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DOCTORAL THESIS

Effects of Swine Manure Biochar on the Bioavailability and Accumulation of Heavy Metals in Rice and Wheat in Soils

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Abstract

With the rapid expansion of intensive livestock and poultry production, the large-scale return of animal manure to farmland can replenish soil nutrients and promote agricultural waste recycling, but may also introduce continuous heavy metal inputs and long-term accumulation risks. Pyrolysis of swine manure into biochar has been regarded as an important pathway for manure detoxification, resource recovery, and remediation of Cd-contaminated farmland. However, the heavy metal input risks associated with livestock and poultry manure, the release of endogenous heavy metals from swine manure biochar during aging, the regulation of long-distance Cd translocation within crops, and the soil microecological responses under long-term field conditions remain insufficiently understood.

This thesis focused on the research chain of “heavy metal input risk identification in livestock and poultry manure—safe conversion and aging stability of swine manure biochar—crop safety control in Cd-contaminated farmland—soil microecological response mechanisms”. Literature-based data integration, accelerated oxidative aging simulation, wheat and rice field experiments, structural equation modelling, and integrated microbiome, metagenomic, metaviromic, and metabolomic analyses were used to systematically evaluate the role and mechanisms of swine manure biochar in the safe utilization of heavy metal-contaminated farmland. The main findings are as follows.

(1) Integrated analyses at global and Chinese scales showed clear element-specific and livestock-type-dependent differences in heavy metal concentrations in livestock and poultry manures. The overall median concentrations followed the order $Zn > Cu > Cr > Ni > Pb > As > Cd > Hg$. Pig manure had the highest Cu and Zn concentrations and exceedance risks, making it a priority target for risk control. Based on provincial livestock statistics in China, the national fresh-weight production of livestock and poultry manure was estimated to be approximately 1.41 billion t. Pig manure contributed 71.7% and 48.4% of the national manure-derived Cu and Zn inputs, respectively. Unit cropland-area loading showed stronger spatial heterogeneity than total input, indicating that heavy metal risk assessment for manure recycling should shift from

concentration-based evaluation alone to an integrated framework considering input flux, cropland carrying capacity, and element-specific toxicity.

(2) Accelerated H_2O_2 oxidation was used to simulate the aging of swine manure biochars produced at different pyrolysis temperatures, revealing their structural evolution and endogenous heavy metal release mechanisms. Oxidative aging induced C and N losses, surface functional group reconstruction, mineral phase transformation, and microstructural degradation, with these processes being strongly regulated by pyrolysis temperature. Biochar produced at 700 °C showed greater structural stability and stronger resistance to oxidative degradation. Heavy metal release exhibited clear element-specific patterns: Cd, Zn, Cu, and Ni showed release peaks under low to moderate oxidation intensity, followed by stabilization or decline; Cr release increased progressively with oxidation intensity; and As showed relatively minor variation. These results indicate that the long-term environmental safety of swine manure biochar should be evaluated by integrating pyrolysis temperature, aging processes, and element-specific release mechanisms.

(3) In an acidic Cd-contaminated wheat field, swine manure biochar increased soil pH, reduced Cd bioavailability, and decreased wheat grain Cd concentration by 10.5%–13.0%, while increasing grain Zn concentration by 4.4%–14.8%. In contrast, direct application of swine manure increased grain Cd accumulation and promoted long-distance Cd translocation. Organ-level distribution, translocation factors, structural equation modelling, and random forest analysis jointly demonstrated that wheat nodes played important roles in Cd and Zn retention, partitioning, and bottleneck control during their transport to grains. swine manure biochar not only reduced Cd availability through soil chemical immobilization, but also promoted Cd allocation to nodes and non-grain spike tissues, thereby restricting Cd transfer to grains. This finding extends the mechanism of biochar-mediated Cd reduction from soil–rhizosphere immobilization to the regulation of inter-organ metal translocation within crops.

(4) A five-year in situ field experiment in acidic Cd-contaminated paddy soil was conducted to compare the long-term effects of lime, swine manure, and swine manure biochar. swine manure biochar continuously increased soil pH, reduced available Cd, decreased Cd

accumulation in brown rice, and maintained or improved rice yield. Brown rice Cd concentration under biochar treatment was reduced by 73.1%–76.5% compared with the control, showing better long-term integrated performance than direct swine manure application and conventional lime treatment. These results demonstrate that swine manure biochar has strong long-term Cd immobilization capacity and practical application potential under field conditions.

(5) Long-term field experiments combined with multi-omics analyses showed that swine manure biochar not only improved the soil chemical environment by increasing soil pH and reducing available Cd and exchangeable Al, but also reshaped prokaryotic, fungal, and viral community structures, regulated metagenomic functional pathways, and altered soil metabolite composition. Metaviromic results further suggested that long-term biochar application may influence microbial functions and metabolic processes by modulating virus–host interactions. Therefore, the long-term remediation effect of swine manure biochar in Cd-contaminated paddy soil is not merely a chemical immobilization process, but involves a microecological cascade response linking soil chemical improvement, microbial community reconstruction, virus–host interaction regulation, and metabolic functional optimization.

In summary, this thesis systematically revealed the internal connections among manure-derived heavy metal input risks, aging stability of swine manure biochar, Cd/Zn translocation control within crops, and long-term recovery of soil microecological functions. The results indicate that swine manure biochar can serve as an important functional material linking livestock waste recycling with the safe remediation of Cd-contaminated farmland. This study provides theoretical support and technical references for safe manure return to farmland, engineering application of swine manure biochar, and the safe utilization of heavy metal-contaminated agricultural soils.

Keywords: livestock and poultry manure; swine manure biochar; cadmium contamination; node-mediated restriction; long-term field experiment; metavirome; soil microecological function

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Chapter 1 Introduction

1.1 Research Background and Thesis Overview

Heavy metal contamination of agricultural soils has become an important threat to soil quality, crop safety, ecosystem functioning, and human health. Metals such as Cd, Cu, Zn, Pb, Cr, As, Ni, and Hg originate from both natural processes and anthropogenic activities, including mining, industrial emissions, wastewater irrigation, fertilizer use, and livestock manure application (Alloway, 2013; Nicholson et al., 2003; Hou et al., 2020; Zhang et al., 2012). Because heavy metals are persistent, their risks depend not only on total concentrations but also on soil pH, organic matter, redox conditions, rhizosphere processes, microbial activity, and crop uptake (Bolan et al., 2014; Hou et al., 2020). Cd is of particular concern because of its high mobility, toxicity, and tendency to accumulate in rice and wheat grains, creating important food-safety risks (Clemens et al., 2013; Uraguchi and Fujiwara, 2012; Abedi and Mojiri, 2020).

Livestock manure is an important source of nutrients and organic matter, but intensive livestock production can enrich manure with Cu, Zn, Cd, As, and other metals, resulting in long-term accumulation risks when repeatedly applied to farmland (Bolan et al., 2010; Chadwick et al., 2015; Nicholson et al., 2003; Zhang et al., 2012). Converting swine manure into biochar offers a potential strategy for combining manure recycling with heavy-metal control. Pyrolysis stabilizes organic carbon and mineral nutrients and can reduce metal mobility, while biochar can further immobilize metals through pH regulation, adsorption, ion exchange, complexation, and precipitation (Cantrell et al., 2012; Ahmad et al., 2014; Uchimiya et al., 2011; Palansooriya et al., 2022). However, endogenous metals remain in biochar, and aging may alter its physicochemical properties and metal-retention capacity. Therefore, this thesis integrates livestock-manure heavy-metal risk assessment, biochar aging and stability, Cd control in wheat and rice, and long-term soil microecological responses to evaluate the environmental safety and remediation potential of swine manure biochar.

1.2 Research Questions

This thesis addresses four interconnected questions covering source risk, material stability, crop safety, and soil ecological mechanisms.

RQ1: What are the concentration characteristics, spatial and temporal patterns, and agricultural input risks of heavy metals in livestock and poultry manure at global and Chinese scales?

RQ2: How are endogenous heavy metals released during the environmental aging of swine manure biochar, and how do pyrolysis temperature and physicochemical changes regulate its long-term stability?

RQ3: Can swine manure biochar reduce Cd bioavailability, crop uptake, internal translocation, and grain accumulation in wheat and rice under field conditions?

RQ4: How does long-term swine manure biochar application influence Cd immobilization and soil health through changes in soil properties, microbial and viral communities, functional genes, and metabolites?

1.3 Research Objectives

1.3.1 General Objective

The general objective is to establish an integrated framework linking heavy-metal risks from livestock manure, the environmental stability of swine manure biochar, Cd migration and accumulation in wheat and rice, and the ecological mechanisms underlying long-term biochar remediation in acidic contaminated farmland.

1.3.2 Specific Objectives

The specific objectives are to establish a standardized database of heavy metals in livestock manure and assess their spatial, temporal, and regional input risks; quantify manure-derived heavy-metal loads in Chinese cropland; investigate the effects of pyrolysis temperature and oxidative aging on the structure and endogenous metal release of swine manure biochar; evaluate its effects on Cd/Zn uptake, translocation, and grain accumulation in wheat; assess its long-term Cd immobilization performance and agronomic effects through a five-year rice field experiment; and integrate microbiome, metagenome, virome, and metabolome analyses to elucidate the mechanisms linking soil chemical improvement, microbial restructuring, functional regulation, and soil ecological recovery.

1.4 Significance of the Study

This study connects livestock-manure risk assessment, manure-derived biochar production, long-term environmental safety, crop Cd control, and soil ecological recovery within a unified framework. It therefore contributes both to the mechanistic understanding of biochar-mediated remediation and to the practical management of contaminated agricultural soils.

1.4.1 Theoretical Significance

The study extends heavy-metal risk assessment of livestock manure beyond concentration-based evaluation by incorporating manure production, land-application rates, cropland carrying capacity, and element-specific toxicity. It also demonstrates that biochar aging is a dynamic process involving structural alteration, functional-group reconstruction, mineral transformation, and metal redistribution. Furthermore, by combining wheat and rice field experiments with multi-omics analyses, the study expands the understanding of biochar remediation from soil chemical immobilization to the broader soil-crop-microbe system, including crop internal metal transport and long-term ecological functional recovery.

1.4.2 Practical Significance

The findings provide a scientific basis for monitoring and managing heavy metals in livestock manure, optimizing manure recycling, and converting swine manure into a functional remediation material. Field evidence further supports the use of swine manure biochar to increase soil pH, reduce Cd availability and grain accumulation, and improve soil ecological functions in acidic contaminated farmland. By integrating waste recycling, pollutant immobilization, soil improvement, and carbon stabilization, this research also contributes to circular agriculture, low-carbon development, and the sustainable management of heavy-metal-contaminated farmland.

1.5 Thesis Structure and Technical Roadmap

The thesis follows the logical sequence of “theoretical basis-risk identification-material mechanisms-field validation-ecological mechanisms.” Chapters 1 and 2 establish the research background and theoretical foundation. Chapter 3 assesses heavy-metal contamination and agricultural input risks associated with livestock manure. Chapter 4 investigates the oxidative aging and endogenous metal-release mechanisms of swine manure biochar. Chapters 5 and 6 evaluate its field performance in Cd-contaminated wheat and rice systems, with emphasis on soil Cd availability, plant uptake and translocation, grain safety, and long-term remediation stability. Chapter 7 integrates microbiome, metagenome, virome, and metabolome analyses to reveal how long-term biochar application regulates soil chemistry, microbial communities, functional pathways, and metabolic processes. Together, these components form an integrated research framework from source-risk identification and biochar conversion to field remediation and ecological functional recovery.

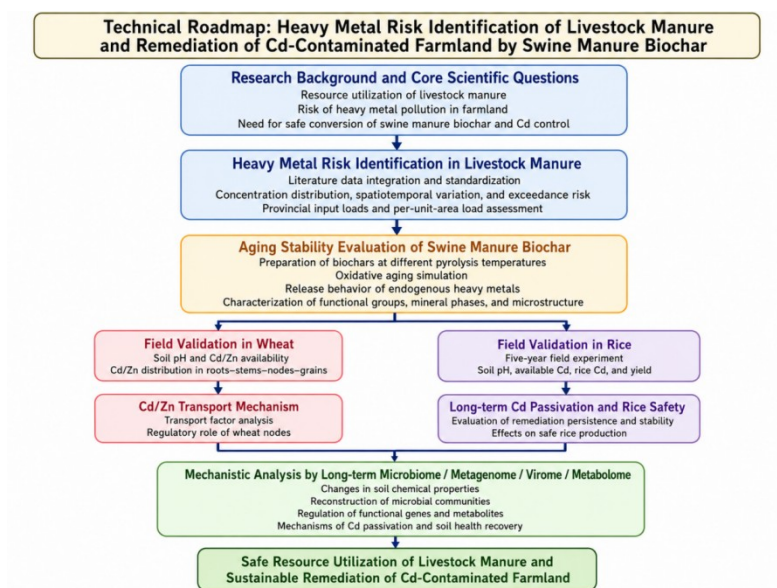


Fig. 1.1. Technical roadmap of this thesis

Chapter 2 Thematic Literature Review

The accumulation of potentially toxic metals in cultivated soils poses substantial risks to ecosystem functioning, agricultural production, food quality, and public health. Elements including Cd, Cu, Zn, Pb, Cr, and As may be introduced into farmland through both natural and anthropogenic pathways. Natural inputs primarily originate from the weathering of parent materials, whereas human-related sources include mineral extraction, industrial releases, atmospheric fallout, wastewater irrigation, and repeated applications of fertilizers and livestock manure (Wuana and Okieimen, 2011; Zhao et al., 2015; Nicholson et al., 2003). In contrast to many organic contaminants, metals cannot be biologically decomposed and may remain in soil for prolonged periods, leading to gradual accumulation and delayed ecological effects. Their potential hazards depend not only on the total amount present but also on the chemical and biological conditions controlling metal mobility and availability. Important regulating factors include soil acidity, organic matter content, oxidation–reduction status, clay minerals, Fe–Mn oxides, and interactions occurring in the rhizosphere (Caporale and Violante, 2016). Therefore, the central challenge in managing heavy metal-contaminated agricultural soils is not merely to reduce total metal concentrations, but to decrease their bioavailability, crop uptake and translocation, and food-chain exposure risks.

