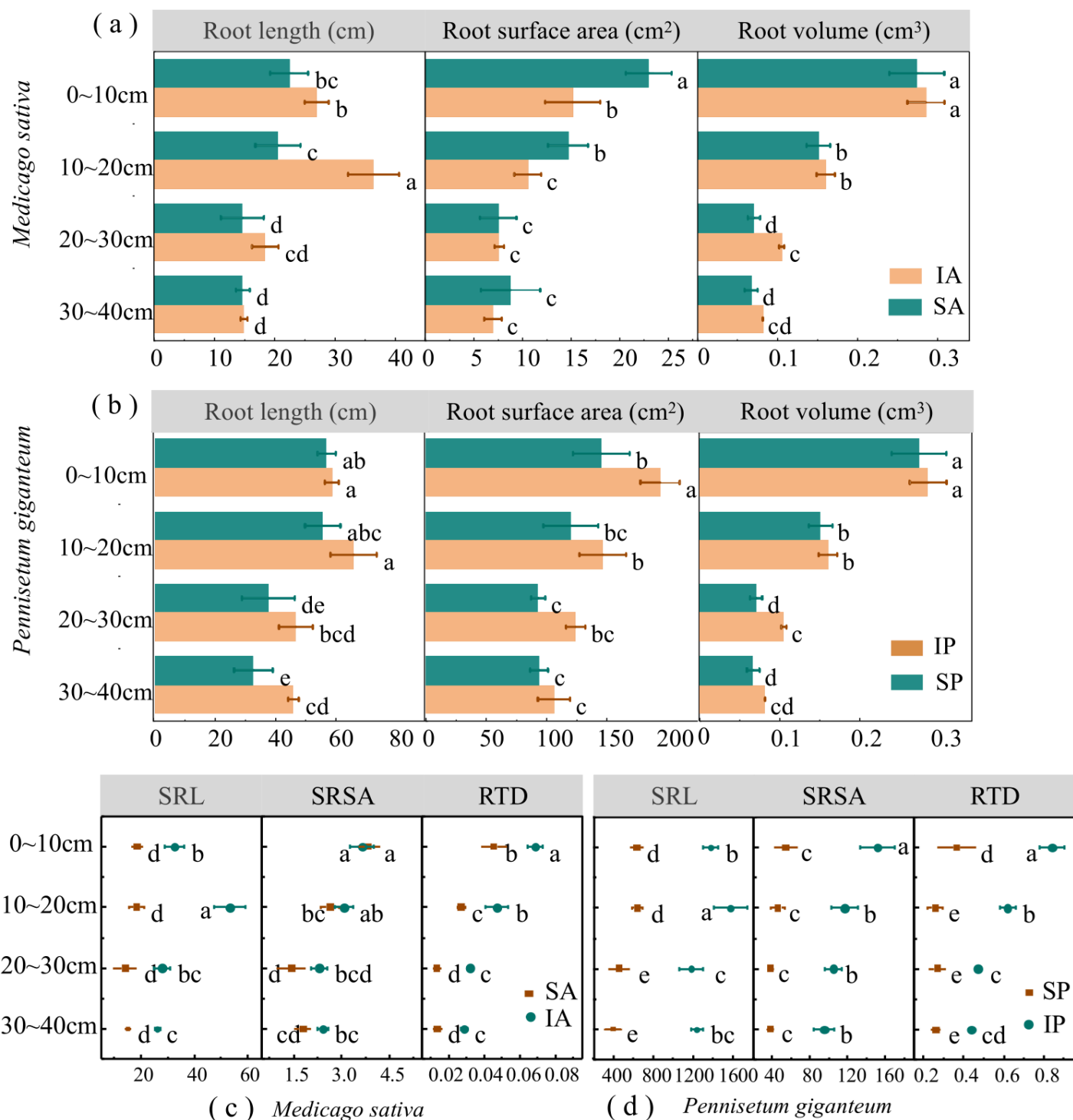


Figure 4

Root morphological traits (a-b) and root architectural characteristics (c-d) of *M. sativa* and *P. giganteum* under different cropping patterns. The same letter labels indicate no statistically significant differences between groups ($p > 0.05$).

