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Ting ZHANG

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

# Soil ridging combined with soil amendments regulates the arsenic-cadmium related geochemical cycles and mitigate its accumulation in rice

Ting Zhang

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Professor Shiming SU (CAAS, China)
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# Abstract

The co-contamination of arsenic (As) and cadmium (Cd) in paddy soils is a significant global issue, threatening food safety due to their high accumulation in rice grains. As a result from their contrasting geochemical behaviors—As being more mobile under anaerobic conditions and Cd under acidic, oxidized conditions—developing an effective, field-applicable strategy to simultaneously reduce their bioavailability in rice cultivation systems remains a critical need. This study aimed to evaluate and mechanistically elucidate the efficacy of an integrated approach combining ridge cultivation with organic (biochar) and inorganic (calcium-magnesium phosphate, CMP) amendments in an acidic paddy field in southern China (pH 5.17), with initial total concentrations of 66.5 $mg \cdot kg^{-1}$ As and 0.41 $mg \cdot kg^{-1}$ Cd.

Field experiments were conducted using two widely cultivated indica hybrid rice varieties (IIyou28 and Ruiyou399), and complemented by controlled microcosm studies. Ridge cultivation improved soil aeration, increased redox potential (Eh), and alleviated soil acidity. When combined with 1% (w/w, dry soil) biochar or 0.05% P (w/w, dry soil) CMP, this approach effectively reduced heavy metal bioavailability and accumulation in rice grains without compromising plant growth or yield. Grain As concentrations were reduced by 38.9% (biochar) and 26.9% (CMP) in IIyou28, and by 39.7% and 35.5% in Ruiyou399, respectively. Grain Cd levels decreased by 38.7% and 37.8% in IIyou28, and by 67.6% and 61.0% in Ruiyou399, compared to ridge-only controls. Microcosm results supported these findings, showing reductions in available soil As by 26.3% (biochar) and 31.2% (CMP), and consistent decreases in soil solution Cd concentrations to 0.13–0.15 $\mu g \cdot L^{-1}$. Arsenic levels in the soil solution were also markedly reduced—by 75.6% with biochar and 82.5% with CMP.

The integrated treatment altered key soil physicochemical properties—especially pH and Eh—resulting in enhanced interactions between Ca, Fe, Mn, and the target heavy metals. Ridge-induced aerobic conditions promoted the formation of poorly and well-crystallized Fe/Al oxides that immobilized As, while Mn (hydr)oxides played a key role in Cd sequestration. Geochemical modeling and Aggregated Boosted Tree (ABT) analysis confirmed that these shifts in elemental interactions were primary drivers of reduced As and Cd bioavailability. Notably, CMP application stimulated nitrification and induced a liming effect that buffered acidification, thereby mitigating Cd risk, while biochar delayed nitrogen oxidation by requiring higher redox thresholds, and promoted Mn-mediated transformations, contributing to co-stabilization of As and Cd.

Metagenomic analyses revealed that microbial redox cycling of Fe, Mn, and N was a critical biogeochemical control. CMP treatments enriched ammonia-oxidizing gene clusters (*amoA/B*), while reducing nitrate-reducing genes, suggesting enhanced nitrification coupled with lowered N reduction potential. Biochar applications upregulated genes involved in Mn (*mntC*) and Fe transport. Among 40 high-quality metagenome-assembled genomes (MAGs), Bradyrhizobiaceae (abundant in Mn and $Fe^{III}$ transport genes), Nitrososphaeraceae (linked to nitrification), and

Caulobacteraceae (Fe transporters) were identified as key microbial taxa contributing to reduced As and Cd mobility.

In conclusion, this study provides a mechanistic and field-validated basis for the integrated application of ridge cultivation with CMP and biochar as a sustainable remediation strategy for As-Cd co-contaminated paddy soils. By improving soil aeration, modifying redox dynamics, and enhancing both abiotic and microbial immobilization processes, this approach effectively reduces heavy metal bioavailability and accumulation in rice, ensuring both environmental and food safety. The findings offer significant implications for large-scale application in similar agroecosystems across Asia and beyond.

# Résumé

La co-contamination des sols rizicoles par l'arsenic (As) et le cadmium (Cd) représente un enjeu majeur pour la sécurité alimentaire mondiale, en particulier en Asie du Sud et du Sud-Est, où le riz constitue un aliment de base. En raison de leurs comportements géochimiques opposés — l'As étant plus mobile en conditions anaérobies et le Cd en milieux acides et oxydés —, il est essentiel de développer des stratégies de remédiation efficaces et applicables sur le terrain. Cette recherche doctorale évalue l'efficacité d'une approche intégrée combinant la culture sur billons avec des amendements organiques (biochar) et inorganiques (phosphate de calcium-magnésium, CMP), ainsi que les mécanismes en jeu, dans une rizière acide typique du sud de la Chine (pH 5,17), présentant des teneurs initiales de 66,5 $mg \cdot kg^{-1}$ en As et 0,41 $mg \cdot kg^{-1}$ en Cd.

Des essais au champ ont été menés avec deux variétés hybrides indica largement cultivées localement (IIyou28 et Ruiyou399), et complétés par des expériences en microcosmes. La culture sur billons a amélioré l'aération du sol, augmenté le potentiel redox (Eh), et atténué l'acidité. En combinaison avec 1 % (m/m, sol sec) de biochar ou 0,05 % P (m/m, sol sec) de CMP, cette stratégie a significativement réduit la biodisponibilité des contaminants sans nuire à la croissance ni au rendement du riz. Les concentrations en As dans les grains ont diminué de 38,9 % (biochar) et 26,9 % (CMP) chez IIyou28, et de 39,7 % et 35,5 % chez Ruiyou399. Les teneurs en Cd ont chuté de 38,7 % et 37,8 % (IIyou28), et de 67,6 % et 61,0 % (Ruiyou399), par rapport au témoin avec billonnage seul. Les microcosmes ont confirmé ces résultats, montrant une réduction de l'As disponible dans le sol de 26,3 % (biochar) et 31,2 % (CMP), et des concentrations de Cd dans la solution du sol maintenues à des niveaux faibles (0,13–0,15 $\mu g \cdot L^{-1}$). L'As en solution a également été considérablement réduit — de 75,6 % avec le biochar et de 82,5 % avec le CMP.

Cette approche intégrée a modifié les propriétés physico-chimiques clés du sol, notamment le pH et l'Eh, renforçant les interactions entre Ca, Fe, Mn et les éléments ciblés. Les conditions oxiques induites par la culture sur billons ont favorisé la formation d'oxydes de Fe/Al (mal et bien cristallisés) qui ont immobilisé l'As, tandis que les oxydes de Mn ont joué un rôle central dans la rétention du Cd. La modélisation géochimique et l'analyse par Aggregated Boosted Tree (ABT) ont confirmé que ces modifications étaient les principaux moteurs de la réduction de la biodisponibilité des métaux. L'application de CMP a stimulé la nitrification tout en exerçant un effet chaulant, atténuant ainsi les risques liés au Cd, tandis que le biochar a retardé l'oxydation de l'azote et favorisé les processus médiés par le Mn, contribuant à la stabilisation conjointe de l'As et du Cd.

Les analyses métagénomiques ont révélé que les cycles redox microbiens du Fe, du Mn et de l'azote jouaient un rôle central dans ces processus. Les traitements au CMP ont enrichi les gènes d'oxydation de l'ammoniac (*amoA*, *amoB*) et réduit ceux associés à la réduction du nitrate, suggérant une nitrification accrue et une réduction du potentiel de réduction de l'azote. Le biochar a activé les gènes de transport du

Mn (*mntC*) et du Fe. Parmi les 40 génomes microbiens assemblés (MAGs), Bradyrhizobiaceae (transport $Mn/Fe^{III}$), Nitrososphaeraceae (nitrification) et Caulobacteraceae (transport du Fe) ont été identifiées comme des taxons microbiens clés impliqués dans la réduction de la mobilité des métaux.

En conclusion, cette étude fournit une base mécanistique et validée sur le terrain pour l'utilisation conjointe de la culture sur billons avec des amendements à base de CMP ou de biochar comme stratégie durable de remédiation des sols rizicoles co-contaminés par l'As et le Cd. En améliorant l'aération du sol, en modulant les dynamiques redox, et en renforçant à la fois les processus abiotiques et microbiens d'immobilisation, cette approche permet de limiter efficacement la biodisponibilité des métaux lourds et garantit une production de riz plus sûre. Ces résultats ouvrent la voie à des applications à plus grande échelle dans des agroécosystèmes similaires à travers l'Asie et ailleurs.

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# List of acronyms

| | |
|---|---|
| CMP | calcium-magnesium-phosphate |
| Eh | redox potential |
| ABT | aggregated boosted tree |
| MAGs | metagenome-assembled genomes |
| methyl-As | methylated As |
| MMAs | monomethylarsinic acid |
| DMAs | dimethylarsinic acid |
| TMAs | trimethylarsine |
| TMAsO | trimethylarsenic oxides |
| FeRB | $Fe^{III}$-reducing bacteria |
| SRB | sulfate-reducing bacteria |
| OM | organic matter |
| SOM | soil organic matter |
| DOM | dissolved organic matter |
| DOC | dissolved organic carbon |
| ROL | radial oxygen loss |
| AOB | ammonia-oxidizing bacteria |

# Chapter 1

## Arsenic and cadmium in soil-rice systems: biogeochemistry, regulatory mechanisms, and co-remediation strategies

# 1. Introduction

Soil, as a non-renewable and indispensable natural resource (Hou et al., 2020), underpins nearly 95% of the food consumed by humans (FAO, 2015). However, up to 90% of global soil resources may be at risk by 2050 due to factors such as soil erosion, excessive use of chemical fertilizers and pesticides, and industrial pollution (FAO, 2015). In 2015, soil pollution has been identified as one of the major threats to global soils by the Global Soil Partnership (GSP) and the Intergovernmental Technical Panel on Soils (ITPS) (FAO and UNEP, 2021). Among various soil pollutants, toxic heavy metals and metalloids are of particular concern due to their widespread presence, resistance to biodegradation, and potential for uptake by crops. These characteristics not only reduce crop productivity but also pose food safety risks and threaten human health. Although certain trace metals, such as manganese (Mn), iron (Fe) and copper (Cu), are essential for biological functions in small quantities, their bioaccumulation in organisms, including crops, can render them toxic within the human food chain (Seregin and Kozhevnikova, 2025). Since toxic metals are non-degradable, they can persist and accumulate in soils over decadal timescales (Hou et al., 2025). Elevated concentrations of heavy metals and metalloids have been reported globally, particularly in the European Union (EU) (Pérez and Eugenio, 2018) and China (Zhao et al., 2015). A study encompassing 27 European countries found that 28% of soils exceeded contamination thresholds (Tóth et al., 2016), while a nationwide survey in China reported that 19% of agricultural soils surpassed national soil quality standards, with arsenic (As, a metalloid, ) and cadmium (Cd) being the primary contaminants (Ministry of Environmental Protection of China, 2014), for writing efficiency, both As and Cd are hereafter referred to as metals.

Arsenic and Cd are two non-essential toxic metals, and their compounds are classified as Group I human carcinogens (International Agency for Research on Cancer, 2025), posing a threat to global human health through human dietary exposure via the food chain (Zhao and Wang, 2020). Compared with other crops, rice was found to have efficient pathways for absorbing silicon (Si)/arsenite ($As^{III}$) (Su et al., 2010), phosphate (P)/arsenate ($As^{V}$) (Kamiya et al., 2013) and manganese (Mn)/Cd (Sasaki et al., 2012), and thus can efficiently absorb As and Cd. Rice is a staple food for more than half of the world's population (Muthayya et al., 2014). Consuming rice as the staple food allows As and Cd as a primary source to enter the food chain (Zhao, 2020). Arsenic and Cd co-pollution of farmland is now widespread worldwide, occurring at multiple points in North America, Europe, and Asia, with varying pollution levels. However, previous studies have primarily focused on the exposure and fate of single elements. Recent studies suggest that under the "Four Per Mille Initiative" —aimed at increasing global soil organic carbon stocks by 0.4% annually—the mobility and risk of As and Cd in soils may further rise by 2050 (Figure 1-1) (Qi et al., 2025). However, due to the opposite biogeochemical behavior of As and Cd in soil, it is quite challenging to remediate As-Cd co-contaminated fields, which is both a hot topic and a difficult problem in global research. Therefore, here we investigated the key factors influencing the biogeochemistry of As and Cd in soil. Here also proposed and summarized the comprehensive strategies and their potential mechanisms for simultaneously mitigating As and Cd in contaminated soils.

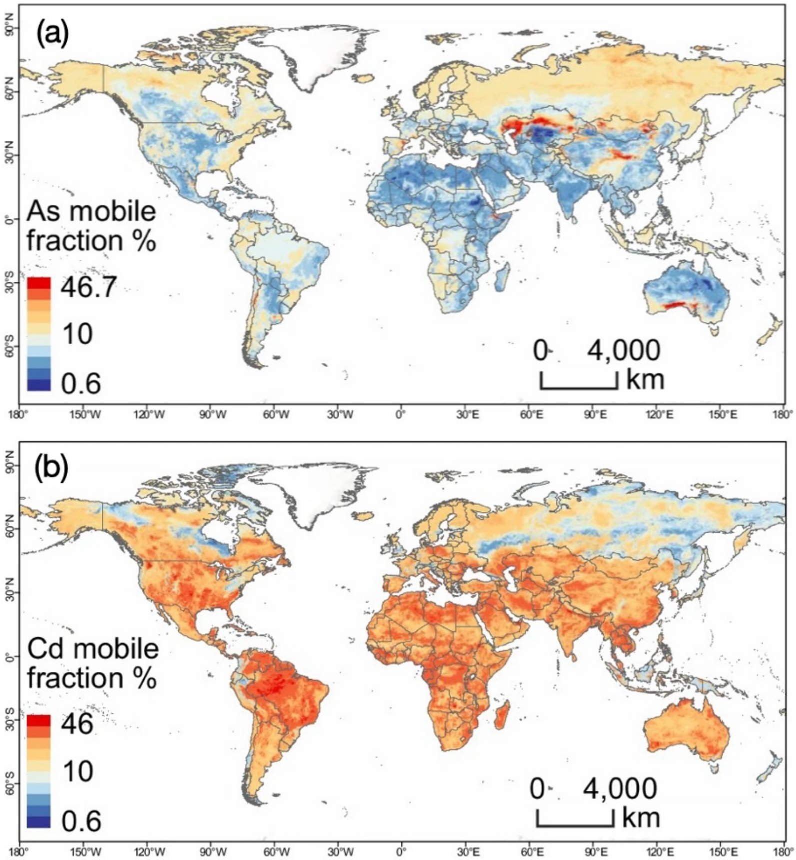



**Figure** 1-1. Predicted global distribution of the mobile fractions of soil As and Cd by 2050. A higher mobile fraction indicates greater mobility, as represented in red. (reproduced from (Qi et al., 2025)).

# 2. The biogeochemistry of As and Cd in soils and their influencing factors

Arsenic is a hazardous metalloid ubiquitous in nature affecting 107 countries. Over 300 million people are at risk of As exposure through the consumption of As accumulated foodstuffs or drinking water (Abbas et al., 2018). Cadmium readily transfers from soil to the food chain (Clemens et al., 2013). More than 5 million individuals are at risk of Cd exposure in over 150 locations worldwide (Rahman and Singh, 2019). Due to natural geogenic and anthropogenic activities, the accumulation of As and Cd in soil resulting in widespread dispersion of environmental contaminants (Jan et al., 2015).

Arsenic and Cd naturally build up in soils from most of the parent materials through pedogenic process, typically in low concentrations rock such as sandstone, igneous rocks and limestones (Loganathan et al., 2012). Human activities, including metal mining and smelting, fossil fuel combustion, industrial wastewater discharge, sewage irrigation, and the overuse of pesticides, herbicides, phosphate fertilizers, wood preservatives and food additives, are all important sources of heavy metals such as As and Cd in agricultural soils (Alloway, 2013). Cadmium in soil is primarily incorporated into sulfide, carbonate, and phosphate rock, and exists in the form of $Cd^{2+}$ in soil solutions (Kubier et al., 2019). Arsenic in soil exists in two main forms: inorganic arsenic (iAs) and oxymethylated arsenic (mAs). Inorganic arsenic (arsenite, $As^{III}$ and arsenate, $As^{V}$) poses greater toxicity to humans than the phytotoxic mAs [monomethylarsinic acid (MMAs), dimethylarsinic acid (DMAs), and trimethylarsenic oxides (TMAsO)] (Li et al., 2024). $As^{III}$ and DMAs have received more attention, as they constitute 85-94% of total As in rice grain (Smith et al., 2008). With advances in detection technology, thioarsenates have been identified and are known to be both phytotoxic (inorganic thioarsenates and methylthioarsenates) and cytotoxic (methylthioarsenates) (León Ninin et al., 2024).

The properties of the anionic metalloid As and cationic metal Cd are distinct. Although the factors influencing their bioavailability share similarities, the physical and chemical effects of the same factors are opposite (Zhao and Wang, 2020). Consequently, the synergistic regulation of As and Cd presents a challenge for current research, yet some avenues for coordinating their regulation have been studied (Honma et al., 2016; Pan et al., 2022). Existing studies predominantly focus on the contamination status, sources, impacts on food safety, speciation, transport, and mitigation strategies of either As or Cd in soil-crop systems (Ali et al., 2020), often overlooking their co-contamination and diverse biogeochemistry on a global scale. This limits our understanding of strategies for mitigating As and Cd simultaneously. The biogeochemical processes of As and Cd primarily differ in efflux, complexation with thiol-rich compounds, vacuolar sequestration and detoxified by complexation with phytochelatins. Redox potential influences the solubility and plays a crucial role in the biogeochemical processes of As and Cd. Both As and Cd possess phytotoxic properties. The potential for their transfer from soil to the food chain is substantial and depends on factors such as soil bioavailability, soil properties, rice genotype and environmental conditions (Zhao and Wang, 2020). Different approaches have been proposed to manage As or Cd contamination in paddy soils, but there remains a lack of comprehensive strategies for simultaneously remediating As and Cd. Urgent attention is needed to develop effective methods for mitigating the excessive accumulation of As and Cd in the edible parts of crops.

The mobility of As and Cd in paddy soils depends on their speciation and chemical form, which significantly affects their uptake by food crops (Figure 1-2). The availability of Cd in soils is primarily influenced by soil Eh, pH, organic matter

(OM) and other elements such as Fe, Mn, zinc (Zn) and sulfur (S) (Huang et al., 2021). Dissolved Cd in the soil solution and exchangeable Cd, which is adsorbed to both inorganic and organic components in the soil, are more readily taken up by plants (Imseng et al., 2019). $As^{V}$ and $As^{III}$ are two dominant species of As in the terrestrial environment. Under oxidizing (aerobic) conditions, $As^{V}$ readily co-precipitates and adsorbs with Fe-oxyhydroxide, whereas under reducing (anaerobic) conditions, $As^{III}$ and arsine ($AsH_3$) may be present (Takahashi et al., 2004; Wang et al., 2014). $As^{III}$ can also co-precipitate or absorb with sulfides ($S^{2-}$) and has an affinity to bind with other S compounds (Roy et al., 2015). However, iAs alone does not fully account for the risks associated with As accumulation in food crops. Excess DMAs in rice husk has been linked to rice straight-head disease (Tang et al., 2020), a condition that poses a significant threat to global rice production, potentially resulting in grain yield losses up to 90% (Yan et al., 2005). DMAs can not be synthesized in plants; instead, it is produced through microbial As methylation processes in paddy soils (Lomax et al., 2012). Various abiotic factors (such as Eh, pH, and the content of Fe, Mn and S) and biotic factors (such as soil microorganisms) can influence the As species to varying degrees in paddy soils (Zhang et al., 2024). Arsenic methylation is commonly considered as As detoxification process (Qin et al., 2006). Soil bacteria have evolved several detoxification mechanisms related to cellular metabolism, including $As^{V}$ respiratory reduction (encoded by *arrA*) (Xiao et al., 2016), detoxication reduction (encoded by *arsC*) (Zhu et al., 2014), $As^{III}$ oxidation (encoded by *aioA* and *arxA*) (Anderson et al., 1992), and methylation (encoded by *arsM*) (Qin et al., 2006). The bioavailability of As and Cd to food crops is primarily influenced by soil Eh (Shaheen et al., 2016), soil pH (Honma et al., 2016), the content of soil minerals (such as Fe/Mn oxides, metal (hydro-)oxides (Wang et al., 2022; Xu et al., 2017), phosphates (Yang et al., 2021), clays (Li and Xu, 2017) and organic/inorganic matters (Chi et al., 2022), as well as tillage management practices (Rizwan et al., 2016). Additionally, other environmental factors such as humidity, rainfall, and temperature can also influence the fate and transport of As and Cd in the soil environment (Madzin et al., 2015).

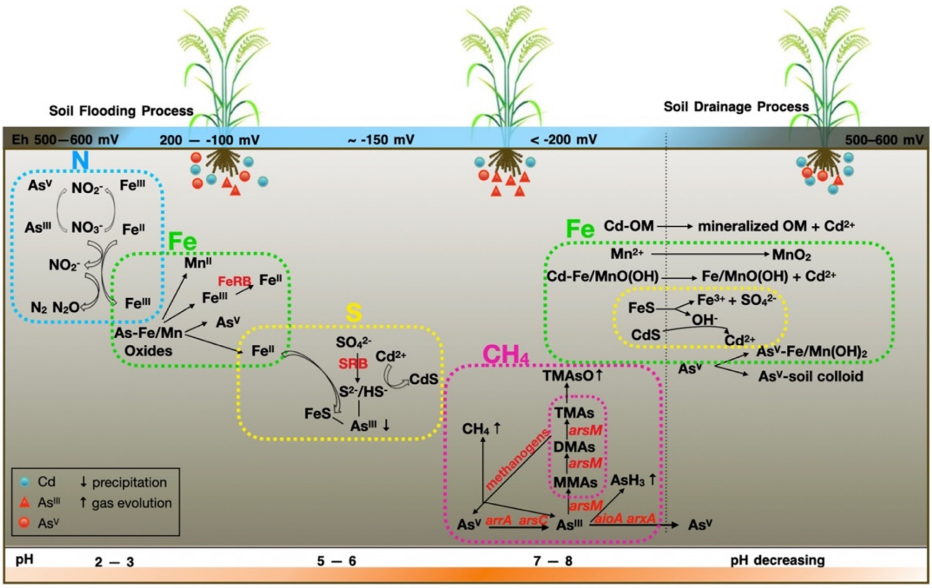



**Figure** 1-2. Biogeochemistry of As and Cd in soils during flooding and drainage cycles. FeRB: $Fe^{III}$-reducing bacteria; SRB: sulfate-reducing bacteria; *arrA*: arsenate respiratory reduction genes; *arsC*: arsenate detoxication reduction genes; *arsM*: arsenite methyltransferase genes; *aioA*: arsenite oxidation genes; *arxA*: arsenite oxidation genes. MMAs: monomethylarsonic acid; DMAs: dimethylarsinic acid; TMAs: trimethylarsine; TMAsO: trimethyl-arsenic oxide. (Original to this study, unpublished.)

## *2.1. Soil redox potential (Eh)*

Paddy fields constitute complex ecosystems wherein the redox potential undergoes significant fluctuations during alternate flooding cycles. The Eh status is heavily influenced by various oxidized compounds such as $SO_4^{2-}$, $NO_3^-$, $Fe^{III}$ and $Mn^{III}$ or $Mn^{VI}$ in flooded paddy fields. These compounds undergo reduction reactions, acquiring electrons to produce reduced substances, consequently lowering the Eh value (Lin et al., 2021). This fluctuation in Eh reduce the bioavailability of Cd while increasing that of As (Honma et al., 2016). Under flooding conditions, As adsorbed onto Fe-Mn oxides can be desorbed and released into the soil solution due to Fe reduction, thereby increasing the bioavailability of As (Shaheen et al., 2016). Subsequently, as Eh further decreases, $As^{III}$ may undergo methylation to form methylated As species (methyl-As) facilitated by sulfate-reducing bacteria (SRB) and other (an)aerobes possessing *arsM*, and further triggers the demethylation of methyl-As by methanogens (Chen et al., 2019). However, reducing conditions promote the formation of insoluble precipitates such as CdS, reducing the bioavailability of soluble Cd (Honma et al., 2016; Rinklebe et al., 2016). The paddy soil under continuous flooding conditions with lower Eh exhibited higher precipitation of $Cd^{2+}$ with $S^{2-}$ compared to soil under 75% field water capacity and wetting-drying cycle regimes, resulting in a more complete movement of Cd towards stable fractions (Wang et al., 2014). Unlike As, Cd is readily released into the soil solution under oxidizing conditions; therefore, Cd availability is increased for uptake by food crops (Rinklebe et al., 2016). The reason is that the hydroxyl free radicals produced in FeS oxidation can promote the oxidative dissolution of CdS and increase Cd mobilization in soils (Huang et al., 2021). As a result, several researches have employed continuous flooding to immobilize Cd in the soil and reduce Cd content in grains (Arao et al., 2009), but this practice enlarges the risk of As accumulation in rice grains.

## *2.2. Soil pH*

Cd mobility and bioavailability increase significantly with reduced soil pH, while that of As decreases (Jiku et al., 2022). Increasing soil pH induces a negative surface charge, potentially promoting the desorption of negatively charged As on the soil surface and increasing its bioavailability. Conversely, Cd in soil is typically positively charged, yielding the opposite response (He et al., 2020). The As-binding species such as Fe-oxyhydroxide compounds are becoming more soluble at extremely low pH levels (pH < 5) and enhance As absorption by plants. Higher soil pH levels (usually pH > 8.5) increase the negative surface charges, such as hydroxyl ions, facilitating the desorption of As from Fe-oxides leading to the mobilization of As near plant roots, thereby enhancing As uptake by plants (Anawar et al., 2013). At pH levels very close to neutral, As is adsorbed simultaneously with the same effect on Fe oxyhydroxides and Fe oxides surfaces. Specifically, $As^{III}$ adsorption is more favored in an alkaline environment, whereas $As^{V}$ adsorption is favored under acidic conditions (Morin and Calas, 2006). However, this is significantly affected by the soil's redox status. A negative correlation between soil pH and Cd phytoavailability has been widely established (Chi et al., 2022). Cd occurs in forms such as carbonates, hydroxides and phosphates, and its solubility and availability lead to higher Cd uptake in paddy soil with low soil pH under field conditions (Wang et al., 2018). In particular, the decrease in soil pH caused the change of Cd between the Fe/Mn-(oxyhydro) oxide fraction and the exchangeable fraction (Wang et al., 2019). Due to the complex soil composition, it is challenging to ascertain the critical pH level that can control the Cd uptake by rice plants in the field. Results indicate that when the soil pH is 5, the critical soil Cd concentration of paddy soil is around 0.18 $mg \cdot kg^{-1}$, while if the pH of the paddy soil is 7, the critical soil Cd concentration increases to 0.9 $mg \cdot kg^{-1}$ (Zhao et al., 2015). Moreover, flooding either elevates pH in acidic soils or decreases pH in alkaline soils, and decreases Eh. These soil changes may increase the negative charge of soil constituents, resulting in increased Cd adsorption and decreased solubility (Li et al., 2014).

## *2.3. Soil organic matter*

Soil organic matter (SOM) appears to act as an effective adsorbent for Cd but a desorbent for As in soils due to the presence of charges that oppositely influence their mobility or availability. SOM can release organic substances into the soil solution, such as humic acid and fulvic acid, which aid in the formation of stable complexes or chelates that decrease the phytoavailability of Cd and its uptake by rice (Zeng et al., 2011). Humic substances compete with As for adsorption sites on the surfaces of Fe and Al hydroxides and participate in the redox cycle of As, increasing As mobility under flooding conditions (Verbeeck et al., 2020). High dissolved organic matter (DOM) may also contribute to retaining Cd in the exchangeable fraction by delivering organic chemicals into the soil solution that can serve as chelates, thereby increasing Cd availability and uptake by plant roots (Impellitteri et al., 2002). DOM in soil may compete with As for the adsorption sites, thereby significantly increases As mobilization (Aftabtalab et al., 2022). Additionally, organic manure application promotes As volatilization and increases the relative abundance of Euryacheota and Planctomycetes carrying *arsM* at the phylum level and Methanocellaceae, Anaerolinea, and Bellinea carrying *arsM* at the genus level, thereby promoting As methylation (Yang et al., 2022) and reducing the toxicity and bioavailability of As in soil (Yan et al., 2020).

## 2.4. Iron Manganese Oxides

As environmental redox conditions fluctuate, the presence of Fe and Mn oxides, hydroxides and oxyhydroxides (oxides hereinafter) influences the dynamics of As and Cd in soil, as well as their uptake and transport by plants (Suda and Makino, 2016; Xu et al., 2017). Under anaerobic conditions, Fe/Mn oxides undergo reductive dissolution, releasing significant amounts of As and Fe, thereby increasing the mobility of As (Takahashi et al., 2004). In more reducing conditions (Eh $<-150$ mV), sulfide ions ($S^{2-}$ or $HS^-$) produced by sulfate reduction react to form precipitates such as As-S or CdS, or form Fe-S compounds with reduced $Fe^{II}$ that subsequently co-precipitate with As (Hindersmann and Mansfeldt, 2014). Dissolved $Mn^{2+}$ may react with $Mn^{III}$/$Mn^{IV}$-(oxygen) oxides to produce mixed-valent minerals (secondary Mn minerals) (Borch et al., 2010). Due to the high surface area and strong affinity of “secondary” Fe-Mn (hydro)oxides for adsorbing metal ions, Cd may be adsorbed or sequestered through co-precipitation (Muehe et al., 2013). When paddy fields are drained or exposed to aerobic conditions, Fe/Mn oxides can oxidize arsenite to less mobile arsenate, thereby reducing As bioavailability (Takahashi et al., 2004). Additionally, Mn oxides can delay the reductive dissolution of As-containing Fe(hydroxy)oxides and the release of As into pore water by maintaining a high redox potential (Xu et al., 2017). Furthermore, the oxidation of FeS generates hydroxyl radicals (OH•), which can directly oxidize CdS, thereby promoting the oxidative dissolution of CdS and increasing Cd mobilization in soil (Huang et al., 2021). Moreover, Fe plaques, composed of iron oxides, on the root surface of aquatic plants can reduce Cd and As concentrations in rice tissues by fixing and sequestering these metals (Zhang et al., 2019). As a divalent ion, Cd typically shares channels or competes transporters with Fe and Mn to uptake into plant cells (Clemens et al., 2013). *IRT1* has been reported as a broad-spectrum transporter for Fe in strategy type I plant, as well as for Mn, Cd, etc. (Dubeaux et al., 2018). *OsNramp5* is responsible for absorbing Fe, Mn and Cd in rice, while *HvNramp5* absorbs Mn and Cd but not Fe in barley (*Hordeum vulgare* L.) (Wu et al., 2016).

