



# Straw and biochar co-application: A strategy to reduce Cd–Zn bioavailability, alleviate microbial nutrient limitations and enhance soil C stability

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**Abstract.** Biochar is widely used to remediate heavy metal-contaminated soils; however, it can increase C limitation in soil microorganisms, potentially compromising soil ecological sustainability. Effective strategies are, therefore, needed to reduce heavy metal bioavailability while maintaining microbial activity and soil fertility. To this end, we aimed to evaluate the effects of different straw–biochar ratios on heavy metal availability, microbial nutrient limitation, C cycling and soil fertility and to identify an optimal remediation strategy for Cd- and Zn-contaminated soil. Cd- and Zn-contaminated soils were amended with varying straw–biochar ratios (S4C0, S3C1, S2C2, S1C3, S0C4 and control) and incubated for 90 days. Soil heavy metal bioavailability, microbial nutrient limitation, CO<sub>2</sub> emissions, enzyme activities and organic C stability were analysed. Compared with the control, the straw–biochar treatment at a 1:3 ratio (S1C3) reduced diethylenetriaminepentaacetic acid–extractable Cd and Zn by 30.22–32.81% and 21.31–23.06%, respectively. S1C3 significantly improved soil organic C content and stability, enhanced available nutrients (N, P and K), increased microbial biomass and elevated enzyme activities related to C, N and P acquisition and alleviated microbial C limitation. The optimized straw–biochar ratio (1:3) provides a novel integrated approach for remediating heavy metal-contaminated agricultural soils while simultaneously enhancing soil C stability, nutrient availability and microbial activity. This strategy offers a practical foundation for sustainable farmland management.

## 1 Introduction

The widespread distribution of Cd and Zn during large-scale mechanical processing, mining and smelting substantially exacerbates soil contamination (Wang et al., 2024a). These metals are highly persistent, bioaccumulative (Kang et al., 2024a). They exhibit high biological toxicity and carcinogenic potential. When present in excess, Cd and Zn seriously endanger both soil ecology and human health (Huang et al., 2024). Additionally, their high mobility poses a major challenge



for complete removal. Thus, mitigating the mobility and toxicity of heavy metals in soil through chemical passivation is an effective strategy for remediating their pollution.

Biochar, a stable C material generated via anaerobic pyrolysis of biomass, is widely applied for soil heavy metal passivation and remediation owing to its high surface area and exceptional adsorption capacity (Ahmad et al., 2024; Wang et al., 2024b).

35 Biochar immobilizes heavy metals through precipitation, chelation and complexation, thereby reducing their bioavailability (Kang et al., 2024b). Several studies have examined the effects of biochar on heavy metal immobilization in soil. Burachevskaya et al. (2023) demonstrated that biochar produced from wood, sunflower and rice husks exhibited high adsorption capacity for Cu, Zn and Pb in soil. Using the soil quality index to assess the impacts of coffee parchment biochar remediation on heavy metal-contaminated soils, Carnier et al. (2023) revealed that biochar enhances both soil fertility and  
40 heavy metal immobilization by improving overall soil quality. Mohan et al. (2024) reported that tea residue biochar and paddy straw biochar simultaneously immobilize Cd(II) and Cr(VI), sequester C and improve soil fertility. However, biochar production is energy-intensive, can release volatile organic compounds and particulates (Ibitoye et al., 2024) and is associated with high cost. Moreover, while biochar alleviates heavy metal stress on soil microorganisms, its stable C structure limits its availability as a microbial C source, potentially exacerbating nutrient limitations (Chen et al., 2022),  
45 thereby affecting microbial activity and overall soil ecology.

In China, the annual output of crop straws is >600 million tons, with approximately 230, 130 and 110 million tons from corn, wheat and rice, respectively. Most straw is returned to fields to supply nutrients and organic C, thereby maintaining farmland production capacity and supporting food security (Guo et al., 2024a; Mo et al., 2024). Straw returned to farmland is beneficial for increasing soil C storage. Straw return also boosts the availability of soil nutrients, including N, P, K, Ca and  
50 Mg, thereby enhancing crop growth and yield (Guo et al., 2024b; Islam et al., 2022). Furthermore, researchers have investigated changes in soil microbial communities, soil CO<sub>2</sub> and other greenhouse gas emissions after straw return (Lin et al., 2023; Peng et al., 2024). However, limited studies have investigated the combined influence of straw and remediation agents on soil heavy metals. In particular, the potential of combining straw and biochar in specific ratios to remediate soil heavy metal pollution, while reducing greenhouse gas emissions, improving soil microbial activity and enhancing soil  
55 productivity, remains inadequately explored.

Thus, in this study, we sought to simulate the combined application of varying proportions of corn straw and biochar in Cd- and Zn-contaminated soil using laboratory incubation experiments. Specifically, we aimed to (1) analyse the impacts of different straw–biochar ratio ratios on heavy metal activity, CO<sub>2</sub> emissions, organic C components, available nutrients, activities of nutrient-acquiring enzymes and microbial biomass; (2) assess microbial nutrient limitation using enzymatic  
60 stoichiometry; (3) examine the relationships among measured indicators and treatments; and (4) evaluate the comprehensive treatment effects on polluted soil.