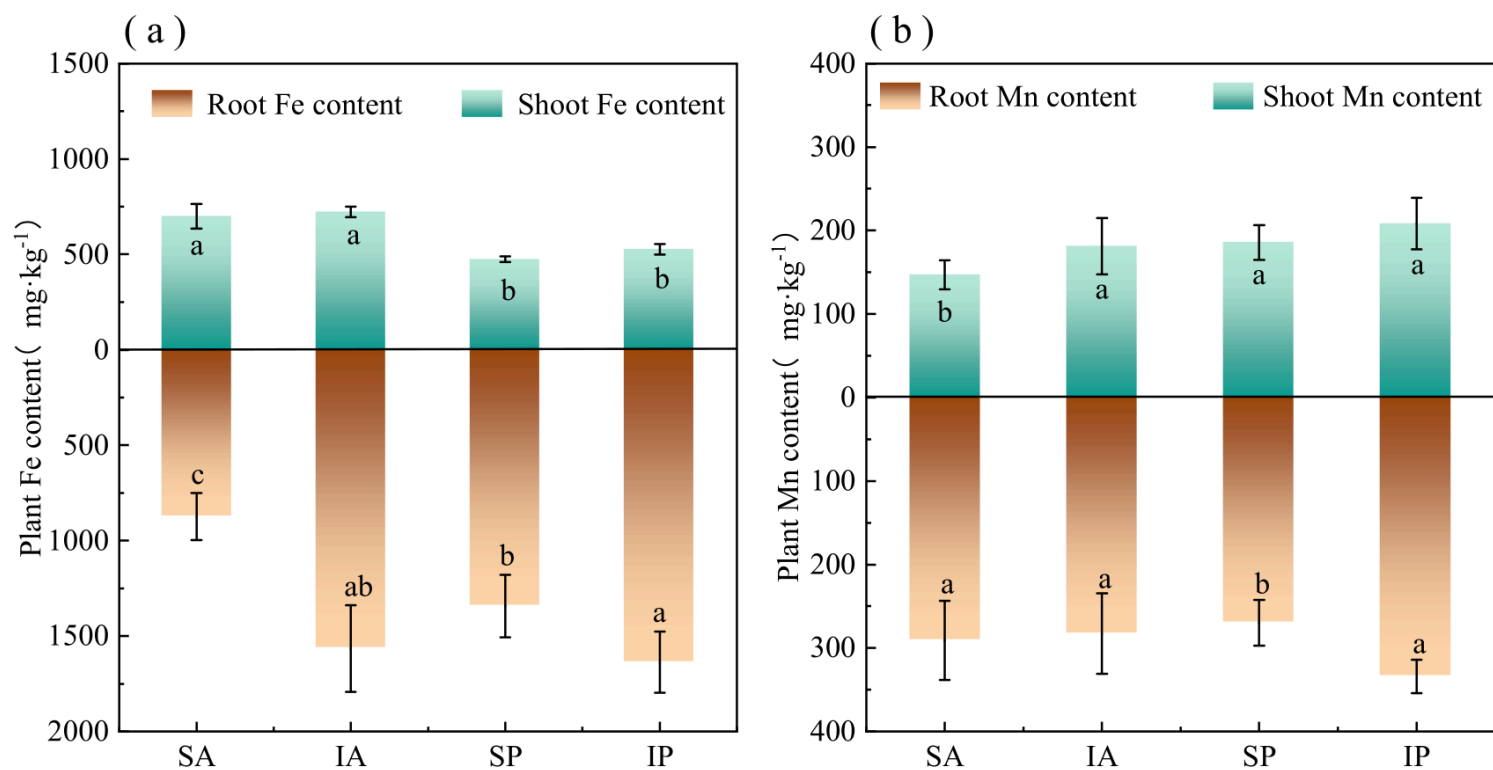


Figure 5

Contents of Fe **(a)** and Mn **(b)** in plants under different cropping patterns. The same letter labels indicate that there is no statistically significant difference between groups ($p > 0.05$).