### 2.5. *Other factors*

Soil texture has been found to be an important factor affecting the solubility of As in soil and the bioavailability of rice plants (Piracha et al., 2022). The silt and clay soils have finer texture, larger surface area, and higher As scavenging potential due to the presence of Fe oxides (Quazi et al., 2011). It has been proved that heavy metals are more mobile in sandy soils than in clayey soils (AL-Oud et al., 2014). Therefore, plants that grow in clayey soils exhibit less toxic effects of As and the phytotoxicity of As is five times more in sandy and loamy soils (Quazi et al., 2011). Moreover, the adsorption of Cd on clay surfaces hinders the movement of Cd and reduces its leaching and loss. In a greenhouse experiment, it was found that the distribution and uptake of soil Cd was largely influenced by soil type (Fayiga and Nwoke, 2017). Soil temperature may affect the change and migration of metals rapidly under paddy field conditions (Li et al., 2013). High temperature enhances and alters the rate of OM decomposition and mineralization which may lead to the formation of organic acids that acidifies the soil and increasing in Cd dissolution and mobility (Onwuka, 2016). With the increase of temperature, the acceleration of soil reduction reaction also accelerated the release of As from the soil solid phase (Weber et al., 2010). This is not surprising, as the activity of soil microorganisms generally increases with increasing temperature, with many bacteria reaching their maximum activity around 25–30 °C (Pietikäinen et al., 2005). Importantly, the effects on the mobilization and bioavailability of As and Cd in soil-crop systems vary with irrigation condition, fertilizer type, OM dosage and the source or form of As and Cd in contaminated areas (Sun et al., 2021). All aforementioned factors are strongly inter-linked and their synergistic effects determine the mobility and bioavailability of As and Cd in soil (Chaali et al., 2022).

# 3. Uptake and regulatory mechanisms of As and Cd in rice

Metal or metalloid elements enter plants through the same pathways as essential elements required for growth (Clemens, 2006). The concept of the "soil-plant triple barrier" indicates that the ability of toxic metals to migrate into the food chain depends on their solubility in the soil, which determines their bioavailability for plant uptake, the capacity of the root surface or root cells to restrict the transport of these elements to the above-ground parts of the plant after root absorption, and the toxic responses generated by the plant itself after uptake, such as reduced yield of edible parts (Chaney, 1980; Li et al., 2023). As a model species, rice has been at the forefront of research, particularly with the advancement of reverse genetics and other methodologies. Rice, as a model species, has remained at the forefront of research due to the development of research methods such as reverse genetics (Kobayashi and Nishizawa, 2012; Salt et al., 2008). Additionally, rice is the most significant source of Cd and As exposure for humans globally, making it a primary focus in studies of crop uptake and accumulation of metal elements. Additionally, as rice is the most relevant source of Cd and As intake for humans globally, it is currently the primary research subject for studying the absorption and accumulation of metal elements in crops.

### 3.1. Interface interactions and uptake mechanisms of As and Cd by rice roots

As the first organ to encounter As and Cd in the soil, rice roots serve as the primary barrier against the toxicity of these metals. Typically, a yellowish iron oxide membrane forms on the root surface of aquatic plants, decreasing from the root tip to the base, known as the iron plaque (He et al., 2024). This iron plaque has been shown to create a natural barrier that protects aquatic plants like rice from heavy metals damage by isolating the root surface from metal ions or adsorbing these ions (Peng et al., 2018). Furthermore, it can benefit the plant by adsorbing nutrients from the soil. The formation of the iron plaque is a spontaneous natural phenomenon influenced by various abiotic and biotic factors, such as soil properties, water management strategies, plant genotype, root radical oxygen loss capacity, root aeration structures, root exudates, and root surface enzyme activity (Lai et al., 2018).

In addition, root hairs and epidermal cells in the mature zone of the root absorb toxic metal ions from the soil. The activity of the root system significantly affects the concentration and speciation of metal ions in the rhizosphere, thereby influencing the root's ability to uptake these ions (Lwin et al., 2025). To maintain charge balance after the absorption of nutrient ions, roots compensate by excreting $H^+$ or $OH^−$ ions. Metal ions can exchange with $H^+$ and $OH^−$ ions produced during plant respiration and adsorb onto the surfaces of root epidermal cells (Lwin et al., 2025). Other factors, such as root surface area, mycorrhizal relationships in the rhizosphere, transpiration pull, and the pattern and extent of root system, also influence the solubility of toxic metal ions in the soil thus affect their uptake by rice. Moreover, rice varieties may affect the solubility of metals in the rhizosphere and their accumulation in grains, with different rice cultivars exhibiting varying capacities for the uptake, transport, and accumulation of Cd and As in reproductive tissues (He et al., 2017).

### 3.2. Molecular mechanisms and transporter differences in As and Cd uptake by rice roots

Multiple As species are typically present in plant tissues (Meharg and Hartley-Whitaker, 2002), and both $As^V$ and the predominant forms of methylated arsenic in soil, MMA and DMA, can be taken up by plants. Among these, $As^V$ is absorbed most efficiently by plant roots (Raab et al., 2007). Therefore, it is essential to distinguish the different pathways through which As is absorbed (Figure 1-3). Arsenate uptake occurs via phosphate transporters due to the chemical similarity between $As^V$ and phosphate (Meharg and Macnair, 1992). Wu et al. (2011) investigated $As^V$ uptake rates in rice mutants deficient in the phosphate transporter *OsPHF1*, as well as in lines overexpressing either the phosphate transporter *OsPht1;8* or the positive regulator of phosphate starvation response *OsPHR2*. Their findings clearly demonstrated that phosphate transporter activity plays a direct role in $As^{V}$ uptake and accumulation.

Under flooded conditions, As predominantly exists in the form of $As^{III}$, and overexpression of phosphate transporters has no significantly impact on As accumulation in rice straw or grains. Arsenite has higher bioavailability than $As^{V}$, which partly explains why the soil-to-plant transfer factor for As in rice is nearly ten times higher than that in other crops (Zhao et al., 2010). In rice, $As^{III}$ is primarily absorbed through the silicon uptake pathway (Ma et al., 2008). The entry of $As^{III}$ into rice roots is mediated by the aquaglyceroporin Lsi1 (*OsNIP2;1*), a nodulin 26-like intrinsic protein (NIP) (Ma et al., 2008). Notably, *Lsi1* is not the only NIP family member capable of transporting $As^{III}$ (Bienert et al., 2008). *Lsi1* also facilitates the uptake of methylated As species such as MMA and DMA (Li et al., 2009). Rice is capable of accumulating silicon to high levels, which contributes to its resistance against both biotic and abiotic stresses (Ma and Yamaji, 2015). The activity of the silicon transport pathway in rice further explains why rice is a major dietary source of As (Zhao et al., 2010). Since aquaglyceroporins mediate facilitated diffusion rather than active transport, *Lsi1* can also facilitate the efflux of $As^{III}$ (Zhao et al., 2009). Some $As^{III}$ is chelated by phytochelatins (PCs), and ATP-binding cassette (ABC) transporters such as *OsABCC1* in rice sequester the As-PC complexes into vacuoles (Song et al., 2014). The remaining $As^{III}$ is transported to the xylem by the efflux transporter *Lsi2* (Ma et al., 2007). To date, transporters responsible for xylem loading of methylated arsenic species have not been identified.

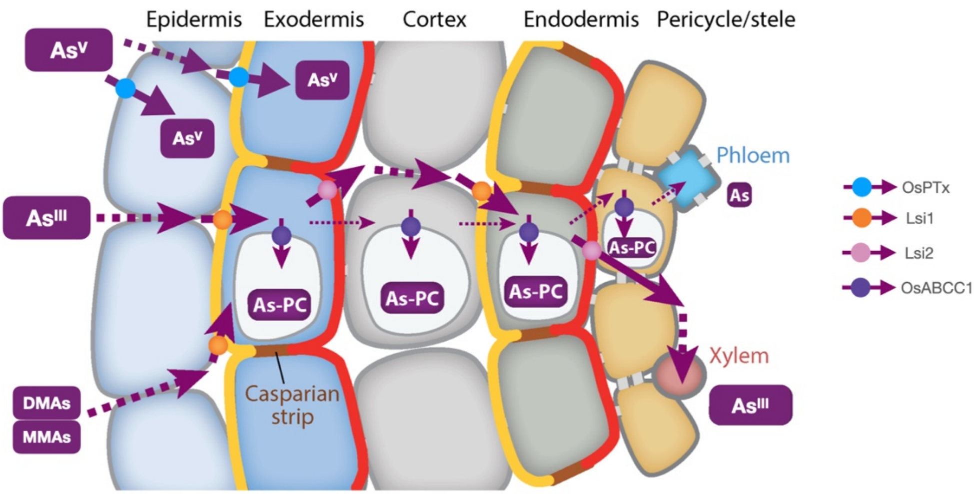



**Figure** 1-3. Radial transport of As in rice roots. $As^{III}$, arsenite; $As^{V}$, arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; *Lsi1*/*Lsi2*, silicon transporters; *OsPTx*, phosphate transporter; *OsABCC1*, *Oryza sativa* ATP-binding cassette transporters; As-PC, arsenic–phytochelatin complexes. Dashed arrows indicate possible passive diffusion pathways, and the thickness of arrows reflects the relative contribution to overall transport. (adapted from (Clemens and Ma, 2016)).

In rice roots, Cd is primarily taken up in the forms of $Cd^{2+}$ and $CdCl^-$ (Figure 1-4). Cadmium uptake mainly occurs through competition with Mn for the Mn transporter *OsNRAMP5* (Sasaki et al., 2012). *OsNRAMP5* is predominantly expressed in rice roots and is polarly localized on the distal plasma membrane of exodermal and endodermal cells (Sasaki et al., 2012). Knocking out *OsNRAMP5* significantly reduces $Cd^{2+}$ uptake by rice roots and decreases Cd accumulation in both the rice shoot and grain (Yang et al., 2014). Other transporters, such as *OsNRAMP1*, iron-regulated transporters (*OsIRT1* and *OsIRT2*), and *OsCd1* (a member of the major facilitator family), also contribute to Cd uptake in rice, but their roles are less significant compared to *OsNRAMP5* (Lee and An, 2009; Takahashi et al., 2011; Yan et al., 2019). The vacuolar membrane-localized *OsHMA3* sequesters absorbed Cd into vacuoles (Miyadate et al., 2011), while *OsHMA2*, localized on the plasma membrane of pericycle cells, is hypothesized to transport Cd from the apoplast into the cytosol, facilitating its translocation via the phloem (Takahashi et al., 2012).

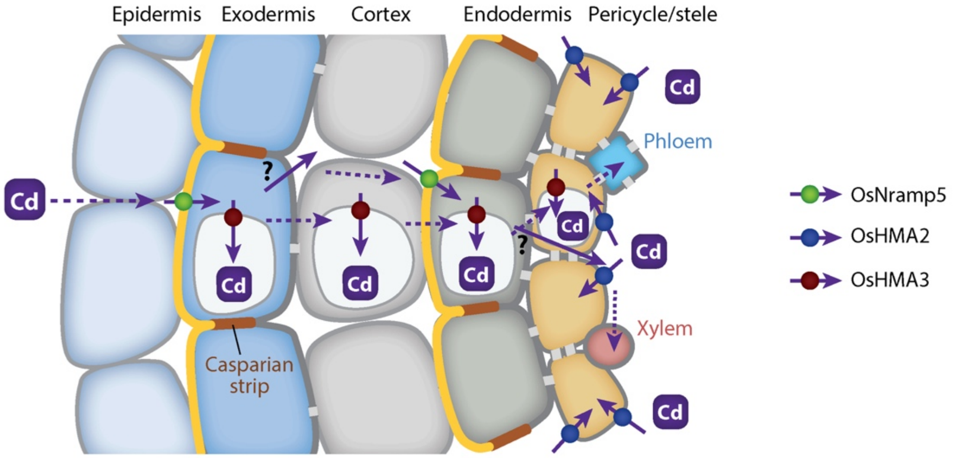
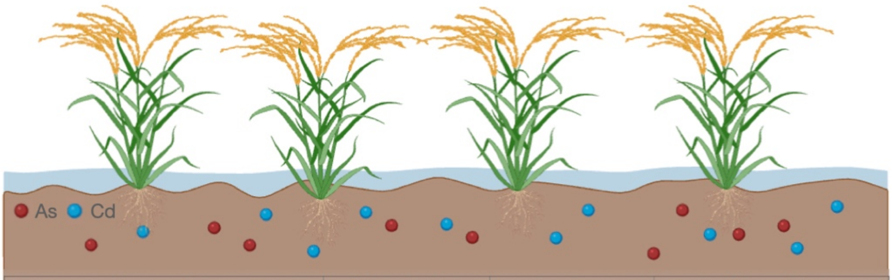



**Figure** 1-4. Transporters involved in Cd uptake and root-to-shoot translocation in rice. *OsNRAMP5*, Mn transporter, is responsible for transporting Cd from the apoplast into root cells. *OsHMA3*, sequesters absorbed Cd into vacuoles. *OsHMA2*, is hypothesized to mediate the transport of Cd from the apoplast into the cytosol to facilitate translocation via the phloem. Dashed arrows indicate possible passive diffusion pathways. (reproduced from (Clemens and Ma, 2016)).

# 4. Current approaches in simultaneously mitigating As and Cd bioavailability in soils

Various approaches have demonstrated potential in reducing the bioavailability of As or Cd in paddy soils, yet few have shown simultaneous remediation effects (Figure 1-5). This section reviews the efficacy and potential mechanisms of employing water management, chemical passivation, foliage dressing, cultivation of low As and Cd uptake crops and phytoremediation to simultaneously decrease As and Cd accumulation in the edible parts of crops.




| Approaches | | iAs reduction | Cd reduction | Key points |
|---|---|---|---|---|
| **Water Regime** | Alternate wet & drainage | 14~61% (Li et al., 2019) | 21~90% (Huang et al., 2022) | The critical periods for the accumulation of Cd and As in rice grains are different. |
| | Ridge cultivation | ~48% (Jiku et al., 2022) | Keep in low level (Jiku et al., 2022) | Adjust soil pH and Eh to a trade off value. |
| **Soil amendments** | Lime | / | 29~51% (Yang et al., 2020) | Adjust pH, $Ca^{2+}$ competes with $Cd^{2+}$ for adsorption sites. |
| | Calcium magnesium phosphate | Need to combine with other materials. | ~ 30% (Zhang, 2018) | Adjust pH, new ions to compete adsorption sites. |
| | Biochar | Weak. | 26~50% (Tang et al, 2022) | Adsorption; affects microbial communities. |
| | Fe-based materials | 33.3-42.7% (Feng et al, 2022) | 26.4~51.6% (Feng et al, 2022) | Adjust soil pH, chemical adsorption. |
| **Crop substitute** | Low accumulation cultivar | 3-34 fold variation (Norton et al., 2012) | 10-32 fold variation (Duan et al., 2017) | Difference in environment and genotype. |

**Figure** 1-5. Summary diagram of current methods and their effectiveness in simultaneously reducing As and Cd accumulation in rice grains (Original to this study, unpublished).

## *4.1. Water management*

Water management practices in paddy fields play a significant role in controlling the bioavailability of As or Cd in the soil-plant system, albeit with contrasting effects As and Cd levels. Soil maintained under aerobic conditions can substantially reduce As content in rice grains by 10-20 fold but may increase Cd content, while the content of DMAs in rice grains also decreases markedly (Arao et al., 2009). Previous studies have demonstrated the efficacy of specific water management strategies, including alternate wetting and drying (Norton et al., 2017), intermittent irrigation (Shrivastava et al., 2020) and sprinkler irrigation (Moreno-Jiménez et al., 2014) in reducing As accumulation, whereas continuous flooding has been found effective in reducing Cd accumulation in edible parts of crops (Carrijo et al., 2022). However, relying solely on water management for simultaneous control of As and Cd availability in paddy soil remains a significant challenge. Adjusting soil moisture to achieve an appropriate Eh and pH trade-off value may keep As and Cd availability at a low level, but these trade-off values vary depending on the type of paddy soil and the concentrations of As and Cd in the soil. Moreover, achieving the desired trade-off situation in practice can be difficult. Soil ridge cultivation, a traditional agronomic practice in cold waterlogged paddy fields, has been utilized to adjust soil Eh and pH to a trade-off situation, thereby simultaneously decreasing As and Cd availability, especially in soil with low Cd content (Jiku et al., 2022). Furthermore, the critical periods for Cd and As accumulation in rice grain differ (Maillard et al., 2015). Cd is predominantly absorbed by rice from the soil during the grain-filling stage and transferred to the grain, whereas As is absorbed by the roots and transported to the grain during the tillering, jointing and heading stages. The accumulation of As in the grain during the filling stage can be negligible (Huang et al., 2022). Therefore, a combination of intermittent irrigation and drainage during the vegetative growth period, from tillering to heading, along with irrigation or delayed drainage during the filling period, can synergistically reduce the accumulation of Cd and As in rice grains (Huang et al., 2022).

### *4.2. Soil amendments*

Passivation with natural or artificially modified materials shows significant potential to simultaneously reduce the bioavailability of As and Cd in paddy soils. Table 1-1 presents the list of reported materials for the simultaneous remediation of As and Cd in paddy soils.

Organic amendments, including crop residues, farmyard manure, composts and various biochar types have been utilized to mitigate the mobility of Cd in agricultural soils. Despite the potential heavy metal content in organic manure, farmyard manure exhibited higher efficacy (36-45% reduction) in reducing Cd phytoavailability compared to rice husk (23% reduction) and straw dust (14% reduction) in *Amaranthus caudatus* (Singh and Prasad, 2014). Biochar application elevates soil pH and facilitates the competition or exchange of $Cd^{2+}$ with $Ca^{2+}$ and $Mg^{2+}$ (Harvey et al., 2011). Moreover, biochar can interact with plant roots, soil organic matter and microorganisms to form organo-mineral-biochar complexes (Joseph et al., 2015), thereby inhibiting Cd mobility. However, the effectiveness of biochar alone in reducing As mobility in the field is generally limited, exhibiting minimal impact (Wang et al., 2015). Inorganic amendments, such as lime, calcium magnesium phosphate fertilizers, silicon fertilizers, vermiculite minerals and iron materials, have been applied individually or in combination to immobilize Cd and As in contaminated agricultural soils. The combined application of limestone and red mud reduced $Ca(NO_3)_2$ extractable As and Cd by 58% and 98%, respectively, resulting in reduced plant uptake and increased microbial activity (Lee et al., 2011). Due to the large specific surface area of Fe bearing minerals, they effectively adsorb or co-precipitate $As^{V}$ and $As^{III}$ (Pan et al., 2016). These can be used alongside passivator materials rich in sulfur, calcium, silicon or functional groups such as -OH, -COOH to synergistically reduce the availability of As and Cd, examples include 2,4,6-Trimercaptotriazine, trisodiumsalt, nonahydrate (TMT) and ferric sulfate (Jiang and Zhou, 2018), titanium gypsum (Zhai et al., 2020), Fe-Ca composite (Yuan et al., 2021), Fe-Si materials (Guo et al., 2018) and iron hydroxyl phosphate (Yuan et al., 2017). When applying chemical passivation, long-term stability and effectiveness, potential toxicity and secondary pollution, cost, and the impact on soil functions, ecosystem services, and environmental concerns should also be taken into consideration.

Emerging technologies have made artificially modified materials promising for the remediation of Cd- and As-co-contaminated soils. These include nanotechnology, microbial technology, and innovative chemical methods. Nanomaterials can effectively adsorb heavy metals due to their large surface area and adjustable physical properties (Ekrami et al., 2022). For example, zinc oxide nanoparticles (ZnONPs) and calcium-based nano-formulations can significantly reduce the bioavailability of Cd and As in soil (Ma et al., 2020; Nazir et al., 2024). Microorganisms regulate mineral concentrations in soil through various mechanisms, including mineralization and immobilization, which directly influence the fate and transport of As and Cd in soil. More importantly, microorganisms can convert both organic and inorganic contaminants into less toxic forms; however, this approach is more commonly used to break down toxic, complex organic compounds into simpler, nontoxic forms (Bala et al., 2022). Nonetheless, the method has limitations, as heavy metals cannot be broken down. Furthermore, the simultaneous remediation of pollution by multiple heavy metals often requires the use of mixed bacterial agents (Peng et al., 2023).

**Table** 1-1. List of potential materials for immobilizing As and Cd in soil simutaneously.

| Materials | Passivation Effect | Possible mechanism/reason for immobilization | |
|---|---|---|---|
| | | Cd | As |
| Limestone + Red mud (Lee et al., 2011). | The combination was applied at a dose of 2% w/w ratio of each amendment, reducing soil available As and Cd by 58% and 98%. | Increased soil pH promotes Cd adsorption, reducing the exchangeable Cd fraction while increasing the carbonate- and Fe/Mn-oxide-bound Cd fractions. | Ca-As precipitation, such as $Ca_4(OH)_2(AsO_4)_2 \cdot 4H_2O$ and $Ca_5(AsO_4)_3 \cdot OH$. |
| Calcium carbonate + metakaolin + fused calcium–magnesium phosphate fertilizer (CMP) (Yang et al., 2017). | The application of 0.2% CMP reduced Cd and As in brown rice by 43.6% and 32.0%. Acid-extractable Cd and exchangeable As was decreased by 12.1% and 37.4%. | Ion exchange, surface complexation, soil colloid sorption, and Cd-cation competition in soil. | Arsenic physiosorption or chemisorption by metakaolin. Arsenic bound-to-Al and bound-to-Ca fractions increased. |
| Zero valent iron (ZVI) + biochar (Qiao et al., 2018). | 1% w/w ZVI-biochar (5% Fe) reduced rice grain Cd by 93% and As by 61%. Soil bioavailable Cd and As decreased significantly. | Immobilized through amorphous Fe and Fe plaques, as well as adsorption onto biochar. | Immobilized by amorphous Fe and Fe plaques, and adsorption by ZVI. |
| Vermicompost (VC) + zero-valent iron ($Fe^0$) (Pan et al., 2022). | 2% VC+$Fe^0$ (1:1, 2:1 by weight) reduced As and Cd bioavailability by 15.5%-30.6% in paddy soil and by 17.4%-21.7% in laterite soil. | VC increased soil pH. VC is rich of organic substances like carboxylic acids, phenolic, and alcoholic hydroxyls, resulting in a strong adsorption capacity and complexation reaction with Cd. | The Fe-(hydro) oxides produced in the process of $Fe^0$ corrosion create additional surface sites for As adsorption. |
| Natural iron-based desulfurization material | The application rate of 0.5%-1.5% reduced soil availability Cd | Elevated Fe and Mn levels in Fe plaques on root surfaces | Elevated Fe and Mn in root surface Fe plaques reduced iAs in grains |

| Materials | Passivation Effect | Possible mechanism/reason for immobilization | |
|---|---|---|---|
| | | Cd | As |
| (Feng et al., 2022). | by 88.0-89.6%, soil availability As by 37.9-69.9%, grain Cd by 26.4-51.6%, and grain inorganic As by 33.3-42%. | reduced Cd accumulation in grains. This occurred through co-precipitation with $Cd(OH)_2$ and ion exchange between released $Fe^{2+}$ and $Cd^{2+}$. | through Fe-As and Ca-As complexation. |
| Fe-Si material (IS) (Guo et al., 2018). | 0.6% IS significantly decreased soil bioavailable Cd and As content by 71.4% and 40%. | IS material increased soil pH significantly, which decreased Cd bioavailability. | Adsorption and co-precipitation of As facilitated by high-dose Fe-Si materials rich in Fe oxides and abundant in Ca. |
| Fe-Ca composite (Yuan et al., 2021). | Application dosage is 0.3%. Soil availabile As and Cd was reduced by 6.3-45.2% and 33.5-47.5%. | Form hydroxylated metal ions with surface hydroxyl groups on iron-containing oxides. $SO_4^{2-}$ in the material reduces to $S^{2-}$ under flooding conditions, leading to $Cd^{2+}$ precipitating as CdS. Increased $Ca^{2+}$ raises soil surface negative charge and colloid formation, enhancing Cd adsorption. | Form hydroxylated metal ions with surface hydroxyl groups on Fe-containing oxides. Fe-Ca materials, rich in Fe and S elements, facilitate Fe oxide surface charge adsorption and hydrogen bonding with oxygen-containing functional groups and bonding with iron oxide surface hydroxyl group form inner surface chelates to passivate As. $Ca^{2+}$ reacts with As to precipitate $Ca(AsO_4)_2$ and $CaHAsO_3$. |
| Fe-Mn binary oxide (FMBO) (Yuan et al., 2021). | Application dosage is 0.3%. Soil available As and Cd decreased by 6.8-27.3% and 18.1-52.8%. | Form hydroxylated metal ions with surface hydroxyl groups of iron-containing oxides. Fe-Mn material elevate soil pH, | Form hydroxylated metal ions with surface hydroxyl groups of iron-containing oxides. Fe-Mn materials are primarily used to passivate As |

| Materials | Passivation Effect | Possible mechanism/reason for immobilization | |
|---|---|---|---|
| | | Cd | As |
| | | enhance $Cd^{2+}$ adsorption, and facilitate Cd hydroxide and carbonate precipitation. Under flooded conditions, $SO_4^{2-}$ in the material reduced to $S^{2-}$ and precipitate $Cd^{2+}$ as CdS. | by adsorbing iron oxides via surface charge, forming hydrogen bonds with oxygen-containing functional groups, and bonding with surface hydroxyl groups of iron oxides to form inner surface chelates. Mn oxides mainly assist in oxidizing $As^{III}$ to $As^{V}$. |
| Hydroxyapatite + zeolite + biochar (HZB) (Gu et al., 2019). | Application of 9000 kg $ha^{-1}$ HZB (2:1:2) reduced soil exchangeable Cd and As by 68.9% and 28.6%. | HZB can adsorb and covalently bind Cd and $As^{V}$ via -OH, -COOH, -Si-O-Si, and $CO_3^{2-}$ groups, forming carboxylates, silicates and carbonates, thus facilitating Cd and As immobilization in the soil solution. | |
| Zeolitic material synthesised from coal fly ash (Querol et al., 2006). | The application rate ranges from 10 to 25 t/ha, with an efficiency in reducing leached amounts of As and Cd by 90 to 99%. | Illite clay minerals adsorb Cd and As. Adding zeolite, which increases soil pH from 3.3 to 7.6, enhances metal adsorption significantly. | |
| Fe-Mn binary oxide (FM) (Lin et al., 2020). | With a 0.60 wt% FM, As and Cd decreased by 42.01% and 34.05% in the rhizosphere soil. | $As^{III}$ is oxidized to $As^{V}$ and immobilized in the rhizosphere soil. FM promotes the formation of Fe/Mn-plaques on root surface, significantly enhancing adsorption of As and Cd. | |
| Titanium gypsum (Zhai et al., 2020). | Applying 0.3% TG reduces soil bioavailable Cd and As by 35.2% and 38.0%, respectively. | CdS formation under flooded soil conditions. | Formation of insoluble arsenic-sulfide species such as orpiment and realgar under flooded conditions. |
| Woody peat + $Fe(NO_3)_3$ (Wang et al., 2019). | The addition of woody peat (5 g/kg dry soil) and $Fe(NO_3)_3$ (16 mmol/kg dry soil) | The pH rose due to $Fe(NO_3)_3$ application, reducing $As^{III}$ and Cd in porewater. Peat and $Fe(NO_3)_3$ applications lowered mobile portions of As and Cd, but increased their immobile portions. $Fe(NO_3)_3$ addition | |

| Materials | Passivation Effect | Possible mechanism/reason for immobilization | |
|---|---|---|---|
| | | Cd | As |
| | significantly reduced As in nonspecifically and specifically sorbed fractions and exchangeable Cd in soils by 78%, 70.6% and 67.0%. | promoted Fe plaques formation and poorly crystalline Fe oxides production, aiding As and Cd immobilization in soil. | |
| Ferric trichloride ($FeCl_3$) modified corn-straw biochar (FCB) (Wang et al., 2020). | 10% FCB exhibited the highest immobilization efficiency for Cd (63.21%) and $As^V$ (95.10%). | The surface oxygen-containing functional groups of FCB, such as -COOH and -OH, sorb Cd, primarily through surface complexation and cation exchange mechanisms. | Fe-modified biochar had fewer negative charges, leading to increased electrostatic sorption of anion contaminants like $As^V$. Fe-O-$As^V$ complexes formed, contributing to the sorption of $As^V$ onto the Fe-modified biochar. |
| Iron hydroxyl phosphate (FeHP) (Yuan et al., 2017). | At 10% FeHP, As and Cd immobilization in soil reached 69% and 44%. | The exchangeable fraction of Cd decreased while the residual fraction increased. | The proportions of exchangeable, carbonate-bound and Fe/Mn oxide-bound As decreased, while residual fractions increased. |
| 2,4,6-trimercaptotriazine, trisodiumsalt, nonahydrate (TMT) and ferric sulfate (Jiang and Zhou, 2018). | At TMT doses of 0.02 to 0.06 $L \cdot kg^{-1}$ with ferric sulfate doses of 48.0 to 80.0 $g \cdot kg^{-1}$, Cd immobilization efficiency exceeds 97%, while As reaches 62%. | The immobilization efficiency of Cd is determined by TMT precipitation and DPTA extraction, involving the transformation of $SO_4^{2-}$ to $S^{2-}$ and Cd precipitation. | The efficiency of As immobilization is determined by the precipitation of $FeAsO_4$. |
| 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate (TMT) | When 200 mL/kg TMT was applied to the soil, soil bioavailable As and Cd decreased | TMT reduced DTPA-extractable Cd and $NaHCO_3$-extractable As by chelating heavy metals with its unique sulfur group. | |

| Materials | Passivation Effect | Possible mechanism/reason for immobilization | |
|---|---|---|---|
| | | Cd | As |
| (Xiao et al., 2019). | by up to 48.0% and 68.9%. | | |
| Iron oxide ($Fe_2O_3$) coated with modified hairs with $CaCO_3$ as the base amendment (Ullah et al., 2020). | Using 10 g of $CaCO_3$ as the base amendment, 1 g of modified hair-$Fe_2O_3$ reduced soil As and Cd contents by 67% and 60%. | Iron oxide accelerated the iron plaque formation, reducing root permeability to As and Cd. The smaller size of Fe oxide and root hairs increased surface area available to HMs, enhancing Cd and As sorption. | |
| Zeolite coated with $KMnO_4$, with $CaCO_3$ as the base amendment (Ullah et al., 2020). | Using 10 g of $CaCO_3$ as the base amendment, 1 g of zeolite-$KMnO_4$ reduced the As and Cd contents in soil by 46% and 50%, respectively. | High levels of Cd and As were adsorbed into the soil, reducing their bioavailable fraction. This occuurred because the coated zeolite with $KMnO_4$ increased surface area and enhanced negative charge. | |

### 4.3. Foliage dressing

Foliage application of Si or Se fertilizers has demonstrated significant potential to alleviate As and Cd accumulation in the edible parts of crops (Ding et al., 2017). Weather conditions and foliar effectiveness are the main challenges for foliar dressing. Silicon is recognized as an essential nutrient for rice plants due to its role in promoting plant growth, enhancing stress resistance, and reducing the uptake of As and Cd in rice (Bogdan and Schenk, 2008; Zhang et al., 2008). Foliar application of Si fertilizers can diminish the uptake of As and Cd by plants through various mechanisms: (a) enhancing the capacity of the roots rhizosphere to produce the apoplatic barrier, which directly contributes to the reduction of Cd uptake. For example, the impact of Si on radial oxygen loss (ROL) and the formation of Mn plaques on the root surface of A. marina seedlings can alleviate Cd toxicity and decrease Cd uptake (Zhang et al., 2013). (b) Formation of precipitates through co-precipitating reactions between silicate ions and Cd and arsenate (Liang et al., 2007). (c) Sequestration of Cd in shoot cell walls (Liu et al., 2009). Foliar dressing has been observed to alleviate Cd stress in rice by enhancing photosynthesis. Moreover, foliar application of Si has been found to increase production and plant height while decreasing As concentrations in soils with low to moderate polluted soils (Gu et al., 2022).

