Environmental Science and Pollution Research (2023) 30:109266–109282
https://doi.org/10.1007/s11356-023-30008-7

RESEARCH ARTICLE

# Coal slime as a good modifier for the restoration of copper tailings with improved soil properties and microbial function

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Received: 23 June 2023 / Accepted: 17 September 2023 / Published online: 28 September 2023

## Abstract

In recent years, the solid wastes from the coal industry have been widely used as soil amendments. Nevertheless, the impact of utilizing coal slime for copper tailing restoration in terms of plant growth, physicochemical characteristics of the tailing soil, and microbial succession remains uncertain.

Herein, the coal slime was employed as a modifier into copper tailings. Their effect on the growth and physiological response of Ryegrass, and the soil physicochemical properties as well as the bacterial community structure were investigated. The results indicated that after a 30-day of restoration, the addition of coal slime at a ratio of 40% enhanced plant growth, with a 21.69% rise in chlorophyll content, and a 62.44% increase in peroxidase activity. The addition of 40% coal slime also increased the content of nutrient elements in copper tailings. Following a 20-day period of restoration, the concentrations of available copper and available zinc in the modified tailings decreased by 39.6% and 48.51%, respectively, with 40% of coal slime added. In the meantime, there was an observed augmentation in the species diversity of the bacterial community in the modified tailings. The alterations in both community structure and function were primarily influenced by variations in pH value, available nitrogen, phosphorus, potassium, and available copper. The addition of 40% coal slime makes the physicochemical properties and microbial community evolution of copper tailings reach a balance point. The utilization of coal slime has the potential to enhance the physicochemical characteristics of tailings and promote the proliferation of microbial communities, hence facilitating the soil evolution of two distinct solid waste materials. Consequently, the application of coal slime in the restoration of heavy metal tailings is a viable approach, offering both cost-effectiveness and efficacy as an enhancer.

**Keywords** Coal slime · Restoration of copper tailings · Microbial community structure

## Introduction

Over the past few years, the coal mining and processing industry has seen a rapid increase, bringing with it a great deal of progress in terms of social, industrial, and human development. Nevertheless, the same industry has also created serious ecological issues due to its associated by-products (Li et al. 2018). As one of the by-products of coal, coal slime (CS) is tailing coal produced during coal flotation, which is characterized by high ash content, low calorific value, and fine particle size (Dong et al. 2021; Zhou et al. 2018). According to the Action Plan for Clean and Efficient Utilization of Coal (2015–2020), the output of coal slime in 2020 is approximately 360 MT (Guo et al. 2021), and they are usually stored in the form of accumulation, which easily causes land occupation and

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dust pollution. The large amount of water in coal slime makes the combustion and relocation difficult, thus limiting their wild application (Wang et al. 2022c). Therefore, there is an urgent need to find a new way to resource utilization of coal slime.

Presently, coal slime is being utilized in various ways, such as mixed combustion (Wang et al. 2022b), co-pyrolysis with biomass (Guo et al. 2021), and incorporation into construction materials (Shen et al. 2022). Among these methods, the addition of coal slime to impoverished soils is regarded as a viable approach for resource utilization (Novo et al. 2013; Rodriguez-Vila et al. 2014; Saleh et al. 2018). On the one hand, the addition of coal slime can improve the soil structure and increase the content of organic matter, nitrogen, phosphorus, potassium, and other nutrients (Yong et al. 2022), and the artificial soil prepared from coal slime has a high organic matter content, and plants planted on coal slime artificial soil grow well and have the potential to be used as topsoil (Weiler et al. 2018). On the other hand, the addition of coal slime can also reduce the heavy metal content and promote plant growth by changing the physicochemical properties of poor soils (Chu et al. 2017). In recent years, the solid wastes from coal industry have been widely used as soil conditioners, such as fly ash and coal gangue. The addition of coal gangue can increase pH, organic matter content, and nutrient content to improve soil conditions and help plant growth (Chu et al. 2020), and the addition of fly ash can reduce the bioavailability of heavy metals to plants (Manyapu et al. 2018). However, the majority of studies primarily concentrate on exploring the influence of solid wastes from coal industry on the physicochemical characteristics of the soil. There is a dearth of research investigating the variables that induce alterations in the composition of soil microbial communities following remediation efforts.

Copper tailings refer to the solid waste produced during the processing of copper ore, constituting a significant proportion of 95–99% of the original copper ore. These tailings are commonly kept in designated areas such as tailing dams or tailing ponds (Beylot and Villeneuve 2017). Nevertheless, it is worth noting that the presence of toxic metallic elements, including copper, zinc, cadmium, and chromium, within copper tailings poses a significant risk of migrating into the adjacent ecosystem (Conesa and Schulin 2010). Consequently, the discharge of metal leachate and acidic wastewater from the disposal process can result in severe contamination of the surrounding environment with heavy metals (Adrianto and Pfister 2022).

Meanwhile, copper tailings are difficult to restore because of their low pH and lack of nutrients, such as nitrogen, phosphorus, and potassium, which are needed for plant growth, and their presence often greatly affects the growth and development of plants in the surrounding ecosystem (Munir et al. 2021). However, the application of coal slime in the restoration of copper tailings is a new practical, and sustainable development method. Using a small quantity of coal slime makes it possible to satisfy the essential nutrient requirements for plant development and expedite the process, therefore compensating for the inadequate nutritional content of copper tailings (Hamid et al. 2020; Kang et al. 2011). Coal slime has excellent ion exchange performance due to its rich pore structure. It can adsorb heavy metals in copper tailings (Guo et al. 2022a). In addition, coal slime has been proven to have a rich microbial community (Wang et al. 2023). Therefore, in combination with phytoremediation, the coal slime-plant system may achieve restoration effects on copper tailings through rhizosphere effects and enhanced microbial diversity (Zhao et al. 2022).

In this study, ryegrass (*Lolium perenne* L.) and different amounts of coal slime were used to improve copper tailings. Ryegrass is an ideal plant for heavy metal restoration, which show great potential for heavy metal tolerance and can produce a large amount of biomass (Ke et al. 2021a; Ke et al. 2021b; Nie et al. 2023). The present study aimed to (1) evaluate the restoration effect of different contents of coal slime on copper tailings through the growth of ryegrass, (2) study the influence of different contents of slime on the physical and chemical properties of copper tailings, and (3) study the relationship between the evolution of microbial community driven by coal slime and environmental factors. The results of this study aim to demonstrate the possibility of coal slime as waste for copper tailing restoration and provide a new idea for green restoration of copper tailings.

## Materials and methods

### Pot incubation experiments

The copper tailings used in this experiment were from Daye, Hubei (114°56′4″E, 30°5′37″N). A portion of the copper tailings was allocated for growing studies, while another portion was subjected to grinding and afterward sieved through a 1mm mesh to assess the physicochemical characteristics of the tailings.

The tailings have a pH value of 7.03 and an electrical conductivity (EC) of 2540 μS/cm. The principal constituents of the tailings are $SiO_2$ and CaO, while the total concentrations of copper and zinc are 489 mg/kg and 594 mg/kg, respectively (Supplementary Table S1). Copper and zinc in the test tailings (standard values of 100 and 250 mg/kg for copper and zinc at pH 7.03) were above the risk control standard for soil contamination of agricultural land (GB 15618-2018) (Hamid et al. 2020).