Among the various heavy metals in agricultural soils, Cd has become a major focus in the safe utilization of contaminated farmland because of its high mobility, strong plant availability, pronounced toxicity to humans, and high potential for accumulation in cereal grains (Clemens et al., 2013; Järup and Åkesson, 2009). Rice and wheat are staple crops in China and worldwide; however, they differ markedly in Cd uptake, rhizosphere regulation, internal translocation, and grain accumulation (Abedi and Mojiri, 2020; Zhao and Wang, 2020). In paddy soils, Cd speciation and mobility are strongly influenced by alternating flooding and drainage and by rhizosphere redox processes; in upland wheat systems, soil pH, available Cd, Cd–Zn competitive uptake, and root–stem–grain translocation play important roles in determining grain Cd accumulation (Clemens et al., 2013; Abedi and Mojiri, 2020). Accordingly, this chapter reviews agricultural soil heavy metal contamination, heavy metal input risks associated with livestock manure application, the environmental safety of swine manure biochar, the mechanisms by which biochar immobilizes heavy metals, and its role in soil–crop–microbe systems. This review provides a theoretical basis for the subsequent investigation of the safe utilization of swine manure and its derived biochar in heavy metal-

contaminated agricultural soils.

Conclusion

Heavy metal contamination in agricultural soils poses a persistent threat to soil quality, crop safety, and human health. Among the major contaminants, Cd is of particular concern because of its high mobility, strong plant availability, and tendency to accumulate in cereal grains. Since heavy metal risks are controlled by metal speciation, soil properties, rhizosphere processes, crop type, and management practices, remediation should focus on reducing bioavailability and limiting transfer from soil to edible crop tissues.

Livestock manure is an important organic resource for nutrient recycling and soil fertility improvement, but intensive livestock production has made manure a potential source of Cu, Zn, Cd, As, and other heavy metals. Long-term manure application may cause cumulative metal inputs and increase farmland contamination risks. Therefore, safer manure utilization strategies are needed, especially for swine manure, which often contains relatively high Cu and Zn concentrations.

Pyrolysis can convert swine manure into biochar with higher stability, alkalinity, mineral nutrient content, and heavy metal immobilization potential. Although total metal concentrations may increase during pyrolysis due to mass loss, metal mobility and bioavailability are often reduced through transformation into more stable fractions. Biochar can immobilize heavy metals through pH regulation, adsorption, ion exchange, functional group complexation, mineral precipitation, and metal fraction transformation.

However, the effectiveness of biochar must be evaluated within the whole soil–crop system. Reduced soil metal availability does not always guarantee lower crop uptake or grain accumulation. Biochar performance is influenced by feedstock, pyrolysis conditions, soil pH, metal type, crop species, water management, aging, and microbial processes. Microorganisms may further contribute to Cd immobilization through biosorption, extracellular polymer binding, biomineralization, and rhizosphere regulation, but they may also remobilize metals under certain conditions.

Overall, swine manure biochar has considerable potential for the safe utilization of livestock waste and the remediation of heavy metal-contaminated farmland. Future research should focus on the long-term stability of biochar-immobilized metals, crop-specific safety outcomes, and the coupled physicochemical–microbial mechanisms controlling heavy metal migration in soil–crop systems.

Chapter 3 Multi-scale Assessment of Heavy Metal Pollution

Characteristics, Spatiotemporal Variation, and Cropland Input

Risks in Livestock and Poultry Manures

Livestock and poultry manure is an important agricultural resource for nutrient recycling and soil fertility improvement, but its repeated application may also introduce heavy metals such as Cu, Zn, As, Cd, Pb, Cr, Ni, and Hg into cropland (Westerman & Bicudo, 2005; Belon et al., 2012; Nicholson et al., 2003). Cu and Zn are generally the most abundant metals because of their widespread use as feed additives and their relatively low absorption efficiency in animals, whereas elements such as Cd, As, Pb, Cr, Ni, and Hg occur at lower concentrations but may pose greater ecological and food-safety concerns because of their toxicity, persistence, and low environmental thresholds (Jondreville et al., 2003; Li et al., 2007; Wang et al., 2014; Alloway, 2013; Farid et al., 2025). The environmental significance of manure-derived metals therefore depends not only on their concentrations, but also on long-term accumulation, bioavailability, and transfer through the soil–crop system (Lopes et al., 2011; Tóth et al., 2016).

Previous studies have examined heavy metal contamination in livestock manure from feed–manure–soil, pollution-load, and agricultural-input perspectives (Wang et al., 2013; Xu et al., 2019; Liu et al., 2020; Leclerc and Laurent, 2017). However, differences in livestock categories, sample types, analytical bases, and reporting units have limited data comparability, while many studies have focused on single species, individual regions, or a limited number of elements. In addition, concentration-based assessment alone cannot fully represent environmental risk because manure production, land-application proportion, cropland area, and regional livestock structure jointly determine total and unit-area metal inputs (Leclerc & Laurent, 2017; Liu et al., 2020).

To address these limitations, this study compiled Chinese and English literature published from 2000 to May 2026 and established a standardized database for Cd, Cu, Zn, Pb, Cr, As, Ni, and Hg in livestock and poultry manures. The study compared metal concentrations among different manure types, evaluated global and provincial spatial patterns and temporal trends, assessed exceedance risks and single-factor pollution indices, and further estimated manure application, total heavy metal inputs, and cropland-area-based loads across China using livestock and cropland statistics. This integrated framework was designed to support safer

manure recycling, source-oriented heavy metal control, and region-specific agricultural risk management..

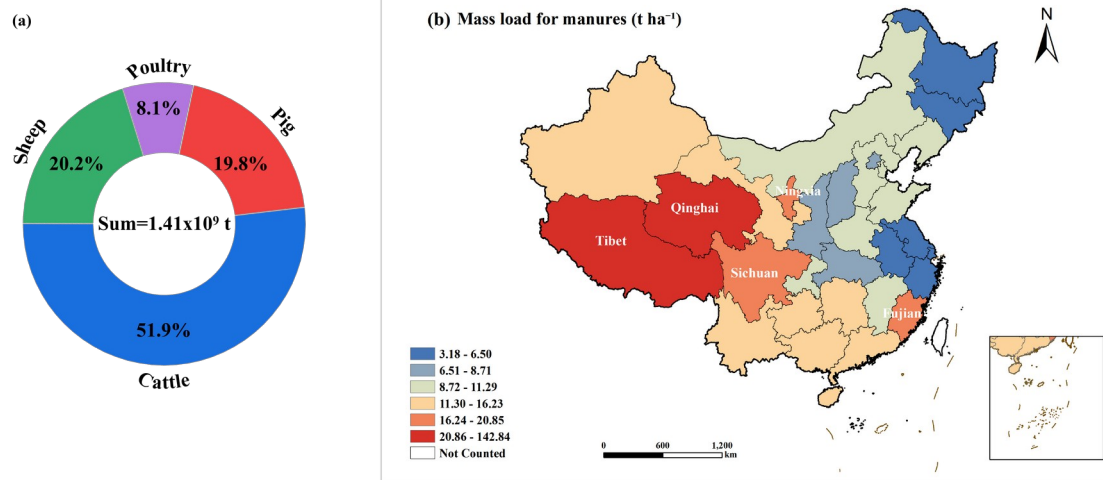


Fig. 3.7. Estimated fresh-weight manure production and manure load of major livestock and poultry in China based on 2024 statistics from the China Rural Statistical Yearbook 2025. (a) Total manure production and relative contribution of different livestock and poultry categories; (b) provincial distribution of unit cropland-area manure load.

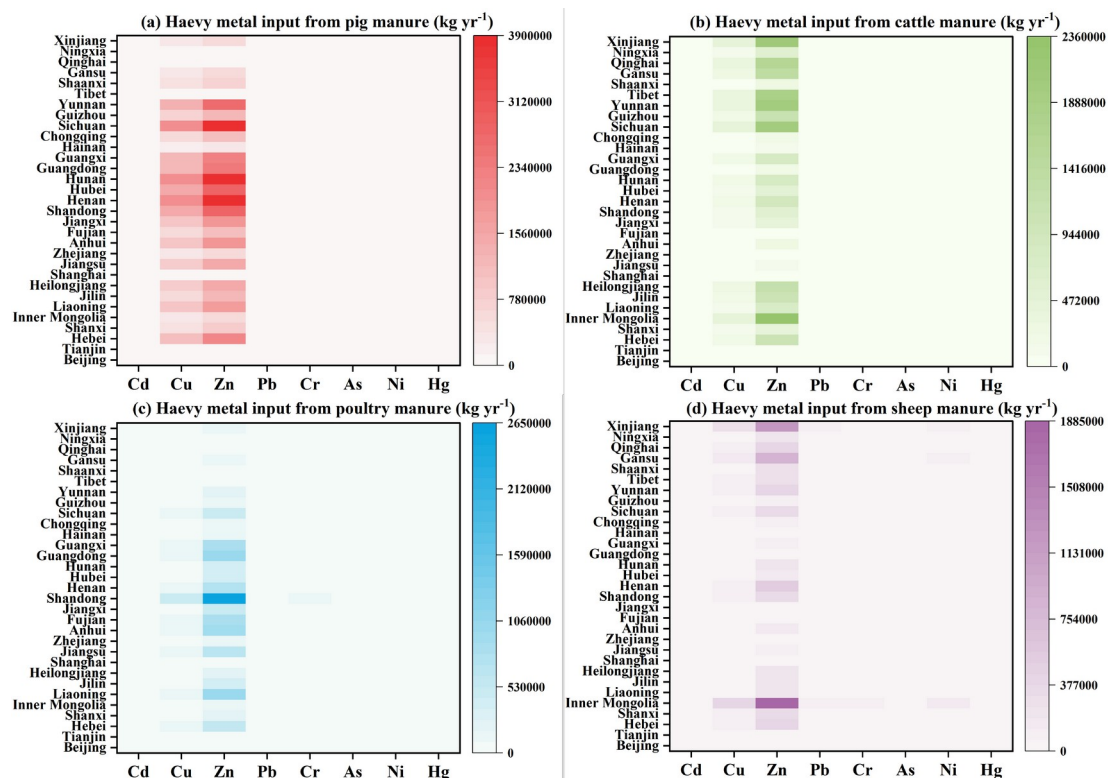


Fig. 3.8. Provincial distribution of heavy metal inputs from different livestock and poultry manures in China, based on 2024 livestock statistics. Heat maps represent estimated heavy metal inputs from (a) pig manure, (b) cattle manure, (c) poultry manure, and (d) sheep/goat manure.

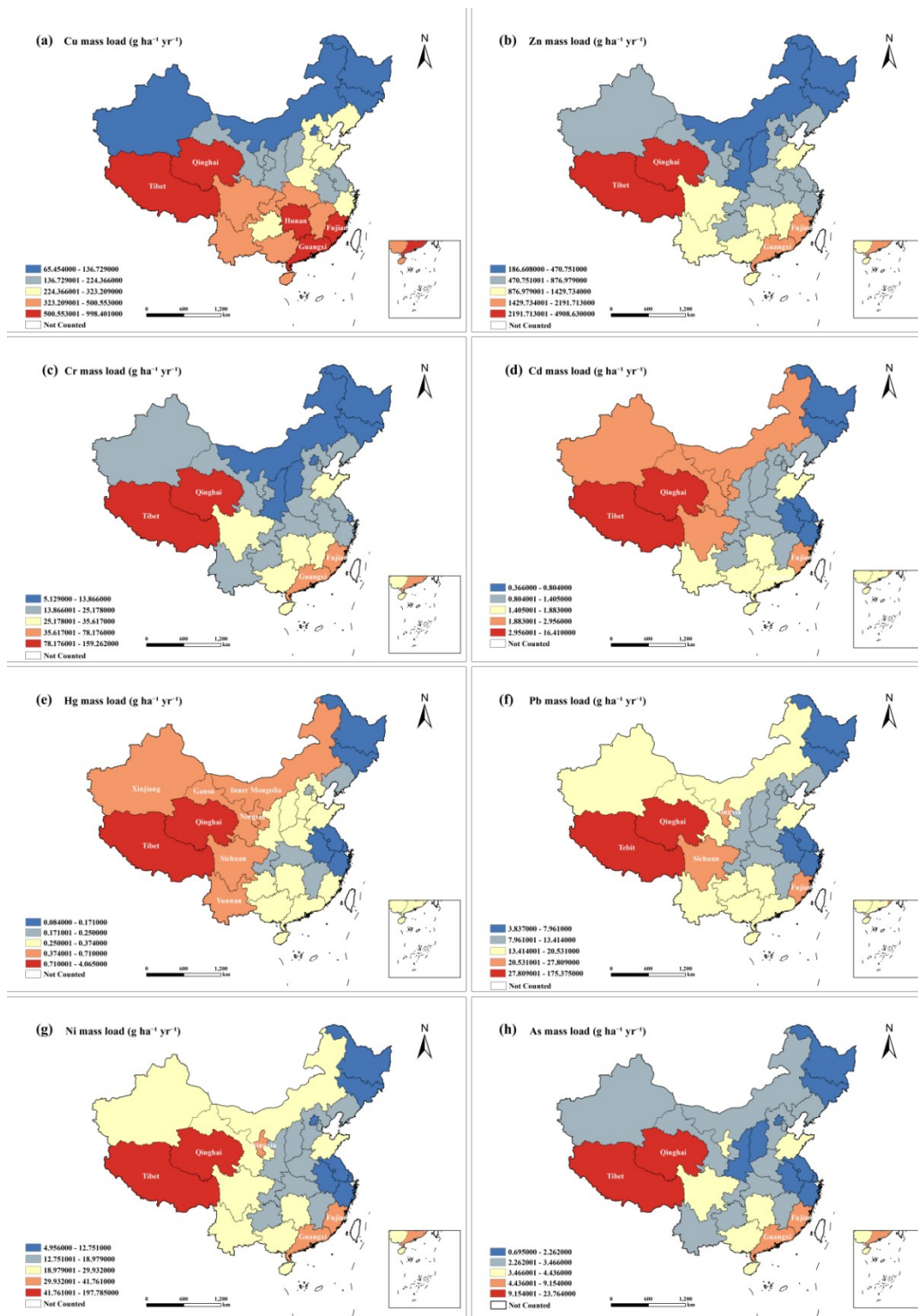


Fig. 3.9. Provincial distribution of manure-derived heavy metal input loads from different livestock and poultry manures in China, based on 2024 livestock statistics.