Proper selenium (Se) levels, including selenite ($Se^{IV}$) and selenate ($Se^{VI}$), have been shown to reduce the uptake of As and Cd in crops. This reduction not only mitigates the toxicity of As and Cd but also maintains rice grain yields and quality (Liao et al., 2016; Lv et al., 2020). Selenium achieves this by: (a) Directly inhibiting the uptake and accumulation of As and Cd (Lv et al., 2020). (b) Regulating antioxidant mechanisms to protect plants against oxidative stress induced by As and Cd (Lv et al., 2020). (c) $Se^{IV}$ demonstrates a stronger ability to reduce the accumulation of As and Cd compared to $Se^{VI}$, possibly due to its chelating activity with As and Cd compounds, thereby reducing their transport within plants (Liao et al., 2016). While foliage dressing shows potential in mitigating As and Cd accumulation in edible crop parts, its applicability may be limited by factors such as climatic and experimental conditions, plant cultivars, spraying techniques, inability to address high metal concentrations, and instability in mitigation mechanisms (Khaliq et al., 2019). To optimize its effectiveness, it is crucial to develop reliable foliar application methods and appropriate dressing materials for mitigating As and Cd accumulation in crops.

### 4.4. Cultivation with crops of low risk of As and Cd accumulation

With the aim of mitigating the risk of accumulating both As and Cd in the human food chain, it is imperative to identify cultivars with low accumulation of these elements in contaminated fields. Previous studies have demonstrated significant genetic variations in the accumulation of As and Cd in edible parts of crops or germplasm, such as rice, wheat and maize (Duan et al., 2017; Lu et al., 2021; Yan et al., 2023). For instance, among 471 locally adapted rice cultivars, variations in As and Cd contents in brown rice grains ranged from 10 to 32-fold and 2.5 to 4-fold, respectively (Duan et al., 2017). Moreover, different rice cultivars exhibit variances in the quantity of As and Cd deposited in the grain, as well as in the relative proportions of organic and inorganic As and Cd contents (Norton et al., 2009). At present, rice varieties with low Cd accumulation stability have been cultivated based on genetic methods with the mutant *lcd1* was found (Zhang et al., 2024). Genotype and phenotype analyses at the genome-wide level have revealed differences in the accumulation of Cd in maize kernels, enabling accurate predictions (Yan et al., 2023). Additionally, the transport capacity of As and Cd in plants varies among different genotypes. For instance, 95 maize genotypes displayed significant differences in Cd accumulation in the grain due to disparities in the transport capacity of the epidermis and xylem vessels, as well as the tolerance of mesophyll cells to Cd (Lin et al., 2022). Therefore, plant varieties with low transfer rates of heavy metals from roots to shoots could be selected for cultivation in contaminated soils. Furthermore, it is critical to identify appropriate low-As/Cd accumulation substitute crops through rational agricultural planning based on the local pollution level and planting system. For instance, a practical approach to simultaneously reduce As and Cd contents in rice is to cultivate low-Cd-accumulating cultivars under aerobic conditions (Arao, 2019). Given the varying ability of crops and their different parts to accumulate As, areas with slight pollution levels could consider planting leafy vegetables, while those with moderate pollution levels may opt for crops such as green tender, pakchoi cabbage, rape, amaranth, etc. In heavily polluted areas, planting maize, tubers or fruit vegetables can help ensure that As content in the edible parts remain within acceptable limits (Zeng et al., 2021).

Previous research has demonstrated significant variations in grain concentrations of As and Cd among rice cultivated under flooded paddy conditions. These variations, reported as 40.7-fold for Cd and 12.1-fold for As, were observed across 1763 rice originating from diverse geographic and genetic backgrounds (Pinson et al., 2015). The substantial genetic diversity present among rice varieties or germplasm offers potential avenues for mitigating As and Cd accumulation in rice grains. However, contrasting findings suggest limited genetic variability in grain As concentrations among rice varieties, with environmental factors exerting a more dominant influence (Ahmed et al., 2011). This underscores the potential significance of environmental selection in shaping differences in As accumulation across species or varieties. Notably, genotype emerges as the primary determinant of Cd concentrations in rice grains (Liu et al., 2021). Specifically, japonica rice varieties such as Koshihikari generally exhibit lower Cd concentrations compared to indica rice varieties when cultivated under similar environmental conditions. Interestingly, upland rice varieties from Africa demonstrate reduced capacity for Cd uptake relative to japonica varieties (Abe et al., 2013), possibly attributable to the presence of the *LAC23* allele at *qlGCd3* on the long arm of chromosome 3, which is associated with diminished Cd levels in grains and inhibits Cd transport from shoots to grains (Abe et al., 2013). In the case of maize or wheat, the accumulation of Cd in grains is substantially influenced by environmental disparities, particularly the Cd content in the soil (Retamal-Salgado et al., 2017). Further comprehensive data analysis is warranted to elucidate the comparative importance of environmental selection versus genetic factors in determining Cd and As co-accumulation.

### 4.5. Phytoremediation with As and Cd hyper-accumulating plant

Hyperaccumulators are plants that typically grow in metal-enriched soils and have abnormally high levels of a certain element or its ions in their tissues (Rascio and Navari-Izzo, 2011). Hyperaccumulators are effective for cleaning up, transferring and stabilizing heavy metals in contaminated soils (Ma et al., 2001). Approximately 500 plant species have been reported to accumulate heavy metals (Krämer, 2010), with several edible plant species, including crops and vegetables, serving as As hyperaccumulators (Chakraborty et al., 2014). However, hyperaccumulative plants typically exhibit an adsorption effect limited to specific metals such as As or Cd (Li et al., 2013). To date, 12 species of ferns in the Pteridaceae family have demonstrated the ability to hyperaccumulate As (Zhao et al., 2009), including *Pteris vittata* L. (Chinese brake fern). Regarding Cd removal, few Cd hyperaccumulators have been reported, including *Thalapsi caerulensis*, Pannycress, *Arabidopsis halleri* (Vassilev et al., 2002), *Solanum nigrum* (Dou et al., 2022) and *Noccaea caerulescens* (Yan et al., 2022). To date, there have been no reports of hyperaccumulative plants capable of simultaneously remediating Cd and As. However, studies have shown that transgenic *Arabidopsis thaliana* can enhance tolerance to and accumulation of heavy metals and metalloids, as well as the detoxification of metalloids in plants, by simultaneously overexpressing *AsPCS1* and *YCF1* (derived from garlic and baker's yeast). When grown in either Cd or As ($As^{V}/As^{III}$) environments, double-gene transgenic lines accumulated 2-10 times more $Cd/As^{III}$ and $As^{V}$ than wild-type or single-gene transgenic lines expressing *AsPCS1* or *YCF1* alone (Guo et al., 2012). This represents a very promising new tool for phytoremediation work. However, many hyperaccumulator plants tend to grow slowly and produce low biomass. As an environmentally friendly phytoremediation strategy, the selection of As and Cd-resistant hyperaccumulative plant species to remediate co-contaminated agricultural soils deserves further exploration.

# 5. Permissible limits of As and Cd in soil and crop

The permissible levels of As and Cd in agricultural soils vary across countries and regions. These differences arise from varying different background levels of soil As and Cd content, as well as diverse land use types and agricultural practices. Consequently, different countries and regions have established thresholds for As and Cd content in soil, often presented as soil quality indicators or screening level guidelines for farmland (Table 1-2). In China, these threshold ranges have been refined based on soil pH. Japan has set requirements for soil As content but has not specified a safety threshold for soil Cd. Instead, it uses a standard based on the Cd content of rice produced in the region, which should not exceed 0.4 $mg \cdot kg^{-1}$. Russia currently lacks formal regulations but has provided temporary thresholds for soil As and Cd content to ensure that environmental factors remain safe and/or harmless to humans, taking soil texture into account. In Europe, due to the generally low levels of soil As, the European Environment Agency (EEA) has not established specific As threshold values. In contrast, the United States and New Zealand have provided ecological safety screening values rather than specific thresholds for agricultural land. The approximate safety thresholds for soil As and Cd are 37-500 $mg \cdot kg^{-1}$ and 0.4-140 $mg \cdot kg^{-1}$, respectively.

The maximum allowable levels for cereals, particularly rice, are largely consistent across various countries, with total As limits ranging from 0.2 to 1 $mg \cdot kg^{-1}$, inorganic As from 0.15 to 0.35 $mg \cdot kg^{-1}$, and Cd from 0.1 to 0.4 $mg \cdot kg^{-1}$ (Table 1-3). Additionally, the United States and World Health Organization (WHO) have clarified the maxium allowable levels of As and Cd in bottled and drinking water, rather than in food, as a direct indicator of potential risks associated with human consumption.

**Table** 1-2. Soil As and Cd screening values in various countries and regions.

| Country/Region | | Name of value | Category | | Arsenic (mg·kg$^{-1}$) | Cadmium (mg·kg$^{-1}$) | Source |
|---|---|---|---|---|---|---|---|
| Australia | | Health investigation level. | Residential A | | 100 | 20 | National Environment Protection Council (NEPC, Australia), 04-2011. |
| | | | Residential B | | 500 | 140 | |
| | | Ecological investigation level. | Areas of ecological significance | | 40 | / | National Environment Protection Council (NEPC, Australia), 2013. |
| | | | Urban residential | | 100 | / | |
| Canada | | Soil quality guideline. | SQG-soil contact Agricultural | | 17 | 10 | Environment Canada, 1999. |
| | | | SQG-soil and food ingestion Agricultural | | / | 3.8 | |
| China | | Risk screening values for soil contamination of agricultural land. | Paddy field | pH⩽5.5 | 30 | 0.3 | Ministry of Ecology and Environment of the People's Republic of China, 2018. |
| | | | | 5.5<pH⩽6.5 | 30 | 0.4 | |
| | | | | 6.5<pH⩽7.5 | 25 | 0.6 | |
| | | | | pH>7.5 | 20 | 0.8 | |
| | | | Farmland | pH⩽5.5 | 40 | 0.3 | |
| | | | | 5.5<pH⩽6.5 | 40 | 0.3 | |
| | | | | 6.5<pH⩽7.5 | 30 | 0.3 | |
| | | | | pH>7.5 | 25 | 0.6 | |
| European | Austria | Soil screening | Warning value | | / | 1-40 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Union | | values. | Action value | / | 10 | |
| | Belgium/ Brussels | | Warning value | / | 1 | European Environment Agency, 08-2022. |
| | | | Action value | / | 2-30 | |
| | Belgium/ Flanders | | Warning value | / | / | |
| | | | Action value | / | 2-30 | |
| | Belgium/ Wallonia | | Warning value | / | / | |
| | | | Action value | / | 1.8-20 | |
| | Bulgaria | | Warning value | / | 1.5-3.5 | |
| | | | Action value | / | 12 | |
| | Czechia | | Warning value | / | 1.5-20 | |
| | | | Action value | / | / | |
| | Denmark | | Warning value | / | 5 | |
| | | | Action value | / | / | |
| | Finland | | Warning value | / | 1 | |
| | | | Action value | / | 10-20 | |
| | Germany | | Warning value | / | 2-20 | |
| | | | Action value | / | 0.1-20 | |
| | Hungary | | Warning value | / | 1 | |
| | | | Action value | / | 10 | |
| | Italy | | Warning value | / | / | |
| | | | Action value | / | 1.5-15 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Lithuania | | Warning value | / | / | |
| | | | Action value | / | 0.75-3 | |
| | Netherlands | | Warning value | / | / | |
| | | | Action value | / | 13 | |
| | Poland | | Warning value | / | / | |
| | | | Action value | / | 1-20 | |
| | Slovakia | | Warning value | / | 0.4-10 | |
| | | | Action value | / | 20-30 | |
| | Slovenia | | Warning value | / | 2 | |
| | | | Action value | / | 12 | |
| | Sweden | | Warning value | / | 0.4-12 | |
| | | | Action value | / | 4 | |
| Japan | | Limit for soil contaminant | / | 15 (10-20) | rice grain Cd < 0.4 | Ministry of the Environment, Japan, 1979. |
| Korea | | Soil contamination warning standard | / | 25 | 4 | Ministry of the Environment, Korea, 2022. |
| New Zealand | | Ecological soil guideline value | Agricultural land | 20 | 3.1 | New Zealand Water and Wastes Association, 2013. |
| Russia | | Hygienic standards and requirements to ensure the safety and/or harmlessness of environmental factors | Sandy and loamy soils | 2 | 0.5 | Ministry of Health of Russia, 2021 |
| | | | Acidic loamy and clayey soils, pH KCl<5.5 | 5 | 1 | |
| | | | neutral and alkaline loamy and clayey soils, pH KCl>5.5 | 10 | 2 | |

| | | | | | |
|---|---|---|---|---|---|
| United Kingdom | Soil guideline values | Residential with home grown produce (CLEA SGV) | 37 | 22 | Environment Agency, 11-Sep-2009. |
| | | Allotment (CLEA SGV) | 49 | 3.9 | |
| | | Agricultural and after sewge sludge application (EC Directive 86/278/EEC) | 50 | 3 | |
| United States | Ecological soil screening levels | Eco-SSL Plants | 18 | 32 | U.S. Environmental Protection Agency (EPA), 2005. |
| | | Eco-SSL invertebrates | / | 140 | |
| | | Eco-SSL avian | 43 | 0.77 | |
| | | Eco-SSL mammalian | 46 | 0.36 | |

**Table** 1-3. Maximum level of As and Cd in food (main rice grain) of current standards.

| Country/ Region | Target foods | Total As ($mg \cdot kg^{-1}$) | Inorganic As ($mg \cdot kg^{-1}$) | Cd ($mg \cdot kg^{-1}$) | Source |
|---|---|---|---|---|---|
| Australia | Cereals (including rice) | 1 | / | 0.1 | Australia New Zealand Food Authority, 2000. |
| Canada | Husked (brown) rice | / | 0.35 | / | Health Canada. 2024 updated. |
| | Polished (white) rice | / | 0.2 | / | |
| China | Cereals (excluding rice) | 0.5 | / | 0.1 | National Health Commission of the People's Republic of China, 2022. |
| | Rice grain | / | 0.35 | / | |
| | Cereals grinding processed products [except brown rice, rice (flour)] | 0.5 | / | 0.1 | |
| | Rice, brown rice, rice (flour) | / | / | 0.2 | |
| | Brown rice | / | 0.35 | / | |
| | Rice (flour) | / | 0.2 | / | |

| | | | | | |
|---|---|---|---|---|---|
| EU | rice (Codex Standard 198-1995) | / | / | 0.15 | Commission Regulation (EU), 25 April 2023. |
| | Non-parboiled milled rice (polished or white rice) | / | 0.15 | / | |
| | Parboiled rice and husked rice | / | 0.25 | / | |
| | Cereals | / | / | 0.1 | |
| | Wheat germ | / | / | 0.2 | |
| | Durum wheat (Triticum durum) | / | / | 0.18 | |
| Japan | Rice (polished rice, brown rice) | / | / | 0.4 | Japan External Trade Organization, 04-2011. |
| Korea | Grains (excluding brown rice) | / | 0.2 (limited to rice) | 0.1 (0.2 for wheat and rice) | Food and Agricultural Import Regulations and Standards Report, Republic of Korea, 2019. |
| New Zealand | Cereals (including rice) | 1 | / | 0.1 | Australia New Zealand Food Authority, 2000. |
| Russia | Food grain, grain legumes seeds and products therefrom, excluding bread and bun products | 0.2 | / | 0.1 | Customs Union Commission, 2011. |
| UK | Non-parboiled milled rice (polished or white rice) | / | 0.2 | 0.2 | Commission Regulation (EU), 2020. |
| | Parboiled rice and husked rice | / | 0.25 | / | |
| United States | Bottled Water | 0.01 | / | 0.005 | US food and drug administration, 2025. |
| FAO | Rice, husked | / | 0.35 | / | Food and Agriculture Organization, Adopted in 2023. |
| | Rice, polished | / | 0.2 | 0.4 | |
| WHO | Drinking water | 0.01 | / | 0.003 | World Health Organization. |

# 6. Scientifc questions and hypotheses

Given the distinct biogeochemical behaviors of As and Cd in paddy field environments, their high uptake efficiency by rice plants, and the urgent need to ensure food safety and sustainable production, this study focuses on rice paddies in China contaminated with As and Cd. Ridge cultivation (Figure 1-6), a traditional Chinese agronomic practice with a history of over 2000 years, has recently shown promising potential in simultaneously regulating the bioavailability of As and Cd in paddy fields. This study explores the application of ridge cultivation to As- and Cd-contaminated rice fields, in combination with other soil amendments, aiming to investigate:

(1) Can agronomic measures be implemented to simultaneously reduce the bioavailability of As and Cd in soil, thereby minimizing their accumulation in rice grains?
(2) How do these techniques influence the biogeochemical cycling of As, Cd, and associated elements in soil?
(3) What are the underlying microbial mechanisms involved, and what role does the microbiome play in these processes?

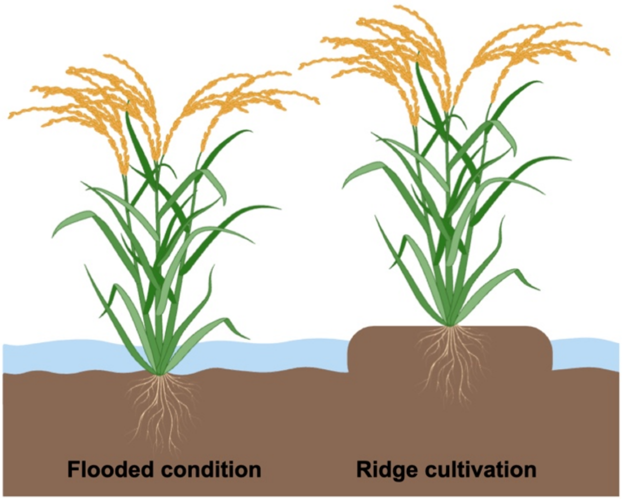



**Figure** 1-6. Ridge cultivation diagram.

## *Hypotheses*

**H1. Simultaneous Reduction of As and Cd Bioavailability (Chapter 2).**

Implementing ridge cultivation that significantly reduces As bioavailability, in combination with organic and inorganic soil amendments effective in reducing Cd bioavailability, can simultaneously decrease the bioavailability of As and Cd in soil, thereby minimizing their accumulation in rice grains.

**H2. Formation of a new rhizosphere habitat by changing soil pH and Eh, ion interactions as key regulators (Chapter 3).**

Changes in soil pH, redox potential (Eh), and the introduction of exogenous ions following ridge cultivation and soil amendments may alter ion interactions and chemical processes in the rhizosphere, potentially leading to the formation of a new soil habitat. Interactions among the induced ions with As and Cd in this newly formed soil habitat may play a crucial role in simultaneously reducing the mobility of As and Cd.

**H3. Microbial activity as the driving force (Chapter 4).**

A new soil redox state is expected to emerge under water and fertilizer management, where the oxidation of nitrogen (N), Fe, and Mn, or the reduction of S, may serve as key regulatory hubs in decreasing As and Cd bioavailability. Microbial activity is hypothesized to be the primary driving force behind redox transformations of key elements, thereby influencing As and Cd bioavailability.

# 7. Overview of the chapters

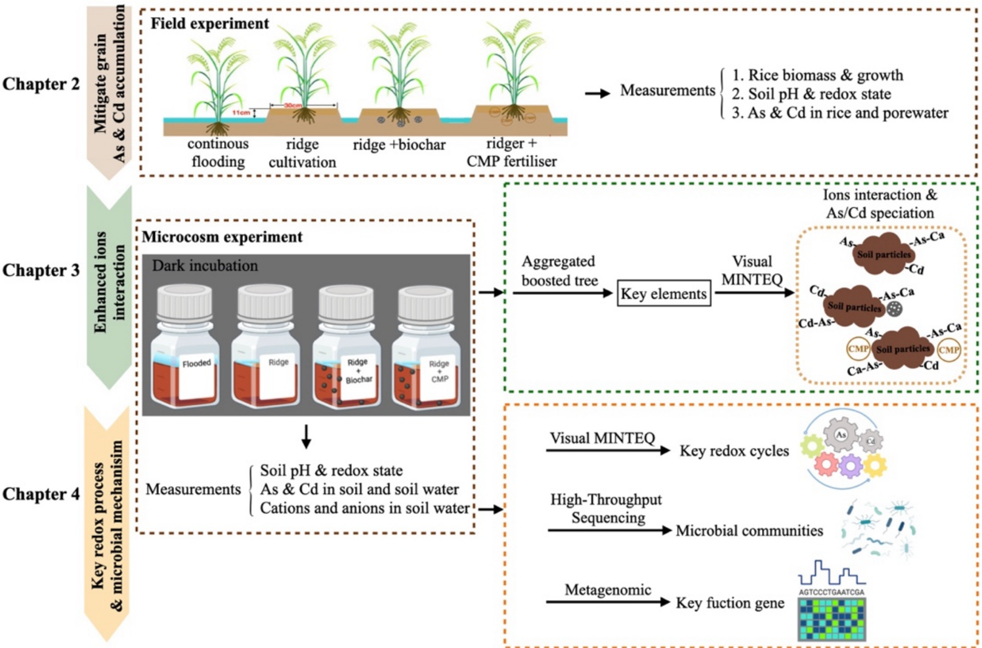



**Figure** 1-7. Technical route.

In this study, we investigated the effectiveness of ridge cultivation combined with the application of biochar or calcium-magnesium-phosphate fertilizer in reducing the bioavailable As and Cd content in soil and minimizing their accumulation in rice grains in As-Cd co-contaminated paddy fields. To elucidate the underlying mechanisms of reduced As and Cd bioavailability, a microcosm experiment was conducted. This included physicochemical analyses, geochemical modeling to assess redox cycling processes, high-throughput sequencing to characterize microbial communities, and metagenomic analysis to identify key functional genes involved in these transformations. The technique route of the research is shown in Figure 1-7.

1. Chapter 1: Reviews the As and Cd in soil-rice systems: biogeochemistry, regulatory mechanisms, and summarizes current remediation strategies for As-Cd co-contaminated farmlands.
2. Chapter 2: Evaluates the effectiveness of ridge cultivation combined with biochar or calcium-magnesium-phosphate fertilizer in reducing As and Cd accumulation in rice grains.
3. Chapter 3: Investigates the physicochemical mechanisms underlying the reduction of As and Cd bioavailability in soil through a microcosm experiment.
4. Chapter 4: Analyzes redox processes using a biogeochemical modeling approach and explores the microbial and metagenomic mechanisms driving As and Cd bioavailability reduction.

# Chapter 2

## Soil ridging combined with biochar or calcium-magnesium-phosphorus fertilizer application maintains grain As and Cd at low levels

Adapted from Zhang, T., Jiku, M.A.S., Li, L., Ren, Y., Li, L., Zeng, X., Colinet, G., Sun, Y., Huo, L. and Su, S., 2023. Soil ridging combined with biochar or calcium-magnesium-phosphorus fertilizer application: Enhanced interaction with Ca, Fe and Mn in new soil habitat reduces uptake of As and Cd in rice. *Environmental Pollution*, 332, p.121968.

# Abstract

This chapter investigates the effectiveness of ridge cultivation combined with biochar and calcium magnesium phosphate (CMP) fertilizer in reducing the co-accumulation of arsenic (As) and cadmium (Cd) in rice grains. A field experiment was conducted in a long-established paddy field in Shimen County, Hunan Province, China. Results showed that applying biochar on ridges reduced As concentrations in rice grains by up to 61.9% and Cd by up to 67.6%, depending on rice cultivar. Similarly, CMP application decreased As by up to 59.3% and Cd by up to 61.0%. Compared to ridging alone, these treatments significantly lowered metal accumulation in rice grains while maintaining soil productivity. This chapter demonstrates that combining traditional ridge cultivation with appropriate soil amendments provides a practical, low-cost, and environmentally friendly strategy for mitigating heavy metal risks in paddy fields. The approach offers a promising solution to ensure food safety in regions affected by moderate Cd and As contamination.

# 1. Introduction

Soil co-contamination with As and Cd is a growing global concern (Zhao and Wang, 2020). Compared with other crops, rice was found to have efficient pathways for absorbing Si/$As^{III}$ (Su et al., 2010), P/$As^{V}$ (Kamiya et al., 2013) and Mn/Cd (Sasaki et al., 2012), and thus can efficiently absorb As and Cd. Consuming rice as the staple food allows As and Cd to enter the food chain and threatens food safety and human health (Zhao, 2020). Therefore, it is imperative to develop or adapt strategies to reduce the bioavailability of both As and Cd in soil and their accumulation in rice grains. However, the anionic metalloid As and cationic metal Cd in paddy soils exhibit opposite biogeochemical behavior, which makes it challenging to remediate in the As and Cd co-contaminated fields (Zhao and Wang, 2020).

In paddy fields, flooding and drainage are common measures for rice cultivation. When paddy soil is flooded, As is mobilized by the reductive dissolution of (Fe)-Mn minerals (Shaheen et al., 2020), while soluble Cd forms precipitates, such as CdS, resulting in high As and low Cd bioavailability (El-Naggar et al., 2018; Shaheen et al., 2016). When the paddy field is drained, the transformation of CdS into soluble $CdSO_4$ increases the mobility of Cd (Sebastian and Prasad, 2014), while As is immobilized by the Fe/Mn-(oxy)hydroxides with the oxidation of FeS or $Fe^{2+}$ (Yu et al., 2016). Adjusting water management strategies in paddy soils to the appropriate Eh and pH trade off value can keep As and Cd availability at a low level (Honma et al., 2016). The trade-off value varies with the paddy soil types and the contents of soil As and Cd. It is often difficult to control the Eh and pH at an optimal trade-off state in practice.

Soil ridge cultivation is a traditional agronomic practice in waterlogged paddy fields and has been implemented in China for over 2000 years (Zheng et al., 2014). Ridging prevents the defects caused by high groundwater level, sufficient rainfall, poor drainage, and enrichment of reducing toxic substances in waterlogged fields (Ren et al., 2016). Usually, the height of the ridge is 10-20 cm, while the width of the ridge surface and furrow adapts to the practical crop production (Xiong et al., 2014). This traditional agronomic practice has been proven to keep As and Cd availability at low levels through the adjustment of the soil Eh and pH to a trade-off situation (Jiku et al., 2022). Due to the elevated Eh, ridge cultivation may increase the risk of Cd for paddy fields, especially with high Cd content (Jiku et al., 2022). Therefore, it may be of added benefit to utilize a form of soil amendments to stabilize Cd on the ridges to reduce Cd release from the soil. However, little information on the effect of soil ridging combined with soil amendments on the As and Cd bioavailability is currently known.

Soil in-situ amendments such as biochar (Rehman et al., 2017), and CMP fertilizers (Jiang et al., 2022; Shi et al., 2022) are effective for Cd immobilization. Both materials are environmentally friendly soil inputs and are compatible with food crops. The objective of this chapter was to test the possibility of combining ridging with soil amendments in rice cultivation to simultaneously reduce the accumulation of Cd and As in grains. Hence, a field trail was carried out to investigate the effects of the combination of ridging and biochar or CMP on grain As and Cd concentrations. The results of this study provide a new method with demonstrated experimental evidence to mitigate Cd and As accumulation in paddy fields to enhance food safety.

# 2. Materials and methods

## 2.1. Materials

Two three-line indica hybrid rice cultivars that are widely planted in the rice producing areas in southern China were selected for the field experiment: IIyou 28 produced by Fujian Fengtian Seed Co. Ltd. (Fuzhou, China) and Ruiyou 399 produced by Sichuan Kerui Seed Co. Ltd. (Chengdu, China). Rice straw biochar was obtained from Beijing PhD Union Academy of Agriculture (Beijing, China). The content of Cd and As in the biochar was 0.12 $mg·kg^{-1}$ and 0.5 $mg·kg^{-1}$. In the given dose of biochar applied to the soil, the content of Cd and As was negligible. CMP fertilizer with the available phosphorus content ($P_2O_5$) of ≥ 12% and As and Cd content below the detection limit was produced by Phosphate Fertilizer Factory in Liuyang East District (Liuyang, China).

## 2.2. Experiment design

The field experiment was conducted in a long-term rice cultivation field located in Shimen County, Hunan Province, China (Figure 2-1). Shimen is situated in south-central China and lies within a subtropical monsoon humid climate zone. The region has a long history of realgar mining, spanning over 1500 years, which has led to severe heavy metal contamination in the surrounding agricultural soils. The field experiment covered an area of 20 square meters (5 meters × 4 meters). The soil in the experimental plot is classified as paddy soil, primarily developed from Quaternary red clay parent material, and is categorized as Hydragric Anthrosol according to the World Reference Base for Soil Resources (WRB). This type of soil is typical and widely distributed in rice-growing regions of southern China. Due to the long-term mining and smelting of realgar mines nearby, the adjacent farmland was polluted by a large amount of As-containing slag or waste water (Jiku et al., 2022). The total As and Cd contents in the field plot was 66.5 $mg·kg^{-1}$ and 0.41 $mg·kg^{-1}$, respectively, and the pH value was 5.17, which failed to meet the safety criteria of agricultural land in China: when the soil pH≤5.5, As limit, 30 $mg·kg^{-1}$; Cd limit, 0.3 $mg·kg^{-1}$ (GB15618-2018).

The experiment treatments included conventional tillage (continuous flooding with a standing layer of ~2cm water, CK) and ridge cultivation above the irrigation level (Figure 2-1). In order to evaluate the effect of the combination of ridge and soil amendments, ridge cultivation is comprised of ridge without soil amendments (R), ridge with rice straw biochar (R+B), and ridge with CMP (R+CMP). The biochar and CMP were thoroughly mixed and applied to the ridge twice over a month before transplanting rice seedlings. The dosage of the biochar and CMP fertilizer are

1%(w/w, dry soil) and 0.5‰P (w/w, dry soil), respectively. The upper width was 30 cm for all ridges. As previous work showed that As and Cd contents in rice grains remained at low levels when the soil ridge height was around 11cm (Jiku et al., 2022). Therefore, the ridge height of 11 cm was adopted in this field experiment. Paddy field water and plant management were the same and according to local practices unless otherwise noted, with continuous flooding during rice growth season and drainage 10 days before harvest. Each treatment was repeated three times.

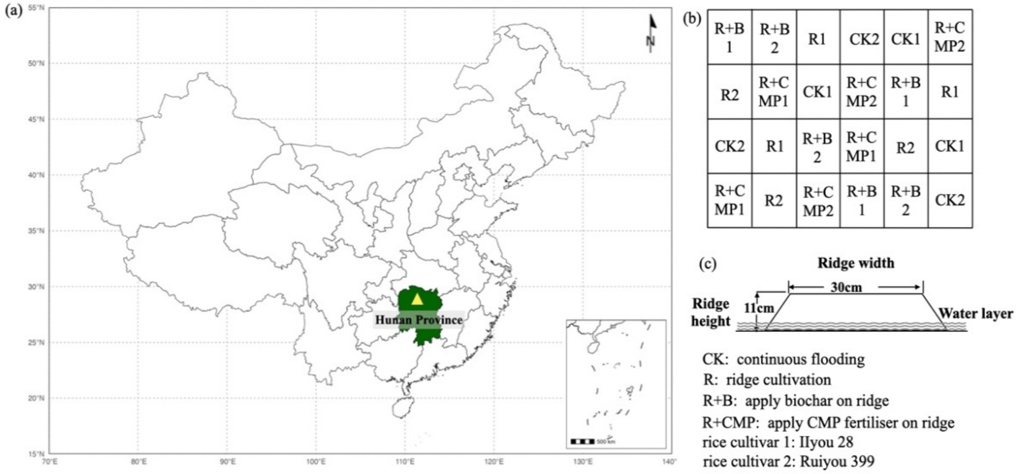



**Figure** 2-1. (a) Location of the experimental field, (b) layout of the experimental plots, and (c) diagram of ridge cultivation.