The coal slime used in this experiment comes from Ordos, Inner Mongolia (110°4′27″E, 39°29′16″N), which is

**Table 1** Effects of content, time, and their interactions on measured parameters based on ANOVA analysis.

| Parameter | Content | Time | Content × Time |
|---|---|---|---|
| Plant height | 9.428*** | 322.07*** | 1.657* |
| Fresh weight | 5.297*** | 539.134*** | 3.948*** |
| Germination rate | 1.827 ns | 2.161 ns | 0.64 ns |
| Chlorophyll | 89.286*** | 130.097*** | 18.03*** |
| Catalase | 4.056* | | |
| Peroxidase | 0.61 ns | | |
| Superoxide dismutase | 0.813 ns | | |
| Root copper content | 2.33 ns | | |
| Leaf copper content | 0.721 ns | | |
| Root zinc content | 9.903* | | |
| Leaf zinc content | 0.63 ns | | |
| pH | 21.717*** | 10.194*** | 2.342* |
| EC | 7.203*** | 10.491*** | 3.277* |
| Available nitrogen | 3.026* | 14.68*** | 2.766* |
| Available phosphorus | 3.989*** | 13.053*** | 4.135* |
| Available potassium | 7.339*** | 1.892 ns | 10.75*** |
| Urease | 1.639 ns | | |
| Phosphatase | 0.303 ns | | |
| Catalase | 11.081** | | |
| DTPA-Cu | 388.928*** | 149.019*** | 13.974*** |
| DTPA-Zn | 4.247** | 29.383*** | 5.295*** |

*ns* non-significance

* Significance level (*F* value): $p < 0.05$. ** Significance level (*F* value): $p < 0.01$. *** Significance level (*F* value): $p < 0.001$

a kind of waste generated after coal flotation, and its physical and chemical properties are shown in Supplementary Table S1. Ryegrass seeds are purchased from Jiangsu Mengyun Seed Co., Ltd.

This experiment was carried out in pots (Chu et al. 2020), and copper tailings were uniformly mixed with different contents of coal slime (0, 10, 20, 30, 40, 50, and 100%), adding organic fertilizer with a mass fraction (w/w) of 1 and 0.1% of nitrogen, phosphorus, and potassium compound fertilizer to ensure the nutritional needs of normal plant growth. Add enough water to make the modified tailings completely wet and leave it for 3 days to make the nutrients spread evenly. In addition, 0% and 100%, respectively, represent copper tailings and coal slime, and copper tailings were used as the control group.

Ryegrass (*Lolium perenne* L.) seeds were sterilized with 10% $H_2O_2$ for 5 min, washed three times with deionized water and then screened with large, plump particles (Li et al. 2022b). Each seedling pot was filled with 150 g of modified tailings and sown with 20 ryegrass seeds, and deionized water was added every 48 h to maintain the 70% water-holding capacity of the modified tailings. Each proportion of modified tailings was set up in four parallel groups and ryegrass was grown in a light incubator at a temperature of 25 ± 1 °C and a light/dark cycle of 14:10. The pot experiment was replicated in three groups and harvested 10, 20, and 30 days after sowing.

## Plant property and enzyme activity analysis

The study involved the assessment of germination rate, plant height, and fresh weight of ryegrass. The washed ryegrass samples were subjected to enzyme deactivation for 15 min in an oven at 105 °C, followed by drying to constant weight at 70 °C to separate the leaves and roots. The pretreated ryegrass samples were treated by the ashing method (Akinyele and Shokunbi 2015), and the ash was dissolved with 1% nitric acid. The solution was analyzed by atomic absorption spectrometry (ZEEnit700, Analyjena, Germany) to determine the Cu and Zn in the leaves and roots of ryegrass.

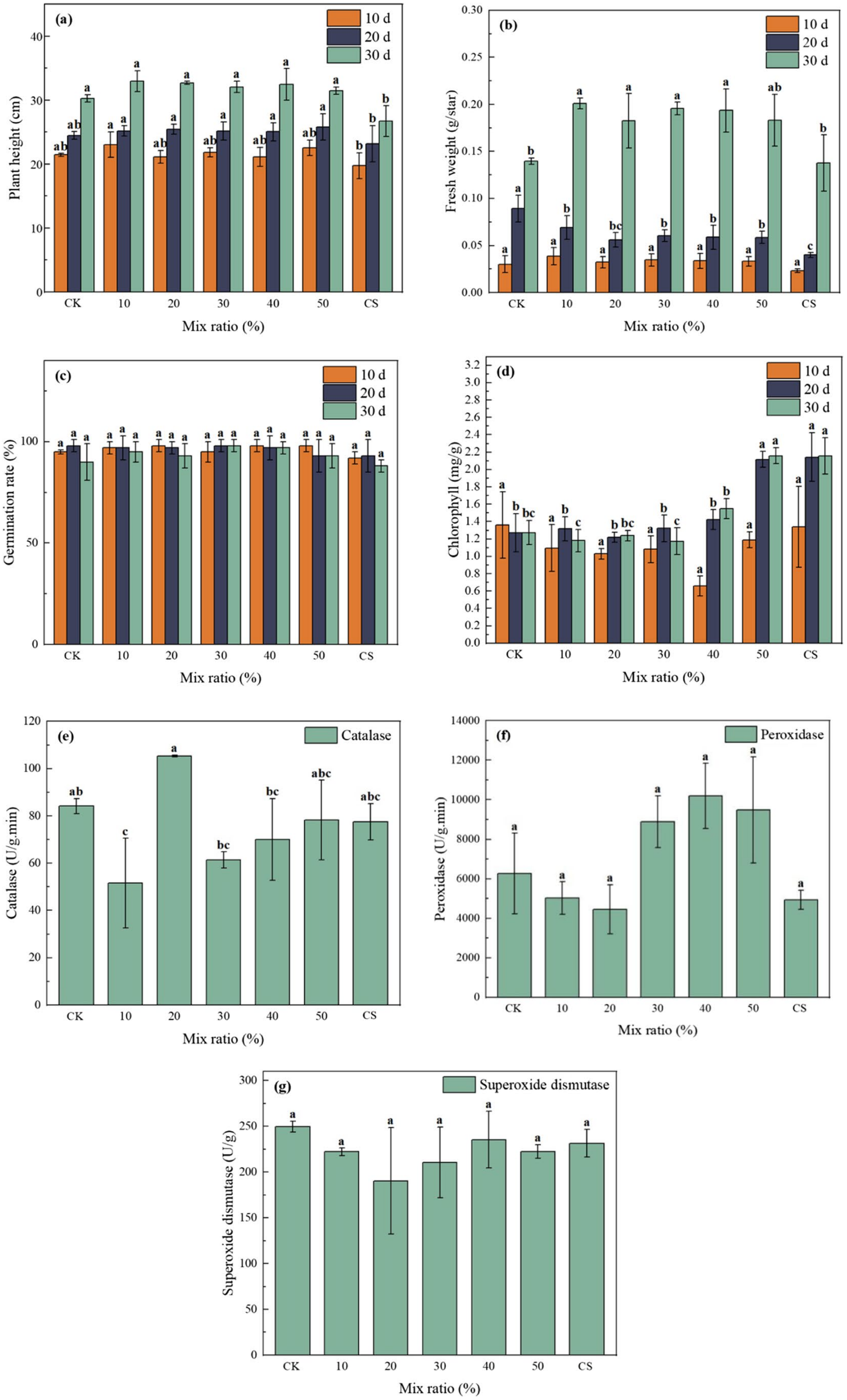
The chlorophyll content of ryegrass was determined by spectrophotometry (Orion Aquamate 8000, Thermo Fisher Scientific, USA). The determination of plant enzymes included catalase, peroxidase, and superoxide dismutase, and the determination of enzyme activity is shown in the supplementary material (Text S2).

## Soil property and enzyme activity analysis

The pH and conductivity were determined using a glass electrode at modified tailings to water ratio (w/v) of 1:2.5 (Yang et al. 2016), the available nitrogen (AN) was determined by alkaline hydrolysis diffusion method, and the available phosphorus (AP) was determined by Mo-Sb colorimetric method (Arif et al. 2018). The available potassium (AK) was determined by flame photometry (Yoo et al. 2018).