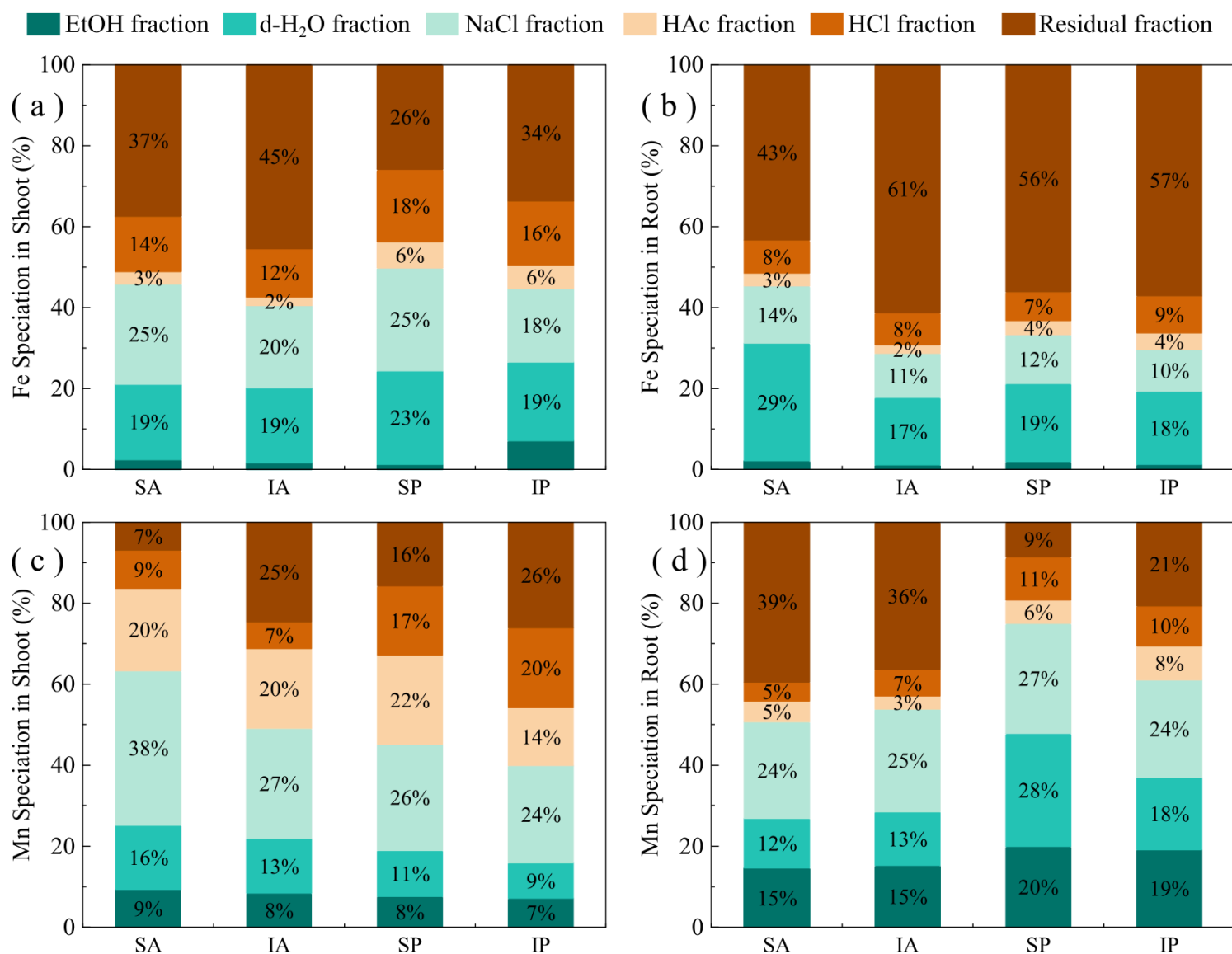


Figure 6

Speciation distribution of Fe and Mn in plants under different cropping patterns. Speciation distribution of Fe in shoots (a) and roots (b), and speciation distribution of Mn in shoots (c) and roots (d).