## *2.3. Sample collection and analysis*

After rice mature, the Eh and pH of the rhizosphere were preliminary in situ determined by an automated ORP depolarization automatic analyzer (FJA-6; Nanjing Chuan-Di Instrument & Equipment Co. Ltd., Nanjing, China). Subsequently, field pore water was collected in the rhizosphere at a depth of 10 cm through a Rhizon soil moisture sampler (Rhizosphere Research Products, Wageningen, The Netherlands). The porewater was stored in the icebox immediately and shipped to the lab for the analysis of As, Cd, Fe, Mn and S concentrations after having been passed through a 0.45-μm syringe filter. Total As concentration was determined by hydride generation-atomic fluorescence spectrometer (HG-AFS, 9120, Beijing Titan Instrument Co., Ltd.). Total Cd, Fe, Mn and S concentrations were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Optima 5300DV; PerkinElmer).

Whole rice plants were collected from each replicate in the field experiment after plant height was measured. All fresh samples were rinsed with tap water and ultrapure water. The plants were separated into husks, grains, straws, and roots without removing the iron plaque on the root surface. All plant organs were oven-dried at 45 °C to constant weight, then weighed, and pulverized for subsequent analysis. Total As concentrations in rice samples were determined by HG-AFS after acid digestion with 4:1:1.5 (v/v/v) $HNO_3$-$HClO_4$-$H_2SO_4$ (Yu et al., 2016). Total Cd concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS, Elan DRC-e, Pekin Elmer, USA) after acid digestion with 9:1 (v/v) $HNO_3$-$HClO_4$ (GB 5009.15-2014).

### 2.4. Quality control

For quality control, certified reference material of rice (GBW10045, Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, Langfang, China) was digested at the same time. The recovery of total As and Cd in the standard sample were for As at 88–99 % and Cd at 95–102 %. CRM water samples (GSBZ 50004-88 for As and GSB 07-1185-2000 for Cd; Institute for Environmental Reference Materials of the Ministry of Environmental Protection, Beijing, China) were mixed among the blank and digested samples at the beginning of each sample test and intervals of 10 samples analysis for quality control of ICP-MS and AFS.

### 2.5. Statistical analysis

Basic statistical analysis was performed using Microsoft Excel 2010 and IBM SPSS Statistics 26.0. Homogeneity of variances was tested first. For data that met the assumption of homogeneity of variances, one-way analysis of variance (ANOVA) was used to assess treatment differences, and significant effects were evaluated using the Least Significant Difference (LSD) test at the 0.05 probability level ($P < 0.05$). For data that did not meet the homogeneity of variances assumption, non-parametric tests were used to evaluate differences among treatments. Specifically, the Kruskal-Wallis test was applied to determine the significance of treatment effects ($P < 0.05$). All data are expressed as the means ± standard error (SE). Prism 9.1.1 (GraphPad Software, San Diego, CA, USA) and 'ggplot' package in *R* were employed for data plotting.

## 3. Results

### 3.1. As and Cd in rice tissues and the biomass change

Ridging alone significantly ($P$<0.05) increased the Cd content of rice grain by 118.3% and 207.0% compared with the control, and similarly, the Cd content of husk, straw and root also increased significantly ($P$<0.05) (Figure 2-2). With biochar application on the ridge, the Cd contents in grain, husk and root of IIyou28 and all tissues of Ruiyou 399 were at the same lower level as that of the control. With CMP application on the ridge, the content of Cd in rice grain was similar to that of CK. The Cd content in straw and root of IIyou28 and husk of Ruiyou 399 significantly ($P$<0.05) increased compared with the control. Compared with ridging alone, application of biochar and CMP on the ridge significantly ($P$<0.05) decreased the grain Cd by 38.7% (IIyou28), 67.58% (Ruiyou399) and 37.8% (IIyou28), 60.98% (Ruiyou399). However, ridge cultivation significantly ($P$<0.05) decreased the As contents in rice grain, husk, straw and root of IIyou28 and Ruiyou399 compared with the control. Applied biochar and CMP on the ridge decreased ($P$<0.05) grain

As by 55.6% (IIyou28), 61.9% (Ruiyou 399) and 46.8% (IIyou28), 59.3% (Ruiyou 399) comparing with the control. The grain As was notably reduced ($P$<0.05) by 38.9% (IIyou28), 39.7% (Ruiyou399) and 26.9% (IIyou28), 35.5% (Ruiyou399), compared with ridging alone.

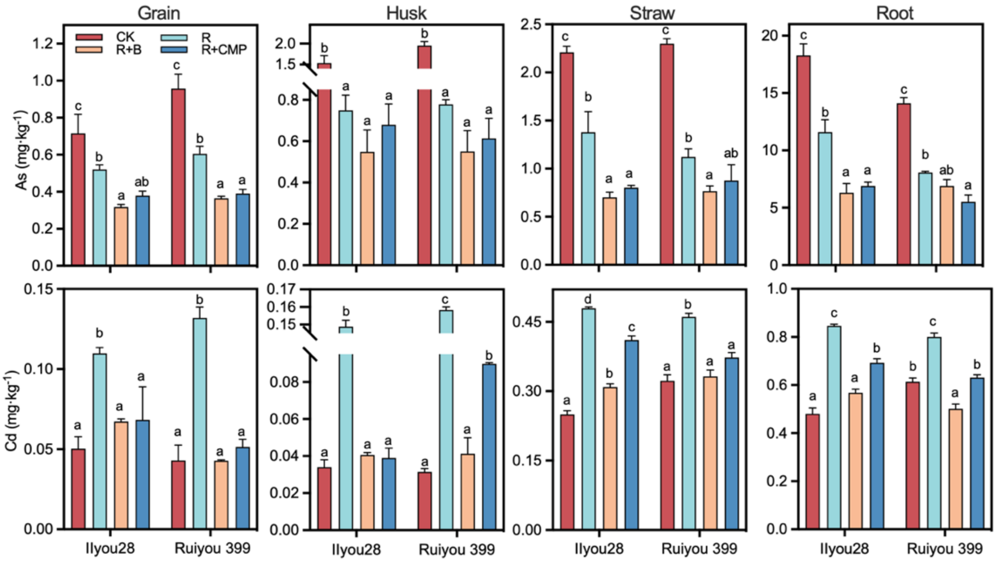



**Figure** 2-2. Contents of total As and Cd of rice grain, husk, straw and root under different treatments. Data are mean ± SE (n = 3). Different letters represent significant differences ($P$ < 0.05, LSD test or Kruskal-Wallis test) between the treatments and the control of the same rice variety. CK, flooded paddy soil; R, ridge cultivation; R+B, biochar application on the ridges; R+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

Application of biochar and CMP on the ridge had a slight effect on the rice thousand kernel weight (Figure 2-3). The plant heights of IIyou28 grown on the ridges and Ruiyou399 grown on the ridges applied with biochar were significantly ($P$<0.05) decreased by 9.3-11.0% and 18.5%, compared with the control. While the plant height of Ruiyou399 grown on the ridges or ridges applied with CMP were at the same level as that of the control.

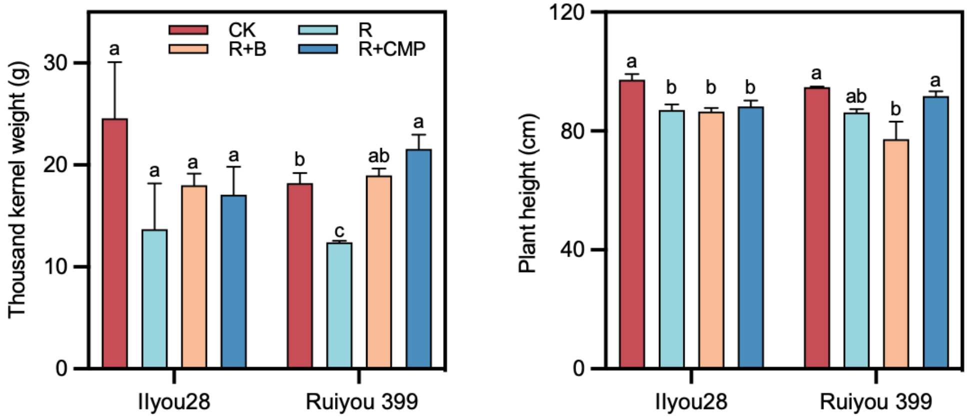



**Figure** 2-3. Contents thousand kernel weight and plant height of rice in the field trail. Data are mean ± SE (n = 3). Different letters represent significant differences ($P$ < 0.05, LSD test or Kruskal-Wallis test) between the treatments and the control of the same rice variety. CK, flooded paddy soil; R, ridge cultivation; R+B, biochar application on the ridges; R+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

## *3.2. Soil pH, Eh, and levels of As, Cd, Fe, Mn, S in porewater*

Soil pH in the control was 5.2 (IIyou28) and 5.0 (Ruiyou 399) (Figure 2-4). Ridging significantly ($P$<0.05) decreased the soil pH to 4 (IIyou28) and 3.7 (Ruiyou 399). By applying biochar on the ridge, the soil pH was raised to the control level. With applied CMP on ridge, the soil pH also increased compared with that of ridge alone, but the rhizosphere of Ruiyou399 was still significantly ($P$<0.05) lower than that of the control. Soil Eh was about -196 ~ -194 mV in the control. Ridging significantly ($P$<0.05) increased the soil Eh, the Eh values in the ridged treatments were in the following order: R+B>R>R+CMP.

Ridging greatly increased the Cd content in soil porewater when compared with the control (Figure 2-5). Having been applied with biochar or CMP, the pore water Cd of IIyou28 decreased to a level comparable to that of the control. The As content was similar in each group without significant difference ($P$>0.05), which were 1.6-2.1 $\mu g \cdot L^{-1}$ (IIyou28) and 2.1-2.2 $\mu g \cdot L^{-1}$ (Ruiyou 399). The content of Mn, Fe and S in soil porewater treated with ridge cultivation were all higher than those of the control, especially Fe and S ($P$<0.05) (Figure 2-6). Application of biochar on the ridge further increased the contents of Mn, Fe and S in the porewater. The contents of Mn, Fe and S in R+CMP of Ruiyou399 were significantly ($P$<0.05) higher than that of R and CK. However, the contents of Mn and Fe in R+CMP of IIyou28 were not significantly ($P$>0.05) different from that of R, and the content of S was significantly ($P$<0.05) lower than that of R but still higher ($P$<0.05) than that of CK.

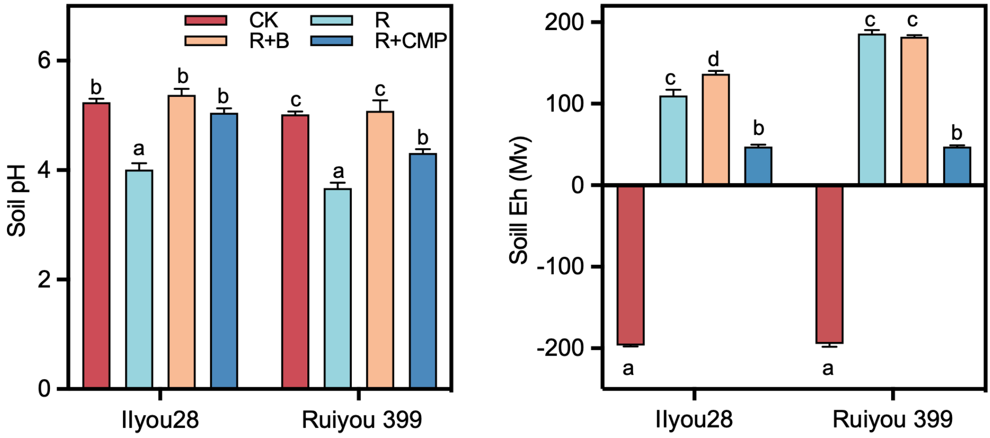



**Figure** 2-4. Soil in situ pH and Eh under different treatments. Data are mean ± SE (n = 3). Different letters represent significant differences ($P$ < 0.05, LSD test or Kruskal-Wallis test) between the treatments and the control of the same rice variety. CK, flooded paddy soil; R, ridge cultivation; R+B, biochar application on the ridges; R+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

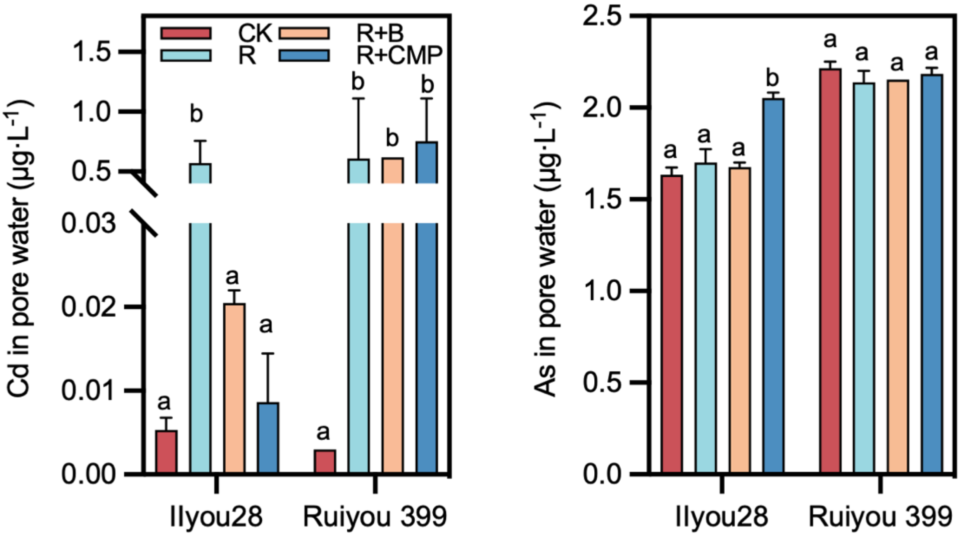



**Figure** 2-5. Content of Cd and As in pore water under different treatments. Data are mean ± SE (n = 3). Different letters represent significant differences ($P$ < 0.05, LSD test or Kruskal-Wallis test) between the treatments and the control of the same rice variety. CK, flooded paddy soil; R, ridge cultivation; R+B, biochar application on the ridges; R+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

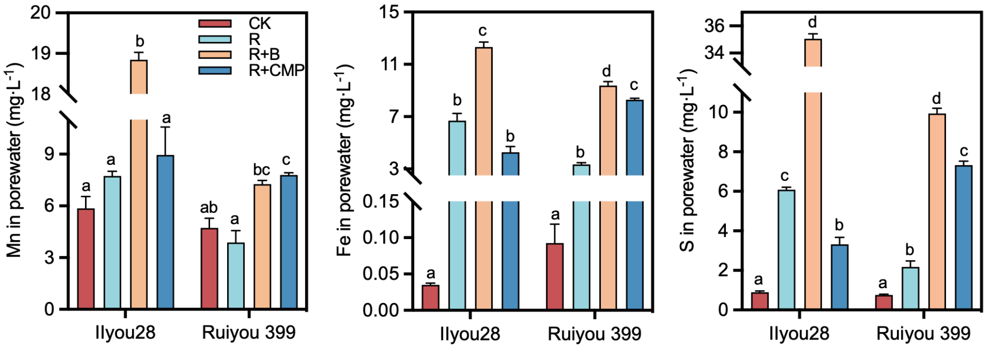



**Figure** 2-6. Content of Mn, Fe and S in pore water under different treatments. Data are mean ± SE (n = 3). Different letters represent significant differences (*P* < 0.05, LSD test or Kruskal-Wallis test) between the treatments and the control of the same rice variety. CK, flooded paddy soil; R, ridge cultivation; R+B, biochar application on the ridges; R+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

# 4. Discussion

Ridge cultivation in paddy fields, combined with the application of biochar or CMP fertilizer, significantly and simultaneously reduced Cd and As concentrations in rice grains. Typically, rice varieties differ in their ability to absorb, transport, and accumulate heavy metals due to genotypic variation, which may be a key factor contributing to differences in the effectiveness of agronomic practices (Feng et al., 2023). In this study, the two rice varieties selected for the field experiment were locally cultivated indica hybrid varieties commonly grown by farmers. Both varieties showed consistent reductions in grain Cd and As compared to the control or ridge cultivation alone. Furthermore, there were no significant differences between the two varieties in As accumulation across different plant organs. For Cd, the only observed difference was in husk Cd accumulation following CMP fertilizer application; there were no significant differences in Cd accumulation in the straws or roots. This may relate to the inherent differences in Cd translocation and storage between the two varieties. However, no studies have conclusively identified the variety-specific sensitivity or preferential accumulation of Cd or As in these two varieties. Additionally, there were no significant differences in thousand kernel weight or plant height between the two varieties, indicating similar growth performance. In summary, the results suggest that the agronomic practices employed in this field experiment were likely the main factors influencing Cd and As accumulation in rice grains.

The mobility of Cd and As is strongly influenced by soil redox potential (Eh) and pH (Honma et al., 2016). Ridge cultivation in acidic soils increases soil Eh while decreasing pH. This may be due to the oxidation process of reduced $Fe^{2+}$ and $Mn^{2+}$ ions releasing $H^{+}$. Soil acidification raises the net positive surface charge of soil particles (Houben et al., 2013), which enhances the adsorption of anionic As and promotes the desorption of cationic Cd (Yang et al., 2018). As a result, ridge cultivation reduces the bioavailability of As but increases that of Cd. When alkaline biochar is applied on the ridges, soil acidification is partially alleviated, while soil Eh remains similar to that observed under ridge cultivation alone. Similarly, the application of alkaline CMP fertilizer also mitigates soil acidification and maintains an aerobic soil environment, although Eh is slightly lower compared to ridge cultivation alone. This may be due to the improved soil conditions and stimulation or enrichment of microbial activity in acidic soils following the application of alkaline amendments (Liu et al., 2020; Tan et al., 2022), which consume oxygen and consequently slow the rise in Eh (Marschner, 2021). The liming effect of biochar and CMP increases soil pH, promoting the precipitation of Cd with carbonate, phosphate, and hydroxide ions, thereby reducing Cd activity (Fang et al., 2024; Wang et al., 2025). Additionally, $Ca^{2+}$ and $Mg^{2+}$ ions released from CMP can form insoluble precipitates with reducible Cd in the soil, further lowering Cd bioavailability (Li et al., 2008). The large amount of phosphate introduced via CMP application likely plays a key role, as it can react with Cd to form $Cd_3(PO_4)_2$ precipitates, reducing the concentration of free Cd ions (Zhou et al., 2022). On the other hand, the addition of $Ca^{2+}$ may lead to the formation of insoluble As compounds such as $Ca_3(AsO_4)_2$ and As–Ca complexes, thereby immobilizing soil As (Nazari et al., 2017). Moreover, biochar increases the number of functional groups, specific surface area, and pore structure, enhancing its ability to immobilize Cd and reduce its bioavailability (Xu et al., 2018). Some studies also suggest that biochar can promote the uptake of essential mineral elements by plants, allowing them to more effectively compete with Cd, thus reducing Cd accumulation in rice grains (Fang et al., 2024). However, no significant difference in porewater As concentration was observed between the control (CK) and ridge (R) in field experiment. This may be resulted from the dilution effect of higher water content in CK. In addition, this study was limited by the relatively low soil Cd content in the selected field plots. As a result, the contents of Cd in rice grains and soil solution were relatively low, which may also be related to the continuous flooding state of the control.

Changes in soil Eh and pH under different moisture conditions can affect redox chemistry in paddy soils (Jiku et al., 2022). Fe, Mn and S also play important roles in controlling the mobility of Cd and As in paddy fields. When paddy fields are flooded, cation exchange drives the reductive dissolution of Fe/Mn-(hydro) oxides, releasing the absorbed As into soil solution, thereby increasing the mobility of As (Takahashi et al., 2004). At the same time, the dissolved $Mn^{2+}$ may compete with $Cd^{2+}$ for adsorption sites (Zhao and Wang, 2020). In this study, Fe and Mn in porewater of treatments without ridging were lower than those of ridging treatment in field experiment and this could be attributed to the dilution effect of high water. When the paddy fields are drained, the transformation of CdS into soluble $CdSO_4$ increases the mobility of Cd (Sebastian and Prasad, 2014), which lead to the potential increasing risk of Cd in the ridging cultivation. While As is immobilized by the Fe/Mn-(oxy) hydroxides with the oxidation of FeS or $Fe^{2+}$ (Yu et al., 2016). In this study, S in porewater of treatments without ridging were far lower than those of ridging treatment in field experiment could be owned to the CdS perciptation formation.

# 5. Conclusion

In the chapter, we demonstrated that ridging combined with biochar or CMP can effectively reduce the content of As in rice grains and maintain the content of Cd in grains at a low level. Ridging is not only a traditional agronomic but also an environmentally friendly practice in waterlogged paddy fields. Biochar is a resource-recycling biological material with carbon sequestration potential, and CMP is a commonly used alkaline fertilizer in acidic soils. The proposed strategy is a new use of traditional measures, which has the advantages of economic cost efficiency, environmentally friendly and resource utilization. Although the Cd and As content of the selected field plots exceeded the Chinese national risk screening values for soil contamination of agricultural land, they were not severely polluted. Nevertheless, it is clear that combined ridging and soil amendments can effectively reduce soil Cd and As availability.

# 6. Acknowledgements

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# Chapter 3

## Enhanced interaction with Ca, Fe and Mn in new soil habitat reduces uptake of As and Cd in rice

Adapted from Zhang, T., Jiku, M.A.S., Li, L., Ren, Y., Li, L., Zeng, X., Colinet, G., Sun, Y., Huo, L. and Su, S., 2023. Soil ridging combined with biochar or calcium-magnesium-phosphorus fertilizer application: Enhanced interaction with Ca, Fe and Mn in new soil habitat reduces uptake of As and Cd in rice. *Environmental Pollution*, 332, p.121968.

# Abstract

This chapter explores the mechanisms by which ridge cultivation combined with biochar and CMP fertilizer reduces the bioavailability of As and Cd in paddy soils. A series of microcosm experiments revealed that these amendments significantly decreased As concentrations in soil solution by 75.6% (biochar) and 82.5% (CMP), while maintaining Cd at low levels (0.13–0.15 $\mu g \cdot L^{-1}$). Aggregated Boosted Tree (ABT) analysis indicated that improvements in soil pH and redox conditions, along with enhanced interactions of Ca, Fe, and Mn with As and Cd, were key factors in reducing metal bioavailability. Ridge cultivation promoted aerobic conditions, encouraging As association with poorly to well-crystalline Fe/Al oxides and Cd with Mn (hydr)oxides. Biochar strengthened the roles of Ca and Mn in Cd immobilization and increased pH-mediated As retention, while CMP enhanced Mn's effect on As reduction and supported Cd stabilization. These findings highlight the synergistic potential of combining ridge cultivation with soil amendments to reshape soil chemistry and reduce heavy metal risks.

# 1. Introduction

In paddy fields, flooding and drainage are common measures for rice cultivation. As soil moisture changes, soil redox state (Eh) or pe (-log $e^-$ activity, pe = Eh (mV)/59.2 (25 °C)) and pH of the soil changes accordingly (Honma et al., 2016). At low pH and high Eh, the increased positive charge on the soil surface facilitates the desorption of Cd, while high pH and low Eh increases the net negative charge on the soil surface resulting in the desorption of As (Bolan et al., 2013; Yao et al., 2022). The activity of Cd and As in paddy fields are strongly affected by the redox of Fe, Mn oxides and S. When paddy soil is flooded, As is mobilized by the reductive dissolution of (Fe)-Mn minerals (Shaheen et al., 2020), while soluble Cd forms precipitates, such as CdS, resulting in high As and low Cd bioavailability (El-Naggar et al., 2018; Shaheen et al., 2016). When the paddy field is drained, the transformation of CdS into soluble $CdSO_4$ increases the mobility of Cd (Sebastian and Prasad, 2014), while As is immobilized by the Fe/Mn-(oxy) hydroxides with the oxidation of FeS or $Fe^{2+}$ (Yu et al., 2016). Therefore, adjusting water management strategies in paddy soils to the appropriate Eh and pH trade off value can keep As and Cd availability at a low level (Honma et al., 2016).

Soil ridge cultivation, a traditional agronomic practice in cold waterlogged paddy fields, has been utilized to adjust soil Eh and pH to a trade-off situation, thereby simultaneously decreasing As and Cd availability, especially in soil with low Cd content (Jiku et al., 2022). Apply biochar or CMP fertilizers on the ridge improve the effect on reducing the accumualation of As and Cd in rice grains. Due to the negatively-charged surface, biochar increases soil pH and net negative charge on the soil surface and reduces the mobility of metal cations (Beesley et al., 2011; Houben et al., 2013). The increased cation exchange sites may also facilitate the competition or exchange of $Cd^{2+}$ with $Ca^{2+}$ and $Mg^{2+}$ (Harvey et al., 2011). As an alkaline fertilizer, when CMP is applied, the surface hydroxyl groups coordinated with metal cations (such as Fe, Al, etc.) on the soil surface were replaced by P ions, and the soil pH increases (Feng et al., 2013). When P fertilizer was applied, Cd was found to be immobilized as precipitates (Bolan et al., 2003).

With the changes in soil pH, Eh and introduction of exogenous ions after ridge combined with soil amendments, the ions relationship and the chemical process in the rhizosphere may change correspondingly. We hypothesize that a new soil habitat will potentially be formed with this change. The strengthened interaction of Fe, Mn, Ca, Mg with As and Cd as above mentioned might be responsible for the simultaneous decrease of As and Cd bioavailability in paddy soils. The objective of this study was to explore the importance of ionic interactions in the new soil habitat. We carried out a soil microcosm experiment with a more controlled environmental condition to investigate whether using paddy soil ridge combined with biochar or CMP can affect the interaction of Fe, Mn, Ca, Mg with As and Cd, and to what extent it plays a role in reducing the bioavailability of As and Cd.

# 2. Materials and methods

## 2.1. Materials

Paddy soil was collected from the same paddy field plots in Shimen County, Hunan province of China, as we mentioned in Chapter 2. The soil is classified as paddy soil, primarily developed from Quaternary red clay parent material, and is categorized as Hydragric Anthrosol according to the WRB. This type of soil is typical and widely distributed in rice-growing regions of southern China. The physico-chemical properties of the experimental soils are shown in Table 3-1. After air drying, sundries were picked out and the soil was ground to pass a 2 mm sieve. Rice straw biochar was obtained from Beijing PhD Union Academy of Agriculture (Beijing, China). The content of Cd and As in the biochar was 0.12 $mg \cdot kg^{-1}$ and 0.5 $mg \cdot kg^{-1}$. In the given dose of biochar applied to the soil, the content of Cd and As was negligible. CMP fertilizer with the available phosphorus content ($P_2O_5$) of ≥ 12% and As and Cd content below the detection limit was produced by Phosphate Fertilizer Factory in Liuyang East District (Liuyang, China).

**Table** 3-1. Physical and chemical properties of the paddy soil.

| Parameters | | Values |
|---|---|---|
| pH | | 5.17±0.07 |
| Alkali hydrolysable nitrogen ($mg \cdot kg^{-1}$) | | 154.4±23.61 |
| Available phosphorus ($mg \cdot kg^{-1}$) | | 0.17±0.02 |
| Avallable potassium ($mg \cdot kg^{-1}$) | | 74±11.14 |
| Soil organic matter ($mg \cdot kg^{-1}$) | | 28.13±3.53 |
| Cation exchange capacity ($cmol(+) \cdot kg^{-1}$) | | 11.67±0.7 |
| Available iron ($mg \cdot kg^{-1}$) | | 249.67±2.77 |
| Avallable manganese ($mg \cdot kg^{-1}$) | | 67.4±2.96 |
| Active aluminum ($mg \cdot kg^{-1}$) | | 2.76±0.22 |
| Extractable $SO_4^{2-}$ ($mg \cdot kg^{-1}$) | | 326.73±11.11 |
| Total cadmium ($mg \cdot kg^{-1}$) | | 0.41±0.02 |
| Total arsenic ($mg \cdot kg^{-1}$) | | 66.53±3.84 |
| Particle analysis (%) | 2–0.2 mm | 2.27 |
| | 0.2–0.02 mm | 17.77 |
| | 0.02–0.002 mm | 51.14 |
| | <0.002 mm | 28.82 |

## 2.2. Microcosm experiment

Microcosms were setup in 100 mL serum bottles. Each serum bottle was filled with 60 g dry soil. According to our pre-experimental results, in order to achieve the same redox and pH conditions for conventional tillage and ridging treatments in the previous field experiment, the soil moisture content of conventional tillage (mCK) and ridging treatments (mR) in this microcosm experiment was set at 75% and 35% ($w_{water}/w_{dry\text{-}soil}$), respectively. To simulate the treatments of biochar (mR+B) and CMP (mR+CMP) combined with ridge cultivation in this microcosm experiment, the water content was set to 35%, and the dosage of biochar and CMP was 1% ($w/w_{dry\text{-}soil}$) and 0.5‰P ($w/w_{dry\text{-}soil}$), respectively.

Soil and amendments were mixed thoroughly one week in advance. The temperature was set to 37°C, and the cells were incubated in the dark for 40 days in a climate chamber. Each treatment was replicated 3 times. The bottles were placed in a randomized, complete block design and weighed every 2 days to keep the soil moisture content the same.

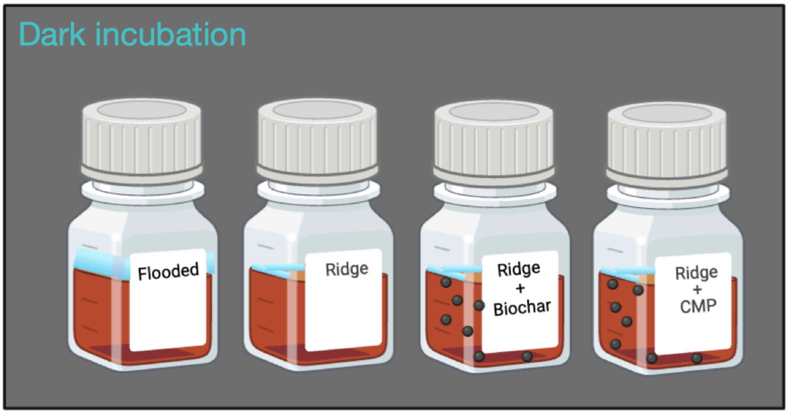


**Figure** 3-1 A scheme diagram of microcosm incubation.

## *2.3. Sample collection and analysis*

In situ Eh and pH was tested on the 1st, 7th, 14th, 20th and 40th day. In situ Eh was measured by a redox micro electrode (Mettler Toledo, Columbus, OH, USA) and in situ pH was measured by the micro pH meter (Mettler Toledo) right after the Eh test. Thereafter, soil samples were collected on the 1st, 20th and 40th day to analyze the concentration of ions in the soil solution and the content of As and Cd in the soil.