Total Cu and Zn in copper tailings and coal slime(CS) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS; Thermo Fisher, Wilmington, DE, USA) (Li et al. 2022a), and the percentage of major element oxides is determined by X-ray fluorescence (XRF; Shimadzu, XRF-1800) for determination (Munir et al. 2018). The available Cu and Zn in modified tailings were measured by DTPA extraction (Zhang et al. 2021). The Cu and Zn components in modified tailings were determined by the European Community Bureau of Reference (BCR) sequential extraction method (Zemberyova et al. 2006).

The determination of modified tailing enzymes included urease, phosphatase, and catalase, and the determination of enzyme activity is shown in the supplementary material (Text S3).


(a)
Plant height (cm)
0
10
20
30
40
10 d
20 d
30 d
CK
10
20
30
40
50
CS
Mix ratio (%)
(b)
Fresh weight (g/star)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
(c)
Germination rate (%)
0
50
100
(d)
Chlorophyll (mg/g)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
(e)
Catalase
Catalase (U/g.min)
0
20
40
60
80
100
120
(f)
Peroxidase
Peroxidase (U/g.min)
0
2000
4000
6000
8000
10000
12000
14000
(g)
Superoxide dismutase
Superoxide dismutase (U/g)
0
50
100
150
200
250
300

◂**Fig. 1** Plant height (**a**), fresh weight (**b**), germination rate (**c**), chlorophyll (**d**), catalase (**e**), peroxidase (**f**), and superoxide dismutase (**g**) under different contents of coal slime treatment. Plant enzyme activity was measured at 30 days of restoration. Different letters above the error bar indicate significant differences among the treatments at $p < 0.05$. ANOVA results are shown in Table 1

### High-throughput sequencing analysis

DNA was extracted from the modified tailings using the MagaBio DNA Kit BSC48L1E-G (Bioer Technology, CHN). After extraction, NanoDrop One (Thermo Fisher Scientific, USA) was used to detect concentration and purity. The V4 region of the bacterial 16s rDNA gene was amplified using the primers 515F (3′-GTGCCA GCMGCCGGTAA-5′) and 806R (3′-GGACTACHVGGG TWTCTAAT-5′). The concentration and length of the PCR products were examined by 1% agarose gel electrophoresis; the fragments were controlled to an average size of about 290–310 bp. E.Z.N.A.® Gel Extraction Kit (Omega, USA) was used to recover PCR mixed products, and TE buffer was used to elution and recover the target DNA fragments. The database building was carried out according to the standard process of NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The amplicons were sequenced using a PE250 (Illumina Nova 6000, USA).

The raw data obtained were used to remove primers from the sequences using qiime2, and the mass numbers were selected for noise reduction. The clustering of OTU was performed based on 97% sequence similarity, and then the OTU was compared with the Silva database to generate species annotations.

### Statistical analysis

The vegan package (version 4.1.1) in R language was used for alpha diversity analysis; principal coordinate analysis (PCoA) based to assess differences in microbial community composition, RDA analysis, Pearson and Spearman correlation analysis were used to evaluate the relationship between microorganisms and environmental factors. PICRUST2 was used to predict the function of bacteria in the modified tailings.

The results are expressed as mean ± standard deviation; statistical analysis was performed using SPSS software (Version 26) and STAMP, and data were tested for independence, normality, and chi-square before ANOVA analysis, significance between means was tested using Duncan's multiple ranges ($p < 0.05$), and plots were performed using Origin (Version 2021) and the R language ggplot2 package (version 4.1.1).

## Results

### Plant growth parameters

#### Plant growth and enzyme activity

Ryegrass exhibits a remarkable capacity to withstand and endure elevated concentrations of heavy metals present in tailings (Li et al. 2020). Consequently, ryegrass was sown in the altered tailings of this study, and the efficacy of the tailings in remediating the environment was assessed based on the growth and physiological reactions of the ryegrass. Plant height and fresh weight increased significantly as restoration time increased. As shown in Fig. 1(a), (b), and (d), after 30 days of restoration, CS application increased ryegrass plant height, fresh weight, and chlorophyll content. The addition of CS resulted in an increase in ryegrass growth (increased plant height and fresh weight). Meanwhile, 50% CS addition significantly increased chlorophyll content by 69.58% compared to the control phase (1.27 mg/g). However, the high percentage of CS also inhibited plant germination, as shown in Fig. 1(c); the germination of plants decreased significantly when the addition amount exceeded 40%. (The germination rate remained at 97% before 40% coal slime content, and dropped to 93% or even lower after the coal slime content exceeded 40%). The results of the ANOVA analysis showed that the physical and chemical properties of ryegrass were significantly affected by restoration time and appropriate CS addition. The experimental results show that the addition of CS can promote plant growth, indicating that it is feasible to use coal slime for copper tailings restoration, but the additional amount should not exceed 40%. The addition of coal slime more than 40% will affect the seed germination.

The study of the physiological reaction of plants to heavy metal stress in modified tailings plays a significant role in assessing the restoration of copper tailings through coal slime. Many studies have concentrated on investigating the impact of heavy metals on the activity of antioxidant enzymes in plants (Chen et al. 2015). Catalase (CAT) is widely distributed inside the cellular structure of plants and functions as a very efficient scavenger of reactive oxygen species. The extent of enzyme activity plays a crucial role in determining the magnitude of oxidative damage inflicted upon the plant (Anjum et al. 2016). As shown in Fig. 1(e), the addition of CS resulted in a reduction of catalase activity in ryegrass as compared to the control group. Peroxidase (POD) is a significant respiratory enzyme found in plants, and its functionality has a strong correlation with the metabolism of phenolic compounds and the resistance mechanisms of plants

(Stefanowicz et al. 2021). As shown in Fig. 1(f), 40% CS treatment boosted peroxidase activity in ryegrass, increasing its activity intensity by 62.44%. Superoxide dismutase (SOD) serves as a scavenger of biological free radicals, playing a crucial role in eliminating excessive superoxide radicals inside the body during stressful conditions and regulating reactive oxygen metabolism (Mondola et al. 2016). As shown in Fig. 1(g), the addition of CS had no significant effect on superoxide dismutase activity in ryegrass when compared to the control.

The findings of the experiments revealed that the addition of coal slime increased the activity of plant peroxidase while decreasing the activities of catalase and superoxide dismutase, which were connected to heavy metal stress (Zhang et al. 2007). When the content of heavy metals in the environment was reduced, the lower activity of enzymes was sufficient to resist oxidative damage (Wang et al. 2018), indicating that the addition of coal slime created a good environmental condition for plant growth.

### Distribution of heavy metals in plants

The addition of CS to ryegrass enhanced the enrichment of heavy metals in both the roots and the leaves, with the concentration in the roots being substantially greater than that in the leaves. As shown in Fig. 2(a) and (b), the copper in the roots showed a trend of increasing and then decreasing with the addition of the CS ratio, with the 30% CS addition having the best effect on copper enrichment, and the copper content increased by 43.49% compared to the control. The enrichment of zinc in roots followed a similar pattern, with zinc content increasing from 70.58 to 191.53 mg/kg when CS was 30%. From the enrichment concentration in leaves, the accumulation of copper in ryegrass showed an increasing trend compared with the control. There was no significant difference in zinc content in leaves, and the heavy metal content in roots was much higher than that in leaves. The experimental results revealed that the addition of CS promoted heavy metal enrichment by plants while inhibiting the upward transfer of heavy metals from plants and protecting the above-ground parts of plants, and heavy metal removal could be accomplished through ryegrass harvesting, enhancing the restoration effect

## Soil environmental factors

### Soil properties and enzyme activity

The addition of coal slime as an improver to copper tailings increased the physical and chemical properties of the tailing soil. In the time dimension, CS exhibited a substantial ($p < 0.05$) influence on the pH, conductivity, available nitrogen, and available phosphorus of the modified tailings.

After 20 days of restoration, the addition of 50% CS had significant effects on the modified tailings pH (Fig. 3a), which increased by 0.83 units (pH 7.87) on the original basis compared to the control pH (7.04). The addition of 50% CS raised available phosphorus (Fig. 3d) by 24.24% compared to the control (AP 2.64 mg/kg), while the addition of 40% CS increased available potassium (Fig. 3e) by 16.29% compared to the control (AK 273.79 mg/kg).