Conclusions

This study systematically integrated concentration data for Cd, Cu, Zn, Pb, Cr, As, Ni, and Hg in livestock and poultry manures at global and Chinese scales, and evaluated their patterns

across manure types, spatial distributions, temporal changes, pollution risks, total metal inputs, and cropland-area-based loads. The results showed clear element-specific and livestock-specific differences in heavy metal contamination. Cu and Zn were the dominant high-abundance elements, particularly in pig and poultry manures, mainly due to their long-term use as feed trace-element additives and their limited absorption efficiency in animals. In contrast, Cd, As, Hg, Pb, Cr, and Ni contributed less to the total metal mass, but some of these elements may still pose ecological and food-safety risks because of their high toxicity, low environmental thresholds, long-term soil accumulation, and potential crop uptake.

The risk profiles varied among manure types. Pig manure was mainly characterized by elevated Cu and Zn concentrations, while poultry manure also showed relatively high levels of some metals. Cattle and sheep manures generally contained lower metal concentrations, but their large manure production volumes mean that their contributions to total metal inputs cannot be ignored. Spatially, high-load regions were commonly associated with intensive livestock production, large manure generation, and limited cropland capacity for manure assimilation. These findings indicate that concentration-based assessment alone is insufficient; manure production, land application rate, and unit-area metal loading should also be considered.

Overall, livestock and poultry manures are both valuable organic fertilizer resources and important potential sources of heavy metal inputs to agricultural soils. Their risks should therefore be assessed using an integrated framework that considers concentration, total input, unit-area loading, and element-specific toxicity. Future work should strengthen source control in animal feed, optimize the use of trace-element additives, improve long-term monitoring across livestock types and regions, and incorporate metal speciation, bioavailability, and soil–crop transfer processes into manure safety assessment systems.

Chapter 4 Effects of aging processes on the potential release behavior of heavy metals from swine manure biochar

The rapid intensification of livestock production has generated large quantities of manure and increased pressure for safe waste management (Parker et al., 2021; Bao et al., 2019). Although manure application can recycle nutrients and organic matter, excessive use may introduce nutrients, pathogens, antibiotics, and heavy metals into farmland, creating long-term risks to soil quality and crop safety (Antonello-Maddox et al., 2020; Ahmad et al., 2020; Zhou et al., 2020; Liu et al., 2020). Pyrolysis offers an alternative management strategy by reducing manure volume, destroying pathogens and organic contaminants, stabilizing carbon, and decreasing the immediate mobility of manure-derived metals (Xu et al., 2018; Meng et al., 2022; Zeng et al., 2018).

Swine manure biochar is particularly relevant for heavy metal remediation because its alkaline properties, porous structure, reactive functional groups, and mineral components can immobilize metals through pH regulation, adsorption, ion exchange, complexation, and precipitation (Chen et al., 2019; Liang et al., 2019; Zhang et al., 2020). However, biochar undergoes environmental aging after soil application, which may alter its surface chemistry, pore structure, mineral phases, and metal-retention capacity (Wang et al., 2021; Xia et al., 2023). H_2O_2 oxidation is therefore commonly used to simulate oxidative aging, although it represents only part of natural field aging processes (Hale et al., 2012; Li et al., 2019). Pyrolysis temperature further influences aging stability, as low-temperature biochars generally retain more labile components and functional groups, whereas high-temperature biochars show greater aromaticity and structural persistence.

Accordingly, this study investigated swine manure biochars produced at 300, 500, and 700 °C under different H_2O_2 oxidation intensities. Carbon and nitrogen losses, release of Cd, Cu,

Zn, Ni, Cr, and As, changes in surface functional groups and mineral phases, and microstructural transformation were evaluated to clarify how pyrolysis temperature and oxidative aging affect the long-term environmental stability and remediation potential of swine manure biochar.

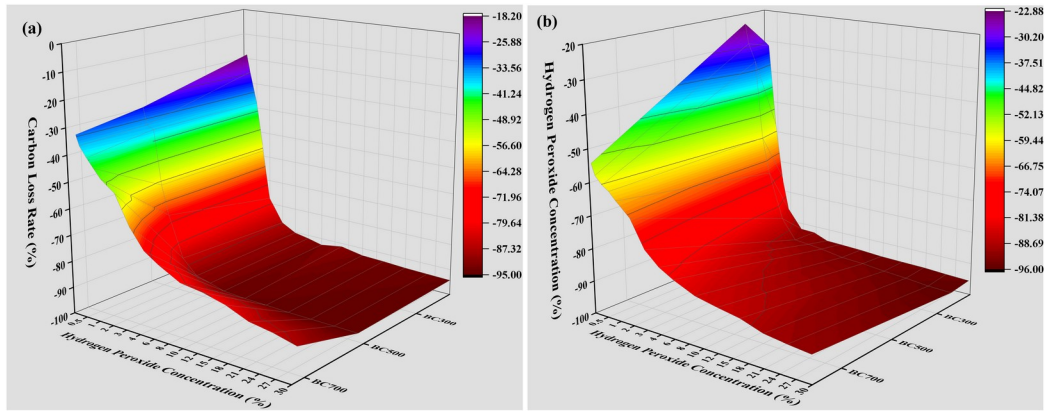


Fig. 4.1. C and N loss rates of swine-manure biochars produced at different pyrolysis temperatures under increasing H_2O_2 concentrations.

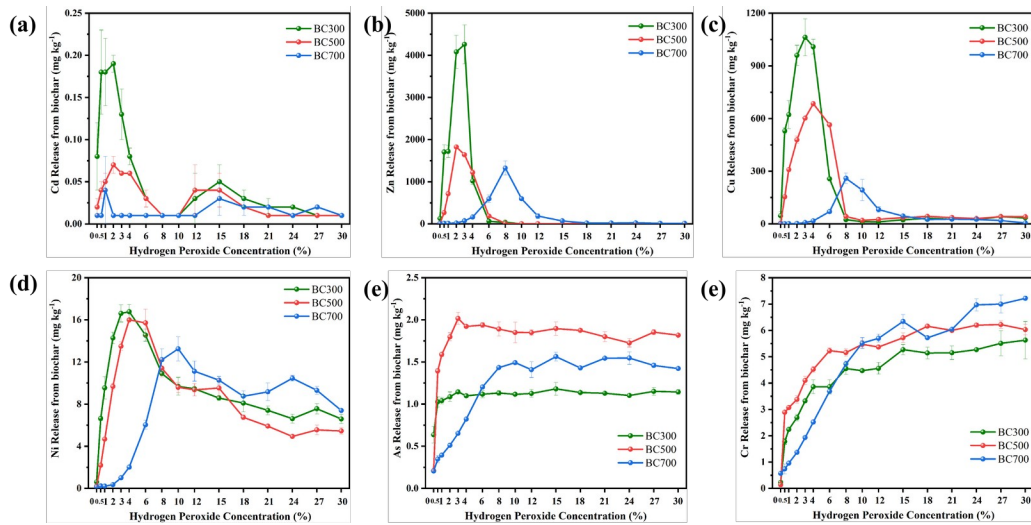


Fig. 4.2. Element-specific release patterns of endogenous Cd, Zn, Cu, Ni, As, and Cr from swine-manure biochars during H_2O_2 oxidation.

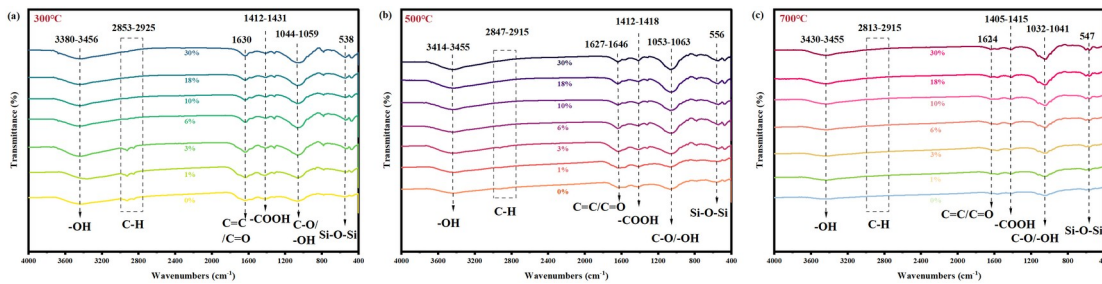


Fig. 4.3. FTIR spectra of biochars at 300 °C, 500 °C, and 700 °C before and after oxidation with different H₂O₂ concentrations.

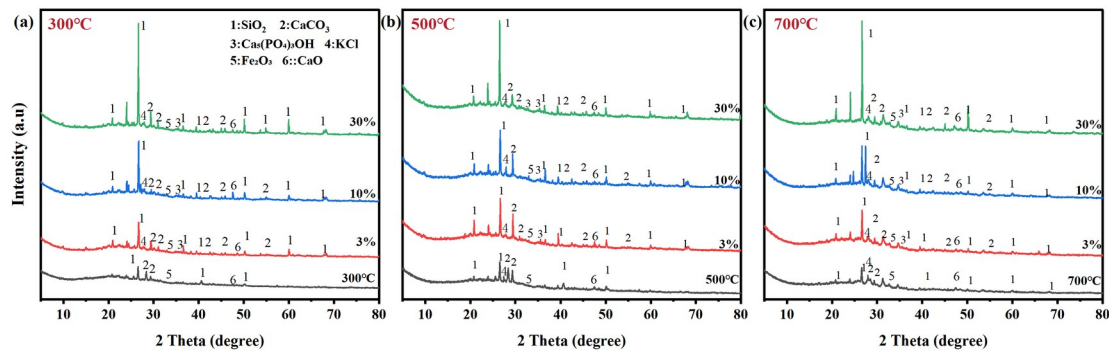


Fig. 4.4. XRD spectra of biochar after oxidation with different concentrations of hydrogen peroxide at 300 °C, 500 °C, and 700 °C.

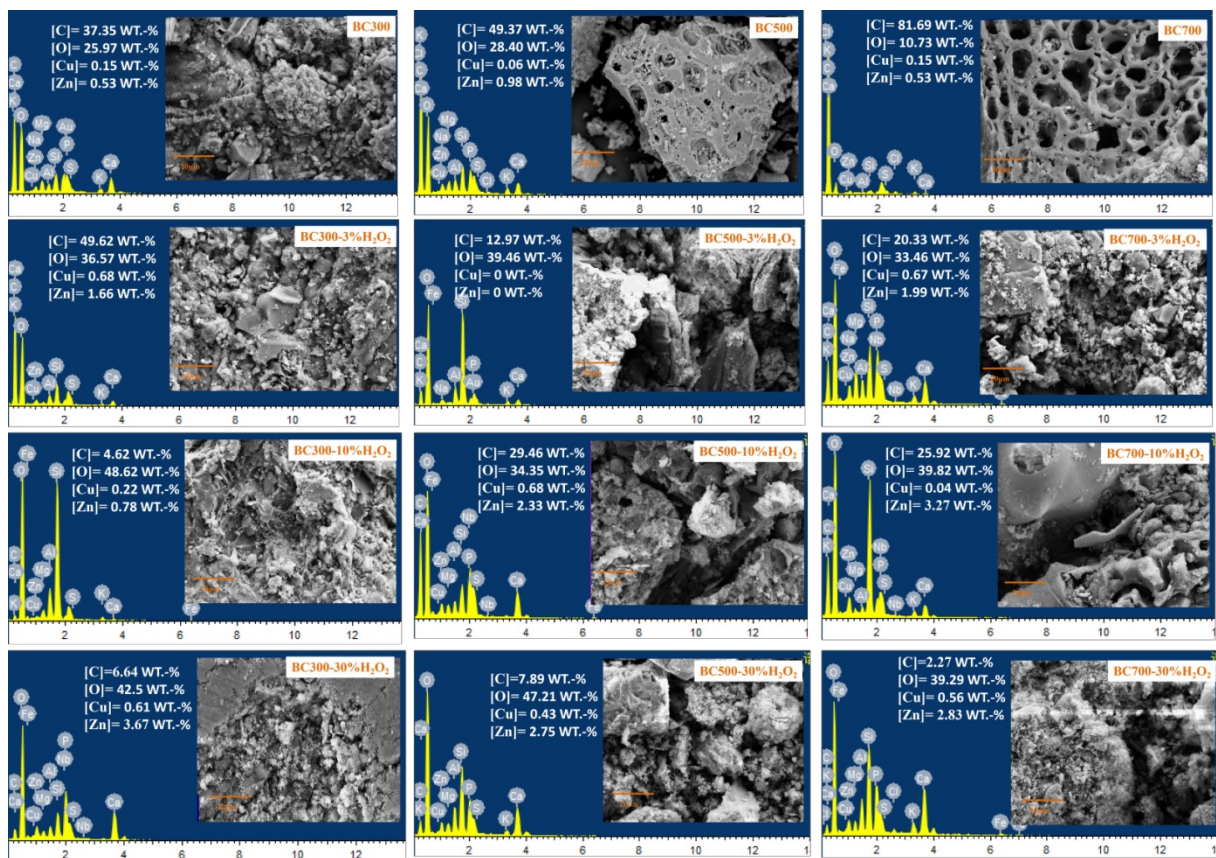


Fig. 4.5. SEM-EDS images of biochar after oxidation with different concentrations of hydrogen peroxide at 300 °C, 500 °C, and 700 °C.

Conclusions

This study systematically investigated the oxidative aging behavior of swine manure biochars produced at 300, 500, and 700 °C using an H₂O₂ concentration gradient as an

accelerated aging approach. The results demonstrated that oxidative aging significantly altered the physicochemical properties of swine manure biochars, including elemental composition, surface functional groups, mineral phases, microstructure, and heavy metal retention behavior. The major conclusions are as follows:

Oxidative aging caused substantial losses of carbon and nitrogen in all biochars, but the magnitude of change depended strongly on pyrolysis temperature. Biochars produced at lower temperatures (BC300 and BC500) exhibited more rapid increases in C and N loss under low to moderate oxidation intensity, whereas BC700 showed relatively slower and more moderate changes throughout the oxidation gradient, indicating stronger resistance to oxidative degradation.