## 2 Materials and methods

### 2.1 Experiment materials



Cd- and Zn-contaminated soil was collected from the 0–20 cm cultivated layer of farmland in Liaocheng City, Shandong  
65 Province, China. After air-drying, the soil was sieved using a 2-mm mesh and thoroughly mixed for the cultivation  
experiment. The soil pH, total Cd, total Zn, organic matter and total N contents were 8.05, 0.83 mg·kg<sup>-1</sup>, 407.00 mg·kg<sup>-1</sup>,  
18.18 g·kg<sup>-1</sup> and 1.52 g·kg<sup>-1</sup>, respectively.

Corn straw was collected from a local farm in Taian City, Shandong Province, oven-dried and then crushed. The crushed  
straw was pyrolysed in a muffle furnace at 500 °C for 120 min with a heating rate of 5 °C min<sup>-1</sup> under a nitrogen flow rate of  
70 300 mL min<sup>-1</sup> to produce biochar. The elemental compositions of corn straw and the resulting biochar are presented in Table  
S1.

## 2.2 Laboratory incubation experiment

Six treatments were established: (1) CK (control; 200.0 g soil, no straw or biochar), (2) S4C0 (200.0 g soil and 4.0 g straw),  
(3) S3C1 (200.0 g soil, 3.0 g straw and 1.0 g biochar), (4) S2C2 (200.0 g soil, 2.0 g straw and 2.0 g biochar), (5) S1C3  
75 (200.0 g soil, 1.0 g straw and 3.0 g biochar) and (6) S0C4 (200.0 g soil and 4.0 g biochar). Each treatment included nine  
replicates. The designated amounts of straw and biochar were incorporated into the soil, mixed thoroughly and placed into  
500-mL plastic culture bottles. The moisture content was then adjusted to 70% of field capacity by adding deionized water.  
The bottles were incubated for 90 d at 25 °C in the dark using an incubator (GXZ-430, Jiangnan Instrument, Ningbo, China).  
Soil moisture was maintained with adjustments every 3 d using the gravimetric method. Destructive sampling was conducted  
80 at 30, 60 and 90 d for each treatment.

## 2.3 Measurements

### 2.3.1 Soil chemical properties

Diethylenetriaminepentaacetic acid (DTPA) was used to extract soil Cd and Zn, which were quantified using an inductively  
coupled plasma optical emission spectrometer (Thermo Scientific CAP 7200, USA). Soil organic carbon (SOC) and alkaline  
85 hydrolysable N (AHN) were determined using the dichromate oxidation–ferrous sulfate titration and alkaline hydrolysis  
dissolution diffusion methods (Hou et al., 2025). The acetate extraction–flame photometry and NaHCO<sub>3</sub> extraction–  
molybdenum antimony colourimetry methods were used to determine soil available P (Olsen-P) and available K (AK).

Organic C fractions were determined using samples collected on day 90. Easily oxidizable organic C (EOC), particulate  
organic C (POC) and mineral-associated organic C (MAOC) were measured following Geng et al. (2022). Dissolved organic  
90 C (DOC) was extracted with deionized water and analysed using a total organic carbon analyser (Acquray TOC, Elementar,  
Hanau, Germany). The three-dimensional excitation–emission matrix (3D-EEM) of soil DOC was obtained using a Cary  
Eclipse fluorescence spectrophotometer (Agilent Technologies, CA, USA).

### 2.3.2 Estimation of CO<sub>2</sub> emissions

The alkali absorption method (Geng et al., 2022) was used to measure the soil CO<sub>2</sub> emission rate. During the incubation  
95 period, the cumulative release of CO<sub>2</sub> was calculated as CO<sub>2</sub>-C in mg·kg<sup>-1</sup> of soil.



### 2.3.3 Microbial biomass assessment

Microbial biomass was assessed using soil samples collected on day 90. The chloroform fumigation method was used to determine microbial biomass C (MBC), microbial biomass N (MBN) and microbial biomass P (MBP), calculated from the difference between the fumigated and non-fumigated samples.

### 100 2.3.4 Soil enzymatic stoichiometry

$\beta$ -Glucosidase (C-acquiring), urease (N-acquiring) and alkaline phosphatase (P-acquiring) activities were measured following the protocols of Silva et al. (2024) and Li et al. (2024). Enzyme activity vector analysis (Moorhead et al., 2016) was used to quantify microbial nutrient limitations (C, N and P). The vector endpoint was defined as (X, Y):

$$X = \frac{C - \text{acquiring enzyme}}{C - \text{acquiring enzyme} + P - \text{acquiring enzyme}} \quad (1)$$

and

$$Y = \frac{C - \text{acquiring enzyme}}{C - \text{acquiring enzyme} + N - \text{acquiring enzyme}} \quad (2)$$

105 The vector length and angle were calculated as follows:

$$\text{Vector length} = \sqrt{X^2 + Y^2} \quad (3)$$

$$\text{Vector angle} = \text{DEGREES}(\text{ATAN2}(X, Y)) \quad (4)$$

The vector length indicates the degree of microbial C limitation, with longer vectors representing stronger C limitation. The vector angle represents the relative N and P limitation experienced by microorganisms. When the vector angle is  $<45^\circ$ , microorganisms are relatively limited by N; when  $>45^\circ$ , they are relatively limited by P. As the angle increases, N limitation decreases and P limitation increases.