After 30 days of restoration, the addition of 40% CS had a substantial impact on pH (Fig. 3a), increasing by 0.66 units (pH 7.63) on the original basis compared to the control pH (6.97). The addition of CS also increased the content of salt ions in tailings. Compared with the electrical

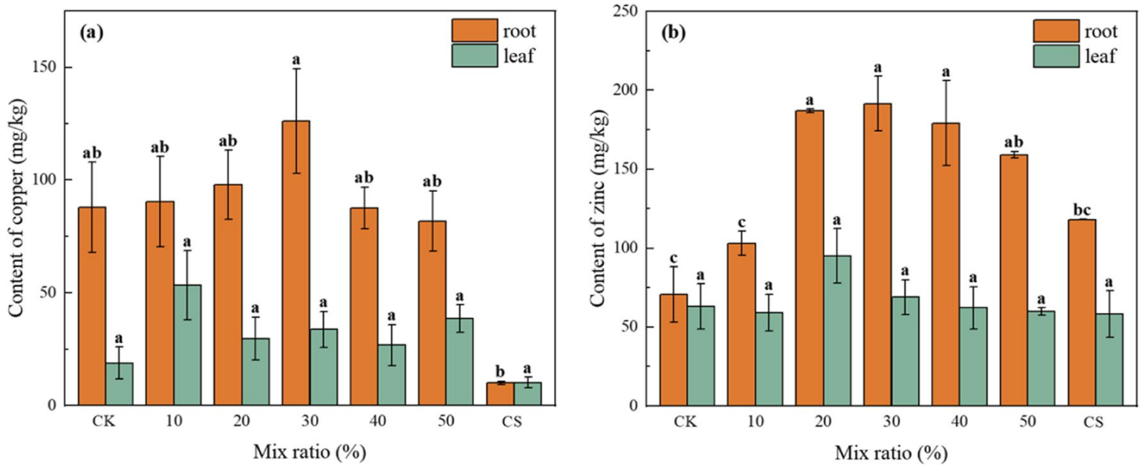


**Fig. 2** Distribution of copper in ryegrass (**a**), and distribution of zinc in ryegrass (**b**) under different coal slime contents after 30 days of restoration. Different letters above the error bar indicate significant differences among the treatments at $p < 0.05$. ANOVA results are shown in Table 1

conductivity (Fig. 3b) of the control (EC 2408 μS/cm), the electrical conductivity increased by 20.56% with the addition of 50% CS. Meanwhile, the 40% CS addition increased the amount of available phosphorus (Fig. 3d) in the tailings by 32.56% compared to the control phase (AP 2.15 mg/kg). Under the same time conditions, the decrease of available nitrogen (Fig. 3c) in the modified tailings was related to the uptake of ryegrass, which has been shown to preferentially uptake and translocate available nitrogen in the soil (Watson 2009), thus causing a greater impact on the available nitrogen in the improved tailings. The results of the ANOVA analysis showed that restoration time and CS content would affect the environmental factors of modified tailings. The experimental findings revealed that adding CS raised the pH and conductivity of the modified tailings, as well as the nutrient content, which aided in boosting the physicochemical properties of copper tailings.

Soil enzyme activity reflected the microbial metabolic intensity and fertility of modified tailings (Aponte et al. 2020). Overall, CS addition had no significant influence on the activity of urease or phosphatase in modified tailings ($p > 0.05$) but had a significant effect on catalase ($p < 0.05$).

Urease was engaged in the conversion of elemental nitrogen and was more susceptible to stress (Cordero et al. 2019). With the addition of CS content, the urease activity (Fig. 3f) in the modified tailings increased, reaching a maximum value of 0.48 mg/g at 40% addition. Phosphatase was involved in the mineralization of organophosphates, thus improving the availability of phosphorus in the soil (Cui et al. 2013). Compared with the control, the phosphatase activity (Fig. 3g) of modified tailings in 40% CS addition was slightly increased, reaching 0.35 mg/g. Soil catalase activity was related to the abundance of microorganisms, which could evaluate the change in environmental quality in a short time (Song et al. 2022). The doping of CS ($p < 0.05$) had a significant effect on the catalase content in the modified tailings. Compared with the control, 40% CS addition enhanced the catalase activity (Fig. 3h) in the modified tailings, and the content reached 2.36 mg/g. The experimental results showed that the addition of coal slime increased the activity of soil enzymes, and the activity of all three enzymes reached the maximum value at the addition level of 40%, which indicated that the addition of coal slime promoted the physicochemical properties of copper tailings.

### Heavy metal bioavailability

Different CS treatments showed a significant influence on the heavy metal concentration of copper tailings ($p < 0.05$). CS treatment might drastically lower the amount of available copper and available zinc in modified tailings when compared to the control.

After 20 days of restoration, 50% CS addition reduced the content of available copper (Fig. 4a) in the modified tailings by 46.05%. In addition, CS also had a significant effect on the content of available zinc, and the content of available zinc (Fig. 4b) in the modified tailings decreased by 48.51% at the addition ratio of 40%. At 30 days of restoration, the 50% CS addition reduced the available copper and available zinc (Fig. 4a and b) in the copper tailings by 45.45% and 12.31%, respectively. In addition, with the same coal slime content, the available copper and zinc in the improved tailings showed an increasing trend over time, which was related to the root exudates of ryegrass. Studies have shown that the root exudates of ryegrass can change the form of heavy metals and transform into an available state conducive to absorption (Sarathchandra et al. 2022).

BCR extraction of heavy metals from modified tailings was used to study the morphological changes of copper and zinc in modified tailings. In the untreated copper tailings, copper and zinc were mainly distributed in the residue state (Cu: 51.07%; Zn: 52.34%) and the reducible state (Cu: 34.92%; Zn: 16.85%).