Heavy metal release during oxidative aging showed clear element-specific patterns. Cd, Zn, Cu, and Ni exhibited release peaks at low to moderate H₂O₂ concentrations, followed by declines or stabilization at higher oxidation intensity. In contrast, Cr release increased progressively with increasing H₂O₂ concentration, while As showed comparatively smaller variation. These results indicate that different metals were retained by distinct mechanisms and responded differently to oxidative changes in biochar properties.

Mineral phases and microstructural integrity played important roles in maintaining long-term immobilization functions. XRD and SEM-EDS results showed that oxidative aging altered ash-derived minerals containing Ca, P, K, and Fe, while also causing surface roughening, cracking, and pore collapse. High-temperature biochar maintained a more intact particle framework and more stable mineral characteristics than low-temperature biochar, suggesting greater long-term structural persistence.

Pyrolysis temperature significantly regulated the long-term environmental stability of swine manure biochar. Although BC700 showed the strongest resistance to oxidative aging, biochar selection for agricultural remediation should not rely solely on structural recalcitrance. Practical performance is expected to depend on the balance between environmental stability, reactive surface functionality, nutrient properties, and interactions with soil ecosystems.

Overall, this chapter demonstrates that swine manure biochar does not remain static after environmental application, but undergoes continuous physicochemical evolution during aging.

The long-term remediation potential of biochar therefore depends not only on its initial properties, but also on how its functional groups, mineral phases, and structural characteristics change over time. These findings provide an important mechanistic basis for evaluating the performance of swine manure biochar in subsequent wheat and paddy field remediation systems.

Chapter 5 Effects of swine manure biochar on the bioavailability and accumulation of heavy metals in wheat plant in soil

Cadmium (Cd) contamination of agricultural soils is a major food-safety concern because of its high mobility, toxicity, and tendency to accumulate in wheat grain (Adrees et al., 2021; Cui et al., 2026; Xia et al., 2023). In China, Cd contamination is widespread, and excessive grain Cd has been reported even in some soils that comply with soil-quality standards, indicating that soil total Cd alone cannot reliably predict crop safety (Li et al., 2019; Li et al., 2025; Wang et al., 2020). Effective control therefore requires not only reducing soil Cd availability, but also restricting root uptake and long-distance transport to edible tissues. Cd behavior in wheat is closely linked with Zn because the two divalent metals may share uptake and transport pathways, although Zn is an essential nutrient while Cd is toxic (Cakmak et al., 2008; Saifullah et al., 2014; Cui et al., 2026). Organic amendments such as compost and biochar can reduce Cd mobility by increasing soil pH and promoting adsorption, complexation, ion exchange, and mineral precipitation (Rahim et al., 2024; Rehman et al., 2018). Biochar has shown considerable potential for reducing wheat Cd accumulation, but its effectiveness depends on feedstock, pyrolysis conditions, soil properties, and application rate. Compared with crop-residue biochar, manure-derived biochar contains more ash, nutrients, alkaline minerals, and endogenous metals, while its effects on simultaneous Cd and Zn redistribution among wheat tissues remain insufficiently understood.

Metal accumulation in wheat grain is governed not only by soil availability but also by internal transport through roots, stems, nodes, internodes, and reproductive tissues. Wheat nodes can act as important junctions for metal retention, redistribution, and xylem–phloem transfer, thereby influencing the final delivery of Cd and Zn to grains (Di et al., 2023; Xia et al., 2023; Zhao et al., 2022). Accordingly, this field study compared swine manure and its biochar at two application rates in acidic Cd-contaminated soil and quantified Cd and Zn in soil and

individual wheat tissues. The objectives were to determine their effects on soil acidity and metal availability, clarify the role of roots in metal uptake and upward transfer, and identify how nodes and internodes regulate long-distance Cd/Zn transport and final grain accumulation, thereby providing a basis for reducing Cd risk while maintaining wheat Zn quality (Cui et al., 2026).

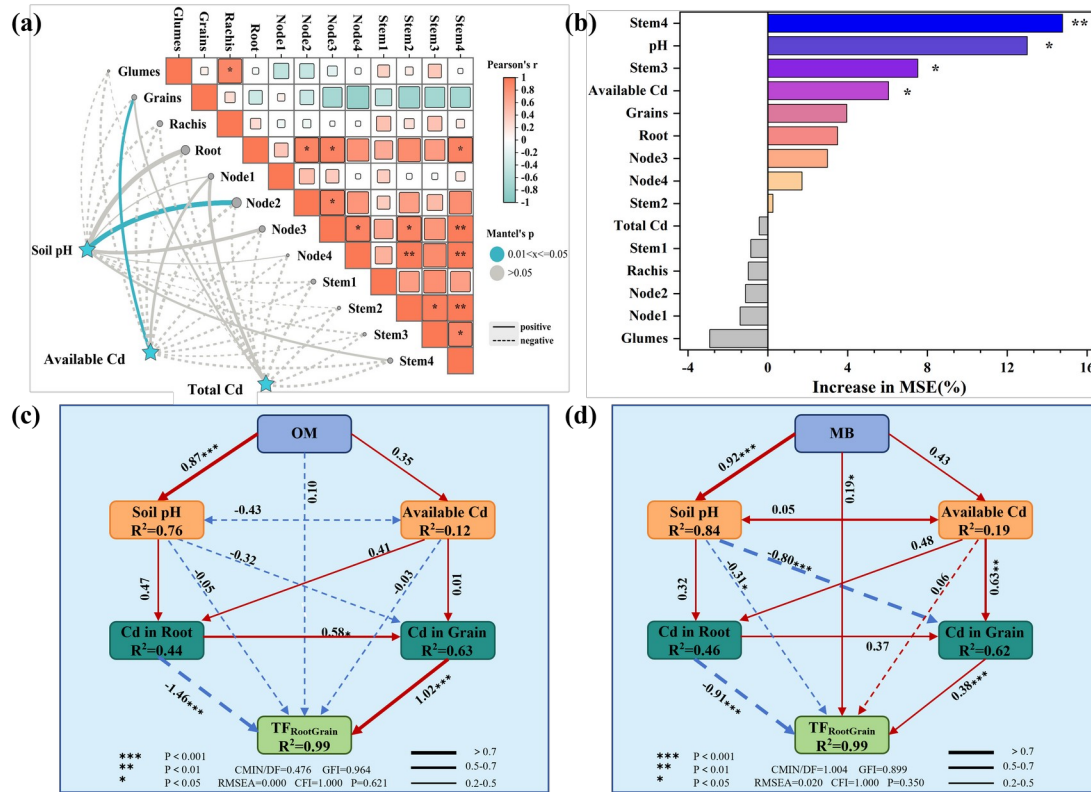


Fig. 5.3. Soil and plant variables associated with grain Cd. a, Pearson correlations between candidate variables and tissue Cd. b, Random-forest ranking of predictors for grain Cd. c, Direct and indirect pathways under swine manure (OM). d, Corresponding pathways under biochar (MB). *, **, and * denote significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively (Cui et al., 2026).**

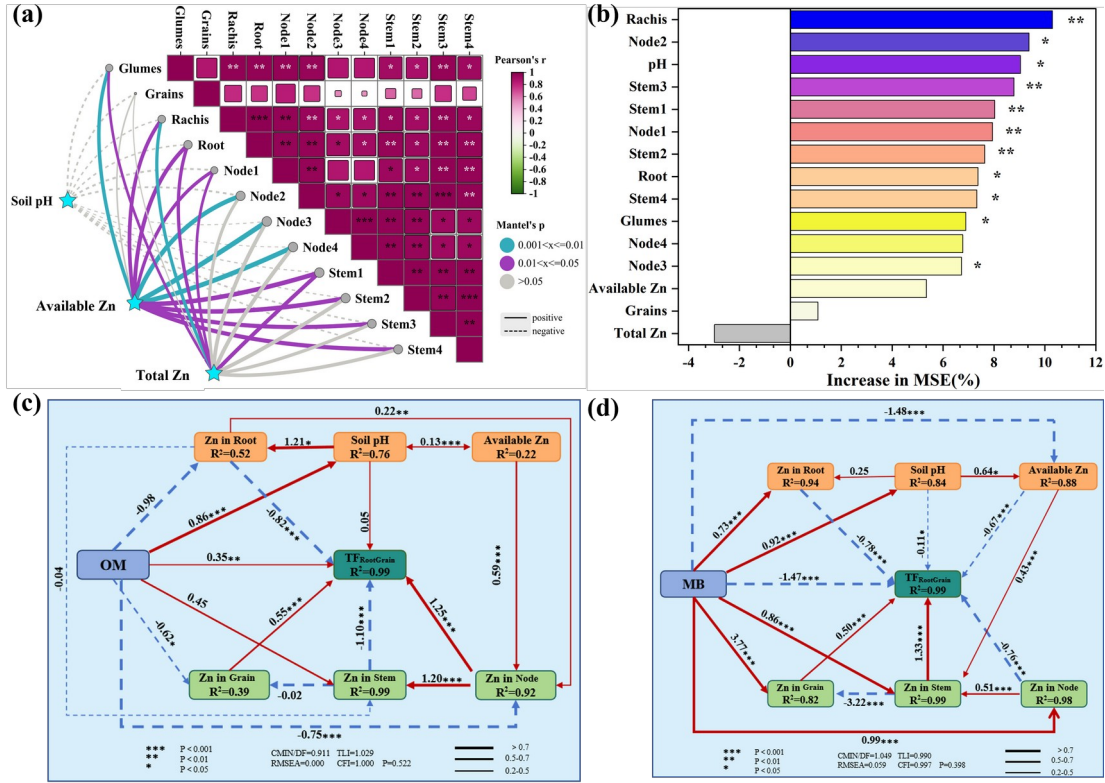


Fig. 5.4. Soil and plant variables associated with grain Zn. a, Pearson correlations between candidate variables and tissue Zn. b, Random-forest ranking of predictors for grain Zn. c, Direct and indirect pathways under swine manure (OM). d, Corresponding pathways under biochar (MB). *, **, and * denote significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively (Cui et al., 2026).**

Conclusions

The field experiment showed that biochar produced from swine manure regulated Cd and Zn more effectively than the original composted manure. MB generated a stronger increase in soil pH, reduced the soil fraction of Cd accessible to roots, lowered Cd accumulation in wheat grain, and increased Zn uptake. OM produced weaker and less consistent responses, particularly at the lower rate of 1.5 t ha⁻¹. The reduction in grain Cd under MB resulted from coordinated regulation in both soil and plant compartments. Biochar decreased Cd availability in the acidic soil and reduced the efficiency of root-to-grain and rachis-to-grain transfer. At the same time, greater amounts of Cd were retained in roots, nodes, stems, rachis, and glumes. These non-edible tissues consequently acted as competing sinks that limited Cd delivery to grain. Biochar also altered Zn partitioning. It enlarged the total Zn pool within wheat and increased grain Zn, although the largest proportional increases occurred in roots, nodes, stems, and other non-grain tissues. Structural equation modeling and random-forest analysis consistently identified nodes

as important regulatory sites for both metals. These tissues controlled the onward passage of Cd while serving as storage and redistribution zones for Zn. Taken together, the findings demonstrate that swine-manure biochar can simultaneously decrease grain Cd risk and improve Zn accumulation by combining soil alkalization, metal immobilization, and regulation of internal transport pathways. Its application therefore offers a practical strategy for improving crop safety and managing metal-contaminated acidic farmland. Future studies should compare biochars derived from different livestock manures and evaluate whether the observed nodal restriction mechanism remains consistent across soil types, crop varieties, amendment rates, and longer field periods (Cui et al., 2026).

Chapter 6 Effects of swine manure biochar on the bioavailability and accumulation of Cd in rice in paddy soil: evidence from a 5-year field experiment

Cadmium (Cd) contamination in paddy soils is a major food-safety concern because of its high mobility, efficient crop uptake, and long biological persistence in humans (Kubier et al., 2019; Nordberg et al., 2015). Rice is particularly vulnerable because alternating flooding and drainage can strongly regulate Cd solubility and uptake (Zhao et al., 2020). In southern China, including Zhejiang Province, acidic paddy soils frequently show excessive soil and grain Cd, highlighting the need for effective long-term mitigation strategies (Liu et al., 2016; Guo et al., 2020). Soil pH is a key factor controlling Cd availability, and although liming can reduce Cd mobility, its effectiveness may vary among seasons and repeated application may disturb soil fertility and nutrient balance (Wang et al., 2019; Zhang et al., 2025).

Biochar has therefore attracted attention as a more persistent amendment because its alkalinity, porous structure, reactive surface groups, and mineral components can immobilize Cd through pH elevation, ion exchange, adsorption, complexation, and precipitation (Kosolsaksakul et al., 2018; Guo et al., 2025; Zhou et al., 2025). Swine-manure biochar is especially relevant because it combines livestock-waste valorization with soil remediation and generally contains abundant ash and alkaline minerals that favor Cd stabilization (Meng et al., 2022; Sui et al., 2023). However, most previous studies have focused on crop-residue biochars or relatively short-term experiments, while the durability of manure-derived biochar under repeated paddy-field redox cycles remains insufficiently understood (Zong et al., 2021; Wang et al., 2021). Aging processes may gradually alter surface chemistry, mineral phases, pore accessibility, and Cd-retention capacity, making long-term field evaluation essential (Long et al., 2024).

To address these uncertainties, a five-year field experiment was conducted from 2019 to 2023 in an acidic Cd-contaminated paddy soil in Fuyang, Zhejiang Province. Lime, swine manure, and swine-manure biochar were compared in terms of soil pH, available Cd, brown-rice Cd concentration, and grain yield. Particular attention was given to the temporal stability of remediation and to differences between repeated low-rate biochar application and a one-time high-dose treatment. The study aimed to determine whether swine-manure biochar could provide more persistent Cd immobilization and safer rice production than conventional amendments under long-term field conditions.