### 110 2.4 Data analysis

Data processing was performed using Microsoft Excel 2024 and SPSS 21. To examine significant differences among treatments, one-way analysis of variance was conducted. For post-hoc comparisons, Duncan's multiple range test at a significance level of  $p = 0.05$  was employed. Figures were prepared using Origin (2025).

## 3 Results and discussion

### 115 3.1 Available heavy metals

DTPA-extractable heavy metals are commonly used to evaluate bioavailability and biotoxicity owing to their high mobility (Kang et al., 2025). Soil DTPA-extractable Cd and Zn were measured to evaluate the effects of straw and biochar additions (Fig. 1). Treatments containing both straw and biochar (S3C1, S2C2 and S1C3) and biochar alone (S0C4) reduced available Cd in soil by 10.60–35.34%, 12.30–37.04% and 10.88–37.73% on days 30, 60 and 90 of incubation, respectively (Fig. 1a).

120 Treatments S2C2, S1C3 and S0C4 also significantly reduced available Zn by 16.57–34.41% (Fig. 1b). S0C4 produced the lowest available Cd and Zn concentrations, which was attributed to the high porosity, abundant oxygen-containing functional



groups and strong adsorption capacity of biochar, thereby immobilizing heavy metals and reducing their bioavailability (Zhang et al., 2025a). This finding was consistent with that of Li et al. (2025a), who reported that rice husk biochar significantly decreased the activities of Zn, Pb, Cu and Cd in sludge compost-amended soil. Regarding soil available Cd, the difference between S1C3 and S0C4 was insignificant at any sampling time, indicating that a straw–biochar ratio of 1:3 provides a comparable effect to biochar alone.

Corn straw alone also lowered available Cd, reaching significance ( $P < 0.05$ ) on day 60. During decomposition, straw releases humic substances, including fulvic acid, humic acid and humin, which contribute to the formation of soil organic matter (Song et al., 2025). Soil organic matter immobilizes Cd, thereby reducing its bioavailability. However, by day 90, this effect was minimal. In S4C0, available Zn gradually increased over time; on days 60 and 90, Zn content was slightly higher than that of CK. This may be due to Zn release from straw during mineralization.

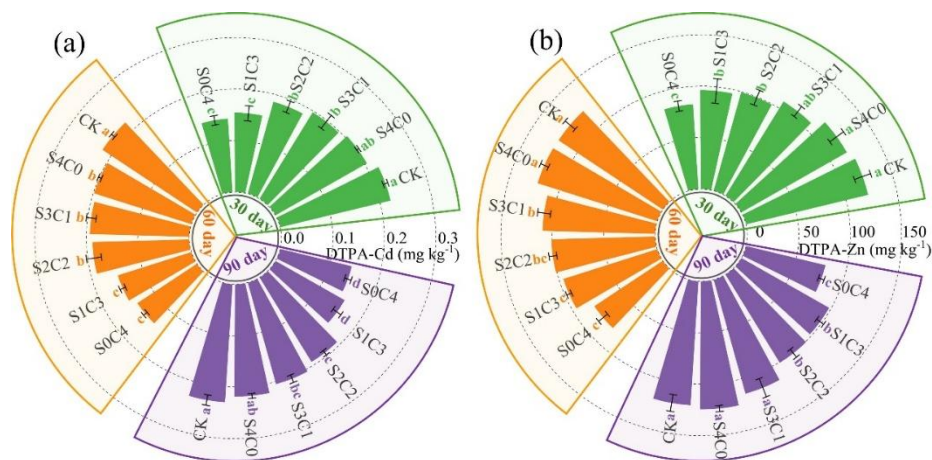


Figure 1. Diethylenetriamine pentaacetic acid extractable (a) Cd and (b) Zn contents of different treatments.

### 3.2 CO<sub>2</sub> flux and accumulation

Soil CO<sub>2</sub> emissions under straw and biochar treatments are shown in Fig. 2. Treatments containing straw markedly increased soil CO<sub>2</sub> flux during the early incubation period (Fig. 2a), while CO<sub>2</sub> release increased with higher straw content (Fig. 2b). By day 90, cumulative CO<sub>2</sub> release from S4C0, S3C1, S2C2 and S1C3 was 4.85, 3.72, 3.06 and 1.85 times higher than that from CK and 4.23, 3.25, 2.68 and 1.62 times higher than that from S0C4, respectively. Straw decomposition supplies labile C, increasing microbial activities and CO<sub>2</sub> emissions (Ding et al., 2025). Moreover, straw inputs promote microbial biomass turnover and substrate utilization (Yan et al., 2024). Furthermore, the physical coverage of straw decreased oxygen contact with SOC, accelerating straw mineralization and decomposition, thereby elevating CO<sub>2</sub> emissions (Yan et al., 2024).

As a soil amendment, biochar has been extensively investigated for pollution remediation, nutrient improvement and greenhouse gas mitigation (Geng et al., 2022). Compared with CK, biochar application alone increased soil CO<sub>2</sub> emission flux by 23.64–51.47% during days 1–10 of incubation (Fig. 2a). By day 90, cumulative CO<sub>2</sub> release in S0C4 was over 20% higher than that in CK (Fig. 2b), likely owing to a priming effect stimulating mineralization of native soil organic matter.



150 tea soils.