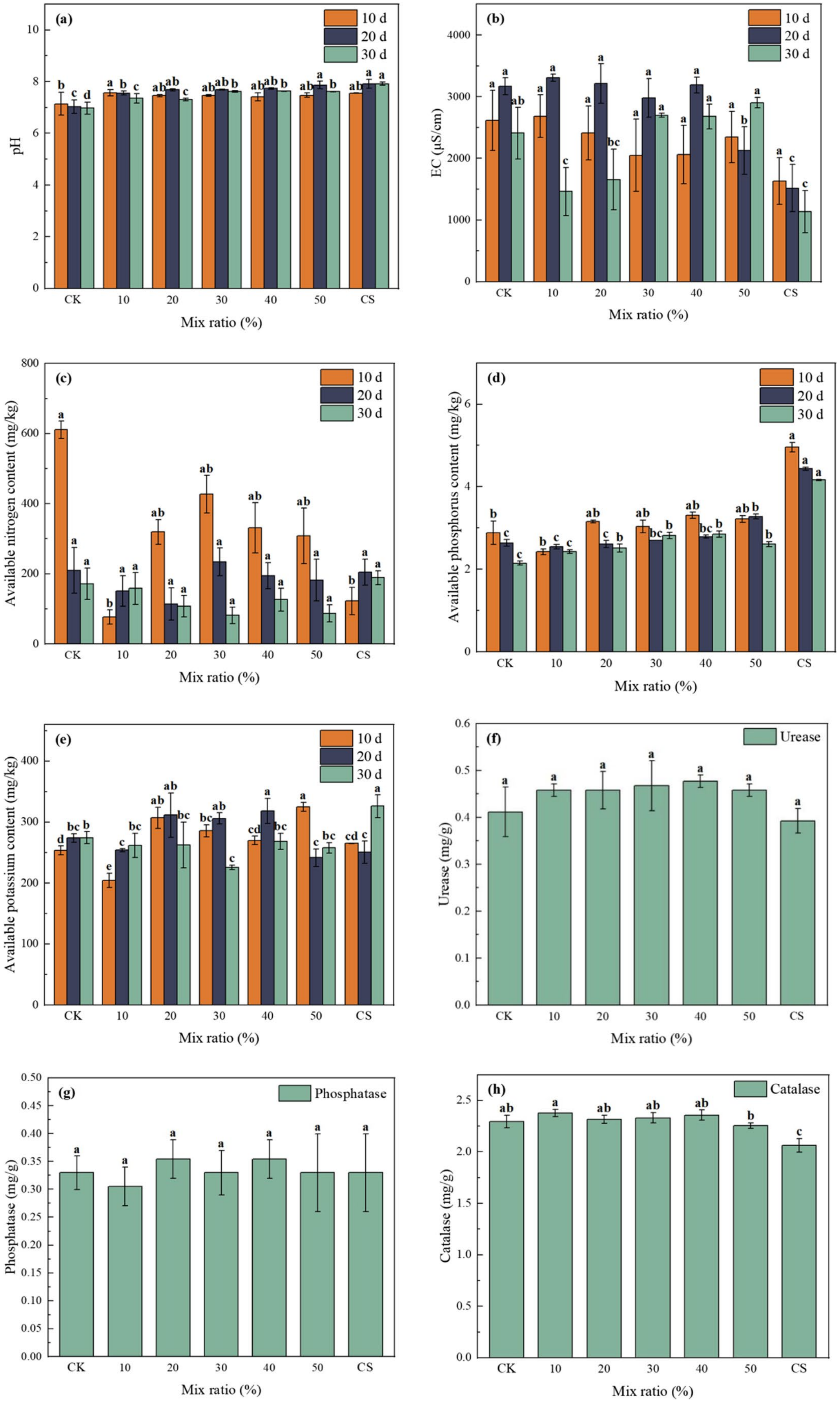
The addition of CS increased the percentage of weakly acid state and oxidizable states and decreased the percentage of reducible and residual states of copper (Fig. 4c) in the modified tailings, and the conversion of reducible and residual states to weakly acid state and oxidizable states was carried out. The addition of CS also resulted in a change in the form of zinc (Fig. 4d), with a decrease in the percentage of residual state and an increase in the percentage of oxidizable, reducible, and weakly acidic states after treatment, and a shift in the residual state towards the other three forms. Although the form of heavy metals changed after adding coal slime, copper, and zinc were still mainly in residue form.

The experimental results showed that the addition of coal slime significantly reduced the content of heavy metals in copper tailings, and the results of ANOVA analysis showed that the content of coal slime, as well as the time, affected the heavy metals, and the heavy metals in copper tailings were mainly in residue state with low migration ability. The application of coal slime in the restoration of copper tailings could solve the heavy metal problems to a certain extent.

## Bacterial community structure and correlation analysis

### Species annotation and diversity

OTU number, Chao1, ACE, Shannon, and Gini-Simpson indexes were used to evaluate the richness and diversity of microbial communities in modified tailings (Table 2). With the change in CS proportion, the species richness of the microbial community (OTU number, Chao1, and ACE)


(a)
pH
10 d
20 d
30 d
Mix ratio (%)
CK
10
20
30
40
50
CS
(b)
EC (μS/cm)
(c)
Available nitrogen content (mg/kg)
(d)
Available phosphorus content (mg/kg)
(e)
Available potassium content (mg/kg)
(f)
Urease
Urease (mg/g)
(g)
Phosphatase
Phosphatase (mg/g)
(h)
Catalase
Catalase (mg/g)

◂**Fig. 3** pH (**a**), EC (**b**), available nitrogen (**c**), available phosphorus (**d**), available potassium (**e**), urease (**f**), phosphatase (**g**), and catalase (**h**) under different contents of coal slime treatment. Soil enzyme activity was measured at 30 days of restoration. Different letters above the error bar indicate significant differences among the treatments at $p < 0.05$. ANOVA results are shown in Table 1

showed a trend of first decreasing and then increasing. On the whole, the addition of CS reduced the species richness of modified tailings. However, the diversity of the microbial community (Shannon and Gini-Simpson index) was improved, and the Gini-Simpson index showed a significant difference ($p < 0.05$). When the CS supplemental level was 40%, the Gini-Simpson index was the highest. Meanwhile, the Shannon index also reached the maximum value of 40%, indicating that the addition of CS increased the species diversity in the modified tailings.

The PCoA method assessed the β-diversity (Fig. S1) of microbial communities in the modified tailings by CS addition, with the first two principal components (PCoA1 and PCoA2) explaining 27.63% and 14.6% of the variation, respectively, with communities with similar structures grouped into the same phase and separated from the other phases. With the addition of the CS ratio, the microbial community evolves along the adulteration ratio in a gradual direction from CK, 10, 20, and 30%, implying a change in the microbial community. Subsequently, succession along the 40% to CS direction and similar spatial location, with relatively similar microbial populations but different richness. As the succession process proceeds, 30 and 40% of the microbial community have a high degree of similarity, but after continued addition, the CS microbial population is similar to 10%, and the community had a large succession. The results show that the more coal slime doping is not the better, the higher the stability of the microbial community at 40%, and the addition of CS has a significant effect on the modified microbial community of tailings.

### Bacterial community composition and abundance

Figure S2 shows the bacterial community structure in modified tailings under different treatments. At the phylum level (Fig. S2a), the dominant phylum includes Proteobacteria (52.37%–69.13%) and Bacteroidota (8.67%–19.90%). Common phyla include Actinobacteriota (3.58%–8.63%), Chloroflexi (1.46%–6.05%), Planctomycetota (1.14%–2.84%), Gemmatimonadota (1.62%–2.49%), Myxococcota (0.53%–1.74%), Verrucomicrobiota (0.18%–3.55%), and Dadabacteria (0.06%–2.34%). Compared with the control, CS supplementation increased the relative abundance of two dominant phyla, Proteobacteria and Bacteroidota, and also increased the relative abundance of three common phyla, Actinobacteria, Chloroflexi, and Myxococcota. At the genus level (Fig. S2b), the dominant bacteria genera included *C1-B045* (1.95%–14.29%), *Pseudomonas* (0.81%–11.09%). Common bacteria genera include Limnobacter (1.65%–9.26%), Hydrogenophaga (0.17%–7.78%), Luteimonas (0.18%–5.79%), Zeaxanthinibacter (0.11%–4.59%), Methylophaga (0.86%–4.66%), and Hyphomicrobium (0.76%–3.92%). Compared with the control, the addition of CS increased the relative abundance of two dominant bacteria genera, *C1-B045* and *Pseudomonas*, and also increased the relative abundance of four common bacteria genera, *Hydrogenophaga*, *Luteimonas*, *Zeaxanthinibacter*, and *Hyphomicrobium*. ANOVA analysis(Supplementary Table S4) showed that the addition of CS had no significant effect on bacterial community richness. Except for some bacteria, different proportions of CS could improve the relative abundance of most bacteria in the modified tailings.

### Correlations between soil environmental factors and bacterial community structure

RDA was used to analyze the relationship between modified tailing bacterial community structure (top 10 phylum level) and environmental factors, and RDA1 and RDA2 explained 82.76 and 10.73% of the differences in bacterial community structure (Fig. S3), respectively. The pH, AN, AK, AP, and available Cu were the main factors influencing the bacterial community at the phylum level in the modified tailings, with the explained percentage of 40.82%, 20.84%, 20.24%, 20.08%, and 15.31%, respectively. The effect of environmental factors on the bacterial community was pH > AN > AK > AP > available Cu.

Spearman correlation (Table 3) was used to analyze the correlation among environmental factors of modified tailings, soil enzymes, and bacterial community. There was a positive effect of available nitrogen on Proteobacteria and a negative correlation between catalase Proteobacteria in the modified tailings. The pH and available phosphorus in the modified tailings are negatively correlated with Bacteroidota, while the available copper is positively correlated with Bacteroidota. Actinobacteriota in modified tailings is negatively correlated with conductivity. In addition, the available nitrogen and available potassium in the modified tailings positively influenced Planctomycetota. There was a positive correlation between Gemmatimonadota and Verrucomicrobiota and available nitrogen. pH and available phosphorus have negative effects on Dadabacteria, while available copper has a positive effect.