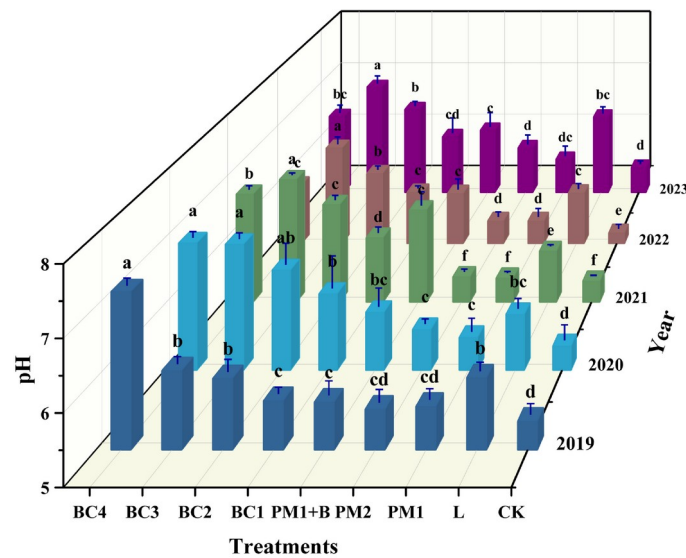


Fig. 6.1. Soil pH under different amendment treatments in a cadmium-contaminated paddy field from 2019 to 2023

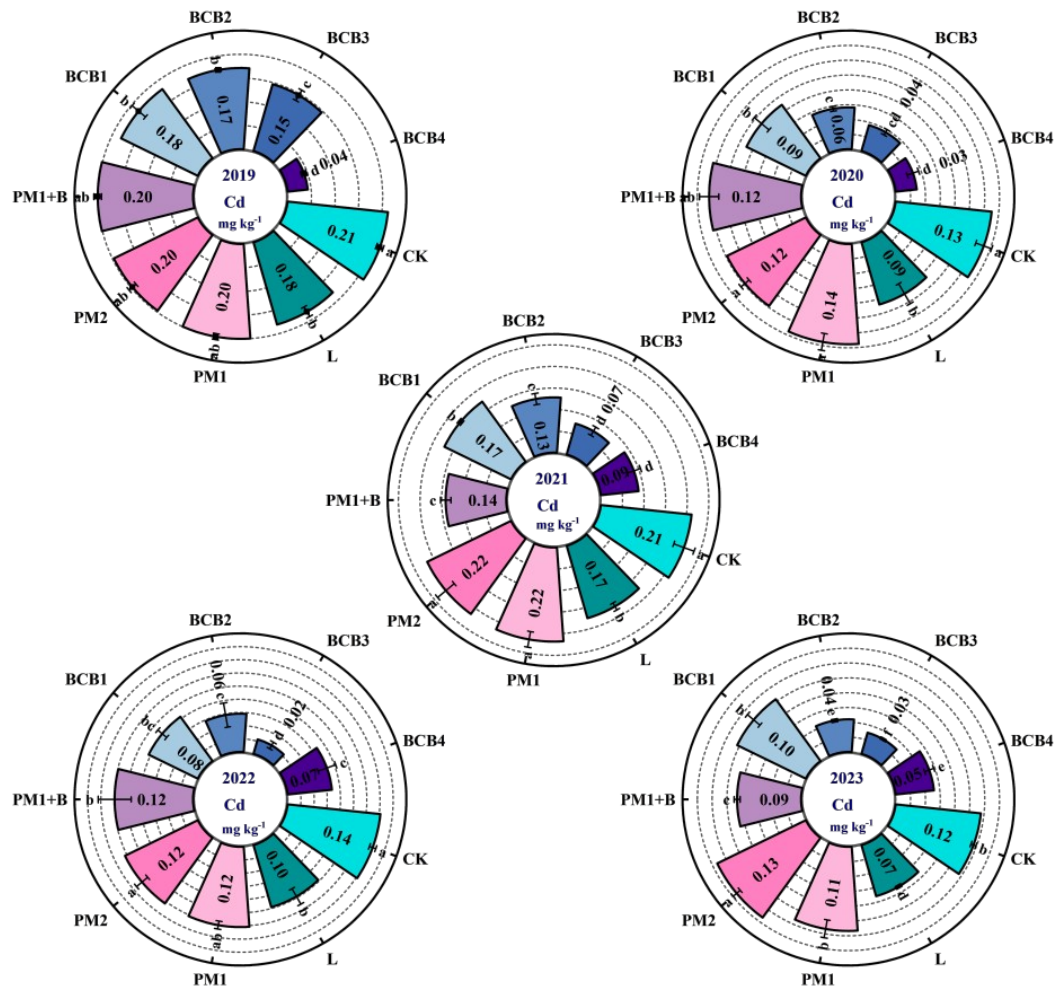


Fig. 6.2. Soil available Cd under different amendment treatments in a cadmium-contaminated paddy field from 2019 to 2023

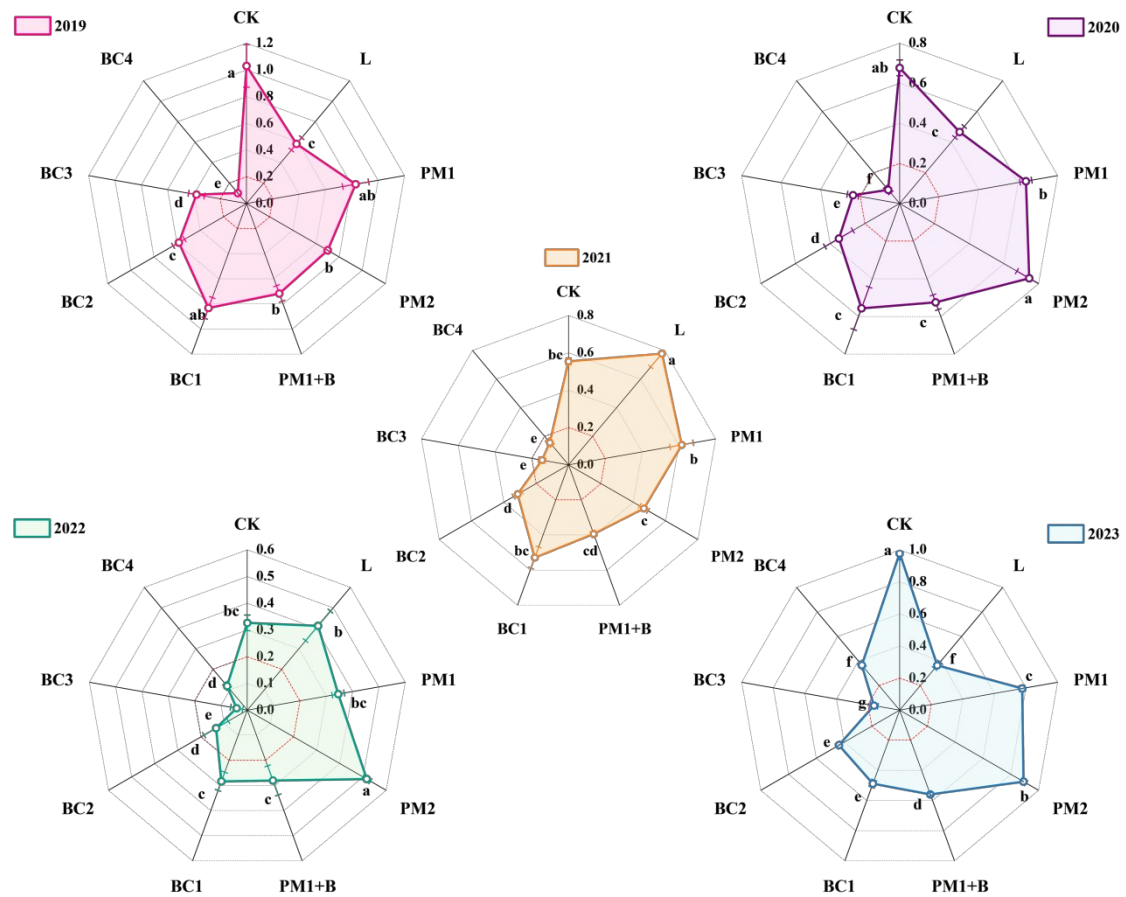


Fig. 6.3. Cd concentration in brown rice under different amendment treatments in a cadmium-contaminated paddy field from 2019 to 2023

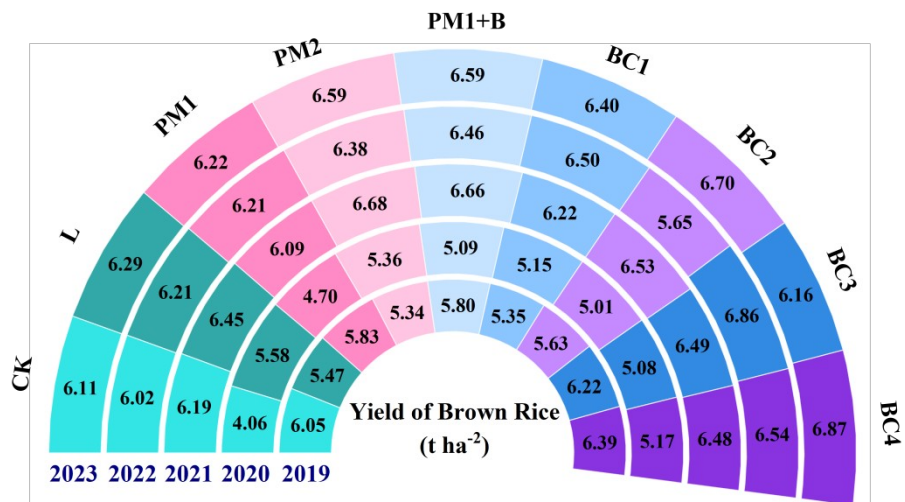


Fig. 6.4. Rice grain yield under different amendment treatments in a cadmium-contaminated paddy field from 2019 to 2023

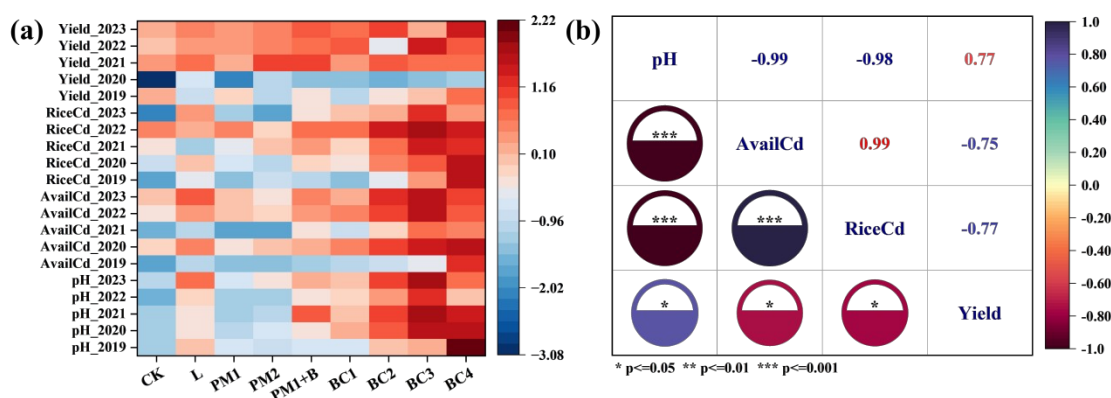


Fig. 6.5. The heatmap (a) and correlation analysis (b) among soil pH, available Cd, brown rice Cd, and grain yield across treatments from 2019 to 2023.

Conclusion

Based on a five-year field experiment (2019-2023) conducted in a Cd-contaminated acidic paddy field in Fuyang, Zhejiang Province, this chapter systematically compared the effects of lime, swine manure, and swine manure biochar on soil pH, soil available Cd, brown rice Cd concentration, and rice yield. The results showed that the CK treatment maintained a low five-year mean soil pH of 5.36, with five-year mean soil available Cd and brown rice Cd concentrations of 0.162 mg kg⁻¹ and 0.714 mg kg⁻¹, respectively. The raw swine manure treatments showed only limited effects on soil acidity regulation and Cd immobilization, with five-year mean soil pH values of only 5.51 (PM1) and 5.57 (PM2), and mean reductions in available Cd of only about 2.5% relative to CK. In contrast, the manure-derived biochar treatments markedly increased soil pH and reduced Cd bioavailability. In particular, the five-year mean soil pH values under BC3 and BC4 reached 6.74 and 6.67, respectively, while the corresponding five-year mean available Cd concentrations decreased to 0.062 and 0.056 mg kg⁻¹, and brown rice Cd concentrations decreased to 0.192 and 0.168 mg kg⁻¹, representing reductions of 73.1% and 76.5% relative to CK.

Clear differences were observed among biochar application strategies. BC4 produced the strongest initial remediation response, with soil pH reaching 7.21 in 2019 and available Cd and brown rice Cd decreasing to 0.04 mg kg⁻¹ and 0.10 mg kg⁻¹, respectively. However, its immobilization effect weakened to some extent in the later stage of the experiment. By contrast, BC3 showed greater long-term stability, with available Cd declining to 0.02 and 0.03 mg kg⁻¹ in

2022 and 2023, respectively, and brown rice Cd reaching 0.04 and 0.16 mg kg⁻¹, the lowest or near-lowest values among all treatments. In terms of agronomic performance, none of the amendments caused obvious yield penalties. The five-year mean yield under CK was 5.69 t ha⁻¹, whereas BC3 and BC4 increased it to 6.16 and 6.29 t ha⁻¹, respectively. Correlation analysis further revealed that soil pH was strongly and negatively correlated with available Cd and brown rice Cd ($r = -0.99$ and -0.98 , respectively), while available Cd was strongly and positively correlated with brown rice Cd ($r = 0.99$), indicating that increasing soil pH and reducing Cd bioavailability were the key mechanisms controlling Cd transfer from soil to rice grain. Overall, BC3 showed the best long-term stability for Cd control, whereas BC4 combined strong early-stage remediation, favorable yield performance, and the practical advantage of one-time application. Therefore, BC4 can be regarded as the preferred option for the safe utilization and large-scale extension of Cd-contaminated paddy soils under practical agricultural conditions.

Chapter 7 Effects of swine manure biochar on soil microbial diversity and community structure in paddy soil

Soil acidification caused by intensive agricultural management can reduce nutrient availability, increase the mobility of toxic metals, and disrupt microbial activity, thereby threatening soil health and crop productivity (Bünemann et al., 2018; Liu et al., 2021; Li et al., 2023; Meng et al., 2026). Lime and livestock manure are commonly used to improve acidic soils, but liming effects may weaken over time, while repeated manure application can introduce pathogens, heavy metals, and other contaminants (Hu et al., 2016a, b; Nicholson et al., 1999; Meng et al., 2026). Biochar offers a more persistent alternative because its alkaline minerals, stable carbon framework, porous structure, and sorption capacity can improve soil pH, nutrient retention, carbon storage, and contaminant immobilization (Biederman and Harpole, 2013; Lehmann, 2007; Yao et al., 2012; Meng et al., 2026).