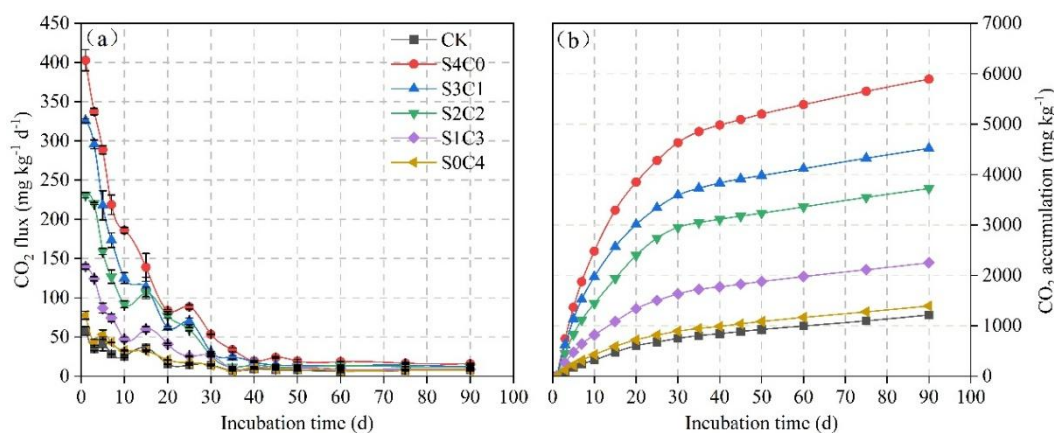


Figure 2. (a) CO<sub>2</sub> flux and (b) accumulation of different treatments.

### 3.3 SOC fractions

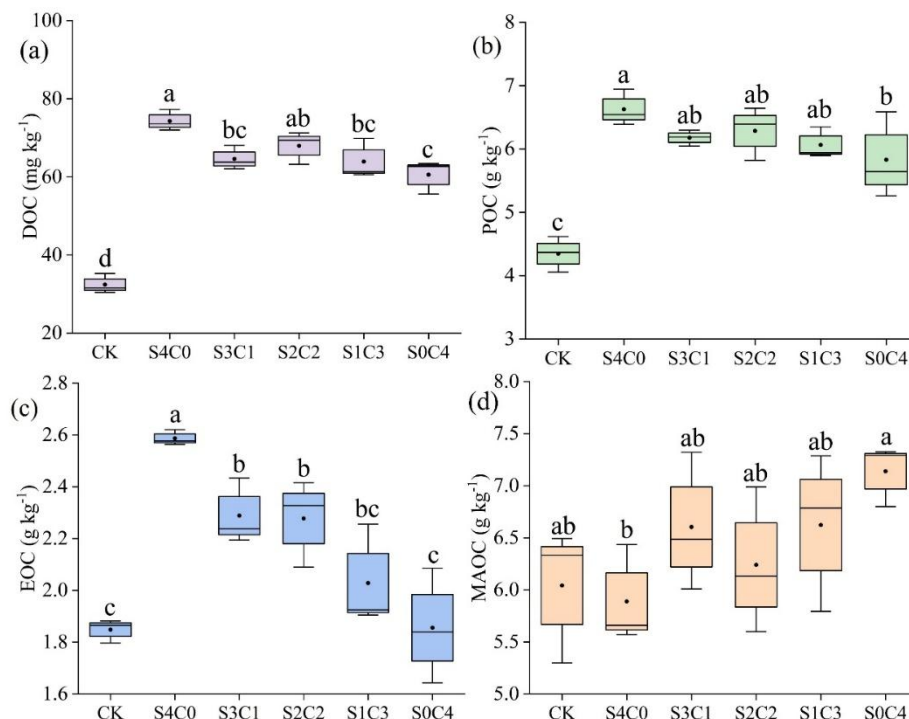
155 SOC storage is crucial for alleviating the global greenhouse effect and enhancing C sequestration (De Rosa et al., 2024). It is  
 a critical element of soil fertility and productivity (Guo et al., 2024c). Its production capacity and stability are strongly  
 affected by agricultural management measures (Chen et al., 2024). Straw returning is an effective conservation tillage  
 practice that ameliorates soil properties and promotes agricultural productivity (Lin et al., 2023), whereas biochar application  
 is increasingly employed as a soil amendment (Jokubè et al., 2025; Zhang et al., 2025b). Returning straw provides lignin,  
 160 cellulose, starch and other compounds that are partly decomposed and transformed into soil organic matter. Biochar contains  
 abundant organic C in stable functional groups such as benzene rings and alkyl groups, which contribute to long-term  
 increases in SOC. Compared with CK, all treatments significantly increased SOC at each sampling time, with increases of  
 18.68–25.72%, 17.89–24.41% and 20.48–24.85% on days 30, 60 and 90, respectively (Fig. S1). These results are  
 comparable to those of Fan et al. (2024), who reported that wheat straw and biochar increased SOC at all sampling times.  
 165 However, no remarkable differences were detected among the various straw and biochar treatments.