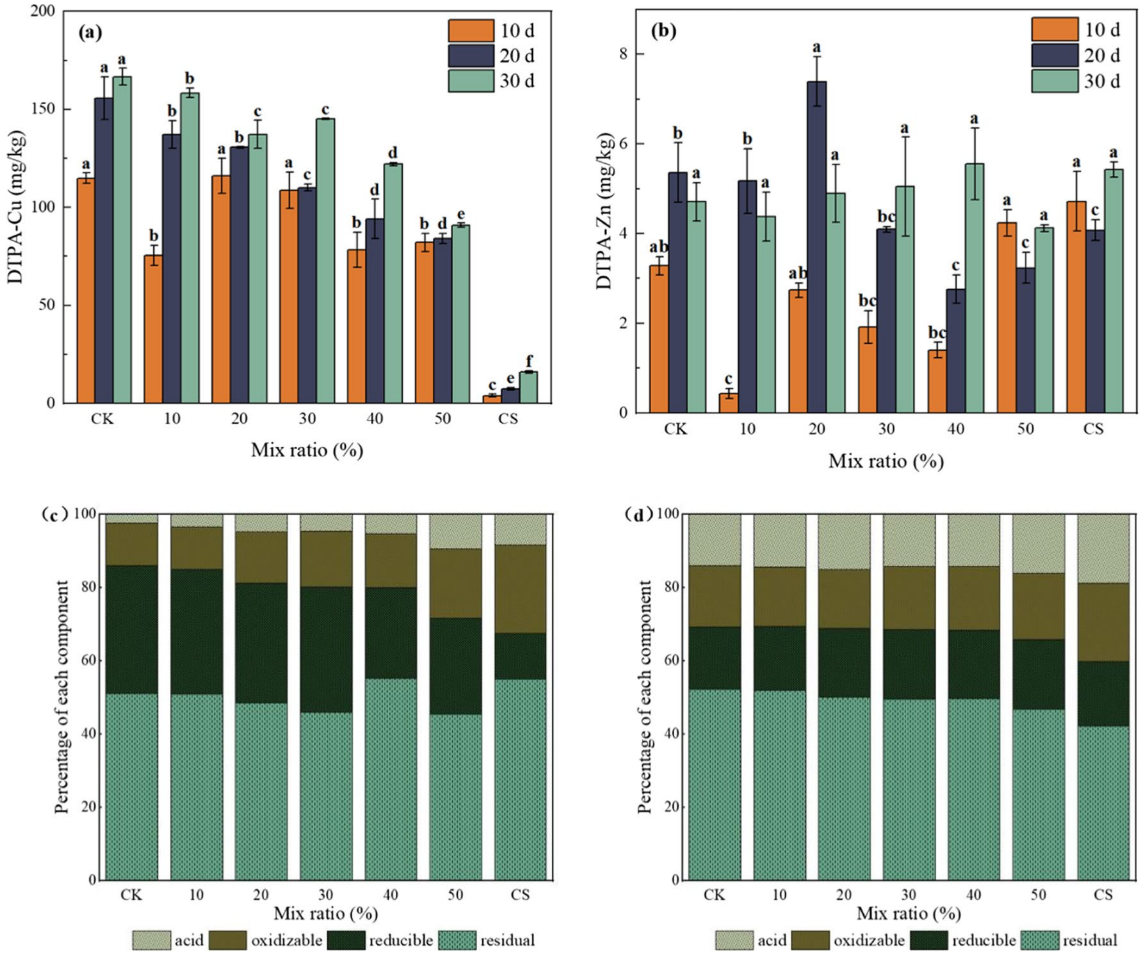


**Fig. 4** DTPA-Cu (**a**), DTPA-Zn (**b**), four fractions change of Cu (**c**), and four fractions change of Zn (**d**) under different contents of coal slime treatment. Four fractions of copper and zinc were measured 30 days after restoration. Different letters above the error bar indicate significant differences among the treatments at $p < 0.05$. ANOVA results are shown in Table 1

**Table 2** Alpha-diversity indices of bacterial communities in modified copper tailing

| Treatment | OTUs | Species richness | | Species diversity | |
|---|---|---|---|---|---|
| | | Chao1 | ACE | Shannon | Gini-Simpson |
| CK | 1269a | 1275a | 1275a | 4.7926ab | 0.9662a |
| 10% | 1225a | 1227a | 1249a | 4.7942ab | 0.9708a |
| 20% | 935a | 943a | 951a | 4.0399b | 0.9312b |
| 30% | 1109a | 1117a | 1127a | 4.8534a | 0.9757a |
| 40% | 1216a | 1222a | 1236a | 4.9682a | 0.9768a |
| 50% | 1037a | 1049a | 1057a | 4.7778ab | 0.9721a |
| CS | 1353a | 1355a | 1370a | 4.8837a | 0.9658a |
| Factor | $p > 0.05$ | $p > 0.05$ | $p > 0.05$ | $p > 0.05$ | $p < 0.05$ |

Different letters in the table indicate significant differences among the treatments at $p < 0.05$. ANOVA results are shown as $p$ values

## Potential functional profiling of microbial community

The data were preprocessed using STAMP; the 401 tested functions were tested for correlation, and a total of 21 functions with significant correlation (Fig. S4a) were obtained in the KEGG database. Among them, metabolic function accounted for 66.67%; cellular process accounted for 28.57%; and genetic information processing accounted for 4.76%. The basic metabolic pathways include energy metabolism, xenobiotic biodegradation and metabolism, lipid metabolism, metabolism of cofactors and vitamins, biosynthesis of other secondary metabolites, and metabolism of terpenoids and polyketides, which dominated the survival of microorganisms. The bacterial chemotaxis, carbon fixation in photosynthetic organisms, and base excision repair were dominant. With the addition of the CS ratio, the function of polycyclic aromatic hydrocarbon degradation (PAHs) was enhanced.

**Table 3** Spearman's correlations of modified copper tailing environmental factors, bacterial taxa abundances (phylum level), and soil enzyme activity

| Variable | pH | EC | AN | AP | AK | DTPA-Cu | Urease | Phosphatase | CAT |
|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria | −0.357 | −0.310 | 0.445* | −0.297 | 0.269 | 0.191 | −0.135 | 0.068 | −0.440* |
| Bacteroidota | −0.676** | −0.203 | 0.290 | −0.619** | −0.174 | 0.513* | −0.006 | −0.402 | −0.170 |
| Actinobacteriota | 0.055 | −0.484* | 0.423 | 0.127 | 0.264 | −0.198 | 0.035 | −0.048 | −0.317 |
| Chloroflexi | −0.136 | −0.105 | 0.145 | −0.169 | 0.089 | 0.212 | −0.296 | −0.138 | −0.044 |
| Planctomycetota | −0.199 | −0.396 | 0.664** | −0.165 | 0.440* | 0.135 | −0.417 | −0.217 | −0.362 |
| Gemmatimonadota | −0.411 | −0.296 | 0.571** | −0.344 | 0.393 | 0.193 | −0.308 | −0.236 | −0.373 |
| Myxococcota | 0.261 | 0.029 | 0.228 | 0.257 | 0.273 | −0.217 | −0.049 | 0.082 | −0.198 |
| Verrucomicrobiota | −0.312 | −0.057 | 0.528* | −0.349 | 0.303 | 0.352 | −0.293 | −0.163 | −0.170 |
| Dadabacteria | −0.822** | 0.105 | 0.055 | −0.716** | −0.174 | 0.672** | 0.147 | −0.026 | 0.124 |
| Others | −0.588** | −0.243 | 0.126 | −0.574** | −0.276 | 0.607** | −0.137 | −0.284 | −0.025 |

*EC* electrical conductivity, *AN* available nitrogen, *AP* available phosphorus, *AK* available potassium

*, $p < 0.05$ **, $p < 0.01$

The Spearman method was used to explore the correlation between phylum-level bacteria and predictive function. As shown in the figure (Fig S4b), bacteria at the phylum level were positively and significantly well correlated with most of the predicted functions, which Proteobacteria were closely related to transport and catabolism, energy metabolism, cell motility, cell growth and death, replication and repair, lipid metabolism, xenobiotic biodegradation and metabolism, metabolism of cofactors and vitamins, and biosynthesis of other secondary metabolites. Bacteroidota are closely involved in the replication and repair and biosynthesis of other secondary metabolites. Actinobacteriota is significantly related to the metabolism of terpenoids and polyketides and the biosynthesis of other secondary metabolites. It can be seen that the addition of CS promotes the change of its function by influencing the bacterial community.