Beyond these physicochemical effects, biochar can reshape soil microbial communities by altering pH, nutrient supply, substrate availability, and habitat structure. Microorganisms are central to nutrient cycling, carbon transformation, and soil ecological functioning, while their responses to biochar depend strongly on application rate and soil conditions (Trivedi et al., 2020; Fierer et al., 2021; Zhao et al., 2024; Meng et al., 2026). However, most previous studies have focused on bacteria and fungi over relatively short periods, whereas the long-term responses of microbial functions, viral communities, and virus–host interactions remain poorly understood (Kerner et al., 2023; Santos-Medellín et al., 2022; Meng et al., 2026). Integrating metagenomics, metaviromics, and metabolomics is therefore necessary to determine whether biochar-induced changes in soil chemistry propagate through microbial communities and ultimately alter ecosystem functions.

To address these questions, a five-year field experiment in acidic paddy soil compared biochar applied at 4.5, 11.25, and 22.5 t ha⁻¹ with lime and swine manure. Soil physicochemical properties and metal bioavailability were analyzed together with prokaryotic and fungal

communities, viral assemblages, metagenomic functions, and metabolite profiles. The study aimed to determine whether long-term biochar application could generate coordinated improvements in soil chemistry, microbial and viral community structure, functional pathways, and metabolic processes, thereby providing a mechanistic basis for understanding the transition from chemical soil improvement to broader ecological recovery and for optimizing biochar application in acidified agricultural soils.

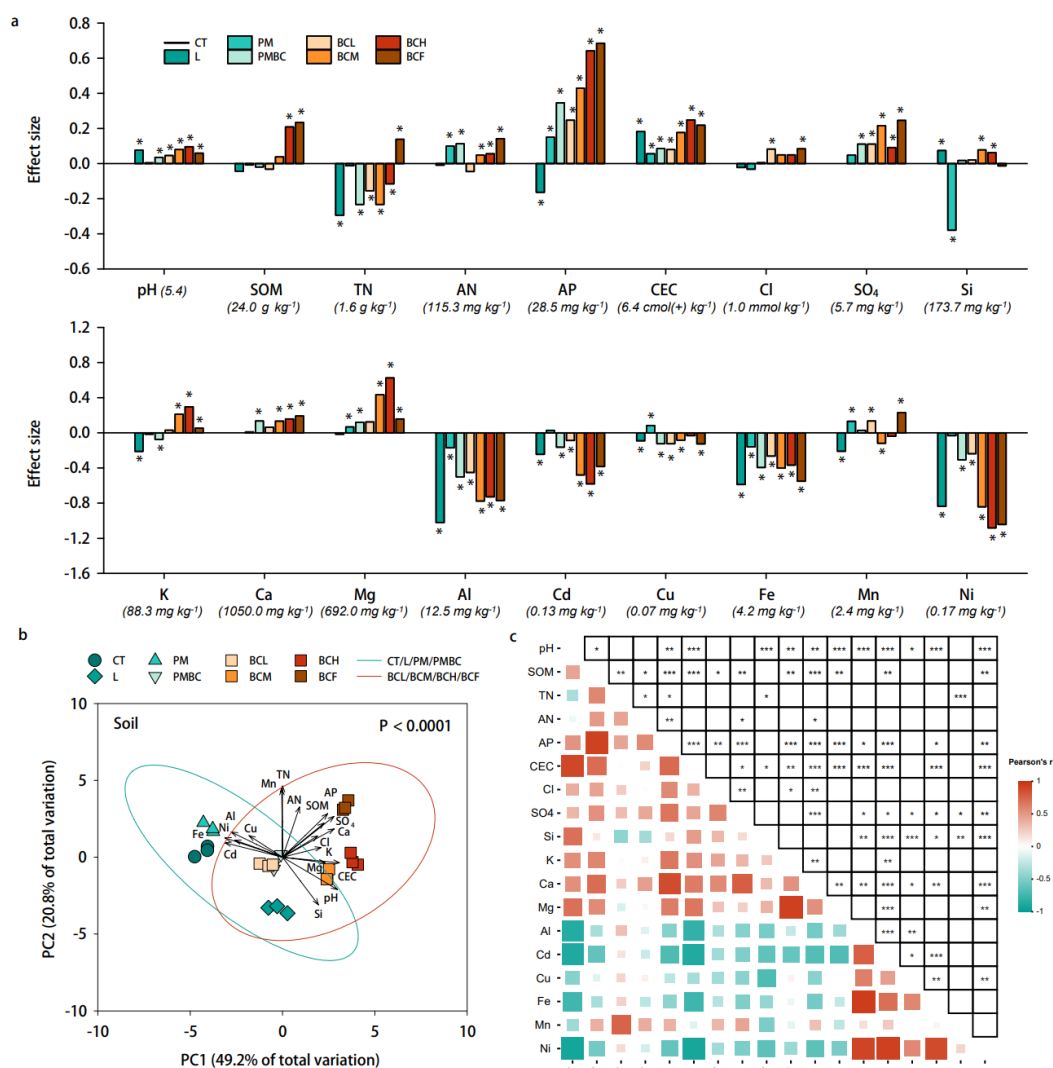


Fig. 7.1. Effects of soil amendments on soil characteristics. a, Soil properties under CT, L, PM, PMBC, BCL, BCM, BCH, and BCF. b, PCA of soil characteristics; ellipses denote 95% confidence regions and vectors indicate explanatory variables. c, Pearson correlations among soil properties; square size and color represent correlation strength and direction, respectively. Asterisks in a indicate significant differences from CT ($p < 0.05$). For correlations: * $p < 0.05$; ** $p < 0.01$; * $p < 0.001$**

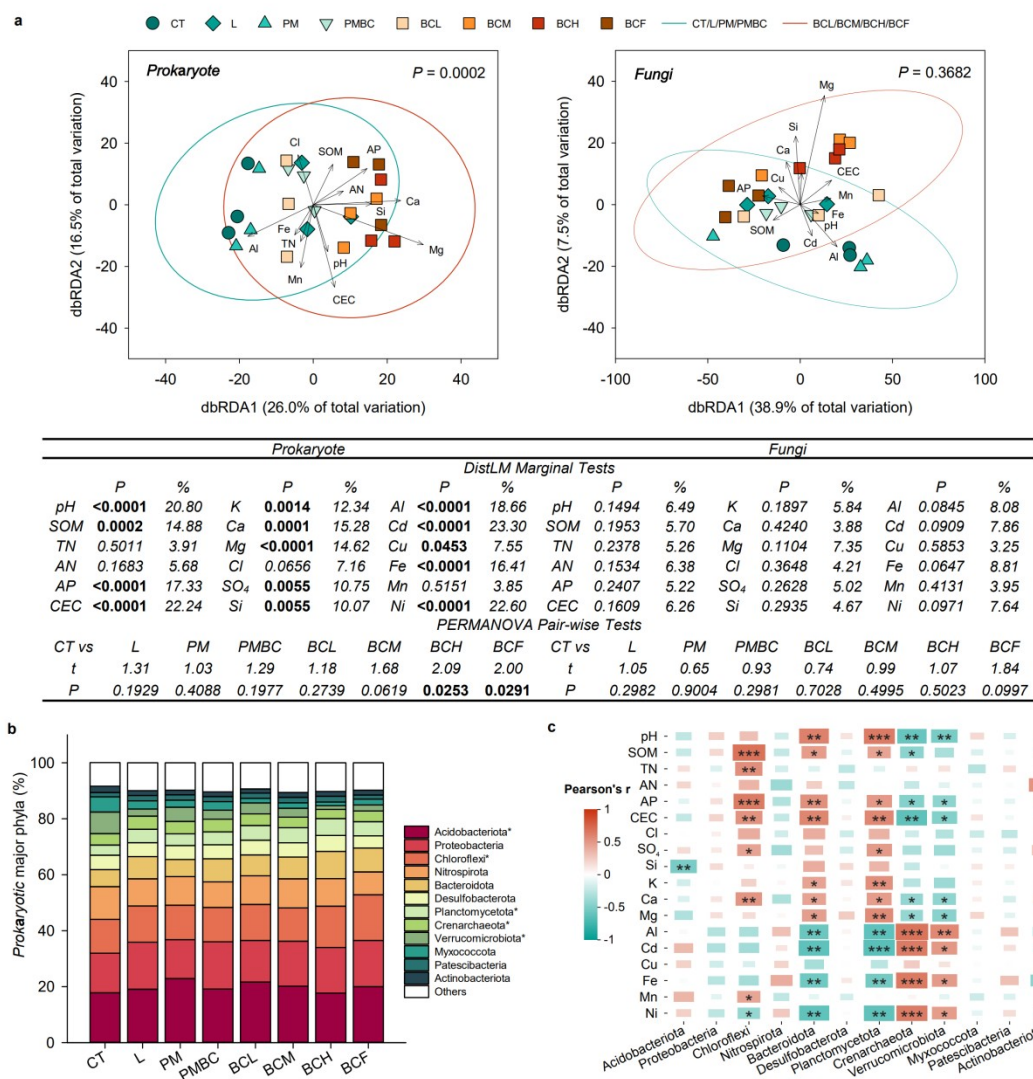


Fig. 7.2. Effects of soil amendments on microbial communities. a, dbRDA of prokaryotic and fungal communities in relation to soil properties. b, Relative abundances of dominant prokaryotic phyla. c, Pearson correlations between dominant prokaryotic phyla and soil properties. Ellipses indicate 95% confidence regions and vectors represent explanatory variables. Asterisks indicate significant treatment differences ($p < 0.05$). For correlations: * $p < 0.05$; ** $p < 0.01$; * $p < 0.001$.**

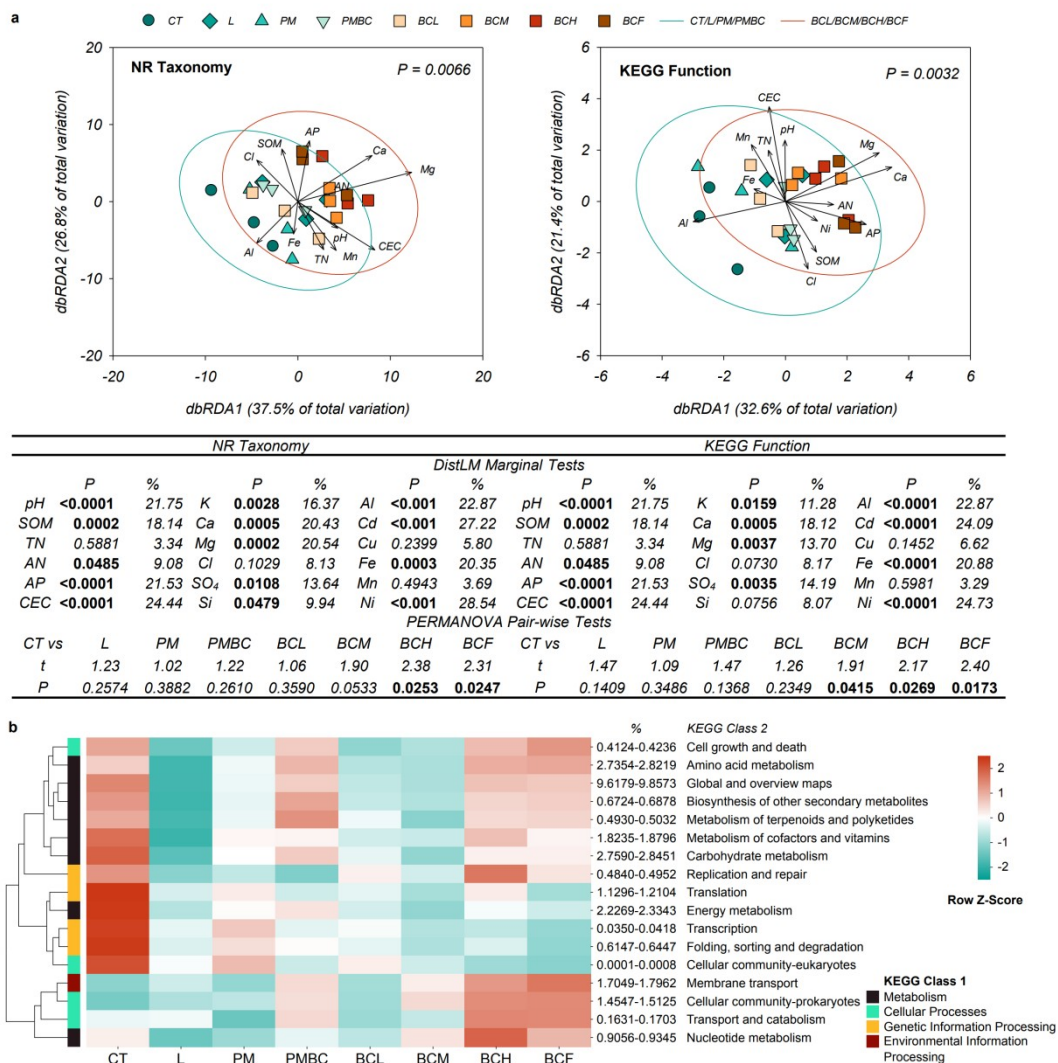


Fig. 7.3. Effects of soil amendments on metagenomic taxonomy and functions. a, dbRDA of metagenomic taxa and KEGG functional profiles. b, Heatmap of significantly different KEGG Class 2 categories. Taxonomy and function were annotated against NCBI-NR and KEGG, respectively. Ellipses indicate 95% confidence regions and vectors represent explanatory soil variables.