SOC mineralization is the principal pathway for C loss; therefore, improving its stability (which largely depends on its  
 composition) aids in reducing mineralization and greenhouse gas emissions. Active fractions (such as DOC, EOC, POC and  
 MBC) are more sensitive to management changes (Chen et al., 2024). MAOC is formed through adsorption of microbial  
 residues, decomposition products and dissolved organic matter onto mineral surfaces and is more resistant to degradation



170 and persists longer in soil (Hu et al., 2025). In the current research, compared with CK, all treatments resulted in significant  
 increases in DOC and POC contents of 86.72–129.05% and 34.16–52.46%, respectively (Fig. 3a and 3b). S4C0, S3C1 and  
 S2C2 also increased EOC by 23.23–23.83% (Fig. 3c). Compared with S4C0, S3C1, S2C2, S1C3 and S0C4 decreased DOC  
 and EOC, whereas S0C4 reduced POC. Furthermore, the MAOC content of S0C4 was 21.21% higher than that of S4C0 (Fig.  
 3d). These findings indicate that, compared with straw addition alone, biochar or straw–biochar combinations enhanced SOC  
 175 stability.

The 3D-EEM spectra was used to further analyse DOC composition under different treatments (Fig. 4a–f). Based on 3D-  
 EEM, DOC can be classified into four components: aromatic protein (I), fulvic acid (II), microbial metabolites (III) and  
 humic acid (IV). Regional integration was performed on each DOC component using Origin (2025). All straw and biochar  
 treatments enhanced the fluorescence intensity of DOC (Fig. S2). Across all treatments, the fluorescence intensity of DOC  
 180 components followed the order of IV > III ~ II > I, consistent with the results of Huang et al. (2025). These results suggest  
 that straw and biochar applications enhance the content of four DOC components without altering the overall composition of  
 soil DOC. The treatments primarily increased soil humic acid, thereby enhancing soluble organic C. Fulvic acid and  
 microbial metabolite contents also increased markedly, whereas protein content showed limited change.



185 Figure 3. (a) Dissolved organic carbon (DOC), (b) particulate organic carbon (POC), (c) easily oxidizable carbon (EOC), and  
 (d) mineral-associated organic carbon (MAOC) contents of different treatments.

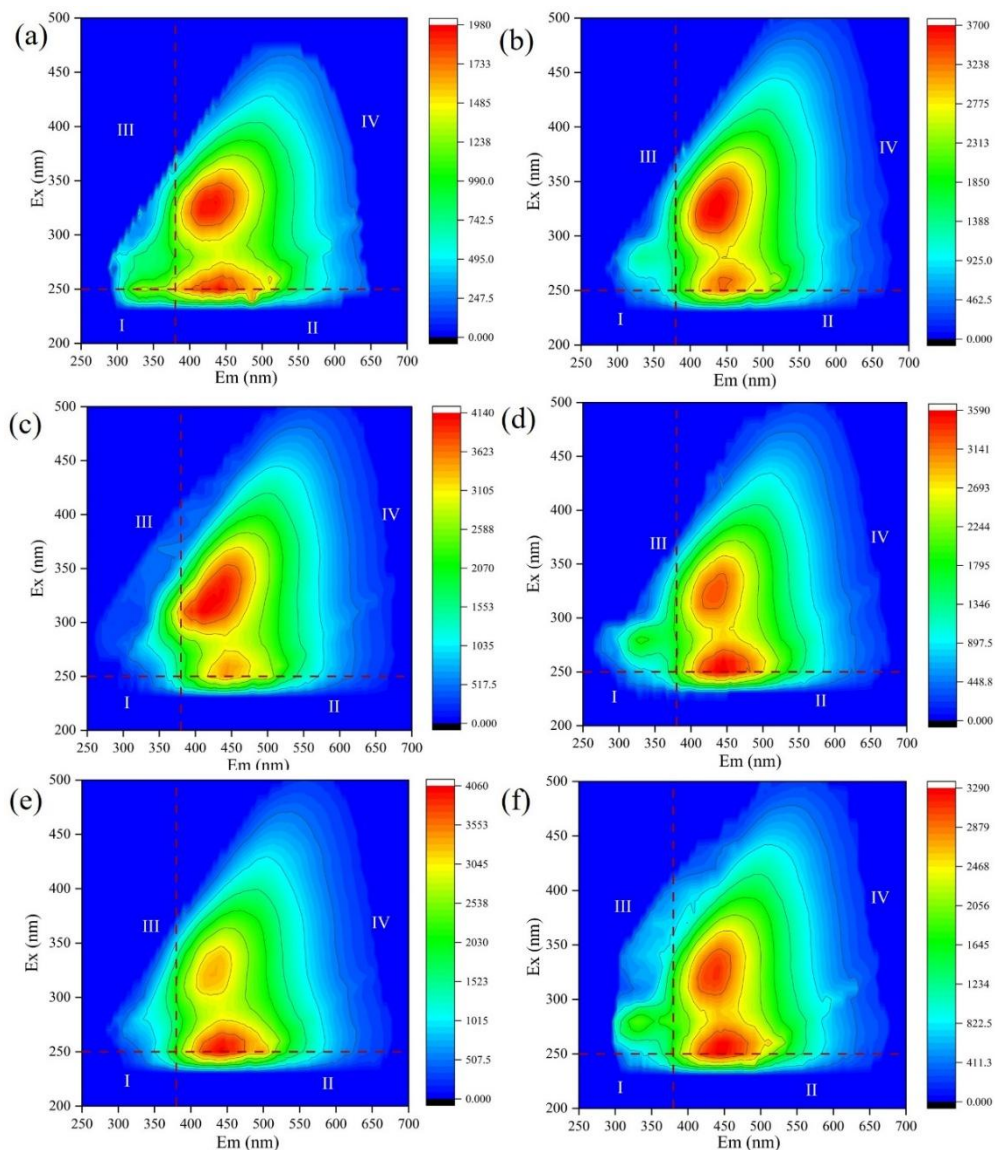


Figure 4. Three-dimensional excitation-emission-matrix spectra of (a) CK, (b) S4C0, (c) S3C1, (d) S2C2, (e) S1C3, and (f) S0C4.