## Discussion

### Coal slime promotes plant growth and heavy metal immobilization

The addition of coal slime promoted the growth of ryegrass, on the one hand, because it provided additional nutrients for the growth of ryegrass. On the other hand, the increase of pH and EC in the modified tailings could improve the nutrient utilization (Hamid et al. 2020). However, under the condition of 30 days of restoration, when the addition of coal slime exceeds 40%, the plant height, fresh weight, and germination rate are inhibited, which could be due to the excess addition of extra toxic metals and soluble salts, which inhibit plant growth (Sun et al. 2015).

In terms of plant enzymes, heavy metals, and salts are common environmental stresses that trigger oxidative stress by inducing the production of reactive oxygen species (ROS) in plants (Zhang et al. 2007). In general, ROS in plants maintains a dynamic balance with the antioxidant system. However, ROS accumulate excessively in plants under heavy metal stress, when enzymes (such as SOD, CAT, and POD) are used in plants to counteract oxidative stress (Imtiaz et al. 2015). SOD is the first line of defense in scavenging ROS, which converts superoxide radicals into $H_2O_2$ and $O_2$, while CAT and POD can catalyze the formation of $O_2$ and $H_2O$ from $H_2O_2$ (Liu et al. 2014). Pearson correlation analysis (Supplement Table S5) showed that the content of available copper and available zinc was positively correlated with the activity of antioxidant enzymes, which was consistent with the results of Wang et al. (2018). In the present study, the decrease in heavy metal content in the modified tailings led to a weakening of CAT and SOD activities, while POD activity increased and remained more effective for the $H_2O_2$ scavenging mechanism, which is consistent with the findings of Chu et al. (2017).

In terms of heavy metal enrichment, the content of heavy metals in plant roots is generally greater than that in leaves (Elloumi et al. 2015), mainly because heavy metals bind to plant root ligands (Li et al. 2009), thus protecting the aboveground parts of the plant. Meanwhile, studies have shown that ryegrass has a high bioenrichment factor for copper and zinc (Sarathchandra et al. 2022), and its root exudates can change the form of heavy metals and increase the absorption of heavy metals by ryegrass. Moreover, the addition of coal slime promoted the growth of ryegrass and increased the enrichment of heavy metals in roots and leaves through growth uptake (Zou et al. 2022), on the other hand, the introduction of coal slime altered the availability of metal elements as well as other nutrients, thus enhancing the accumulation of heavy metals by plants (Rees et al. 2015).

The experimental results showed that the addition of coal slime significantly improved the physicochemical properties of the plants and enhanced the enrichment effect of heavy metals. Under the condition of 30 days of restoration, the plant height, fresh weight, germination rate and peroxidase reached the maximum value when coal slime was added at 40%, and the plant growth was inhibited at more than 40%, thus indicating that the use of coal slime for copper tailing restoration is feasible, but the addition should not exceed 40%.

## Coal slime improves physical and chemical properties of copper tailings

The experimental results showed that the physicochemical properties of the copper tailings were improved after the use of coal slime, and the pH value increased significantly, mainly due to the "alkalinization" effect of the carbonate minerals in the coal slime (Park et al. 2013). In addition, the presence of alkaline cations such as magnesium, calcium, and potassium in the coal slime may also lead to an increase in pH in the modified tailings (Chuncai et al. 2014). Since the coal slime is rich in nutrient elements such as available phosphorus and available potassium, the content of available phosphorus and available potassium in the modified tailings can be increased after the coal slime is used to restoration copper tailings. The environment of copper tailings can be improved by the addition of coal slime, which provides favorable conditions for the growth of plants and microorganisms in the modified tailings (Chu et al. 2020; Rodriguez-Vila et al. 2014; Tang et al. 2023).

Soil enzymes are mainly derived from the physiological activities of microorganisms and their plants, and their activities can reflect the direction and intensity of biochemical reactions in the modified tailings. On the one hand, plants release organic/inorganic compounds and their coenzymes into the inter-rooted soil, thus directly or indirectly affecting soil enzyme activity (Gao et al. 2011), and on the other hand, soil enzyme activity is closely related to heavy metals such as Cu and Zn (Tang et al. 2020). Copper and zinc in the modified tailings inhibit the activities of urease, phosphatase, and catalase (Tang et al. 2022), and the addition of coal slime reduces the amount of available copper and zinc in the modified tailings, thus promoting enzyme activity. Pearson correlation analysis showed (Supplementary Table S6) that the content of available copper was negatively correlated with urease and phosphatase, which was consistent with the above research results. In addition, the heavy metal components in the modified tailings also affect the enzyme activity. The oxidizable state of copper and zinc improves the enzyme activity of soil by reducing the utilization rate of heavy metals by microorganisms (Zhang et al. 2018). In this experiment, the coal slime increases the oxidizable state content of copper and zinc in the modified tailings, further promoting the improvement of soil enzyme activity.

After adding coal slime, the content of available copper and available zinc in modified tailings decreased. Relevant studies showed that the availability and fluidity of heavy metals in modified tailings were closely related to the type of tailings and pH value (Ahmad et al. 2018; Peng et al. 2018). When the pH in the modified tailings increases, $H^+$ separates from carbonyl, carboxyl, hydroxyl, and phenolic groups, thus enhancing the affinity for metal cations and leading to their precipitation by the compound (Rodríguez-Vila et al. 2015).

BCR extraction can explain the availability and fluidity of heavy metals in modified tailings. Weak acid states are mainly metals bound as ions or with carbonates, and in the present study, the percentage of weak acid states increased for Cu and Zn, a result that can be attributed to the interaction of coal slime with ryegrass root secretions, forming carbonates bound to heavy metals and thus increasing the weak acid state content (Sarathchandra et al. 2022; Zhang et al. 2021). The reducible state is mainly a component bound to oxides or hydroxides of Fe/Mn. The introduction of coal slime can increase the active surface sites of bound metals, leading to partial binding of zinc to Fe/Mn oxides (Chuncai et al. 2014), and the alkaline pH of coal slime itself can also promote the formation of metal oxides and increase the proportion of the reducible state (Fe/Mn) (Jiang et al. 2012). The oxidizable state refers to the component combined with organic matter, and the addition of coal slime increases the percentage of the oxidizable state of copper and zinc in modified tailings, because coal slime introduces its own organic matter, and the zinc-copper/organic matter binding state related to organic matter is affected (Chu et al. 2020).

The experimental results showed that the addition of coal slime between 40 and 50% would promote the physicochemical properties of copper tailings, the content of nutrient elements increased, the content of heavy metals in the tailings decreased, and most of the heavy metals existed in the form of residue state, and the activities of urease, phosphatase, and catalase reached the maximum at the addition amount of 40%. The addition of coal slime alleviated the problems of nutrient depletion and heavy metals in copper tailings to some extent.