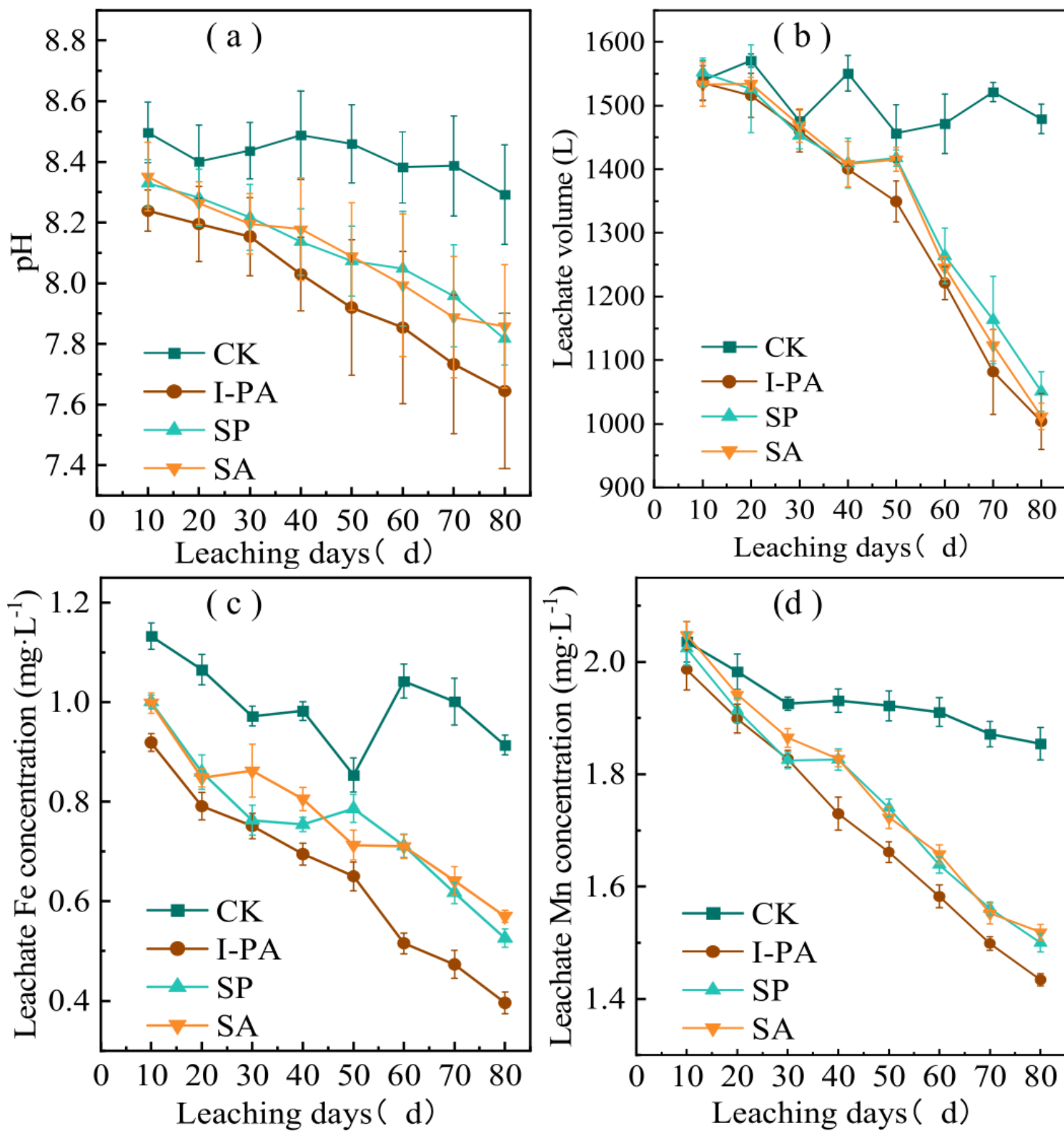


Figure 7

Variation characteristics of leachate volume (a), pH (b), Fe content (c), and Mn content (d)