### 190 3.4 Soil available nutrients

Compared with CK, S4C0, S3C1 and S1C3 increased soil AHN content on day 30. All straw and biochar treatments increased AHN content on day 60, whereas S3C1, S2C2, S1C3 and S0C4 increased AHN content on day 90 (Fig. 5a). During the early stage of cultivation, straw addition enhanced soil available N. However, as cultivation progressed, AHN content in straw-treated soil gradually decreased. On day 90, AHN content in S2C2, S1C3 and S0C4 was 14.79%, 24.52% and 18.53% higher, respectively, than that in S4C0. This could be attributed to straw decomposition, which releases a large



amount of available N. However, this N was unstable and lost through volatilization as ammonia or N oxidized. In later stages, as straw decomposition slowed, soil AHN content declined. Additionally, Cong et al. (2025) reported that straw return is more effective than biochar at improving soil quality in the short term.

On day 30, compared with CK, S2C2, S1C3 and S0C4 significantly increased Olsen-P content by 23.63%, 25.90% and 34.95%, respectively (Fig. 5b). All straw and biochar treatments increased Olsen-P content on days 30 and 60, with S1C3 and S0C4 showing greater improvements than other treatments. Soil AK content exhibited a similar trend to Olsen-P (Fig. 5c): S2C2, S1C3 and S0C4 significantly increased AK content on days 30 and 60. By day 90, all straw and biochar treatments increased soil AK content by 8.50–30.94%. Between S1C3 and S0C4, no significant differences were detected in soil AHN, Olsen-P and AK across all sampling periods.

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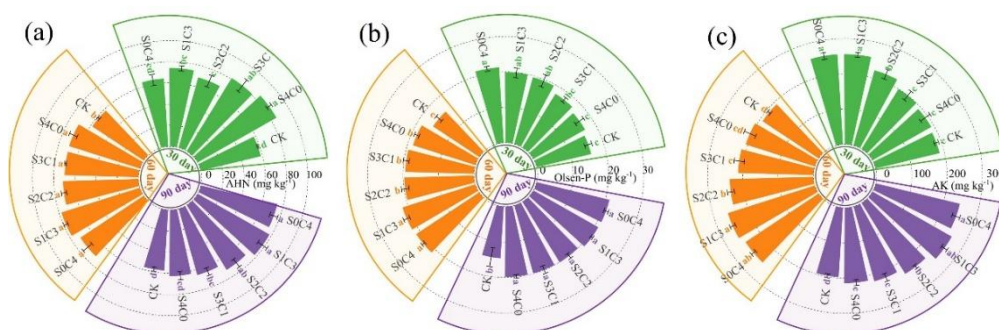


Figure 5. (a) Alkali hydrolysable nitrogen, (b) available phosphorus, and (c) available potassium contents of different treatments.

### 3.5 Soil microbial biomass

After completion of the cultivation experiment, treatment with straw, biochar or their combination increased soil MBC, MBN and MBP by 24.73–61.41%, 20.77–58.60% and 23.76–47.61%, respectively (Fig. S3a–c). The MBC content of S1C3 was significantly higher than that of S4C0, S3C1, S2C2 and S0C4. Compared with S4C0, soil MBN in S3C1, S2C2, S1C3 and S0C4, increased by 18.87–31.32%. The soil MBP content of S1C3 differed significantly from that of S4C0. Overall, S1C3 produced the highest MBC, MBN and MBP.

Biochar improved soil available nutrients at the end of cultivation (Fig. 5). Its large specific surface area and high porosity provide ample habitat for microorganisms. However, because biochar C is structurally stable and not readily utilized by microorganisms, it imposes C limitations on microbial reproduction. In contrast, straw supplies abundant available C and nutrients for microorganisms. Thus, combining straw and biochar, especially at the S1C3 ratio, was most effective in promoting microbial growth and increasing soil microbial biomass.

### 3.6 Enzymatic activity and stoichiometry

Soil enzymes derived from microorganisms are important drivers of soil microbial activity. They decompose dead plant and microbial cells, depolymerize macromolecules and generate soluble nutrients that can be assimilated by microorganisms. As



225 catalysts of most biochemical reactions, enzymes are instrumental in material cycling and energy conversion. Soil enzyme activities are closely related to microbial growth and activity and can reflect soil health (Das et al., 2025). This study selected  $\beta$ -Glucosidase, urease and alkaline phosphatase as C-, N- and P-acquiring enzymes, respectively.  $\beta$ -glucosidase is a vital component of the enzyme mixture required for lignocellulose degradation, catalysing the conversion of cellulose disaccharides into glucose (Wu et al., 2025). Urease catalyses the conversion of urea into ammonia and water, while phosphatase promotes soil organic phosphorus mineralization and enhances the bioavailability of soil phosphorus (Kang et al., 2025).