## Coal slime changes microbial community structure

The richness and diversity of the microbial community are one of the sensitive indicators reflecting the quality and health of the modified tailings (Xu et al. 2022), and a high level of microbial diversity is beneficial to the stability of the improved tailing system (Maron et al. 2018). In this study, the addition of CS weakened the microbial richness in the improved tailings, which may be due to the environmental changes caused by coal slime addition (Yan et al. 2017). On the other hand, the Gini-Simpson index indicated the richness of microbial species in the coal slime, which enhanced the diversity of microbial communities in the modified tailings during the introduction of coal slime. In addition, the richness and diversity of microbial communities in the modified tailings showed a trend of decreasing and then increasing, which may be related to the stability of the microbial community structure. PCoA analysis showed that the modified tailing community had a significant succession when the addition level was 10 to 20%, and the richness and diversity of the microbial community declined. With the addition of coal slime, the microbial community structure tended to be stable, and the richness and diversity of the microbial community were improved. Differential changes in the clustering structure of the bacterial communities also indicated that the addition of coal slime affected the metabolic activity and composition of the modified tailing flora.

At the same time, the addition of coal slime also caused changes in the composition of the bacterial community. The microorganisms in the modified tailing system mainly come from coal slime. At the phylum level, Proteobacteria, Bacteroidota, Actinobacteriota, and Chloroflexi were widely present in coal samples in the improved system (Strąpoć et al. 2011), and they played an important role in the degradation of organic compounds (Hanada 2002). The combined action of coal slime and ryegrass stimulates the growth of these bacteria and accelerates the utilization of organic compounds in coal slime (Zhao et al. 2022). The abundance of Myxococcota is driven by the concentration of copper (Dell'Anno et al. 2021), and its metabolism is capable of utilizing $H^+$ while producing hydroxides (Cho et al. 2021). Spearman analysis showed (Table 3) that the decrease in available copper content promoted the abundance of Myxococcota, and the change in pH also created a favorable environment for Myxococcota.

At the genus level, *C1-B045*, *Pseudomonas*, *Luteimonas*, and *Zeaxanthinibacter* were found in the combined biochar-ryegrass system. *C1-B045*, *Pseudomonas* can promote the degradation of polycyclic aromatic hydrocarbons in coal slime (Wang et al. 2022a), while the Hydrogenophaga is involved in the utilization of hydrocarbons (Wei et al. 2014). The decomposition of organic matter and the conversion of soil nutrients are dominated by *Luteimonas*, improving the soil fertility of modified tailings (Guo et al. 2017). Meanwhile, the combined action of Hyphomicrobium and ryegrass was able to immobilize heavy metals in the modified tailings (Chen et al. 2023; He et al. 2019). The change of bacterial community in the modified tailings could be attributed to the irregular surface of the coal slime (Li et al. 2020), which created a favorable environment for microbial proliferation; on the other hand, it could be attributed to the fact that the root system of ryegrass could pass through the surface and macropores of the coal slime (Jia et al. 2020), providing nutrients or a diversified ecological niche for the bacteria on the surface.

RDA and Spearman correlation analysis were used to evaluate the relationship between environmental factors of modified tailings and microbial diversity at the phylum level (top 10 phylum level). The results show that pH is the main driving force of the microbial community, which is consistent with some research results of Naz et al. (2022) and Sun et al. (2020). The rise of pH of modified tailings has a negative impact on the microbial community, resulting in a decline in microbial richness (OTU number, Chao1, and ACE) indices. Available nitrogen and potassium in modified tailings drove the positive development of the microbial community, while available phosphorus had a negative effect on the microbial community (Cui et al. 2020; Xiao et al. 2021). For heavy metals, most phylum level microorganisms have no significant effect on the content of heavy metals, but the abundance of Dadabacteria is positively correlated with the available copper, and the abundance of Bacteroidota has a high correlation with the bioavailability of available copper (Guo et al. 2022b), and the related species of copper is more than that of zinc (Hao et al. 2019), which is consistent with the results of Guo et al. (2022b) and Hao et al. (2019).

According to the function prediction of PICRUSt2, the effect of coal slime on microbial function was studied. The bacterial chemotaxis, carbon fixation in photosynthetic organisms, and base excision repair processes dominate, and the addition of coal slime weakens these processes, possibly because the addition of coal slime reduces the environmental pressure. Bacteria are oriented towards or away from chemicals through chemotactic processes. Under the condition of malnutrition, bacteria will accelerate the expression of this function to ensure faster adaptation to the environment, while under the condition of adequate nutrition, it will be weakened (Zhang et al. 2017).On the other hand, bacteria fight genomic damage through the base excision repair (BER) pathway (Hindi et al. 2021), and under environmental stress, the abundance of genes related to genetic information processing, such as BER, will be increased as a way to protect cells from harmful effects (You et al. 2019). The expression of carbon fixation in modified tailings is mainly derived from the Calvin-Benson-Bassham cycle and Arnon-Buchanon cycle in Proteobacteria (Ward and Shih 2019), and the way of carbon fixation is driven by phosphorus and pH (Zheng et al. 2022). According to the Spearman correlation (Table 3) analysis, the rise in phosphorus and pH will have an impact on the carbon fixation function. At the same time, the addition of coal slime also enhanced the expression of metabolic function, in which the degradation of polycyclic aromatic hydrocarbons (PAHs) in xenobiotic biodegradation and metabolism played a dominant role, providing more nutrients such as carbon and sulfur in the tailing system. In this study, the relative abundance of bacteria using organic matter was significantly increased, and their presence dominated the process (Zhao et al. 2022).

The experimental results showed that the addition of coal slime could enhance the microbial diversity in the modified tailings, and the main phylum level species of microorganisms in the modified tailings are the same as those in coal, and the microorganisms mainly come from the coal slime. When the additive amount was 40%, the microbial community stability was good. The dominant strains in the modified tailings had strong organic matter utilization ability, and their existence enhanced the expression of the PAH degradation function. In addition, coal slime drove the microbial community changes by changing the environmental factors of the modified tailings, among which pH was the main factor driving the microbial community changes.

## Conclusions

We discovered that the use of coal slime in the restoration of copper tailings is practicable by the improvement experiment of copper tailings. The 40% coal slime boosted the nutritional content of the modified tailings and increased the activity of soil enzymes (urease, phosphatase, and catalase). Following a 20-day of restoration, the concentrations of available copper and available zinc in the modified tailings decreased by 39.6% and 48.51%, respectively, when 40% coal slime was added. Furthermore, after 30 days of restoration, 40% coal slime addition enhanced plant growth, a 21.69% rise in chlorophyll content, and a 62.44% increase in peroxidase activity. The changes in community structure and function in the modified tailing system were primarily driven by variations in pH value, available nitrogen, phosphorus, potassium, and available copper. The presence of a coal slime-ryegrass system enhanced the relative abundance of Proteobacteria, Bacteroidota, Chloroflexi, and other bacteria, and enhanced the function of organic matter utilization and PAH degradation. The results showed that the 40% coal slime addition brought the physicochemical properties and microbial community evolution of copper tailings to an equilibrium point under the combined effect of ryegrass, and the addition of coal slime as an improver to copper tailings provided an environmentally friendly idea for the green restoration of tailings.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-023-30008-7.

**Author contribution** Zhou Zhou: formal analysis, writing — original draft, and methodology. Ling Xia: conceptualization, writing — review and editing, and project administration. Xizhuo Wang: methodology. Chenyu Wu: formal analysis and investigation. Jiazhi Liu: formal analysis and investigation. Jianbo Li: project administration. Zijing Lu: project administration. Shaoxian Song: project administration. Jiang Zhu: project administration. María Luciana Montes: project administration. Mostafa Benzaazoua: project administration

**Funding** This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 32061123009 and 52074203) and Hubei Sanxin Gold Copper Limited Company.

**Data Availability** All data is available and the author can be contacted if necessary.

## Declarations

**Ethics approval** Not applicable

**Consent to participate** Not applicable

**Consent for publication** Not applicable

**Competing interests** The authors declare no competing interests.

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