Soil solutions were extracted by water extractions by shaking 5 g of incubated fresh soil with 50 mL of deionized water for 24 hours (Faulkner et al., 2001). The slurry was centrifuged at 8000 rpm for 10 min, decanted and the solution was filtered with a 0.22 μm syringe filter (Faulkner et al., 2001; Hobson et al., 2020). The dissolved organic carbon (DOC) was determined using carbon and nitrogen analyzer (Multi N/C 3100, Analytik jena, German). The $As^{III}$, $As^{V}$, $SO_4^{2-}$, $PO_4^{3-}$(not detected), $Cl^-$, $CO_3^{2-}$ and $NO_3^-$ levels were determined using high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS, PerkinElmer NexION 300X) (Yan et al., 2020). The content of Cd, Fe, Mn, Ca and Mg was analyzed by ICP-OES (Optima 5300DV; PerkinElmer) (Cruz et al., 2015). The analytical results including pH, temperature, DOC, anions and cations were imported into the Visual MINTEQ model 3.1 to calculate the As and Cd species in soil solutions (Gustafsson, 2020).

Two different sequential extraction methods were used to determine the As and Cd fractions in soil samples from the microcosm experiment. A classical Tessier scheme modified by Hobson et al (Hobson et al., 2020) was chosen for Cd determination (Table 3-2). The method established by Wenzel et al (Wenzel et al., 2001) was used for As analysis (Table 3-3). Total As and Cd concentrations in soil samples were determined by inductively coupled plasma mass spectrometry (ICP-MS, Elan DRC-e, Pekin Elmer, USA) after samples were extracted using $HNO_3$ and HCl in a ratio of 1:3 (Desrosiers et al., 2008).

**Table** 3-2. The scheme for Tessier sequential extraction method (Hobson et al., 2020).

| Step | Fraction | Extractant | Reaction Times and Conditions |
|---|---|---|---|
| 1 | readily exchangeable | $NH_4Cl$ | 2 h |
| 2 | carbonate-bound | $NH_4OAc$ | 24 h, pH=5 |
| 3 | Mn oxide-bound | $NH_2OH{\cdot}HCl$ | 30 min, pH=2 |
| 4 | Fe oxide-bound | $NH_2OH{\cdot}HCl$-$CH_3COOH$ | 6 h, 95 ◦C |
| 5 | organic matter-bound | NaOH | 24 h |
| 6 | residual | $HNO_3$-HCl-$H_2O_2$ | 100 ◦C |

**Table** 3-3. The scheme for Wenzel sequential extraction method (Wenzel et al., 2001).

| Step | Fraction | Extractant | Reaction Times and Conditions |
|---|---|---|---|
| 1 | Non-specifically sorbed | $(NH_4)_2SO_4$ | 4h, 20°C |
| 2 | Specifically sorbed | $NH_4H_2PO_4$ | 16h, 20°C |
| 3 | Amorphous and poorly crystalline hydrous oxides of Fe and Al | $NH_4^+$- oxalate buffer | 4h, dark, 20°C, pH=3.25 |
| 4 | Well-crystallized hydrous oxides of Fe and Al | $NH_4^+$–oxalate buffer + ascorbic acid | 30min, 96°C, pH=3.25 |
| 5 | Residual | Aqua regia (HCl/$HNO_3$) | 16h digestion |

## *2.4. Quality control*

For quality control, certified reference material of soil (GBW07429) was digested at the same time. The recovery of total As and Cd in the standard sample were for As at 85–90 % and Cd at 83–106% for soil. The recovery rates in soil sequential extraction were calculated as the sum of each fraction divided by the total content determined by digestion, of which Cd was 102-157% and As was 78-116%. CRM water samples (GSBZ 50004-88 for As and GSB 07-1185-2000 for Cd, GBW08666 for $As^{III}$, GBW08667 for $As^{V}$, BWZ6877-2016 for $CO_3^{2-}$, BWZ8035-2016 for $Cl^-$, GBW(E)080264 for $NO_3^-$, GBW(E)080264 for $SO_4^{2-}$ and GBW(E)083180 for $PO_4^{3-}$) were mixed among the blank and digested samples at the beginning of each sample test and intervals of 10 samples analysis for quality control of ICP-MS and ICP-OES.

### *2.5. Statistical analysis*

Basic statistical analysis was performed using Microsoft Excel 2010 and IBM SPSS Statistics 26.0. Homogeneity of variances was tested first. For data that met the assumption of homogeneity of variances, one-way analysis of variance (ANOVA) was used to assess treatment differences, and significant effects were evaluated using the Least Significant Difference (LSD) test at the 0.05 probability level ($P < 0.05$). For data that did not meet the homogeneity of variances assumption, non-parametric tests were used to evaluate differences among treatments. Specifically, the Kruskal-Wallis test was applied to determine the significance of treatment effects ($P < 0.05$). All data are expressed as the means ± standard error (SE). Aggregated boosted tree (ABT) analysis was processed by the "gbmplus" package in *R* (R Development Core Team, Vienna, Austria). Prism 9.1.1 (GraphPad Software, San Diego, CA, USA) and 'ggplot' package in *R* were employed for data plotting.

## 3. Results

### *3.1. Cd and As in incubated soil solution*

The Cd content in the soil solution was the highest (0.38-0.66 $\mu g \cdot L^{-1}$) on the 1st day, the lowest (0.07-0.13 $\mu g \cdot L^{-1}$) on the 20$^{th}$ day, which reached the level of 0.13-0.15 $\mu g \cdot L^{-1}$ in each group on the 40$^{th}$ day (Figure 3-2). Compared with the control, ridging significantly ($P<0.05$) increased the Cd content in the soil solution, but the increase was gradually weakened with the change of culture time. After applying biochar or CMP, the Cd content was lower than that of ridge alone on the 1st day, but still higher than that of the control, until it dropped to the same level as the control on the 40th day. The analysis result of Visual MINTEQ showed that during the entire process of incubation, the organic complexes (Cd-DOM) were the main fraction of Cd in the soil solution (Figure 3-10).

The content of As in the soil solution of the control showed a trend of increasing in the first 20 days and then decreasing in the next 20 days (Figure 3-2). The lowest content was 7.4 $\mu g \cdot L^{-1}$ on the 1st day, and it increased to a maximum of 71.4 $\mu g \cdot L^{-1}$ on the 20th day, then decreased to 38.9 $\mu g \cdot L^{-1}$ on the 40th day. All ridging treatments showed the opposite trend. On the 1st day, the As content was the highest, which was 287.2% (mR), 652.9% (mR+B) and 916.6% (mR+CMP) higher than the control, respectively. On the 20th day, they all decreased to the lowest level and were 97.7% ($P<0.05$) (mR), 87.3% ($P>0.05$) (mR+B) and 93.6% ($P<0.05$) (mR+CMP) lower than the control, respectively. On the 40th day, the As content increased to 6.5-9.2 $\mu g \cdot L^{-1}$, but it was still lower than that of the control by 83.3% ($P<0.05$) (mR), 75.6% ($P>0.05$) (mR+B) and 82.5% ($P<0.05$) (mR+CMP). The analyzing result of Visual MINTEQ showed that the major speciation of inorganic As in the control is $H_3AsO_3$ and that the major speciation of inorganic As in the ridged treatments are $HAsO_4^{2-}$ and $H_2AsO_4^-$ (Figure 3-11).

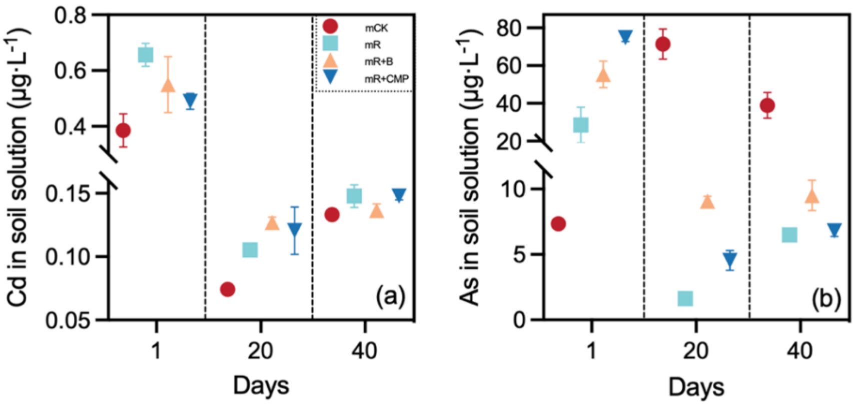


**Figure** 3-2. Contents of Cd and As in soil solution. Data are mean ± SE (n = 3).

## *3.2. The pH, Eh and the ions correlated with As and Cd in soil solution*

In the microcosm experiment, the soil pH decreased by 9.5%-28.6% after ridging compared with the control (Figure 3-3). With the application of biochar or CMP on the ridge, the soil pH decreased by 0.1%-34.0% and 0.3%-25.1%, respectively, compared with the control. The soil Eh indicated a distinct increase under all ridging treatments, with the changes of 50.8%-275.5% (mR), 22.0%-233.5% (mR+B) and 51.4%-275.8% (mR+CMP), respectively. The concentrations of all tested ions are listed in Table 3-S1.

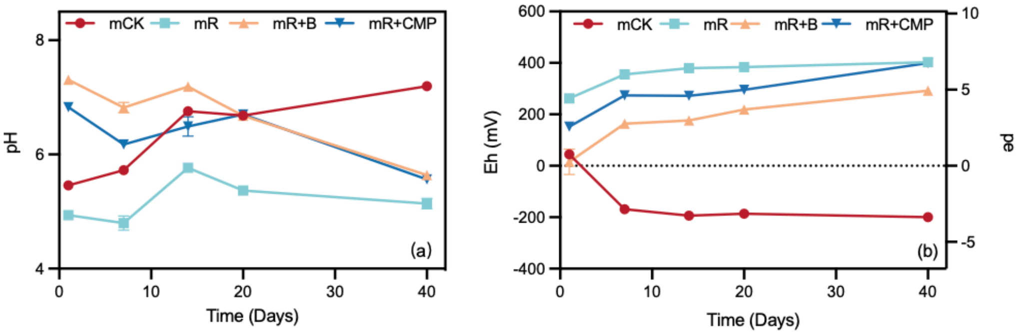


**Figure** 3-3. Temporal changes of soil pH and redox potential during 40 days incubation. mCK, flooded paddy soil; mR, ridge cultivation; mR+B, biochar application on the ridges; mR+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

The correlation analysis (Figure 3-4) showed that the content of iAs was positively correlated with $Mn^{2+}$($P$<0.01), $CO_3^{2-}$($P$<0.01), pH ($P$<0.05) and negatively correlated with $NO_3^-$($P$<0.01) and pe ($P$<0.01). The $Cd^{2+}$ content in the soil solution was positively correlated with $Mn^{2+}$($P$<0.01), $Ca^{2+}$($P$<0.05), $Mg^{2+}$($P$<0.05), $SO_4^{2-}$ ($P$<0.05) and negatively correlated with DOC ($P$<0.01) and $NO_3^-$ ($P$<0.05).

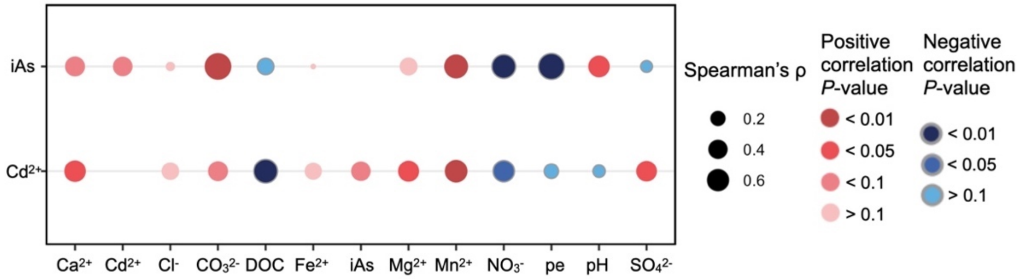


**Figure** 3-4. Spearman correlation of iAs and Cd with ions and environmental factors in soil solutions. The size of the bubble represents the correlation levels. The color of the bubble represents the significant levels. Red: positive correlation. Blue: negative correlation.

Aggregated boosted tree (ABT) analysis was used to analyze the relative importance of ions content, pe and pH in affecting Cd and iAs contents in soil solution. As shown in Figure 3-5, the main factors contributing to the influence of Cd content in soil solutions were $Mn^{2+}$ and $NO_3^-$, with relative influence rates of 29.3% and 28.6% respectively, much higher than other indicators. The primary factors affecting iAs were pH, pe, $CO_3^{2-}$ and $Mn^{2+}$, with relative influence rates of 23.3%, 15.3%, 13.5% and 12.1%, respectively. Further analysis indicated that the primary factors that affected the Cd and iAs contents in soil solutions varied with the treatments (Figure 3-6 and 3-7). For Cd, the primary factors in mCK were iAs, $Mn^{2+}$ and $SO_4^{2-}$, whereas in mR were iAs, $NO_3^-$ and $Mn^{2+-}$, in mR+B are $Ca^{2+}$, iAs and $Mg^{2+}$ and in mR+CMP are iAs, $Ca^{2+}$ and $NO_3^-$. Cd was the primary factor affecting iAs content in mCK. The primary factors affecting iAs were $Ca^{2+}$, $Mn^{2+}$ and pH in different ridging treatments.

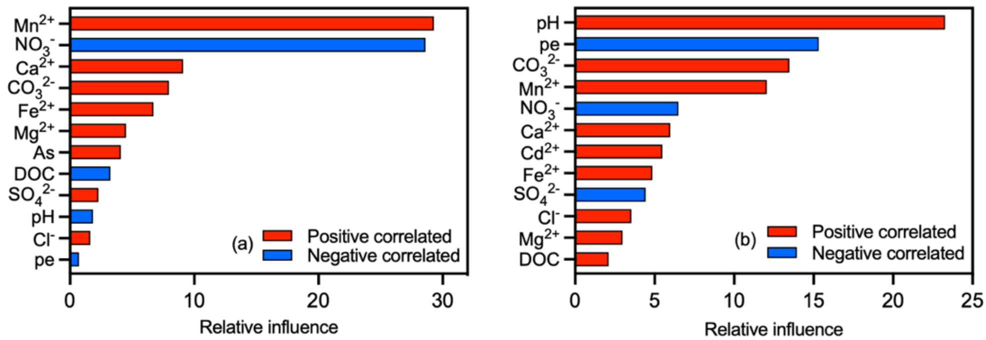


**Figure** 3-5. Aggregated boosted tree analysis for relative importance of elements for Cd and iAs in soil solution.

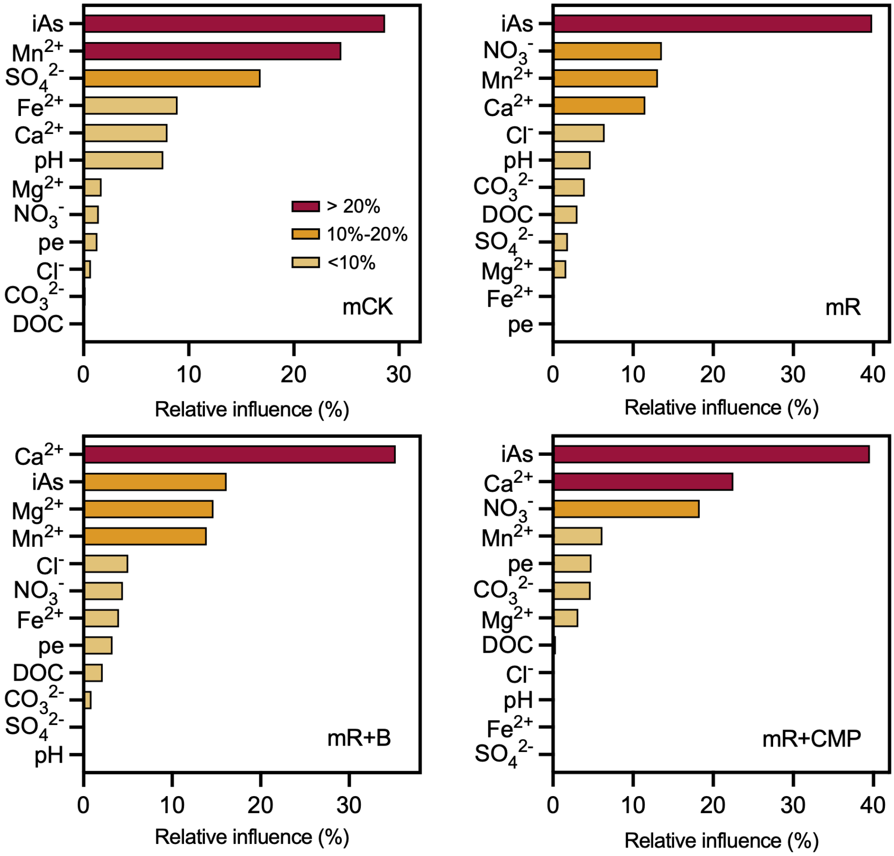


**Figure** 3-6. Aggregated boosted tree analysis for relative importance of elements for Cd in soil solutions under different treatments. Colors indicate different levels of relative importance. mCK, flooded paddy soil; mR, ridge cultivation; mR+B, biochar application on the ridges; mR+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

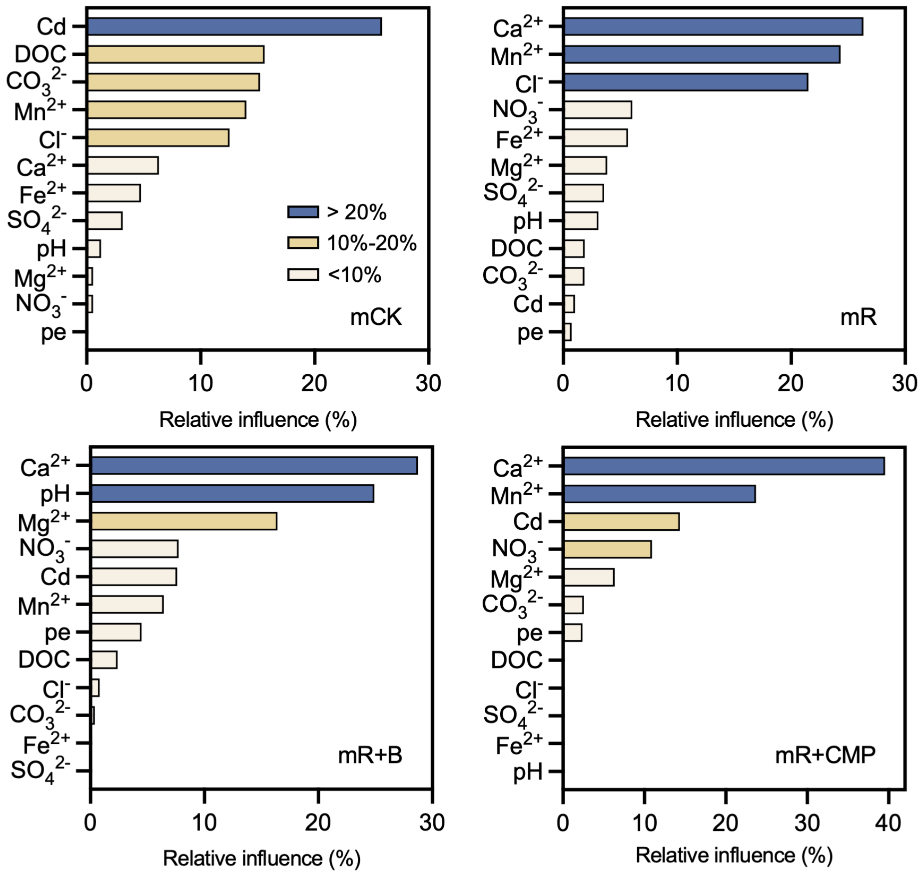


**Figure** 3-7. Aggregated boosted tree analysis for relative importance of elements for iAs in soil solutions under different treatments. Colors indicate different levels of relative importance. mCK, flooded paddy soil; mR, ridge cultivation; mR+B, biochar application on the ridges; mR+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

## *3.3. Cd and As fraction in soil solid phase*

Based on Tessier sequential extraction, in the control, Cd was predominant (61.2%) readily exchangeable, followed by that associated with Fe oxide fraction (21.2%) on the 1$^{st}$ day (Figure 3-8). While on the 20$^{th}$ and 40$^{th}$ day, the Fe-oxide fraction was the main factor (41%). Ridging significantly increased the exchangeable fraction by up to 102.7% ($P$<0.05) and decreased the carbonate bound or Fe oxide-bound fraction by up to 54.9% or 67.3% ($P$<0.05) on the 20$^{th}$ and 40$^{th}$ day. The Cd associated with Mn-oxide was significantly increased by 36.7% ($P$<0.05) on the 20$^{th}$ day and decreased by 11.0% ($P$>0.05) on the 40$^{th}$ day. With applied biochar on the ridge, the readily exchangeable Cd and Fe oxide bound fraction significantly decreased by up to 45.7% and 61.2%, while the Cd associated with carbonate and Mn-oxide significantly increased by up to 283.9% and 134.0%, compared with the control. CMP application on the ridge increased the readily exchangeable Cd, carbonate bound, Mn oxide-bound and residual fraction by up to 40.1%, 62.1%, 155.3% and 32.8% respectively, and also decreased Fe oxide-bound fraction by up to 62.9%. The organically bound Cd fraction significantly decreased by 79.7% on the 1$^{st}$ day and decreased by up to 33.3% on the 20th and 40th days, but the difference was not significant.

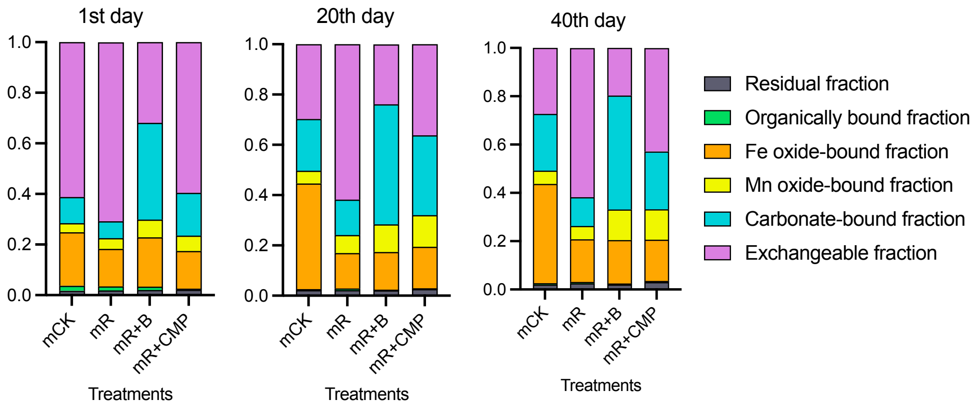


**Figure** 3-8. Sequential extraction by Tessier method of Cd on the 1st day, 20th day and 40th day incubation. Total metals are divided into F1: exchangeable, F2: carbonate bound, F3: Mn oxide-bound, F4: Fe oxide-bound, F5: organically bound, and F6: residual fraction. mCK, flooded paddy soil; mR, ridge cultivation; mR+B, biochar application on the ridges; mR+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

Based on Wenzel sequential extraction, in the control, the portion of non-specifically sorbed As and the specifically sorbed As increased to 3.2% and 23.49%, respectively, on the 20$^{th}$ day and then decreased to 2.83% and 17.5%, respectively, on the 40$^{th}$ day (Figure 3-9). However, the non-specifically sorbed As and the specifically sorbed As in all ridging treatments showed a downward trend, and they were significantly ($P$<0.05) lower than that of the control on the 20$^{th}$ and 40$^{th}$ day. Ridging primarily increased the As associated with poorly-crystalline Fe/Al. The poorly-crystalline Fe/Al was increased by 91.6% when ridging alone, 114.7% when applying biochar on the ridge and 109.8% when applying CMP on the ridge on the 40$^{th}$ day. The As associated well-crystalline Fe/Al also increased after ridging. The change ratio was 30.5% for ridging alone, 23.5% for applying biochar on the ridge and -2.3% for applying CMP on the ridge on the 40$^{th}$ day. Compared with the control, there was no significant difference in the residual fraction As.

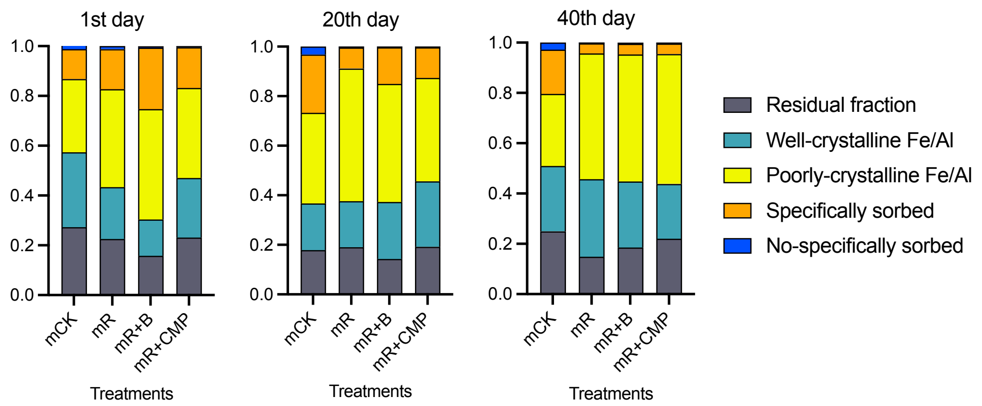


**Figure** 3-9. Sequential extraction results by Wenzel method of As on the 1st day, 20th day and 40th day incubation. Total metals are divided into F1: no-specifically sorbed, F2: specifically sorbed, F3: poorly-crystalline Fe/Al, F4: well-crystalline Fe/Al, and F5: residual fraction. mCK, flooded paddy soil; mR, ridge cultivation; mR+B, biochar application on the ridges; mR+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

# 4. Discussion

In soil science or ecological analyses with limited sample sizes, Aggregated Boosted Trees (ABT) analysis is often preferred when the goal is to identify key driving factors and quantify feature importance (Glenn, 2007). Compared to Random Forests, which use a "bagging" approach that involves random sampling of both samples and features—potentially diluting the contribution of important variables—ABT is more sensitive to the influence of critical factors (Wang et al., 2020). This makes it particularly well-suited for identifying key factors affecting the behavior of Cd and As ions. In this work, the strong contribution of Ca in reducing As and Cd availability in soil solution was observed after ridge cultivation, especially combined with biochar or CMP addition. This could be explained by the following reasons: (I) $AsO_3^{3-}$ and $Ca^{2+}$ can form precipitates in the soil solution and reduce the availability of As (Nazari et al., 2017), especially with the increase in soil pH after applying biochar or CMP on ridges; (II) Calcium ions ($Ca^{2+}$) and Cd have similar ionic radius and are both divalent cations (Hawkesford et al., 2012). $Ca^{2+}$ and $Cd^{2+}$ may compete for Ca transporters, channels or binding sites on root surface (Wu et al., 2021). Biochar provides more cation exchange sites in soil system, facilitating the exchange of $Cd^{2+}$ with $Ca^{2+}$ (Harvey et al., 2011); (III) Ca has the function of protecting cell membrane stability and cell integrity and may change the negative charge on the membrane surface, thereby hindering the flux of Cd into the plant cell and reducing the Cd in rice grains (Kanu et al., 2019). In addition, although studies have shown that the exogenous input of Mg reduces the Cd content in rice grains (Kikuchi et al., 2008), there is little report on the competition mechanism between Mg and Cd uptake by plants, which may be of future research interest.

In this study, after ridging, the association of As with poorly/well-crystalline Fe/Al increased due to the strong adsorption between Fe-(hydro) oxides and $As^{V}$ (Goldberg, 2002). The latter was easily produced from the $As^{III}$ oxidation under the oxygenated soil condition (Takahashi et al., 2004). FeS dissolves and generates hydroxyl free radicals (OH·), which may oxidize CdS to mobilize Cd (Huang et al., 2021). Furthermore, our results showed that the transformation of the Cd associated with Fe/Mn-(hydro)oxides into the exchangeable fraction leads to the increase in Cd solubility after soil reoxidation. A similar phenomenon was also observed in the work of Wang et al. (Wang et al., 2019). Mn is strong abiotic oxidants of $As^{III}$ (Feng et al., 2006). Moreover, Mn oxides can delay the reductive dissolution of As-containing Fe-(hydro) oxides and the release of As into pore water by maintaining the Eh at a relatively higher level (Ehlert et al., 2014). In this work, after ridging, the Cd associated with Mn oxides increased. A large amount of dissolved $Mn^{2+}$ may react with $Mn^{III}/Mn^{IV}$-(oxy)oxides to form heterovalent minerals. Due to its large surface area and strong adsorption affinity for metal ions, secondary Mn minerals can sequester Cd by adsorption or co-precipitation (Wang et al., 2022).

Biochar also has a certain adsorption effect on As and Cd, even though the negatively charged surface makes it weaker to adsorb As than Cd (Wang et al., 2015). CMP brings a large amount of P, which combines with Cd to form $Cd_3(PO_4)_2$ precipitation, thereby reducing the free Cd. Furthermore, with the increase in Cd/As or As/Cd concentration ratio at the mineral interface, the interaction mechanism of Cd and As changes from electrostatic adsorption to the formation of interface-As-Cd ternary complexes, and then becomes the formation of surface co-precipitation (Jiang et al., 2013; Tao et al., 2022). In this study, the co-adsorption between Cd and As was also observed as analyzed by ABT. Since the concentration of As was much higher than that of Cd, As and Cd may interact on soil colloidal particles mainly through the formation of surface precipitation. Further study on this point is needed in the future.

After ridging, the observed increase in the impact of N may be due to its indirect effects on the bioavailability of As and Cd by adjusting soil pH and related microbial activities. Reaeration after soil ridging promotes nitrification, which helps to reduce soil pH (Papirio et al., 2014), resulting in a decrease in As activity and an increase in Cd activity (Zhao and Wang, 2020). In addition to the adsorption of Cd, the addition of biochar can restore the activity of ammonia-oxidizing bacteria (AOB) poisoned by Cd, and promote nitrification by neutralizing the protons generated by nitrification through the lime effect while avoiding more acidification of the soil (Zhao et al., 2020). This effectively addresses the risk of increased Cd bioavailability after ridging. Similarly, alkaline CMP also bufferes soil acidification through the lime effect to reduce the risk of Cd. Furthermore, CMP drives *Thiobacillus* and *Ignavibacteriae* to reduce nitrate to ammonium which affects the Cd uptake by plants (Cheng et al., 2020). Nitrate promotes the accumulation of Cd in rice more than ammonium (Wu et al., 2018). This may be another reason for the decrease of Cd in rice tissues after CMP application on the ridge.

# 5. Conclusion

In the study, we demonstrated that the aerobic soil habitat after ridging promotes the association of As with poorly/well-crystallized Fe/Al and the association of Cd with Mn-(hydro)oxides. Remarkably, the new soil habitat resulting from this new measure changes the soil pH and Eh and enhanced the interaction of Ca, Fe and Mn with Cd and As in the soil solution, which caused a synergistic decrease in the bioavailability of As and Cd. The synergistic remediation effect of the proposed strategy can play an important role in paddy soils with even higher As and Cd content by adjusting the dosage of soil amendments, which still needs to be further evaluated in the future.

# 6. Acknowledgements

The authors are grateful for financial support from the National Foundation of Natural Science of China; Grant No. 42077139, the Project of Guizhou Provincial Science and Technology Foundation (Grant No.[2020]4Y032), and the Science Innovation Project of the Chinese Academy of Agricultural Science (CAAS-ASTIP-2016-IEDA).