230 At all three sampling times, treatment with straw or biochar alone or their combination increased the activities of  $\beta$ -glucosidase, urease and alkaline phosphatase by 44.51–131.16%, 50.64–219.97% and 50.62–251.67%, respectively (Fig. 6). The activity levels of enzymes involved in nutrient acquisition reflect microbial nutrient demand in relation to substrate utilization efficiency (Li et al., 2025b). In this study, the increase in nutrient-acquiring enzyme activities was attributed to the increased availability of enzymatic substrates following straw and biochar application. S4C0 had the most pronounced impact on enzyme activities because microorganisms are more responsive to straw, which provides readily utilizable nutrients, than to biochar. C derived from straw increased microbial demand for N and P, thereby stimulating urease and alkaline phosphatase activities.

240 Li et al. (2022) reported similar results, showing that increased straw application enhanced soil enzyme activities associated with the uptake of C, N and P. Although biochar is generally considered stable and resistant to decomposition, its application also significantly enhanced the activities of C-, N- and P-acquiring enzymes. This effect depends on the properties of both the biochar and the soil. For example, Liao et al. (2022) demonstrated that biochar produced at temperatures  $<500$  °C increased enzyme activities, whereas biochar produced at  $\geq 500$  °C exhibited less pronounced effects. Moreover, increased enzyme activity may be related to reduced heavy metal toxicity, as heavy metal pollution inhibits microorganisms and nutrient-acquiring enzymes.

245 Overall, the effects of straw addition on enzyme activities, particularly  $\beta$ -glucosidase and urease, gradually weakened over time (Fig. 6), likely owing to depletion of enzyme substrates as the easily decomposable straw mineralized. By contrast, S0C4 treatment resulted in no significant change in enzyme activities over time because biochar is stable and non-biodegradable.

250 In terrestrial ecosystems, microbial nutrient limitation is widely recognized as an important factor controlling C cycling (Cui et al., 2022). Enzymatic stoichiometry aids in evaluating nutrient limitations in soil microorganisms and provides insight into microbial nutrient equilibrium (Zhang et al., 2025c). In this study, enzymatic stoichiometry was used to evaluate the effects of straw and biochar additions on microbial nutrient limitations in heavy metal-contaminated soil (Table S2). On days 30, 60 and 90, vector length values of CK were highest, indicating strong microbial C limitation. S0C4 reduced vector length on day 30 but showed no significant difference on days 60 and 90. These findings indicate that biochar alone does not provide an adequate C source for microorganisms.



255 S4C0, S3C1, S2C2 and S1C3 reduced vector lengths on days 30 and 60. Compared with S0C4, vector lengths of S3C1 and S2C2 were reduced on day 30, while those of S3C1, S2C2 and S1C3 were reduced on day 60. Therefore, combined straw and biochar mitigated microbial C limitation. However, this effect decreased over time as straw mineralized. On all sampling days, vector angle values were  $<45^\circ$ , indicating that soil microorganisms were relatively N-limited. On days 30 and 90, treatments had no significant effect on vector angles, suggesting no change in microbial N–P balance at the initial and final stages. On day 60, however, S4C0, S3C1, S2C2 and S0C4 significantly reduced vector angles. This may be due to

260 increased soil P availability (Fig. 5b), which altered the N–P balance and intensified microbial N limitation.

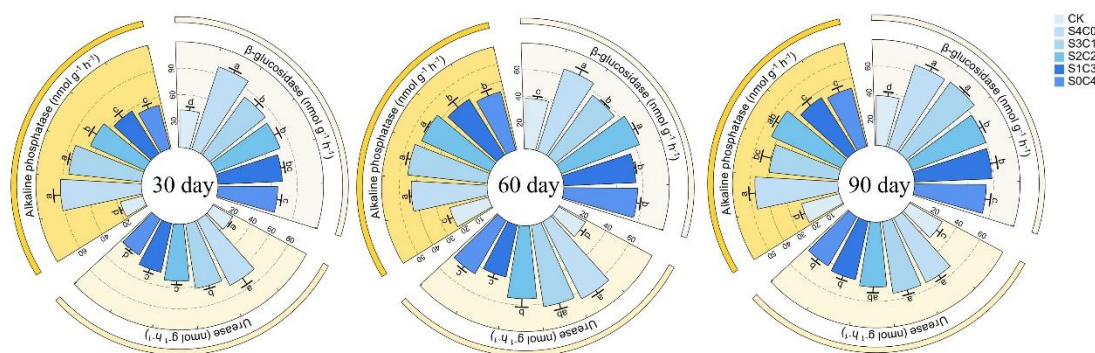


Figure 6.  $\beta$ -Glucosidase, urease and alkaline phosphatase activities of different treatments.