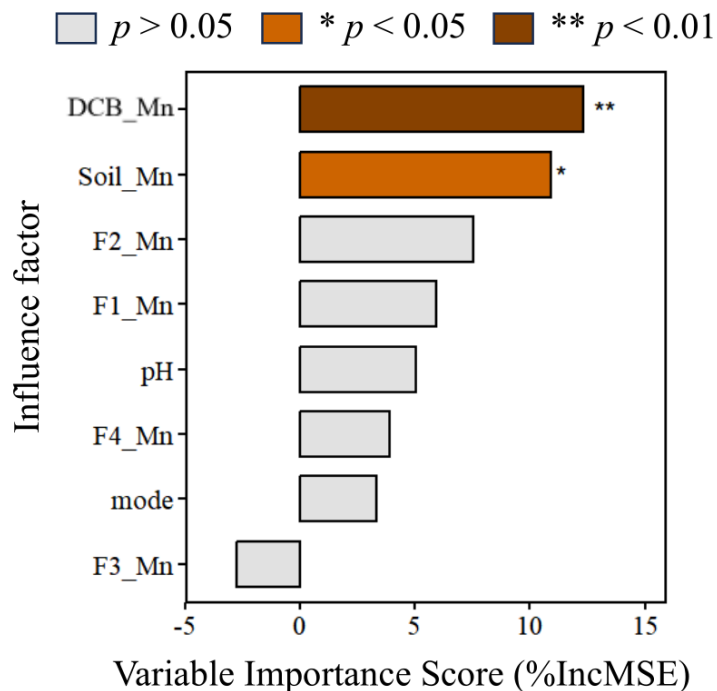
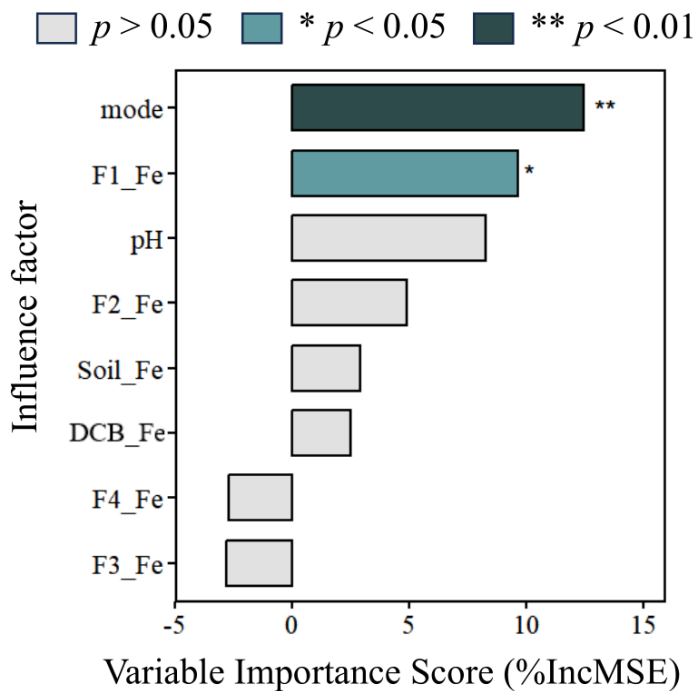


Figure 8

Variable Importance Assessment Based on an Enhanced Random Forest Model: Analysis of Influencing Factors for Fe Content (**a**) and Mn Content (**b**) in the Leachate. "Mode" represents different cropping patterns; "pH" denotes the pH value of the leachate; "Soil_Fe" indicates the total Fe content in coal gangue soil; "DCB_Fe" refers to the Fe content in iron plaque on root surfaces; F₁_Fe to F₄_Fe denote the acid-extractable, reducible, oxidizable, and residual fractions of Fe, respectively. The chemical speciation of Mn (F₁_Mn to F₄_Mn) follows the same classification.