# 7. Supplementary Figures

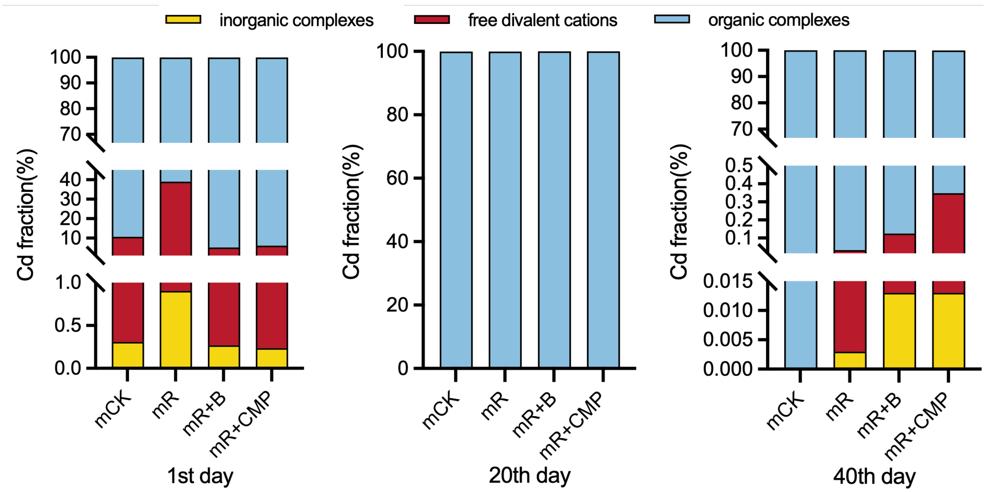


**Figure** 3-10. Results of Visual MINTEQ 3.1 calculation of Cd fraction in soil solutions. mCK, flooded paddy soil; mR, ridge cultivation; mR+B, biochar application on the ridges; mR+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

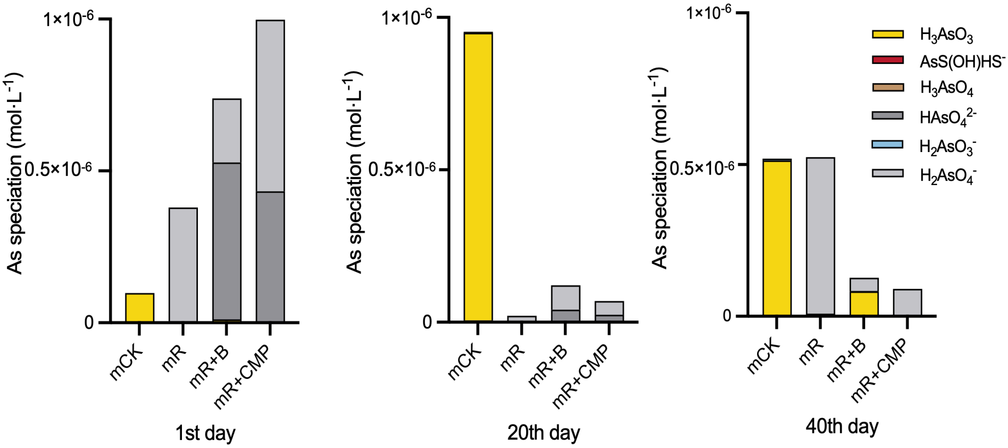


**Figure** 3-11. Results of Visual MINTEQ 3.1 calculation of iAs speciation in soil solutions. mCK, flooded paddy soil; mR, ridge cultivation; mR+B, biochar application on the ridges; mR+CMP, application of calcium-magnesium-phosphorus fertilizer on the ridges.

# Chapter 4

## Water-fertilizer regulation drives microorganisms to promote iron, nitrogen and manganese cycling to mitigate As and Cd bioavailability

Adapted from Zhang, T., Sun, Y., Parikh, S.J., Colinet, G., Garland, G., Huo, L., Zhang, N., Shan, H., Zeng, X. and Su, S., 2024. Water-fertilizer regulation drives microorganisms to promote iron, nitrogen and manganese cycling: A solution for arsenic and cadmium pollution in paddy soils. *Journal of Hazardous Materials*, *477*, p.135244.

# Abstract

In this chapter, we conducted a series of microcosm experiments, using flooding and drainage, alongside fertilization treatments to emulate different redox environment in paddy soils. The results showed that soil As significantly reduced in drained conditions following applications of biochar or CMP fertilizers by 26.3% and 31.2%, respectively, with concurrent decreases in Cd levels. Utilizing geochemical models, we identified the primary redox cycles dynamically altering during flooding (Fe and S cycles) and drainage (Fe, Mn, and N cycles). PLS-SEM elucidated 76% and 61% of the variation in Cd and As through Mn and N cycles. Functional genes implicated in multi-element cycles were analyzed, revealing a significantly higher abundance of assimilatory N reduction genes (*nasA*, *nirA*/*B*, *narB*) in drained soil, whereas an increase in ammonia-oxidizing genes (*amoA*/*B*) and a decrease in nitrate reduction to ammonium genes were observed after CMP fertilizer application. Biochar application led to significant enrichment of the substrate-binding protein of the Mn transport gene (*mntC*). Moreover, Fe transport genes were enriched after biochar or CMP application compared to drained soils. Among 40 high-quality metagenome-assembled genomes (MAGs), microbial predictors associated with low Cd and As contents across different treatments were examined. Bradyrhizobacea harbored abundant Mn and $Fe^{III}$ transport genes, while Nitrososphaeraceae carried nitrification-related genes identified as microbial predictors in the mRF treatment. Two MAGs affiliated with Caulobacteraceae, carrying diverse Fe transport genes, were enriched in biochar-applied soils. Therefore, applying CMP fertilizer or biochar in aerobic rice fields can synergistically reduce the bioavailability of Cd and As by specifically enhancing the circulation of essential elements.

# 1. Introduction

Due to both natural geological processes and anthropogenic activities such as mining and smelting, the co-occurrence of Cd and As in global farmland is widespread (Khan et al., 2021; National Toxicology Program (NTP), 2000). These compounds, classified as Group I human carcinogens (International Agency for Research on Cancer, 2025), are known to pose serious threats to human health through the food chain (Zhao et al., 2023). In paddy fields, the competent accumulation of Cd and As in rice can lead to various diseases, including itai-itai disease and cardiovascular diseases (Smith et al., 2000). Under anoxic conditions, Cd primarily exists as CdS, an insoluble compound with low bioavailability (Liu et al., 2022), while As exists in the form of cytotoxic inorganic As (iAs) oxides such as $As^{III}$ and $As^{V}$, as well as relatively less toxic phytotoxic organoarsenic oxides (oAs) such as DMA (Zhao et al., 2013; Zheng et al., 2013). Arsenic is most soluble under reducing conditions (anoxic), primarily as $As^{III}$, whereas Cd is most soluble in oxidizing conditions (oxic) as $CdSO_4$ (Honma et al., 2016). These opposing geochemical properties present challenges in efficiently managing Cd and As bioavailability in paddy soils. Therefore, urgent attention is needed to develop effective methods to synergistically remediate farmlands co-contaminated with Cd and As, as well as to elucidate the critical mechanisms for reducing Cd and As bioavailability.

Arsenic and Cd cycling in soil is coupled with the redox cycles of N, Fe, Mn, S and other elements, which further affect their solubility, toxicity and bioavailability (Afzal et al., 2019; Yin et al., 2024). Moreover, these processes are impacted by soil moisture, mediated in paddy soils by flooding and drainage, due to the different redox potentials (Eh) and pH values these processes facilitate (Honma et al., 2016; Jiku et al., 2022). When paddy fields are flooded, the reductive dissolution of Fe and Mn (hydro)oxides and the reduction of $As^{V}$ results in an increase in $As^{III}$ mobility and bioavailability (Suda and Makino, 2016). Upon drainage, Cd is mobilized from Fe/Mn-(hydro) oxides resulting in a substantial dissolution into soil porewater (Wang et al., 2022). With sulfate reduction, Cd precipitates as CdS (Huang et al., 2021). Arsenite can also co-precipitate or adsorb with sulfides ($S^{2-}$) (Roy et al., 2015). Additionally, the formation of inorganic thioarsenates and methylthioarsenates may also be facilitated by sulfate reduction (Chen et al., 2019; León Ninin et al., 2024). Furthermore, both ammonium oxidation and nitrate reduction are coupled with As redox reactions, closely intertwined with Fe redox reactions (Zhang et al., 2022, 2017). Ammonium nitrogen may promote Cd accumulation in plants by increasing Cd bioavailability in soil and regulating chelation and transport (Chai et al., 2018). Due to the difference in the thermodynamics of electron acceptors, the onset of the redox reactions of each element is different. These intricately coupled chemical cycles profoundly affect the bioavailability of As and Cd in heterogeneous soil systems. In rice fields, regular flooding and draining are essential to ensure yields, as well as the application of organic and chemical fertilizers. During the late tillering stage of rice growth, drainage is performed to promote effective tillering, and then chemical fertilizers are applied to provide nutrients for later jointing and heading. The changes of soil moisture and the increased ion concentration brought by the fertilizers would both be involved in regulating the bioavailability of Cd and As (Yang et al., 2021; Zhao and Wang, 2020). Moreover, fertilization in drained co-contaminated rice fields can effectively and synergistically reduce the availability of Cd and As (Zhang et al., 2023). Frequent redox transitions make the biogeochemical cycles of elements in the rice field systems very active (Zhang et al., 2024). However, there is still a lack of comprehensive understanding of its potential link with As and Cd availability in this system.

The soil microbiome plays a critical role in soil ecosystem services, especially in nutrient cycling and biogeochemical processes (Aryal et al., 2022; Schaeffer et al., 2017). Microbial communities in paddy soils is profoundly influenced by moisture regulation and the application of both organic and chemical fertilizers (Han et al., 2023; Li et al., 2018). However, compared with taxonomy-based approaches, the assemblage of functional genes is considered to provide a better reflection of the functional response of microbial communities to environment factors (Liu et al., 2023), enabling a deeper understanding of the factors influencing elemental cycles (Mohapatra et al., 2021). Interspecific interactions among functional microorganisms in rice fields contribute to potential coupling in the biogeochemical cycles of N, Fe, Mn, S, Cd, and As (Chen et al., 2021, 2019; Maguffin et al., 2020; Xue et al., 2020). Nonetheless, our comprehension of microbial-driven element cycling in rice fields under temporal dynamics remains limited, and there exist knowledge gaps regarding the specific ecological roles of key indigenous soil microbes and their metabolic potentials associated with elements cycling.

In this chapter, we conducted a series of microcosm experiments to simulate various soil Eh and pH conditions under typical rice production moisture management and incorporating organic and chemical soil amendments. In situ soil conditioners such as biochar (Shi et al., 2022) and calcium magnesium phosphate fertilizers (Rehman et al., 2017) are commonly used acidic amendments that effectively regulate the bioavailability of soil Cd and As. These amendments were selected to represent both organic and chemical fertilizers. We hypothesize that a new redox state of soil is formed under the regulation of water and fertilizer, whereby the oxidation of N, Fe, Mn, or the reduction of S, might serve as critical regulatory hubs for reducing the bioavailability of As and Cd. Additionally, microbial activity is proposed to be the primary driving force behind these processes. The objectives of this study were to (1) assess the impact of modulating multi-element cycling networks on mitigating Cd and As bioavailability in soils; and (2) identify the potential key microbiota and functional genes involved in the key biochemical processes under different fertilization regimes. With this information elucidated, we anticipate that this study can provide the groundwork to develop a feasible strategy for the remediation of polymetallic co-pollution in the future.

# 2. Materials and methods

## *2.1. Soil sampling and physicochemical characterization*

Paddy soil was collected from five different areas within one established paddy field (half a century old), which is the same as that in Chapter 3. The surrounding field has an As and Cd content of approximately 66.5 $mg{\cdot}kg^{-1}$ and 0.41 $mg{\cdot}kg^{-1}$ respectively, rendering the soil in this region an ideal natural laboratory for studying the interaction of natural microbiota and major element cycles with As and Cd co-contamination (Zhang et al., 2023). Prior to incubation, selected soil properties were measured on air-dried and sieved (<2 mm) soil samples. These properties include pH, organic carbon content, total Cd/As content, as well as available P, K, Mn and Fe contents, which were analyzed using standard methods described previously, are shown in Table 3-1.

## *2.2. Soil incubation experiments*

Soil incubation experiments were conducted to simulate flooded paddy soil (mCK), aerobic paddy soil (mR), and applying biochar (mRB, organic) or CMP fertilizer (mRF, inorganic) in aerobic paddy soil. Each treatment was replicated three times at 3 sampling time points. The Cd and As contents of the selected commercial rice straw biochar and CMP fertilizer (with $P_2O_5$ content greater than 12%) were found to be lower than the detection limit. For the incubation, 60g of paddy soil contaminated with both Cd and As, with a pH of 5.17, was added into 100mL serum bottles covered with aluminum foil. Deionized water was added at 75% and 35% (wwater/wdry-soil) to simulate flooded and aerobic conditions, respectively, mimicking field conditions. Weigh the bottles every two days to ensure that the soil moisture content is constant at 75% and 35%. One week prior to the experiment, the soil and materials were mixed evenly, and the application rates (by weight) of materials were equivalent to local agricultural practices, specifically 1% biochar (Beijing PhD Union Academy of Agriculture, Beijing, China) and 0.5‰P of calcium-magnesium-phosphate fertilizer (Phosphate Fertilizer Factory in Liuyang East District, Liuyang, China). All samples were then incubated in the dark in a climate chamber at 37°C for 40 days to simulate the single moisture condition of rice from transplanting to the heading stage.

## *2.3. Soil sampling and chemical analysis*

Subsamples were collected on days 1, 20, and 40 of the incubation periods. In-situ pH and Eh were tested before sampling, and soil solution was extracted as previously described (Zhang et al., 2023). The available concentrations of As and Cd were extracted with 1M $KH_2PO_4$ (Xu and Fu, 2022) and 0.01M $CaCl_2$ (Houba et al., 2000), respectively. The content of $As^{III}$, $As^{V}$, DMA, MMA, $SO_4^{2-}$, $Cl^-$, $CO_3^{2-}$ and $NO_3^-$ in the soil solution was quantified using high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS, PerkinElmer NexION 300X) (Yan et al., 2020). The Cd, Fe, Mn, Ca and Mg concentrations in the soil solution were determined by ICP-OES (Optima 5300DV;

PerkinElmer) (Cruz et al., 2015). The dissolved organic carbon (DOC) was analyzed using a carbon and nitrogen analyzer (Multi N/C 3100, Analytik Jena, Germany).

## 2.4. Geochemical modeling

The modeling of microcosm aqueous geochemistry was conducted using Visual MINTEQ3.1. For the geochemical modeling, parameters including temperature, pH, DOC (modeled using NICA-Donnan Model), trace elements, anions and cations were incorporated into the geochemical model. It was assumed that the concentrations of tested elements, namely Fe, Mn, Cd, Ca and Mg, are equivalent to their divalent ionic forms ($Fe^{2+}$, $Mn^{2+}$, $Cd^{2+}$, $Ca^{2+}$ and $Mg^{2+}$) (Maguffin et al., 2020). Furthermore, several redox couples associated with As and Cd bioavailability were included in the model. These couples comprised $HS^-/SO_4^{2-}$, $Fe^{2+}/Fe^{3+}$, $Mn^{2+}/Mn^{3+}$, $NH_4^+/NO_3^-$ and $NO_2^-/NO_3^-$, with the equilibrium constants (logKs) determined within the model (Gustafsson, 2020):

$$SO_4^{2-}+9H^++8e \Leftrightarrow HS^-+4H_2O \qquad logKs= 33.66$$

$$Fe^{3+} + e \Leftrightarrow Fe^{2+} \qquad logKs=13.032$$

$$Mn^{3+} + e \Leftrightarrow Mn^{2+} \qquad logKs= 25.35$$

$$NO_3^-+10H^++8e \Leftrightarrow NH_4^++3H_2O \qquad logKs=119.077$$

$$NO_3^-+ 2H^++2e \Leftrightarrow NO_2^-+4H_2O \qquad logKs=28.57$$

## 2.5. DNA extraction and Illumina Miseq sequencing

Total DNA from 36 soil samples (3 replicates × 4 treatments × 3 time points) was extracted using the Fast DNA SPIN Kit for Soil DNA (MP Bio) following the manufacturer's protocols. The concentration and quality of the extracted DNA were assesed using a NanoDrop One spectrophotometer (Thermo Scientific, Wilmington, NC, USA). Subsequently, the DNA was utilized to amplify and sequence the bacterial 16S rRNA gene which targeted the variable V3-V4 region (forward primer, 338F-5′-ACT CCT ACG GGA GGC AGC AG-3′; reverse primer 806R-5′-GGA CTA CNV GGG TWT CTA AT-3′). Amplicons were sequenced based on the Illumina Miseq platform of Allwegene Technology Co. Ltd (Beijing, China). An operational taxonomic unit (OTU) table was then constructed using the UPARSE pipeline (Edgar, 2013). Briefly, reads were truncated at 300 bp and quality-filtered using a maximum expected error threshold of 0.5. Following the discarding of replicates and singletons, the remaining reads were clustered into OTUs at a 97% identity level threshold. This process resulted in a 16S rRNA OTU table encompassing 36 samples × 2742 OTUs (1,758,066 reads). The number of high-quality sequences per sample ranged from 21,479 to 84,722. Lastly, representative sequences for each OTU were selected and taxonomically classified against the RDP 16S rRNA database (Wang et al., 2007).

## 2.6. Bioinformatics analysis of 16S rRNA gene profiling

To standardize sequencing depth for subsequent bacterial community analysis, each sample was rarefied to the smallest sample size (21,479) using *R* software through the GUniFrac package (function: Rarefy). Alpha diversity was assessed using a richness index through the VEGAN packages (function: diversity). Weighted Unifrac distances between treatments were calculated using the GUNIFRAC package in *R* and visualized via principal coordinate analysis (PCoA) with the ggplot2 package, illustrating differences in bacterial community structures across all soil samples. Differences in community structure between treatments were assessed using permutational multivariate analysis of variance (PERMANOVA), performed with VEGAN package in *R* (function: adonis), utilizing 9999 permutations. The relative abundance of each taxonomic group per sample was calculated as the number of sequences assigned to that group divided by the total number of sequences. A Random Forest model was constructed using the R package RANDOMFOREST to identify key bacteria species that correlated with As and Cd content. The effect of different treatments on the abundance of individual OTUs was assessed using the DESEQ2 package (function: DESeq), employing negative binomial generalized models. Size shrinkage effects were evaluated in each contrast using the lfcShrink() function. To account for multiple testing, a false discovery rate correction (Benjamini-Hochberg procedure) was applied to adjust *P*-values. Additionally, groups of differentially abundant OTUs exhibiting similar changes were identified through hierarchical clustering (Ward's algorithm) on shrunken log fold changes with the hclust() function (Santos-Medellín et al., 2021).

## 2.7. Metagenome analysis

Twelve soil samples (3 replicates × 4 treatments) were collected on the 40th day of incubation. Shotgun metagenomic sequencing was performed on Illumina Hiseq 2500 platform using a paired-end (PE150) sequencing strategy, yielding an average 10Gb of data per sample. Raw sequencing data were trimmed, filtered assembled by MEGAHIT. Contigs longer than 300bp were retained for subsequent gene prediction and annotation.

The Prodigal program (https://github.com/hyattpd/Prodigal) was used to predict the open reading frames (ORF). ORFs with lengths exceeding 200bp were translated to generate a non-redundant gene catalog, employing criteria of 95% sequence identity and 90% coverage. The longest sequence was used as the representative sequence for each cluster through the CD-HIT program (http://www.bioinformatics.org/cd-hit/). Gene depth and relative abundance were computed using the Bowtie2 program. Subsequently, unigenes were annotated against the non-redundant protein sequence database (Version: 2021.11) of the National Center for Biotechnology Information (NCBI) for taxonomy (e-value ≤ 0.0001). Additionally, annotation against the Kyoto Encyclopedia of Genes and Genomes (KEGG) for functional classification (e-value ≤ 0.0001) was performed using the DIAMOND program (https://github.com/bbuchfink/diamond).

The contigs assembled from each sample were individually separated into bins using the Vamb program (https://github.com/RasmussenLab/vamb). The quality assessment of these bins was conducted utilizing the ChekM program. Bins meeting the criteria of an estimated genome completeness 70% and contamination below 5% were retained as high-quality metagenome-assembled genomes (MAGs). Ultimately, 40 high-quality MAGs were obtained for subsequent analysis. The abundance of each MAG across samples was determined as fragments per kilobase of exon per million reads mapped (FPKM) using Salmon. Functional genes within individual MAGs were predicted using Prodigal and annotated against the KEGG database using DIAMOND (with an e-value threshold of ≤ 0.0001). Taxonomic classification of all MAGs was performed using GTDB-Tk's classify_wf module.

### *2.8. Statistical analysis*

All statistical analyses were conducted using *R* software (version 3.6.0). Statistical significance was defined as $P< 0.05$ for all tests performed in this study. Unpaired t-tests and two-way analysis of variance (ANOVA) were utilized to assess significant differences. Spearman's rank correlation coefficients between ions and relative abundance of OTUs and As and Cd content were calculated using *R*. *P* value adjustments for multiple comparisons were implemented using false discovery rate (FDR) correction (Benjamini and Hochberg, 1995). Heatmap analysis was conducted using the pheatmap package in *R* (function: pheatmap). Partial least squares structural equation modeling (PLS-SEM) was performed using SmartPLS4.0 software. Data plotting was carried out using Prism 9.1.1 (GraphPad Software, San Diego, CA, USA) and the 'ggplot2' package in *R*.

## 3. Results

### *3.1. Geochemical properties of the incubated soils*

The average soil bioavailable As content was 3.34±0.06 $mg\cdot kg^{-1}$ in mCK at the conclusion of the incubation period (Figure 4-1A). This value significantly decreased ($P<0.05$) by 27.8%, 26.3% and 31.2% in treatments mR, mRB and mRF, respectively. The concentrations of iAs and DMA in the soil solution exhibited similar trends, with significantly lower concentrations observed in treatments mR, mRB and mRF compared to mCK on day 40 (Figure 4-1B, Figure 4-2). There were no significant differences observed in the available Cd content in the soil and soil solutions among the different treatments. However, the average soil bioavailable Cd content decreased in the following order: mCK>mR>mRF>mRB. Spearman's correlation analysis revealed that, apart from $SO_4^{2-}$ ions, the ions present in the soil solutions of mR, mRB and mRF showed stronger correlations with As and Cd compared to those in mCK (Figure 4-1C). In addition to the two major environmental factors, pH and Eh, we computed the sum of absolute values of correlation coefficients between all chemical species and As and Cd. The top three ions in each group with the highest correlation with As and Cd were as follows: $SO_4^{2-}$>DOC>$CO_3^{2-}$ in mCK, $CO_3^{2-}$>$Mn^{2+}$>$SO_4^{2-}$ in mR, $NO_3^-$>$Mn^{2+}$>$CO_3^{2-}$ in mRB, and $Mn^{2+}$>DOC>$NO_3^-$ in mRF.

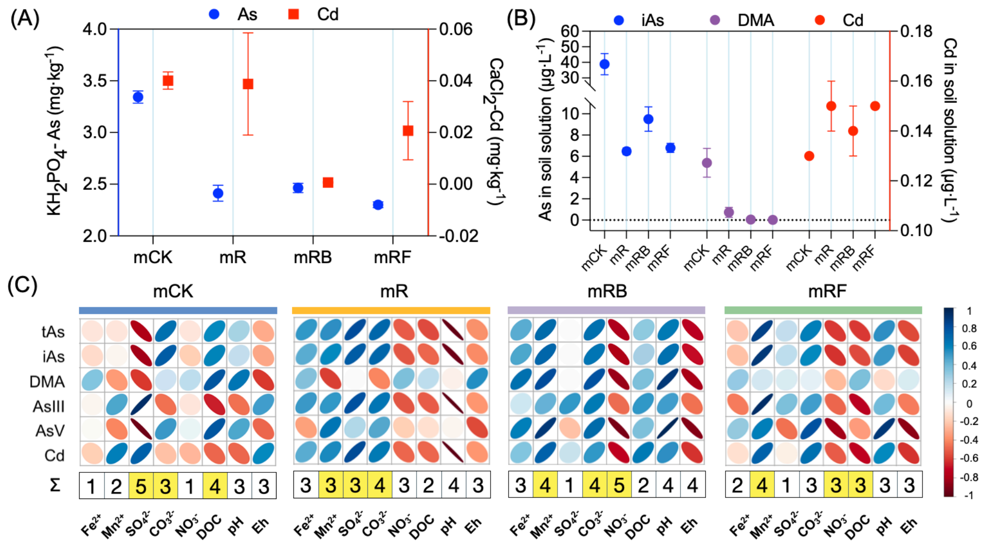

**Figure** 4-1. The available concentrations of As and Cd in soil (A) and the total As and Cd in soil solution (B) on the 40th day. (C) Spearman correlation of As, Cd and ions in soil solution. Data are mean ± standard error (n = 3). ∑ represents the sum of the absolute value of the Spearman correlation coefficients in the corresponding column. The sum of top three absolute values except those of pH and Eh were highlighted. The area and color of the bubbles represent significant levels. The narrower the bubble, the denser the confidence interval and the stronger the correlation. Red: negative correlation. Blue: positive correlation. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

## *3.2. Geochemical modeling*

Equilibrium geochemical modeling analysis was employed to investigate the influence of primary redox-active elements on controlling As and Cd concentrations in the soil solution (Figure 4-3). The redox speciation of N, Mn, Fe and S is shown in Figure 4-4 and 4-11. The onset of Mn reduction occurs at a redox potential of ~550 mV and lower, whereas N reduction is associated with a redox potential of ~360 mV and lower. The initiation of Fe and S reduction is associated with redox potentials of approximately 50 mV or lower and around -200 mV or lower, respectively. In the soil solution of the mCK sample on the first day of incubation (Eh at 44mV), the concentrations of As and Cd were primarily associated with Fe and Mn redox, rather than S reduction. In the later stages of the incubation, as the Eh further decreased to between -190 and -200mV, the occurrence of S redox and continued redox of Fe led to a significant increase in the concentration of As and a notable decrease in the concentration of Cd in the soil solution. In the soil solutions of treatments mR and mRF, As and Cd were predominantly associated with Mn and N redox, while in treatment mRB, they were primarily related to redox process involving Fe, Mn and N. As the Eh levels increased, surpassing 50 mV, the reduction process of Fe and Mn shifted towards oxidation, resulting in simultaneous decrease in the concentrations of As and Cd in the soil solutions of treatments mR, mRB and mRF. Further elevation of Eh levels, exceeding 360 mV, accompanied by a decrease in pH, led to a slight increase in the concentrations of As and Cd in the soil solutions of treatments mR and mRF, possibly due to N oxidation processes.