### 265 3.7 Correlation and principal component analysis

The correlation between the 17 indices and the amounts of straw and biochar applied was analysed (Fig. 7a). DTPA-Cd showed a significantly positive correlation with DTPA-Zn and a negative correlation with AHN, AK, MAOC, MBN and MBP. This indicated that heavy metals might inhibit the formation of stable SOC. Kang et al. (2025) also reported a negative correlation between the availability of heavy metals and that of nutrients after biochar application. Positive correlations were

270 observed between available nutrients, microbial biomass, enzyme activities and active organic C components. The amount of straw was positively correlated with CO<sub>2</sub> emissions, EOC, urease and alkaline phosphatase. C in straw is easily decomposed and utilized. Straw addition provided a substantial quantity of active C for soil microorganisms, enhanced soil respiration and increased microbial demand for N and P nutrients. The amount of biochar applied was significantly positively correlated with soil available nutrients, MAOC and MBN, suggesting that biochar improves SOC stability and enhances soil nutrient

275 availability. Additionally, the amount of biochar applied showed a significantly negative correlation with soil DTPA-Cd and DTPA-Zn contents, indicating that biochar effectively immobilized heavy metals in soil and reduced their toxicity.

Principal component analysis (PCA) was employed to analyse differences among treatments and relationships among the 17 indices (Fig. 7b). The contribution rates of PC1 and PC2 were 53.3% and 29.3%, respectively; the cumulative contribution rate reached 82.6%. Thus, PC1 and PC2 explained most of the variation in soil properties among treatments. The points

280 representing straw and biochar treatments were clearly separated from those of CK. The CK points were distributed on the



left side of the plot, whereas the straw and biochar treatment points were on the right, demonstrating that straw, biochar or their combined application effectively altered the properties of Cd- and Zn-contaminated soil. Moreover, the distribution of different straw and biochar treatment points varied significantly. Consistent with the correlation network heatmap, the PCA plot also showed negative correlations between soil available heavy metal contents and AHN, AK, MAOC, MBN and MBP.

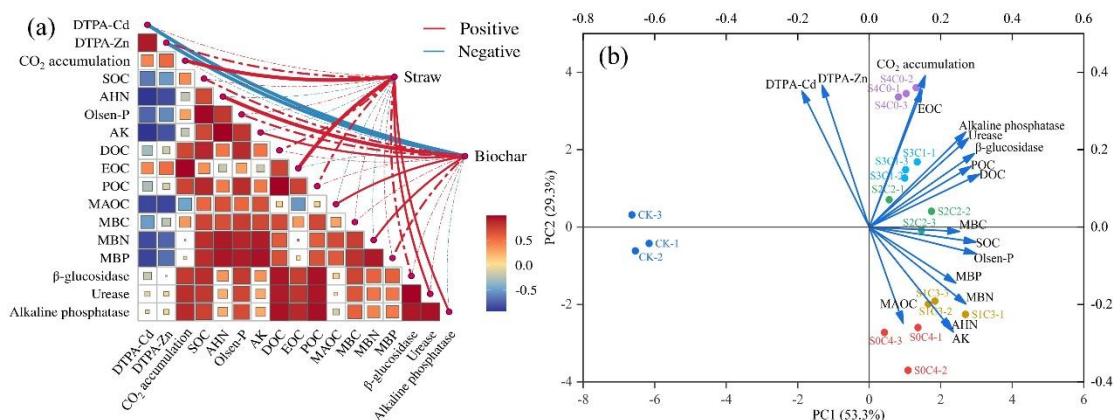


Figure 7. (a) Correlation network heatmap and (b) principal component analysis.

### 3.8 Comprehensive evaluation

After standardizing the key indices, PCA was performed to comprehensively estimate the improvement effects of different treatments on Cd- and Zn-contaminated soil. The cumulative variance contribution of PC1 and PC2 was 90.901% (Table S3).

290 Based on the PCA coefficients for each indicator, membership function values and comprehensive scores were calculated (Sun et al., 2022). The results showed that S1C3 achieved the best comprehensive score (1.7513), ranking first among all treatments (Table 1). Applying straw and biochar at a 1:3 (straw:biochar) ratio effectively decreased the bioavailability of Cd and Zn and produced the greatest overall improvement in contaminated soil.

**Table 1.** Comprehensive score of different treatments

	Score	Rank
CK	-4.0727	6
S4C0	0.0020	5
S3C1	0.3244	4
S2C2	0.8012	3
S1C3	1.7513	1
S0C4	1.1938	2

## 295 4 Conclusion



The combined application of straw and biochar effectively reduced heavy metal pollution and greenhouse gas emissions, improved soil nutrient availability and alleviated microbial nutrient stress. Overall, the SIC3 treatment (straw–biochar = 1:3) achieved the greatest comprehensive improvement in Cd- and Zn-contaminated soil. SIC3 reduced available Cd and Zn while improving SOC stability and nutrient availability. In addition, SIC3 enhanced soil C-, N- and P-acquisition activities, 300 alleviated microbial C limitations and increased microbial biomass. This study contributes to the development of remediation strategies and provides a theoretical basis for the comprehensive treatment of heavy metal-contaminated soil.

### Data availability

The datasets of this study are available at <https://data.mendeley.com/datasets/pg4d42wjwf/1>.

### Author contributions

305 XK: Investigation, Data curation, and Writing–original draft. NG: Investigation and Data curation. ZY: Formal analysis. YL: Investigation. Hui Wang: Software. HP: Validation. QY: Methodology. YL: Conceptualisation, Writing–review & editing and Funding acquisition. YZ: Conceptualisation, Writing–review & editing and Supervision.

### Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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