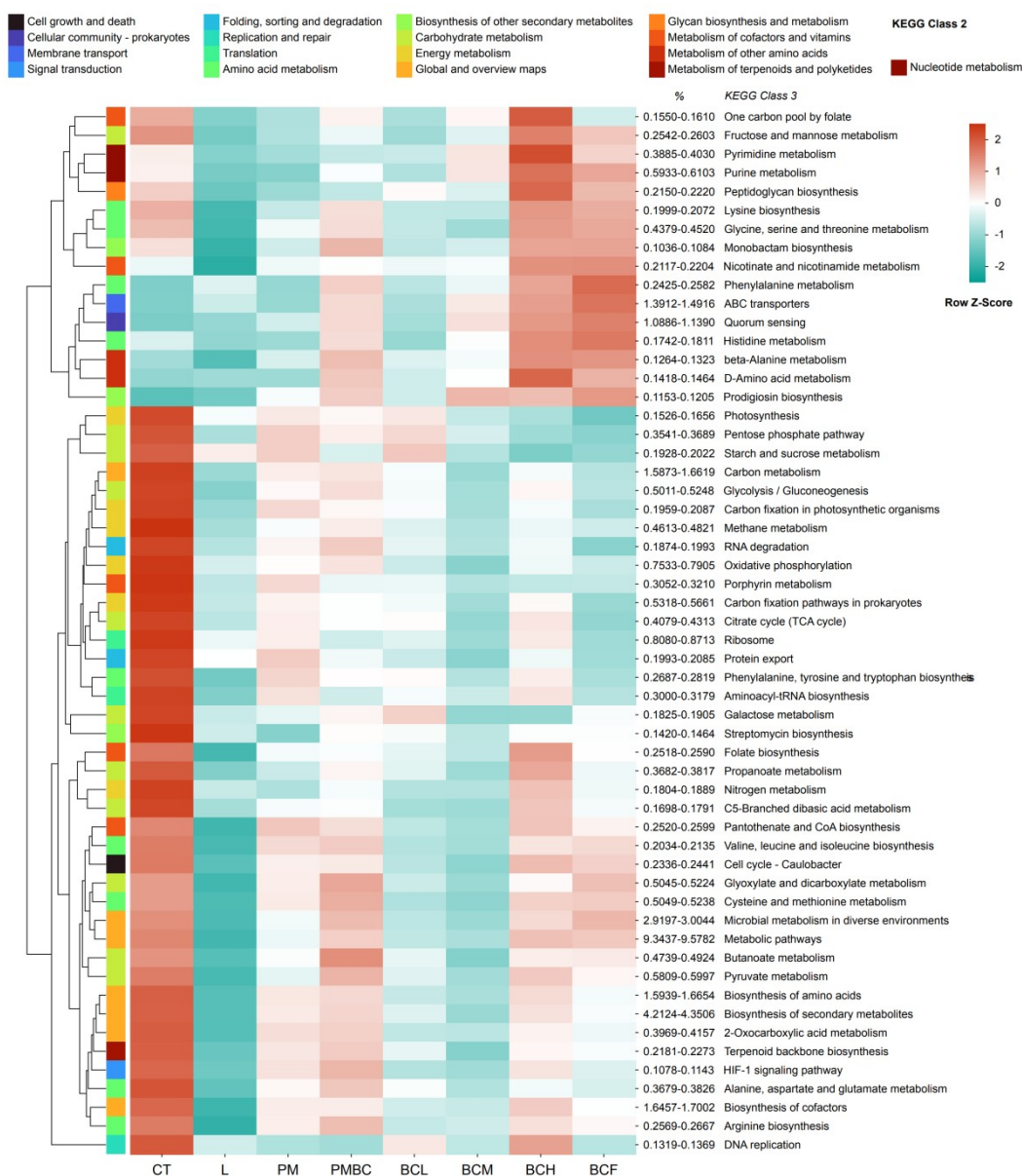


Fig. 7.4. Heatmap of KEGG Class 3 metagenomic functions differing significantly among CT, L, PM, PMBC, BCL, BCM, BCH, and BCF.

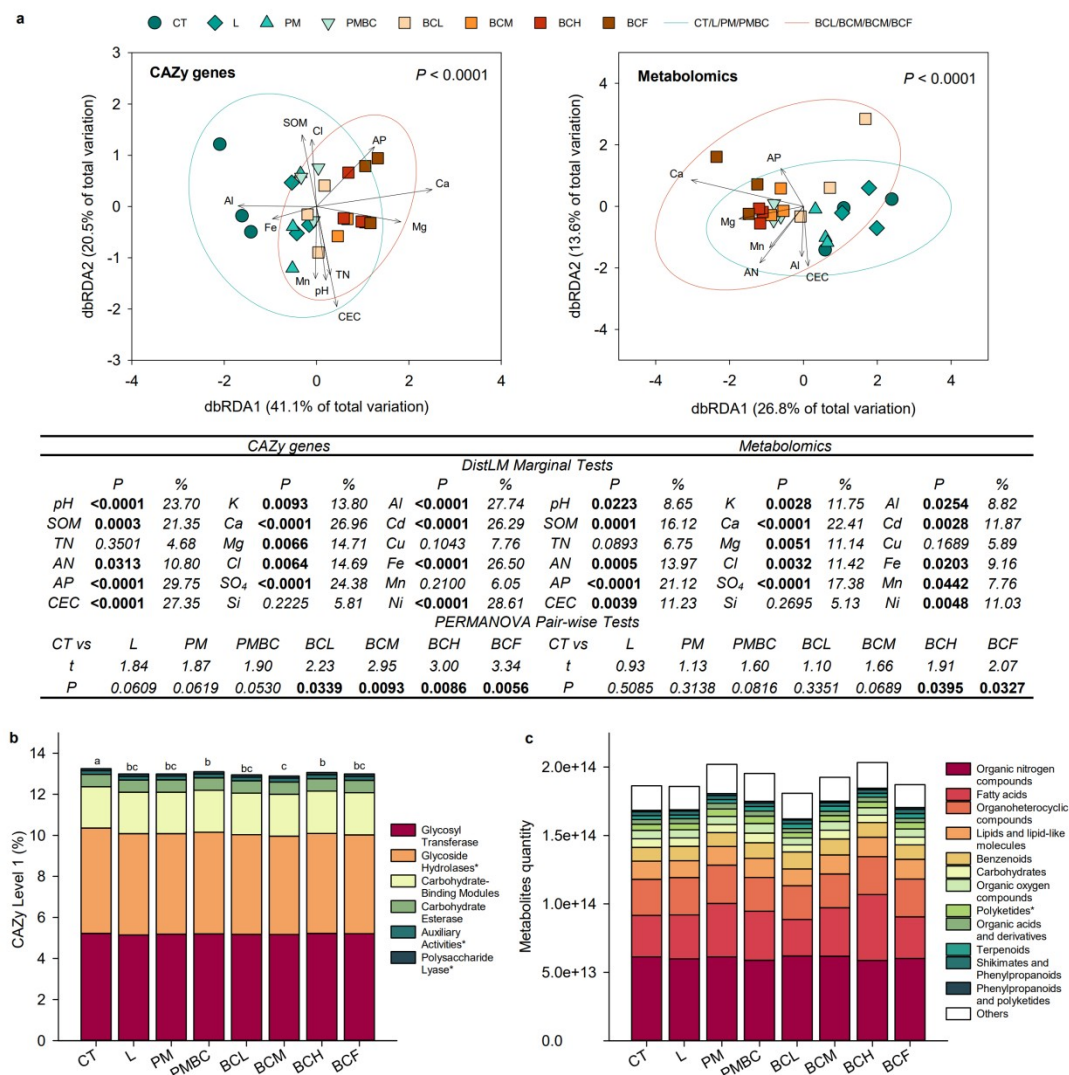


Fig. 7.5. Effects of soil amendments on CAZy functions and metabolite profiles. a, dbRDA of CAZy gene families and soil metabolites. b, Relative abundances of Level 1 CAZy categories. c, Numbers of metabolites grouped by major superclass. Ellipses denote 95% confidence regions, vectors indicate explanatory soil variables, and asterisks identify significant treatment differences ($p < 0.05$).

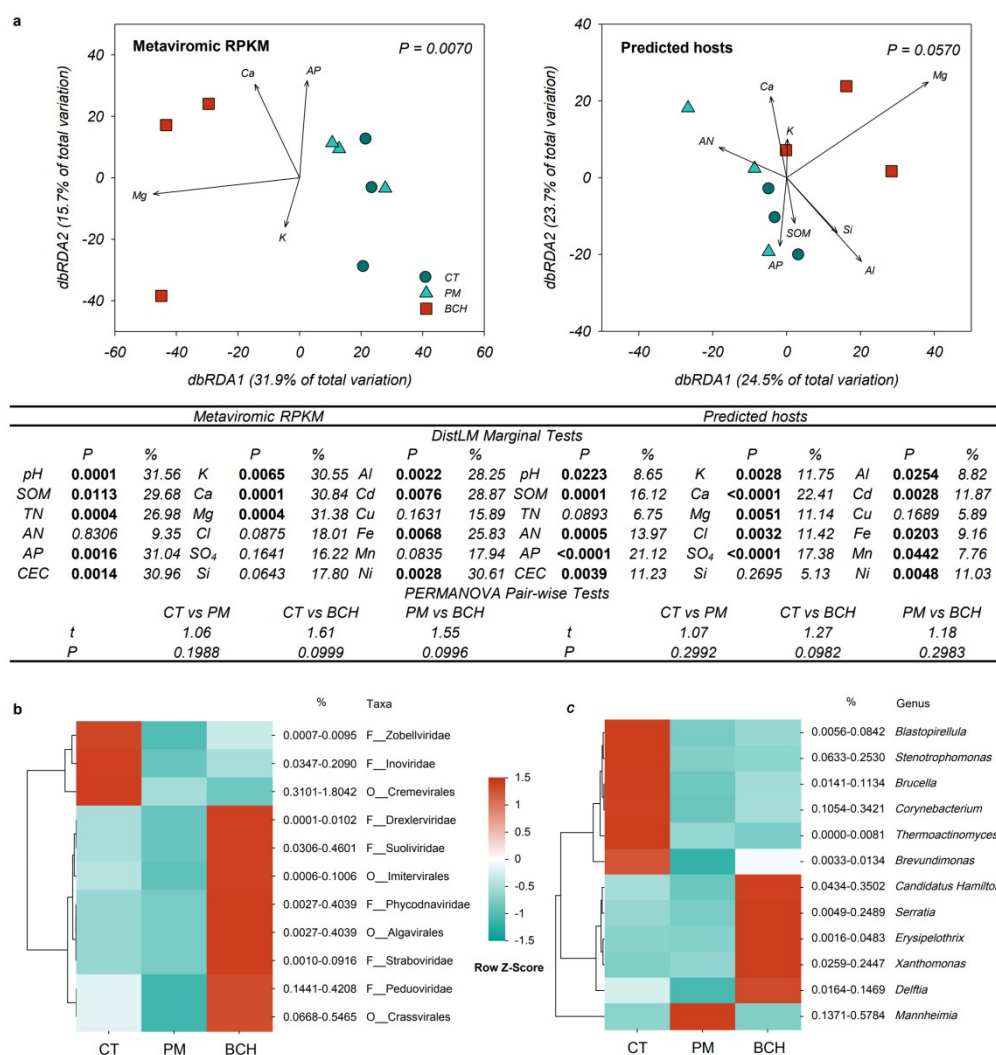


Fig. 7.6. Effects of CT, PM, and BCH on soil viral communities and predicted hosts. **a**, dbRDA of RPKM-normalized viromes and predicted bacterial hosts. **b**, Differential viral taxa at the order and family levels. **c**, Differential predicted hosts at the genus level. Vectors represent explanatory soil variables.

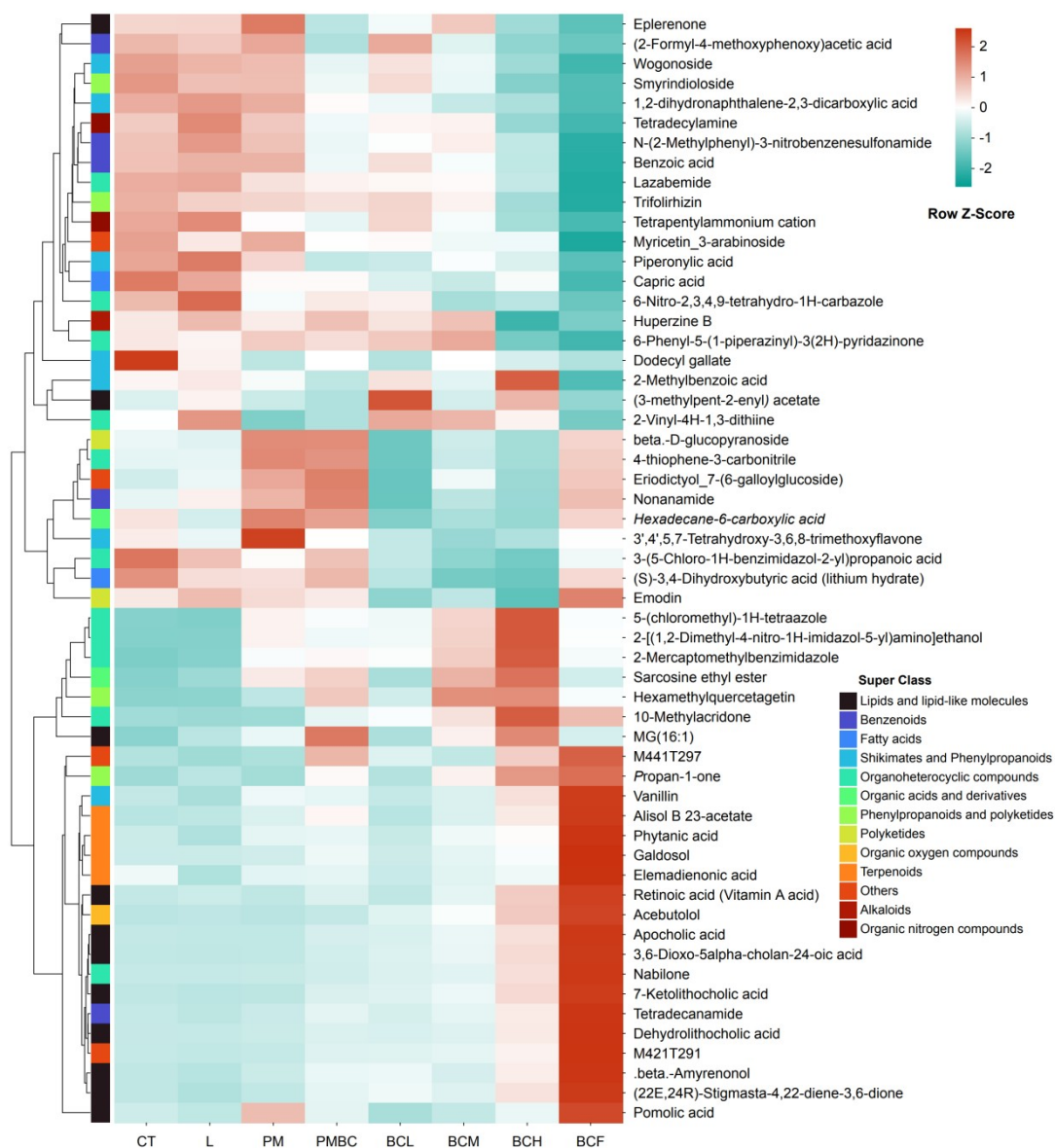


Fig. 7.7. Heatmap of soil metabolites differing significantly among CT, L, PM, PMBC, BCL, BCM, BCH, and BCF.