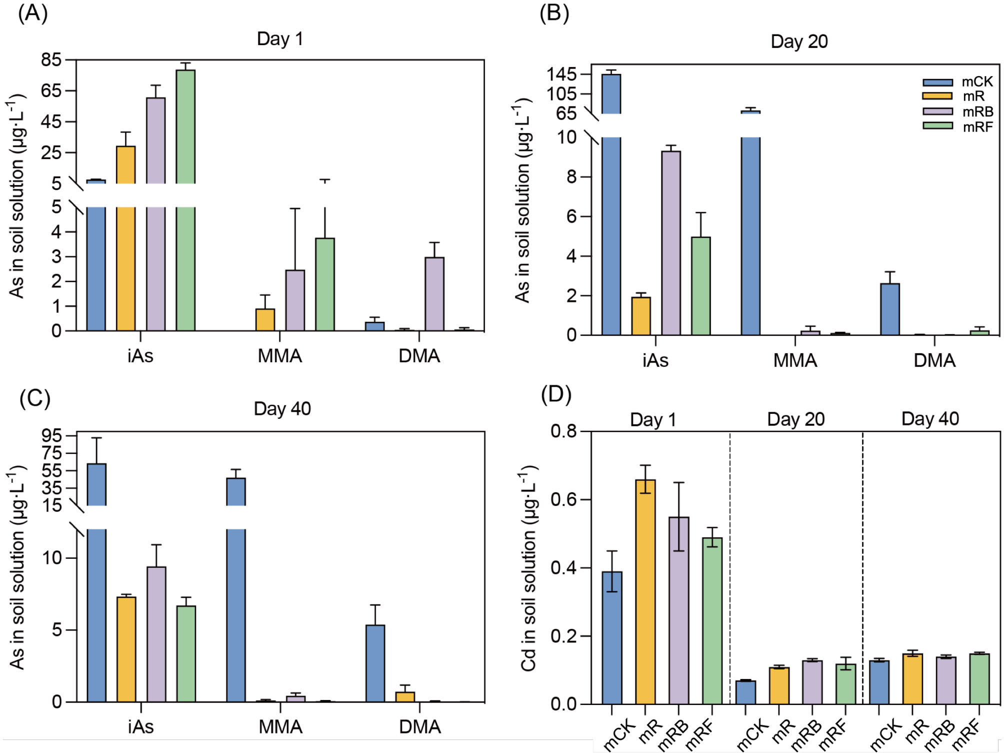


**Figure** 4-2. The content of As (A-C) and Cd (D) in soil solution. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

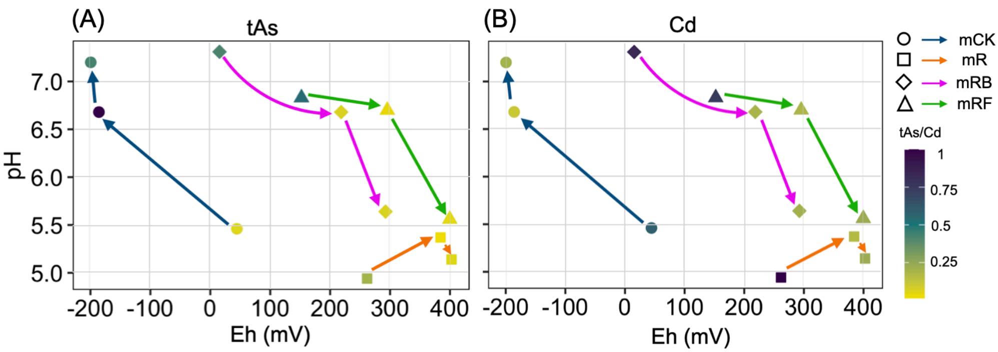


**Figure** 4-3. The ratio of the measured content to the maximum content of dissolved total As (A) and Cd (B) plotted as a function of Eh and pH. The three points in each treatment sequentially connected by arrows are samples on day 1, 20, and 40, respectively. The tAs/Cd content indicated by the color bar was calculated as the ratio of the measured content to the maximum content of As or Cd. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

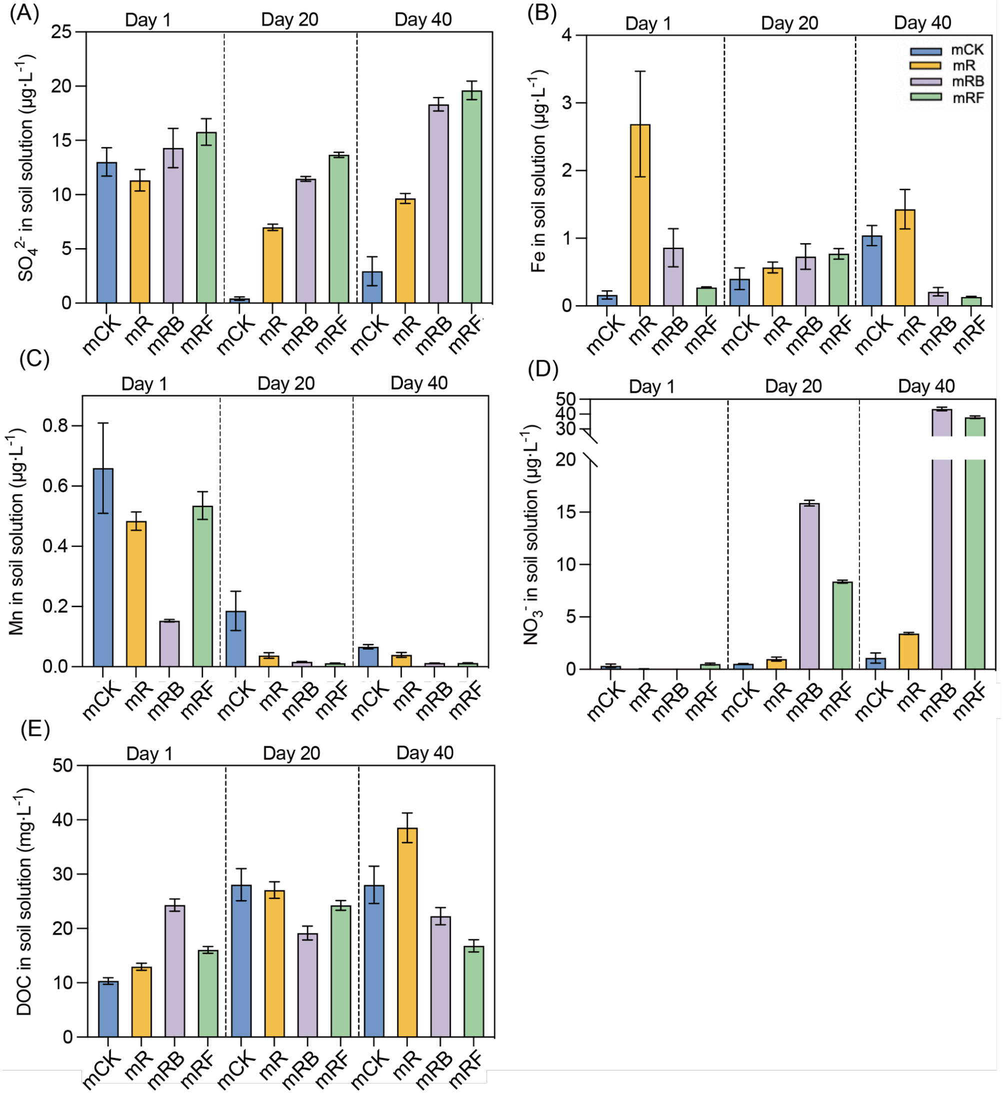


**Figure** 4-4. The content of S (A), Fe (B), Mn (C), N (D) and DOC (E) in soil solution. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

## *3.3. Abundance of functional genes involved in multi-element cycles*

To better elucidate how microbial community functions impact the biochemical turnover of key ions, we conducted a focused investigation on various genes involved in element cycles, such as Fe, Mn, S and N (Figure 4-5 and 4-6). Here we found significant differences ($P< 0.05$) in the abundance of functional genes within KEGG and eggNOG L2 pathways across different treatments. Moreover, we observed distinct modules between treatments mRB and mRF (Figure 4-12 and 4-13). Specifically, regarding the N cycle, mCK exhibited a significantly higher abundance of *nifH/nifD/nifK* genes associated with N-fixation for ammonia-related metabolic pathways. Conversly, the relative abundance of *amoA* and *amoB* genes involved in ammonia-oxidation was significantly increased in the mRF treatment. The relative abundance of the dissimilatory N reduction gene *napA* was significantly greater in the mCK treatment, whereas the relative abundance of assimilatory N reduction genes *nasA, nirA/B*, *narB* was significantly higher in the mR treatment. In general, it appeared that mRF was associated with an increase in ammonia-oxidizing genes and a decrease in nitrate reduction to ammonium (Figure 4-5A). For Mn, the *mntC* gene, encoding the substrate-binding protein of the Mn transport system, was significantly enriched in the mRB treatment. Similar trends were observed in the functional genes *mntD*, *troC* and *troD*, which encode permease protein of the Mn transport system. Regarding the Fe and S cycles, we found that genes related to the Fe transport system exhibited higher abundances in mRB and mRF treatments compared to the mR treatment (Figure 4-6). However, genes involved in dissimilatory and assimilatory sulfate reduction were significantly more abundant in mCK than in other treatments, such as *dsrA* encoding the sulfite reductase α subunit and *dsrB* encoding sulfite reductase β subunit (Figure 4-6).

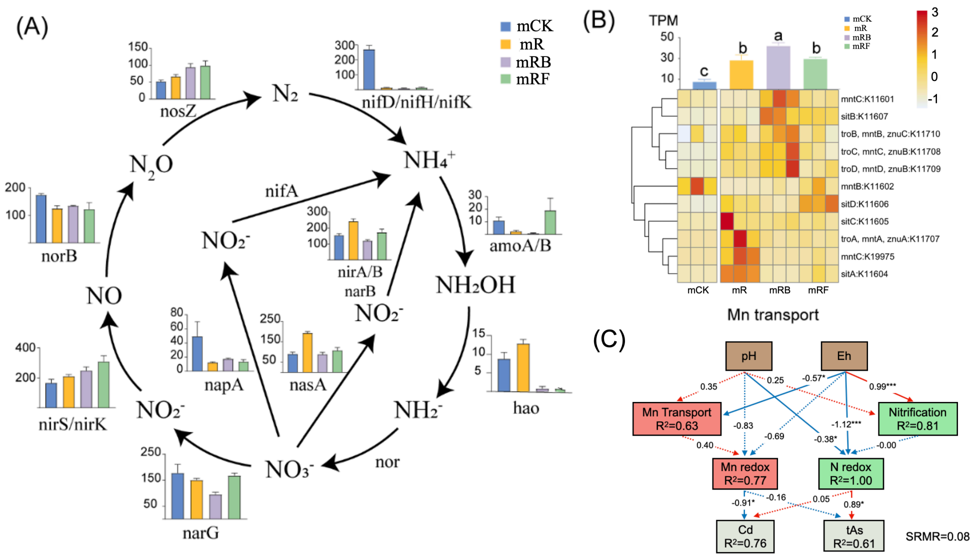


**Figure** 4-5. Differences in the relative abundance (transcripts per kilobase million) of functional genes related to (A) N cycling, (B) Mn cycling and (C) the PLS-SEM model of linkage between crucial genes and Cd and tAs content in soil. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

Further statistical analysis showed that PLS-SEM elucidated 76% and 61% of the total variation in soil Cd and tAs contents, respectively (Figure 4-5C). It was observed that pH and Eh exerted effects on the abundance of functional genes and elemental redox processes. Furthermore, genes associated with Mn and N metabolism were found to influence soil Cd and tAs levels by modulating Mn and N redox states (path coefficient = -0.91 and 0.89, $P < 0.05$). These results suggest that microbial-driven redox transformation of Mn and N play a pivotal role in regulating Cd and tAs content in contaminated soils.

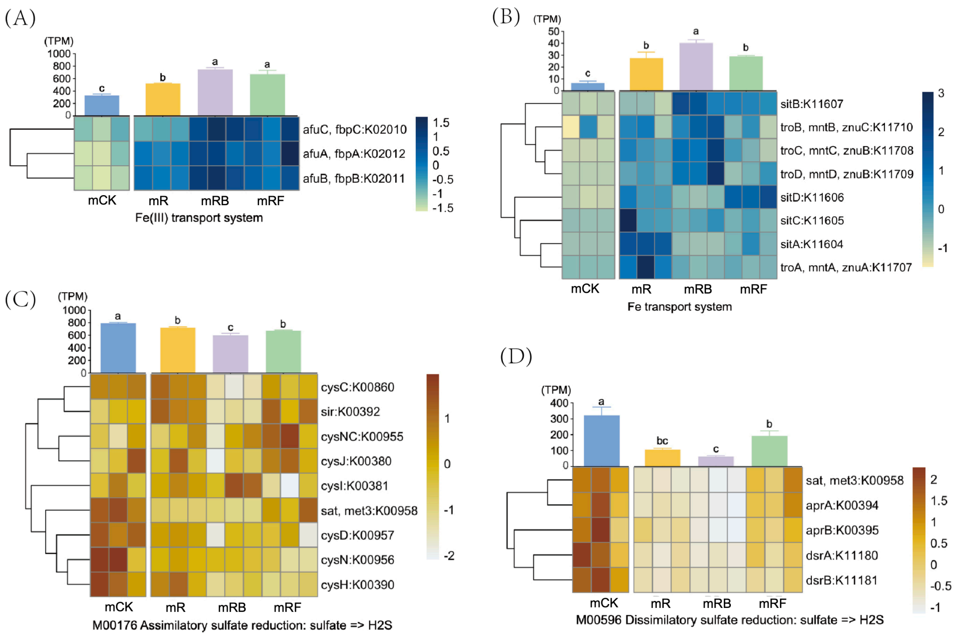


**Figure** 4-6. Differences in the relative abundance of functional genes related to Fe transport and S cycling. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

### *3.4. Bacterial community diversity and composition variation*

To elucidate how shifts in microbial diversity and composition correspond to functional changes, we conducted sequencing of the bacterial 16S rRNA gene across all samples. Overall, a significantly higher bacterial richness index was observed in the mRF treatment compared to the mR and mRB treatments (ANOVA, $P< 0.05$, Figure 4-7). Principal coordinate analysis (PCoA) revealed significant differences in bacterial community structure among mCK, mR, mRB and mRF soil samples at 1, 20 and 40 days (PERMANOVA, $P< 0.001$, Figure 4-7). The PCoA plot illustrated distinct clustering of bacterial communities from different time points along the first component (PCoA1), while the separation of mCK soil communities from those of mR, mRB, and mRF soils was evident along the second component (PCoA2). The bacterial community composition results were consistent with the findings from PCoA analysis. On day 40, a greater relative abundance of Saccharibacteria and Acidobacteria was evident in the mR soil compared to the mCK soil. Moreover, relative to other treatments, the mRB treatment significantly elevated the relative abundance of Bacteroidetes, while the mRF treatment significantly increased the relative abundance of Proteobacteria (Figure 4-8).

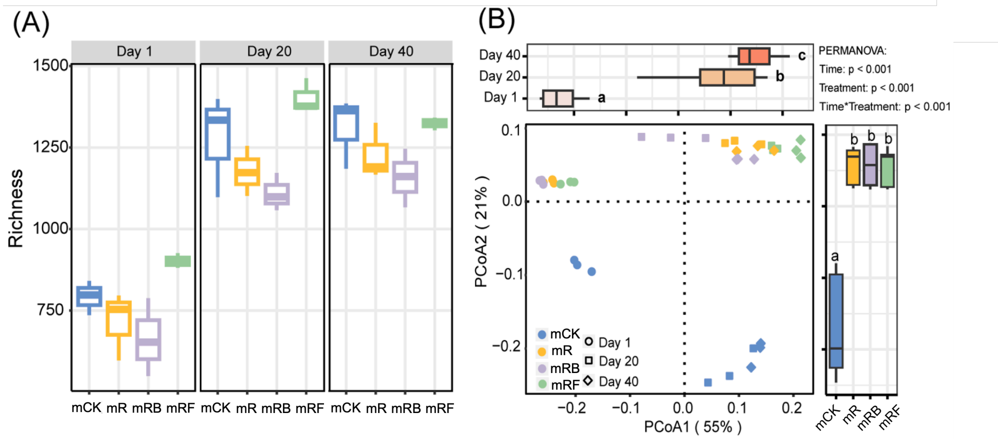


**Figure** 4-7. (A) Bacterial richness among all soil samples (mean ± standard error). (B) Principal coordinate analysis (PCoA) ordinations of bacterial community composition based on Weighted UniFrac distance metric. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

To identify the main microbial predictors of low Cd and As contents, the abundance of individual OTUs was fitted to negative binomial models and pairwise compared to Wald tests' contrasting control and treatment at each timepoint. A total of 1382 OTUs were affected by treatments at least at one timepoint ($P_{FDR} < 0.05$). After discarding low-abundance OTUs with relative abundance < 0.01% in all soil samples, random forest models and Spearman's correlation analysis showed that 20 OTUs and 24 OTUs were negatively correlated with Cd and As content, respectively (Figure 4-9A). Bradyrhizobiaceae, Sphingomonadaceae, Caulobacteraceae, Comamonadaceae and Thiobacillaceae were the top five families in terms of relative abundance (Figure 4-9B). Caulobacteraceae and Comamonadaceae were mainly enriched in mRB treatment soils, while Bradyrhizobiaceae, Sphingomonadaceae, Thiobacillaceae and Chitinophagaceae were primarily enriched in mRF treatment soils.

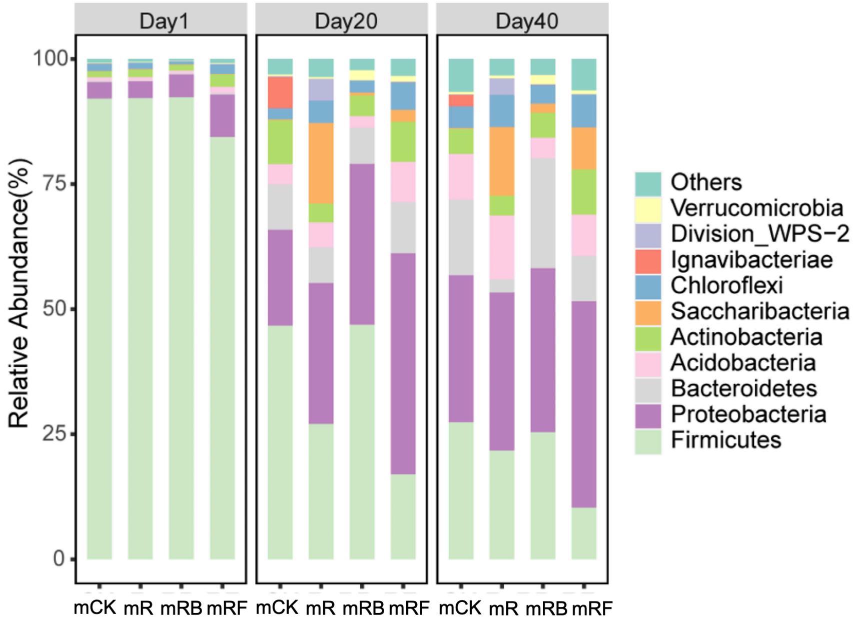


**Figure** 4-8. The relative abundance of different bacterial phyla in all soil samples. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

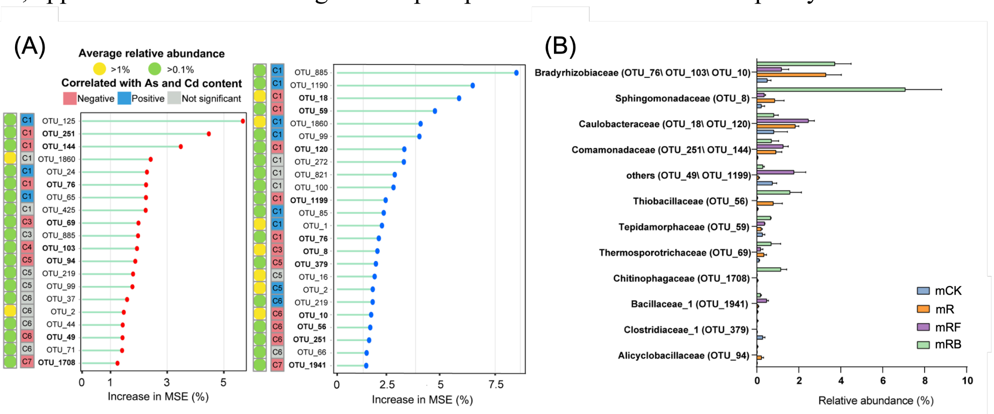


**Figure** 4-9. (A) Random forest mean predictor importance (percentage of increase in mean square error) of the model selection of abundant operational taxonomic units (OTUs) as drivers for the As and Cd content in soil. Percentage increases in the mean squared error (MSE) of variables were used to estimate the importance of these predictors, and higher MSE% values imply more important predictors. Color circles represent the relative abundance of OTUs and color squares show the spearman correlation between OTU abundance and As and Cd content in soil. (B) Bar plot showed the relative abundance of main microbial predictors in different treatments. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

Hierarchical clustering of $\log_2$-fold changes computed across all groups was conducted to further identify coherent patterns of differentially abundant OTUs in soils. Ten clusters were distinguished to illustrate the contrasting trends between treatments (Figure 4-14). We observed that 'Cluster 1' emerged as the most significant cluster for predicting both As and Cd content in soils. 'Cluster 1' comprised 98 OTUs, with their total relative abundance significantly increasing from day 1 to day 40 in mRF treatment soils. This suggests that OTUs enriched in mRF treatment soils served as more significant predictors.

## *3.5. Functional genes annotation of high-quality MAGs*

We assembled and binned 40 high-quality MAGs (integrity > 10% and contamination < 5%) spanning 10 phyla. Consistent with the community composition revealed by 16S rRNA gene sequencing, over half of these MAGs were affiliated with the dominant bacterial phyla Proteobacteria, Acidobacteriota and archaeal phyla Thermoproteota. Functional annotation confirmed their versatile potential for multi-element cycling (Figure 4-10). Specifically, the majority of MAGs harbored functional genes involved in nitrate reduction (67.5%), denitrification (72.5%), nitrification (27.5%), sulfate reduction (92.5%), thiosulfate oxidation (70%), Mn transport (77.5%) and Fe transport (100%). We further investigated the functional genes of representative MAGs belonging to the Bradyrhizobiaceae and Chitinophagaceae families, identified as microbial predictors in the mRF treatment. Bradyrhizobiaceae exhibited a higher abundance of Mn and $Fe^{III}$ transport genes, while Chitinophagaceae harbored more genes involved in denitrification. Two MAGs belonging to Caulobacteraceae were enriched in mRB soils and carried diverse Fe transport genes. Additionally, four MAGs belonging to Nitrososphaeraceae were predominantly enriched in mRF soils, indicating a potential role of archaea in soil nitrification.

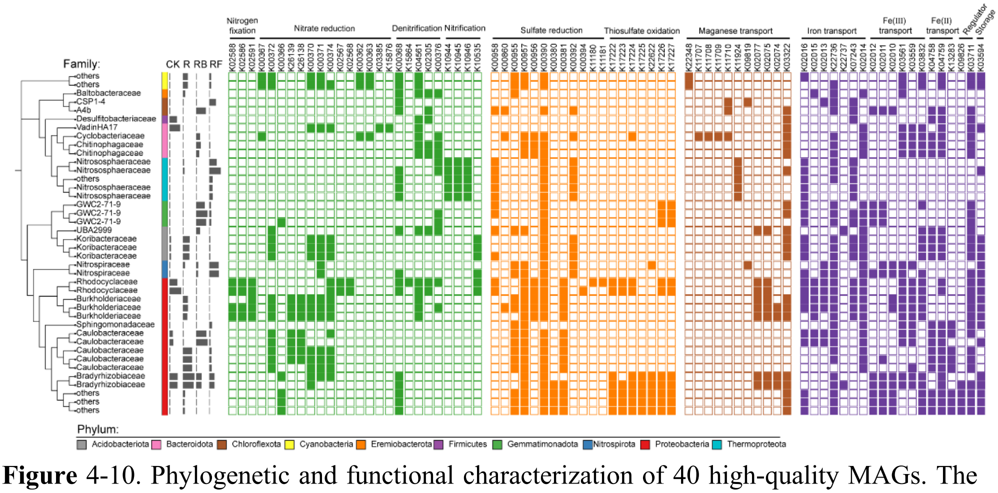


**Figure** 4-10. Phylogenetic and functional characterization of 40 high-quality MAGs. The construction of the maximum likelihood tree was based on a concatenated alignment of 40 marker genes from GTDB-Tk. The bar plot showed the relative abundance of each MAG at four different treatments. The presence (colored) and absence (blank) of protein-encoding genes are represented by the heatmap. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

# 4. Discussion

Flooding and drainage, as well as applying chemical or organic fertilizers, are two of most common water management and fertilizer application practices in rice cultivation. The dynamic trends of soil pH and Eh were observed to be completely opposite when the soil was flooded or in aerobic conditions, regardless of whether fertilizers were applied. Currently, computer-based speciation models are widely utilized for predicting metal element speciation in soil or water. Those models offer lower data requirements and time costs compared to traditional analysis methods (Khalid et al., 2023). In this study, the Cd and As content in contaminated soil and soil solution was successfully reduced by mediating the predominant redox microbial processes of these elements under different conditions. Targeted enhancement of key redox cycles and their corresponding functional microbial activities may be crucial for synergistically reducing the bioavailability of Cd and As.

Under flooded conditions, the As content in the soil solution increased, while the Cd content decreased, consistent with findings demonstrating a gradual decline in soil Eh due to increased irrigation in rice fields (Hu et al., 2015). Our investigation revealed that the predominant redox processes in the soil solution were associated with the Fe and S cycles, resulting in elevated concentrations of $HS^-$ and $Fe^{2+}$ in the soil solution under flooded conditions. These may be the two main factors contribute to the dissolution of As and Cd. Specifically, arsenic was mobilized through the reductive dissolution of Fe-(hydro) oxides (Suda and Makino, 2016). As the Eh decreased to approximately -200mV, the S redox was initiated. Our findings indicated enhanced expression of *aprA/B* and *dsrA/B* genes encoding the dissimilatory sulfate reduction pathway under these conditions. In the cytoplasm, dissimilatory sulfite reductase (DsrAB) is a key protein in microbial sulfate reduction, which is generally used as a functional marker for this process in the environmental and genomic studies (Neukirchen et al., 2023). Enriched sulfate reducing bacteria (SRB) in paddy fields (Wakao and Furusaka, 1973) likely play a crucial role in the generation of $S^{2-}$ and $HS^-$ ions (Huang et al., 2023). This process is implicated in the formation of CdS, which typically leads to a reduction in Cd availability. Additionally, the concurrent presence of $Fe^{2+}$ ions could facilitate co-precipitate with $As^{III}$ in the presence of FeS (Roy et al., 2015). This phenomenon might account for the reduction in As concentration observed in the soil solution following a decrease in Eh to -200 mV. In our study area, fertilizers are typically applied to paddy fields after drainage to maximize their effectiveness. Although we did not apply biochar or calcium-magnesium-phosphate fertilizers under flooded conditions, previous studies in similar acidic paddy fields have shown that biochar application delays the decline of Eh and inhibits reduction reactions under flooding (Li et al., 2023). The application of calcium-magnesium-phosphate fertilizers had little to no effect on Eh (Luo et al., 2020). Over time, the redox states of these treatments were similar to those observed in fields subjected to flooding alone.

In contrast, under aerobic conditions in paddy fields, Fe, Mn and N redox cycling were dominant. As Eh increases, the oxidation of soluble $Fe^{2+}$ led to a decrease in its aqueous concentration; however, this reduction does not fully explain the increase in $Fe^{3+}$ content in the soil solution. We hypothesize that $Fe^{2+}$ was oxidized to form solid Fe-(hydro)oxides, which subsequently adsorb As. Our previous findings support this hypothesis, suggesting that the increased As content associated with Fe oxides may be attributable to this process (Zhang et al., 2023). Since the highest value of the Eh range in this study remained lower than the onset of Mn oxidation in the geochemical model, dissolved Mn predominantly existed in the form of $Mn^{2+}$. Nevertheless, the content of $Mn^{3+}$ increased after reoxygenation compared to flooding conditions. Prior studies have demonstrated that heterovalent Mn minerals in mixed valence states will be formed in the oxidation state, which exhibit strong adsorption affinity for metal ions such as dissolved As and Cd (Wang et al., 2022). Mn oxides production in the environment is believed to be predominately driven by biological activity, as microorganisms oxidize $Mn^{II}$ at kinetically faster rates than many abiotic reactions (Tebo, 1991). Due to the dearth of research on intracellular Mn oxidation in microorganisms, we were unable to annotate specific Mn oxidation genes. However, operative Mn transport system always expression in $Mn^{II}$-oxidizing bacterial (Learman and Hansel, 2014). The presence of enriched *mntC*, *sitaA/B/C* and *troA/B/C/D* genes in the aerobic soil suggests their potential contribution to the Mn uptake pathway, hinting at microbial involvement in the Mn oxidation process (Wang et al., 2022).

Here, we found that the potential functional taxa of Bradyrhizobiaceae family accumulated in low As and Cd content soils may carry multiple Mn and $Fe^{III}$ transport genes. Recent studies have also reported a relatively high abundance of bacteria within the Bradyrhizobiaceae family, suggesting a potential association with Mn-oxidizing bacteria (Matsushita et al., 2018). Observations indicate that when the Eh reaches approximately 400 mV and the pH decreases to 5.5 and lower, N oxidizes. However, this dynamic N reaction leads to the generation of a large amount of $H^+$, which compete with $Cd^{2+}$ ions for adsorption sites, potentially resulting in increased availability of Cd. Conversely, As tends to be adsorbed by the soil, consequently reducing its content in the soil solution.

Varied soil ecological functions are always provided by different taxonomic compositions (Zhang et al., 2024). Applying biochar or CMP fertilizers in aerobic soil can improve soil nutrients levels and influence the soil microbial structure, affecting both bacterial alpha diversity and beta diversity. Interestingly, mRF and mRB showed significantly different microbial richness. Chemical fertilizers can rapidly increase the content of available nutrients in the soil, thereby enhancing the diversity of microbial communities in a short period. However, the difference in microbial diversity between mRB and mRF decreases over time. The decomposition of nutrients in organic matter requires more time to be released (Tang et al., 2023). These alternations subsequently influence the redox cycles of Fe, Mn and N, resulting in both beneficial and detrimental outcomes. The contents of both $Fe^{2+}$ and $Fe^{3+}$ in the soil solution were observed to be lower compared to the non-fertilized drained treatment, potentially indicating an intensified process of Fe oxidation leading to the formation of solid minerals.

Moreover, the distinct physicochemical properties of inorganic and organic fertilizers themselves also play a role in shaping these processes. After applying biochar, Eh did not reach the onset of N oxidation, but the N-related processes were observed, potentially attributed to the biochar addition simulating the activity of relevant microorganisms. There is a feedback mechanism between Cd and soil nitrification: $H^+$ ions with a larger hydrated ion radius, produced by nitrification, desorb $Cd^{2+}$ from soil particles, increasing its mobility. Conversely, Cd inhibits the nitrification process and proton ($H^+$) production by poisoning the growth of AOA and AOB communities, thereby inhibiting its own migration. After applying biochar, the reduction of Cd toxicity and improvement in soil conditions accelerate the recovery of AOA and AOB and nitrification due to biochar's adsorption effect. However, due to the slow calcification of biochar, the enhanced nitrification will not induce acidification (Zhao et al., 2020). However, these processes exhibited lower activity compared to mR or mRF treatments. Moreover, the higher pH observed in mRB treatments may result in elevated iAs levels in the soil solution (Jiku et al., 2022). In addition, applying biochar as an organic amendment increased the content of DMA, known for its high toxicity to rice, potentially contributing to the occurrence of rice straight head disease.

After applying calcium magnesium phosphate fertilizer, the ammonia-oxidizing genes (*amoA*/*amoB*) were significantly more abundant, potentially attributed to the presence of CaO and MgO in the inorganic fertilizer. After liming, significant alterations were observed in the AOA and AOB communities in the field, subsequently impacting gross nitrification activity (Zhang et al., 2017). Notably, the representative MAGs affiliated with Nitrososphaeraceae (AOA) and Nitrospiraceae (AOB) were identified in the mRF treatment, suggesting that the related nitrification gene-carrying microorganisms are active under this condition (Liu et al., 2023; Zhen et al., 2023). The $NH_4^+$ input and aeration can also facilitate nitrification (Blaud et al., 2018). The liming effect of CMP neutralizes soil acidification, thereby reducing the risk of increased available Cd. Although phosphorus fertilizer may be an important factor affecting the bioavailability of Cd and As, we did not detect $PO_4^{3-}$ in the soil solution samples using HPLC-ICP-MS. Therefore, we speculate that phosphorus plays a very small role in the element redox cycle in the soil solution and is more likely to play a role in fixing Cd by forming cadmium phosphate precipitates (Matusik et al., 2008). The increase in $Mn^{3+}$ content may be attributed to enhanced oxidation processes, which led to the amplification of the impact of Mn in mRB treatment. Our results highlight the important role of biogeochemical cycles in reducing As and Cd availability. While the role of microorganisms should not be overestimated, our finding suggest that the bioremediation should be taken into account in future research and global sustainable development goals.

# 5. Conclusion

In this study, we explored the effects of water regime and the application of both organic and inorganic fertilizers on As and Cd co-contaminated environments, highlighting the importance of the redox cycles chain and microbial ecological functions in mitigating the As and Cd bioavailability. Geochemical modeling and shotgun metagenomic analyses showed that the redox chain was primarily composed of Fe and S cycles under flooding. These cycles played a role in elevating As risk and decreasing Cd risk. Conversely, under aerobic conditions, the redox chain was predominantly driven by the "cogs" representing Fe, Mn and N redox processes. The oxidation of Fe and Mn minerals facilitated the adsorption of Cd and As, thereby reducing their contents in the soil solution. However, the intensified N cycle resulted in soil acidification, increasing the risk of Cd but further decreasing As contents. Despite the promotion of nitrification with the application of CMP fertilizer, the liming effect induced by CMP mitigated the risk of Cd associated by soil acidification. While the application of biochar led to the amplification of the impact of Mn and effectively delayed N oxidation, which typically requires a higher Eh onset. Results of 16S rRNA gene sequencing further revealed that diverse field water management and fertilization practices differently influence microbial diversity, composition, and the abundance of key species harboring functional genes, thereby initiating various elemental cycle reactions.

Utilization of CMP fertilizer or organic fertilizer in aerobic rice cultivation not only ensures optimal yield but also enhances essential elements cycling, synergistically reducing the bioavailability of Cd and As. In addition, biochar exhibits environmentally friendliness characteristics and contribute to carbon sequestration, rendering it a promising material. Future field trials across diverse soil types and climatic conditions are warranted to ascertain broader applicability.

# 6. Acknowledgements

This work was financially supported by the National Foundation of Natural Science of China, Grant No. 42077139; the Science Innovation Project of the Chinese Academy of Agricultural Science (CAAS-ASTIP-2021-IEDA); and the special fund for Science and Technology Innovation Teams of Shanxi Province (20230405100101).