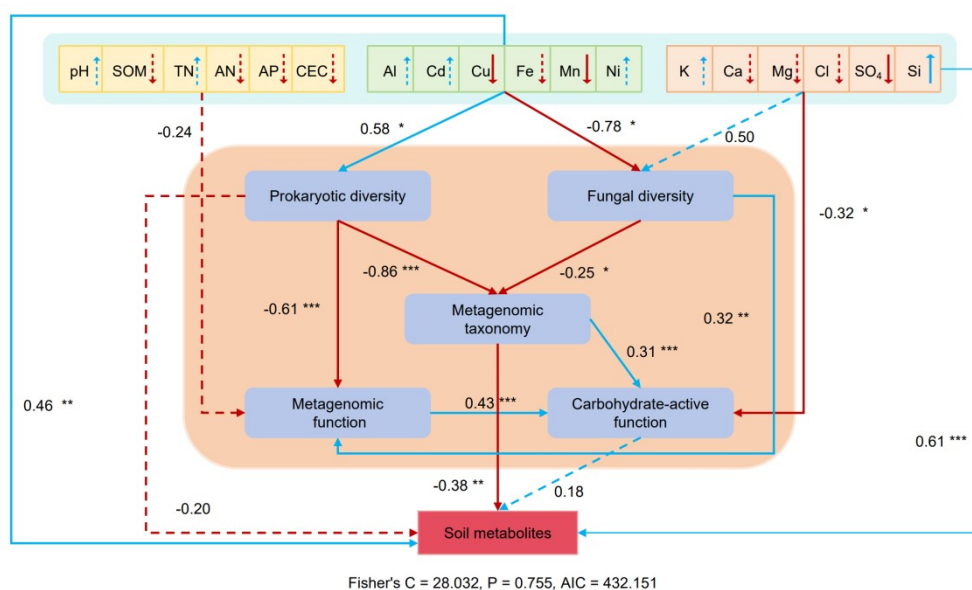


Fig. 7.8. Structural equation model linking soil properties, microbial communities, metagenomic functions, and metabolites across amendment treatments. Red and blue arrows represent positive and negative pathways, respectively; dashed arrows indicate nonsignificant relationships. Values beside arrows are standardized path coefficients. * $p < 0.05$; ** $p < 0.01$; * $p < 0.001$.**

7.5 Conclusions

The five-year field experiment demonstrated that high-rate biochar was more effective than lime and swine manure in alleviating soil acidity and improving fertility in acidic paddy soil. Its long-term advantage resulted from a coordinated ecological cascade that was not reproduced by the conventional amendments. Improvements in soil chemical conditions induced by biochar initiated changes in prokaryotic and viral community composition, which were subsequently associated with shifts in metagenomic functions and soil metabolite profiles. Lime mainly generated a rapid increase in soil pH but did not produce comparable coordinated biological responses. Swine manure supplied readily available nutrients and organic carbon, although its influences on microbial communities and ecological functions were less persistent. Biochar, by contrast, simultaneously regulated soil chemistry, microbial and viral assemblages, functional genes, and metabolic processes. This integrated response supported the long-term maintenance of nutrient availability, metal immobilization, microbial functioning, and crop productivity. The findings therefore indicate that biochar should not be considered solely an amendment for neutralizing soil acidity. Rather, it can serve as an ecosystem-restoration

material capable of coordinating interacting physical, chemical, and biological processes. The mechanistic evidence obtained in this study provides a scientific basis for optimizing biochar application in degraded acidic farmland and for enhancing the long-term resilience and sustainability of crop-production systems.

Chapter 8 Conclusions and Perspectives

8.1 General Summary

This thesis focused on the identification of heavy metal risks associated with livestock and poultry manure, the safe conversion of swine manure into biochar, and the mechanisms by which swine manure biochar can be used for the remediation of Cd-contaminated farmland. Through literature-based data integration, laboratory aging simulation, field experiments, and multi-omics analyses, this thesis systematically evaluated the pollution characteristics and cropland input risks of heavy metals in livestock and poultry manures, revealed the release behavior of endogenous heavy metals during the aging of swine manure biochar, and verified its effectiveness in reducing Cd bioavailability and grain Cd accumulation in wheat and rice systems. Furthermore, by integrating soil microbial community profiling, metagenomics, metaviromics, and metabolomics, this thesis clarified the regulatory mechanisms by which long-term swine manure biochar application affects soil ecological functions in acidic Cd-contaminated paddy fields.

Overall, this thesis links source-oriented risk identification of livestock manure, environmental safety assessment of biochar, heavy metal immobilization in farmland, and soil microbial ecological recovery. The findings provide theoretical support and practical references for the safe resource utilization of livestock manure and the sustainable production of crops in Cd-contaminated farmland.

8.2 Main Conclusions

8.2.1 Heavy metal pollution characteristics of livestock and poultry manures and their cropland input risks

Heavy metal concentrations in livestock and poultry manures showed clear element-specific and livestock-type-dependent differences. Overall, Zn and Cu were the most abundant elements, whereas Cd, As, and Hg occurred at relatively lower concentrations but had greater toxicological significance. Pig manure showed the highest Cu and Zn concentrations and exceedance risks, making it a priority target for heavy metal risk management in manure

recycling. Although cattle and sheep manures generally contained lower heavy metal concentrations, their large production volumes may still contribute substantially to regional total metal inputs.

Spatiotemporal analysis indicated that heavy metal concentrations in manure were jointly influenced by feed additives, livestock production systems, regional environmental backgrounds, and management policies. Arsenic concentrations in pig manure have declined markedly in recent years, whereas Cu and Zn remain at relatively high levels. Provincial load estimation in China further showed that regions with intensive livestock production and limited cropland carrying capacity may face relatively high unit-area metal loading pressures. Therefore, the safe utilization of livestock and poultry manure should consider not only metal concentrations, but also manure production, land application ratios, and cropland carrying capacity.

8.2.2 Release and stabilization mechanisms of endogenous heavy metals during the aging of swine manure biochar

During oxidative aging, swine manure biochar underwent pronounced carbon and nitrogen losses, surface functional group reconstruction, mineral phase transformation, and microstructural evolution. Its stability was strongly regulated by pyrolysis temperature. High-temperature biochar showed greater structural stability and stronger resistance to oxidative degradation.

The release behavior of different heavy metals during aging was highly element-specific. Cd, Cu, Zn, and Ni showed release peaks under low to moderate oxidation intensities, followed by stabilization or decline, whereas Cr release increased progressively with oxidation intensity. In contrast, As showed relatively minor variation. These results indicate that the long-term safety of swine manure biochar should not be evaluated solely on the basis of its initial heavy metal concentrations. Instead, pyrolysis temperature, aging processes, and element-specific release mechanisms should be considered together.

8.2.3 Effects of swine manure biochar on Cd/Zn uptake, translocation, and grain safety in wheat

In the acidic Cd-contaminated wheat field, the incorporation of swine manure biochar raised soil pH and lowered the fraction of Cd available for plant uptake. These changes were accompanied by a significant decline in Cd accumulation in wheat grain and an increase in grain Zn concentration. By contrast, direct application of unpyrolyzed swine manure provided weaker control of Cd mobility and could enhance Cd availability and its subsequent transfer into grain.

The tissue-distribution results further demonstrated that wheat nodes served as important regulatory compartments during the long-distance transport of Cd and Zn. Biochar increased Cd retention within nodes and non-edible spike tissues, thereby decreasing the proportion transported into grain. Therefore, the reduction in wheat grain Cd was not solely caused by Cd immobilization in soil; it also resulted from the biochar-mediated redistribution of metals among roots, stems, nodes, spike tissues, and grain.

8.2.4 Long-term immobilization effects of swine manure biochar in acidic Cd-contaminated paddy soil

Evidence from the five-year field experiment showed that swine manure biochar produced persistent improvements in the acidic Cd-contaminated paddy soil. Biochar application maintained a higher soil pH, reduced available Cd and Cd accumulation in brown rice, and preserved or enhanced rice yield across successive growing seasons. Compared with lime and direct swine manure application, biochar simultaneously alleviated soil acidity, supplied or retained nutrients, and immobilized Cd, thereby exhibiting greater long-term stability and stronger potential for practical agricultural use.

These findings indicate that swine manure biochar can sustain the control of heavy-metal risks under field conditions rather than producing only a short-term immobilization response. Its agronomic value extends beyond decreasing Cd exposure because it also contributes to the improvement of soil quality and the maintenance of stable crop production.

8.2.5 Regulation of soil microecological functions by long-term swine manure biochar application

Long-term application of swine manure biochar not only improved soil chemical properties, but also altered microbial community structure, functional gene composition, viral community characteristics, and metabolite profiles. Biochar may influence nutrient cycling, carbon stabilization, and plant-growth-promoting metabolic processes by increasing soil pH, reducing heavy metal stress, reshaping microbial communities, and modifying virus–host interactions.

These findings suggest that the long-term remediation effect of swine manure biochar in Cd-contaminated paddy soil is not merely a chemical immobilization process, but also involves the recovery and reorganization of soil microecological functions.

8.3 Major Innovations

8.3.1 Clarifying the physical–physiological mechanism of node-mediated partitioning and retention in the crop vascular system driven by exogenous biochar

Conventional studies on biochar-assisted heavy metal immobilization have mainly focused on soil and rhizosphere processes, whereas the regulation of inter-organ metal translocation after heavy metals enter the plant has received less attention. Based on a field wheat experiment, this study revealed that swine manure biochar can improve soil physicochemical conditions and further regulate the retention of Cd and the partitioning of Zn in wheat nodes. This finding extends the mechanism of biochar-mediated heavy metal control from external soil immobilization to internal organ-level redistribution within plants, providing a new physiological explanation for safe wheat production with low Cd accumulation.

8.3.2 Introducing metaviromics to investigate long-term biochar remediation effects and revealing a prokaryote–virus–metabolite cascade response

Previous studies have mainly focused on bacterial and fungal communities and soil physicochemical properties, whereas viral communities and their ecological roles remain insufficiently understood. By integrating long-term field experiments with metagenomic, metaviromics, and metabolomic analyses, this study incorporated viral communities into the

mechanistic framework of long-term biochar remediation. The results suggest that swine manure biochar may regulate virus–host interactions, thereby influencing microbial functional pathways and soil metabolite composition. This finding expands the understanding of how biochar promotes the recovery of ecological functions in contaminated soils from the perspective of microecological networks.

8.3.3 Demonstrating the long-term Cd control effect of swine manure biochar in acidic paddy soil based on a five-year in situ field experiment

Evidence concerning the long-term stability of amendments used for heavy-metal immobilization in farmland remains insufficient because most previous studies have relied on short-duration pot trials or field experiments covering only one growing season. To address this limitation, a five-year in situ experiment was conducted in an acidic Cd-contaminated paddy field to compare swine manure biochar with lime and untreated swine manure in terms of soil pH regulation, available Cd, Cd accumulation in rice grain, and crop yield. The results demonstrated that swine manure biochar maintained a sustained reduction in Cd bioavailability and grain Cd accumulation while simultaneously improving soil properties and preserving rice productivity. This long-term field evidence supports the practical use of swine manure biochar for the safe agricultural utilization of Cd-contaminated paddy soils.

8.4 Limitations

First, the livestock and poultry manure heavy metal database used in this thesis was compiled from studies conducted in different countries and regions. Differences in sampling methods, manure types, analytical procedures, and dry-/wet-weight conversions may have affected data comparability and load estimation accuracy.

Second, the aging of swine manure biochar was mainly simulated using accelerated H_2O_2 oxidation. Although this approach can reveal structural changes and heavy metal release patterns under oxidative conditions, it does not fully represent long-term aging processes under real field conditions.

Third, this study mainly focused on Cd contamination and Cd/Zn interactions. The synergistic control mechanisms and trade-offs under combined contamination scenarios, such as Cd–As and Cd–Pb co-contamination, remain insufficiently explored.

Fourth, the wheat and rice field experiments were limited by experimental location, soil type, crop cultivar, and management practices. The applicability of swine manure biochar across different ecological regions and cropping systems requires further validation.

Fifth, the multi-omics analyses mainly revealed correlations among microorganisms, viruses, and metabolites. The causal roles of key functional microorganisms, virus–host relationships, and metabolic pathways in Cd immobilization and soil functional recovery require further verification.

8.5 Future Perspectives

First, long-term field observation platforms for swine manure biochar should be established across multiple ecological regions and cropping systems to clarify its aging process, effective duration, and reapplication interval under real field conditions. Second, functionalized biochars suitable for Cd–As and other co-contaminated farmlands should be developed, with emphasis on improving the simultaneous immobilization of multiple heavy metals while minimizing potential element mobilization risks. Third, the interactions among biochar, microorganisms, viruses, and metabolites should be further investigated. Stable isotope tracing, metatranscriptomics, targeted metabolomics, and microcosm experiments should be used to verify key ecological processes. Fourth, multi-site, multi-crop, and long-term field experiments should be conducted to optimize the application rate, frequency, combination strategy, and suitable conditions for swine manure biochar. Fifth, source control of heavy metals in livestock manure, pyrolytic conversion, product quality assessment, and safe utilization in contaminated farmland should be integrated to develop regional management systems for livestock manure recycling and farmland remediation.

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