# 7. Supplementary Figures

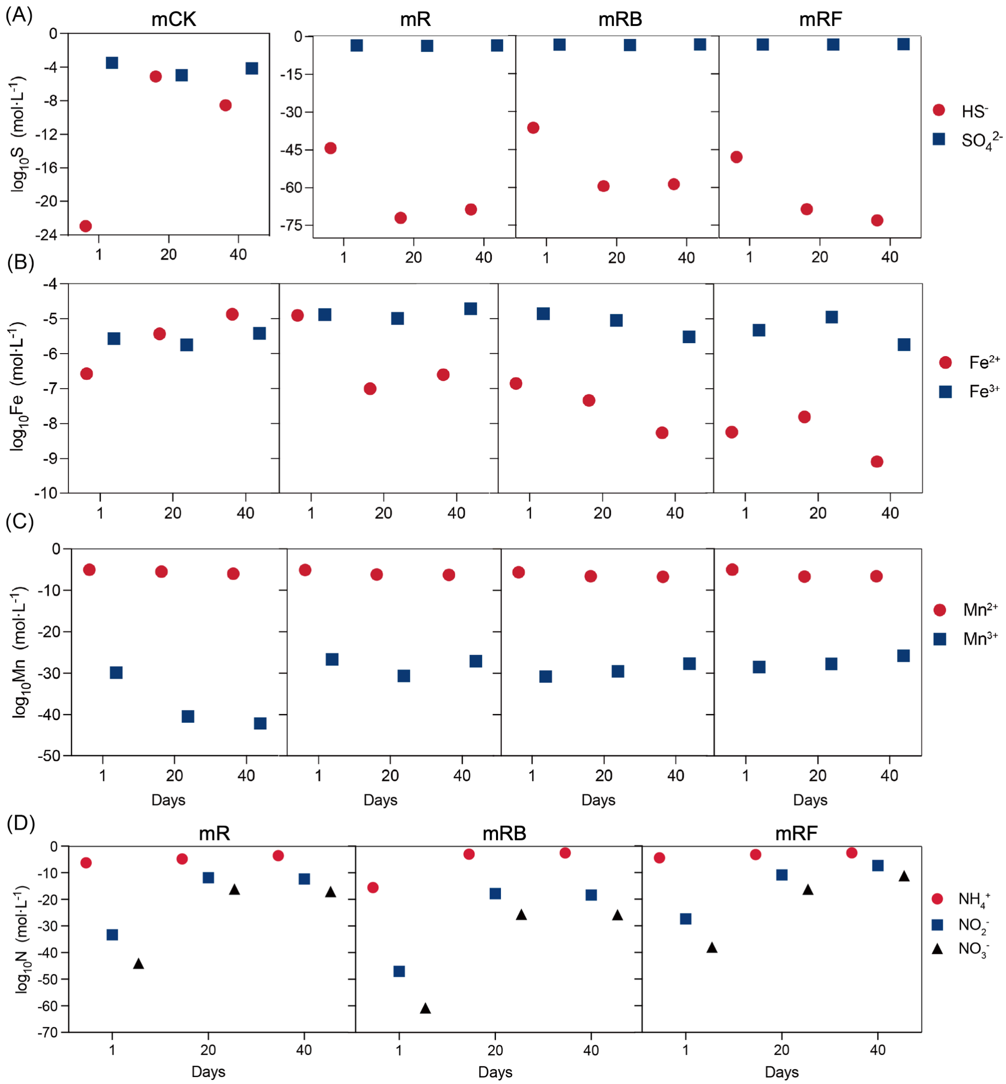


**Figure** 4-11. Logarithm of molar oxidized and reduced S (A), Fe (B), Mn (C) and N (D) in soil solution. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

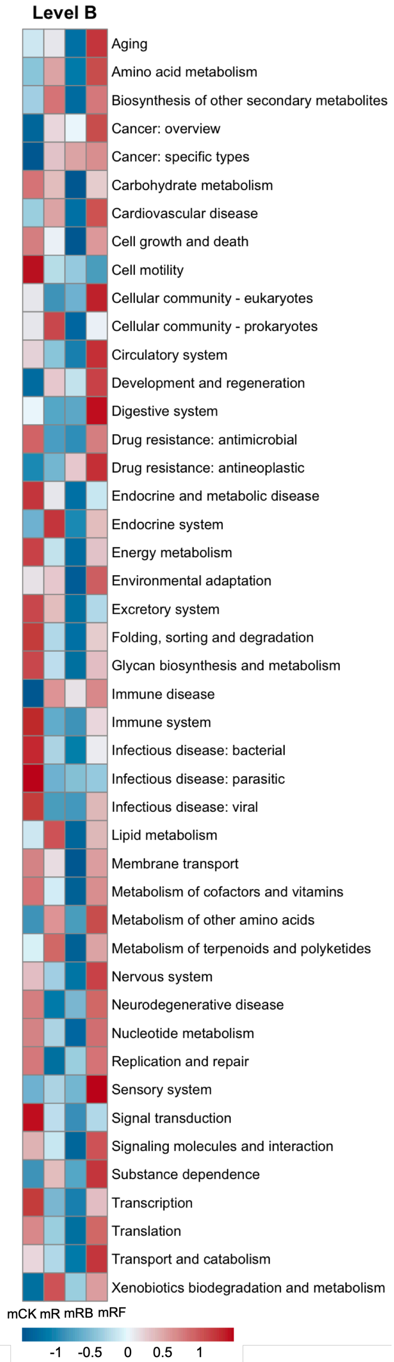


**Figure** 4-12. L2 pathway abundance base on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

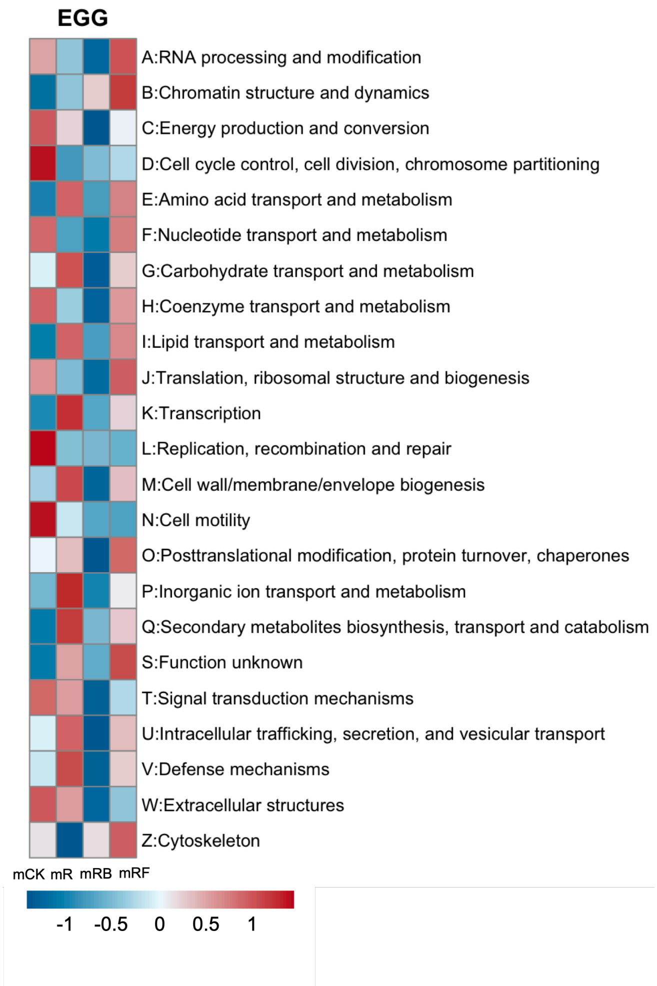


**Figure** 4-13. L2 pathway abundance base on the eggNOG database.

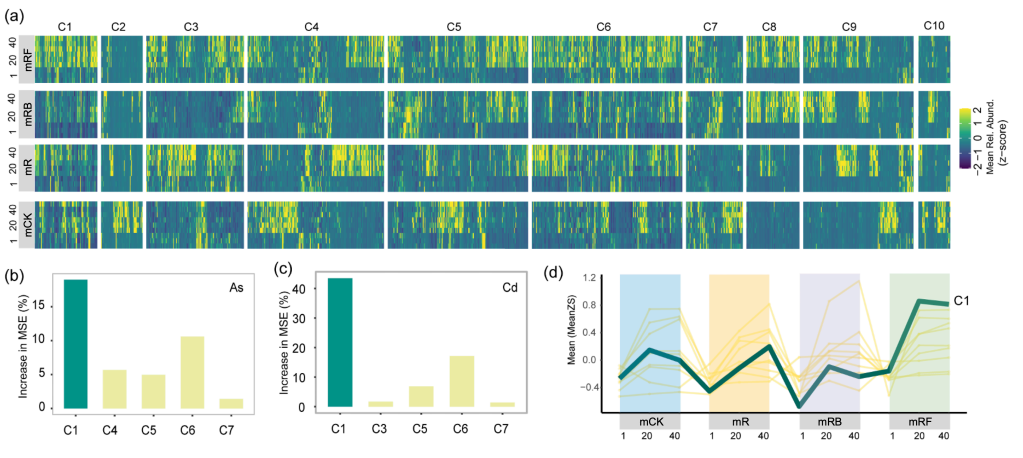


**Figure** 4-14. Microbial community properties of different treatment for tree timepoints. (a) Heatmap of log2 fold changed in taxa relative abundance between mCK and other treatments from three timepoints. Each column represents a differentially abundant OTU detected as significant (Wald test, FDR < 0.05) in at least one pair-wise comparison. Vertical facets indicate each of the modules detected through hierarchical clustering. (b, c) Random Forest mean predictor importance (percentage of increase in mean square error) of the model selection of OTUs as drivers for the content of As and Cd in soil. (d) line diagram shows the change in the total relative abundance of OTUs in each cluster. mCK, flooded paddy soil; mR, aerobic paddy soil; mRB, biochar application in aerobic paddy soil; mRF, application of calcium-magnesium-phosphorus fertilizer in aerobic paddy soil.

# Chapter 5

## General discussion, conclusions and perspective

# 1. General discussions

## *1.1. Synergistic remediation of As and Cd co-contaminated paddy soil via ridge cultivation and soil amendments*

Arsenic and Cd co-contamination in paddy fields poses a significant global challenge. The efficient uptake of As and Cd by rice, coupled with the frequent redox fluctuations in paddy environments and the contrasting geochemical behaviors of these two elements, complicates remediation efforts (Zhao and Wang, 2020). Given that rice serves as a staple food for approximately 60% of the global population, addressing the co-contamination of As and Cd in paddy fields has emerged as a critical area of research.

Soil ridge cultivation is a traditional agronomic practice in waterlogged paddy fields and has been implemented in China for over 2000 years (Zheng et al., 2014). Ridging can solve the defects caused by high groundwater level, sufficient rainfall, poor drainage, and enrichment of reducing toxic substances in waterlogged fields (Ren et al., 2016). Recently, this traditional agronomic practice has been proven to keep As and Cd availability at low levels through the adjustment of the soil Eh and pH to a trade-off situation (Jiku et al., 2022). Due to the elevated Eh, ridge cultivation may increase the risk of Cd for paddy fields especially with high Cd content (Jiku et al., 2022). Consequently, this study aimed to investigate if the application of soil amendments in ridged rice fields could further decrease the Cd bioavailability. We selected an organic amendment, biochar, and an inorganic soil conditioner, CMP fertilizer, both of which synergistically reduced the bioavailability of As and Cd in paddy soils and their accumulation in rice grains.

The experimental field selected for this study is a typical paddy field in southern China, characterized by red paddy soil classified as Hydragric Anthrosol under the World Reference Base for Soil Resources. The soil has a slightly acidic pH of 5.17. Ridge cultivation on acidic soils leads to an increase in soil Eh and a decrease in pH. The enhanced reaeration condition promotes the oxidation of reduced metal species such as Fe and Mn, which in turn releases large amounts of $H^+$ ions, resulting in further acidification of the soil (Jiku et al., 2022). This acidification increases the net positive charge on soil particle surfaces (Houben et al., 2013), thereby enhancing the adsorption of the anionic As and the desorption of the cationic Cd (Yang et al., 2018). As a result, ridge cultivation tends to reduce the bioavailability of As while increasing the bioavailability of Cd. The application of alkaline biochar or CMP fertilizer to the ridge can mitigate soil acidification while maintaining an oxidative soil environment. These amendments exhibit a liming effect, raising soil pH and promoting the precipitation of Cd as carbonate, phosphate, and hydroxide complexes, thereby decreasing Cd mobility and bioavailability (Fang et al., 2024; Wang et al., 2025). Additionally, the $Ca^{2+}$ and $Mg^{2+}$ ions released from CMP fertilizer can bind with reducible Cd in the soil to form insoluble precipitates, further reducing Cd bioavailability (Li et al., 2008). The Ca component may also promote the formation of insoluble compounds such as $Ca_3(AsO_4)_2$ and Ca–As complexes, contributing to the immobilization of As (Nazari et al., 2017). Furthermore, biochar improves Cd immobilization through its high content of functional groups, increased specific surface area, and developed pore structure (Xu et al., 2018).

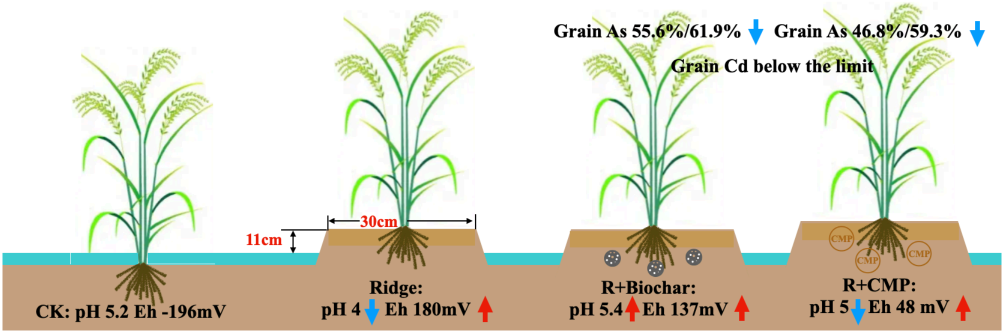


**Figure** 5-1. Synergistic remediation of As and Cd co-contaminated paddy soil via ridge cultivation and soil amendments.

In this study, the As and Cd concentrations in the paddy soil were 66.5 $mg\cdot kg^{-1}$ and 0.41 $mg\cdot kg^{-1}$, respectively. Ridge cultivation combined with the application of biochar or CMP fertilizer proved effective in simultaneously reducing the bioavailability of both As and Cd. This method is broadly applicable to mildly acidic paddy fields or wetlands in China, and potentially in similar climatic zones worldwide. However, for other climate conditions, soil types, or higher contamination levels, further research is needed to optimize ridge height and amendment dosage. Compared with regular wetting-drying alternation methods, ridge cultivation avoids frequent irrigation and drainage, thus saving water resources and reducing agricultural input costs. CMP fertilizer is a commonly available input that can also help ameliorate soil acidity, while biochar contributes to long-term carbon sequestration. This approach is cost-effective and environmentally friendly. Given that ridge cultivation requires more labor than conventional direct seeding or transplanting, its large-scale adoption will depend on the development of integrated machinery capable of simultaneous ridge formation and rice transplanting. Such mechanization could reduce labor intensity, improve efficiency, and yield better results with less effort.

## *1.2. Varietal response of rice to ridge cultivation combined with soil amendments for As and Cd Reduction*

In this study, ridge cultivation in paddy fields combined with the application of biochar or CMP fertilizer significantly and simultaneously reduced As and Cd concentrations in rice grains. Typically, different rice cultivars exhibit genotypic differences in their capacity to absorb, transport, and accumulate heavy metals, which is a key reason why agronomic practices may yield variable outcomes (Feng et al., 2023). The two rice cultivars used in this field experiment were both conventional indica hybrid varieties that have been widely cultivated locally for many years. Under all conditions in this study, both varieties showed consistent reductions in As and Cd accumulation in grains compared to the control or ridge cultivation alone. No significant differences in As accumulation were observed between the two varieties across the same plant organs. Differences in Cd accumulation were limited to husks under CMP fertilizer application, while no significant differences were found in Cd accumulation in straws or roots. This may be related to intrinsic differences in Cd translocation or storage capacity between the two varieties; however, no previous studies have reported that these two cultivars have specific sensitivity or accumulation characteristics for As or Cd. Moreover, no differences were observed in key growth indicators such as thousand-kernal weight and plant height between the two cultivars. Taken together, these results suggest that the agronomic practices applied in this field experiment were likely the primary factors influencing As and Cd accumulation in rice grains.

With advances in rice germplasm development, new low-Cd accumulation resources such as Luohong 4A have been used as donor parents in marker-assisted selection to breed low-Cd rice cultivars, such as "Xizi 3", which has shown stable expression of its low-Cd accumulation trait (Wang et al., 2025). This provides a novel approach and pathway for the coordinated decrease of As and Cd accumulation in rice grains. However, to date, no rice cultivars with stable low-As accumulation have been identified. Since ridge cultivation can effectively reduce As bioavailability in paddy fields, future strategies could explore combining this practice with low-Cd accumulating rice varieties to achieve simultaneous reduction of As and Cd in rice grains. Depending on the degree of soil contamination, site-specific measures such as ridge cultivation, application of soil amendments, or planting of low-Cd accumulation rice varieties—either individually or in combination—can be adopted to ensure the safe use of paddy soils and sustainable rice production.

## *1.3. Mechanisms underlying the effects of ridge cultivation and amendments on As, Cd, and related ion interactions in microcosm simulations*

Microcosms serve as simplified models that simulate complex and heterogeneous natural ecosystems (Cao et al., 2021). While microcosm conditions are not entirely controllable, the Eh and pH levels in our experiments closely mirrored those found in the field. We believe that the mechanisms by which soil amendments applied to ridges reduce the bioavailability of As and Cd can be effectively explored through these simulations. The soil used in this study was sourced from the same As-Cd contaminated plots in the field, where the content of As and Cd are approximately 66.5 mg $kg^{-1}$ and 0.41 mg $kg^{-1}$, respectively. This makes the region an ideal natural laboratory for investigating the interactions between natural microbiota and the biogeochemical cycles of major elements in the context of As and Cd co-contamination (Zhang et al., 2023). Currently, computer-based speciation models are widely employed to predict the speciation of metal elements in soil and water, offering advantages in terms of reduced data requirements and lower time costs compared to traditional analytical methods (Khalid et al., 2023).

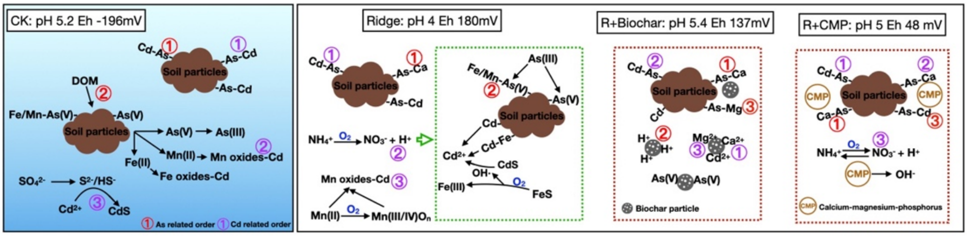


**Figure** 5-2. The importance order of ion interaction among treatments. **CK**: **Cd**: ① co-absorption with As, ② adsorption on Mn-oxides, ③ CdS precipitation. **As**: ① co-absorption with Cd, ② form As-DOM complex, ③ elevated pH mobilize As. **R**: **Cd**: ① co-absorption with As, ② decreased pH mobilize Cd, ③ adsorption on Mn-oxides. **As**: ① $Ca^{2+}$ increased As adsorption, ② adsorption on Mn-oxides. **R+B**: **Cd**: ①, ③ ion competition, ②co-absorption with As. **As**: ①, ③$Ca^{2+}$/$Mg^{2+}$ increased As adsorption, ② elevated pH mobilize As. **R+CMP**: **Cd**: ①co-absorption with As, ② ion competition, ③ elevated pH immobilize Cd and ammonium ration increased; **As**: ①$Ca^{2+}$ increased As adsorption, ② adsorption on Mn-oxides, ③co- co-absorption with Cd. The green square means the enhanced reactions after ridging cultivation. The red square means after adding biochar or CMP on the ridge, the additional enhanced reactions besides ridging alone.

The application of soil amendments on ridges alters soil pH, Eh, and introduces new ions, leading to modifications in the ionic relationships and chemical processes within the original soil habitat. Typically, pH and Eh are critical environmental factors influencing the bioavailability of As and Cd, often overshadowing other effects (Tao et al., 2022). A decrease in soil pH results in an increase in the net positive charge on the soil surface (Houben et al., 2013), which accelerates the adsorption of As anion and the desorption of Cd cation (Yang et al., 2018). Consequently, ridge cultivation may reduce the risk of As accumulation while increasing the risk of Cd accumulation. However, ABT analysis (Glenn, 2007)—commonly employed when sample sizes are limited and the goal is to identify key drivers and quantify feature importance—revealed that the application of biochar or CMP fertilizer on ridges significantly enhanced the role of $Ca^{2+}$ in reducing the bioavailability of both As and Cd. Calcium ions can precipitate with $As^{V}$, thereby reducing As mobility. Due to their similar atomic radius and valence to Cd, $Ca^{2+}$ compete with Cd for adsorption sites on the soil surface (Wu et al., 2021); however, biochar provides additional adsorption sites, effectively diminishing Cd bioavailability (Harvey et al., 2011). Furthermore, $Ca^{2+}$ contributes to the stability of cell membranes and cellular integrity, altering the negative charge on the membrane surface, which hinders Cd entry into plant cells and reduces Cd accumulation in rice (Kanu et al., 2019).

The effects of Fe and Mn were also significantly enhanced following the application of biochar or CMP fertilizer on the ridges. The strong adsorption between Fe-(hydro)oxides and $As^{V}$ accounts for the increased presence of As associated with poorly crystalline and well-crystalline Fe/Al oxides (Goldberg, 2002). Although the oxidation associated with ridge cultivation may lead to the decomposition of CdS and increase Cd mobility (Huang et al., 2021), we observed that the increase in Cd associated with Mn oxides may play a crucial role in reducing Cd risk following the application of soil amendments. Secondary Mn minerals possess a large surface area and a strong adsorption affinity for metal ions, enabling the immobilization of Cd through adsorption or co-precipitation (Wang et al., 2022).

### *1.4. Microbial-driven redox cycles regulate the availability of As and Cd*

In this study, the bioavalability of As and Cd in contaminated soil was successfully decreased by mediating the predominant redox microbial processes of the elements under different conditions. Targeted enhancement of key redox cycles and their corresponding functional microbial activities may be crucial for synergistically reducing the bioavailability of Cd and As.

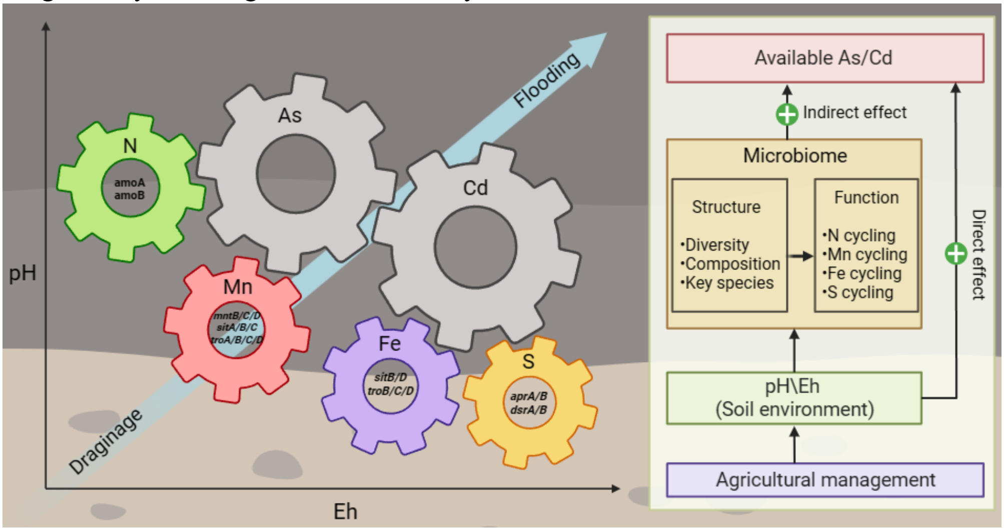


**Figure** 5-3. A scheme diagram of microbial-driven redox cycles regulate the bioavailability of As and Cd.

Following the application of biochar or CMP fertilizer on the ridges, geochemical model analysis indicated that the key redox cycles influencing the bioavailability of As and Cd primarily involve Fe, Mn and N cycles. Although the maximum Eh value remains below the onset of Mn oxidation, the concentration of $Mn^{3+}$ increased after applying biochar or CMP fertilizer on the ridges compared to flooding conditions. The production of Mn oxides in this study is believed to be primarily driven by microbial activity, as the kinetic rate of microbial oxidation of Mn oxides surpasses that of abiotic reactions (Tebo, 1991). Microorganisms from the Bradyrhizobiaceae family, enriched in ridged soil, have been identified as potential functional groups that may harbor multiple Mn and Fe transport genes, indicating potential associations with Mn-oxidizing bacteria (Matsushita et al., 2018). However, due to the limited research on intracellular Mn oxidation in microorganisms, we are unable to annotate specific Mn oxidation genes. Nonetheless, efficient Mn transport systems are consistently expressed in Mn-oxidizing bacteria (Learman and Hansel, 2014). The presence of *mntC*, *sitB*, and *troB/C/D* genes enriched in ridged soils treated with biochar or CMP fertilizers suggests their potential involvement in Mn uptake pathways, indicating that microorganisms play a role in the Mn oxidation process (Wang et al., 2022). The mixed valence Mn minerals formed in the oxidized state exhibit a strong adsorption affinity for dissolved metal ions such as As and Cd (Wang et al., 2022).

Nitrogen cycling was significantly enhanced following the application of CMP fertilizer on the ridges, with a notable increase in ammonia oxidation genes (*amoA/amoB*), likely linked to the presence of CaO and MgO in the fertilizer. Following the lime effect, significant changes in the communities of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were observed in the field, impacting overall nitrification activity (Zhang et al., 2017). Additionally, representative metagenome-assembled genomes (MAGs) from Nitrososphaeraceae (AOA) and Nitrospiraceae (AOB) were identified in the ridge treatment, indicating the activity of microorganisms carrying relevant nitrification genes (Liu et al., 2023; Zhen et al., 2023). Although the nitrification process may lead to a decrease in pH and an increase in Cd mobility, the liming effect of CMP fertilizer mitigates soil acidity, thereby reducing Cd risk. While the role of microorganisms should not be overestimated, our finding suggest that the bioremediation should be taken into account in future research and global sustainable development goals.

# 2. Conclusions

Co-contamination of As and Cd in soils is increasingly becoming a global concern. Due to the significant differences in the geochemical behaviors of As and Cd, as well as their high accumulation efficiency in rice, it is crucial to remediate As-Cd co-contaminated paddy fields in a coordinated manner. This study was conducted in a typical rice-growing region in southern China, one of the world's major rice-producing countries. The soil in the study area had a pH of 5.17, with As and Cd concentrations of 66.5 $mg\cdot kg^{-1}$ and 0.41 $mg\cdot kg^{-1}$, respectively. An adaptive modification of China's traditional ridge cultivation technique was implemented, setting ridge height at 11 cm. This was combined with the application of 1% (w/w, dry soil) biochar and 0.05% (w/w, dry soil) CMP fertilizer, aiming to reduce the bioavailability of As and Cd in the soil and minimize their accumulation in rice grains. In addition, microcosm experiments were conducted to further investigate the mechanisms underlying the effectiveness of this combined approach. The main conclusions are as follows:

1. The integrated strategy of ridge cultivation combined with organic (biochar) and inorganic (CMP fertilizer) amendments created an oxidizing soil environment and improved soil acidity conditions. This approach effectively reduced As accumulation in rice grains while maintaining low Cd levels, without negatively impacting rice growth or yield—thus ensuring safe rice production.

2. The combined treatment altered the soil environment, notably pH and Eh, which in turn enhanced ionic interactions between Ca, Fe, Mn and the heavy metals As and Cd in the soil solution. These changes synergistically decreased the bioavailability of both As and Cd. The aerobic conditions created by ridge cultivation promoted the association of As with poorly and well-crystallized Fe/Al oxides, and Cd with Mn (hydr)oxides, effectively reducing their mobility.

3. Under the aerobic conditions induced by the combined treatment, the redox cycling of key elements—particularly Fe, Mn, and N—was primarily microbially driven. Enhanced oxidation of Fe and Mn minerals increased their adsorption capacity for As and Cd, lowering their concentrations and mobility in the soil solution. The increased abundance of assimilatory N reduction genes (*nasA*, *nirA*/*B*, *narB*) indicated enhanced N cycling, which, while promoting acidification and potentially increasing Cd risk, also contributed to reduced As bioavailability. Although the use of CMP fertilizer stimulates nitrification, its liming effect helps buffer soil acidity, thereby mitigating Cd-related risks. In contrast, biochar application strengthened Mn-mediated processes and delayed the onset of N oxidation—requiring higher Eh—thus further contributing to the co-reduction of As and Cd bioavailability.

In conclusion, ridge cultivation combined with the application of CMP fertilizer or organic soil amendments such as biochar not only ensures safe rice production but also enhances the elemental cycling necessary for the synergistic reduction of As and Cd bioavailability. CMP, as a commonly used and cost-effective fertilizer, is easy to apply, while biochar offers environmental benefits and contributes to carbon sequestration—making both materials ideal for sustainable agricultural development.

# 3. Perspectives

This study innovatively integrates traditional ridge cultivation with modern technology to achieve a breakthrough in the synergistic reduction of bioavailable As and Cd in paddy fields. It reveals the mechanism of remediation from multiple dimensions, including soil chemistry and microbial ecology. The proposed remediation strategy is both economically and ecologically sustainable, aligning with the principles of sustainable agricultural development. It is easy for farmers to accept and promote, has clear application scenarios, and great potential for widespread adoption. This provides crucial technical support for ensuring food security and soil ecological restoration

## *3.1. Future Research*

1. **Expand the scope of the technology's applicability**. Our study proposes an economically efficient and environmentally friendly sustainable agricultural practice, where ridge cultivation combined with soil amendments can effectively reduce the bioavailability of As and Cd. However, we have only verified this in acidic paddy fields in southern China. Paddy fields with similar climate zones globally, which are contaminated with both As and Cd, could benefit directly from this approach. Future research should expand to other climate zones, soil types (such as neutral and

alkaline soils), and paddy fields with higher As and Cd levels, conducting optimization trials on ridge height and amendment application rates. This would clarify the technical boundaries and adjustment schemes, thus expanding its range of application.

2. **Develop supporting agricultural machinery.** To address the increased labor burden due to ridge cultivation, we recommend collaborating with agricultural machinery research institutions to develop integrated machinery for ridge formation and rice transplanting. This would improve operational efficiency, reduce labor intensity, and provide hardware support for the large-scale promotion of the technology.

3. **Explore the synergistic application with low-accumulation varieties.** Combining existing low-Cd accumulation rice varieties (e.g., "Xizi 3") with ridge cultivation and soil amendments, we could conduct trials to explore their synergistic effects in reducing As and Cd levels in grains. This could provide more precise remediation solutions for soils with varying levels of contamination.

### *3.2. Recommendations for policymakers*

The global co-contamination of As and Cd in agricultural land is showing a trend of multi-point growth. With global warming and other climate changes, the potential risks of multi-metal composite pollution to farmland or human health are likely to increase. The spatial and geographical distribution of this pollution is uneven, significantly influenced by geological and environmental conditions. Due to differences in the geochemical behavior of As and Cd, factors such as soil pollution level, soil moisture content, pH, Eh value, and the selection of suitable crops for local conditions all play a key role in the effective remediation strategies for co-contaminated agricultural land. Solving the issue of co-remediation of As and Cd is a major challenge. Existing potential remediation measures, such as water management strategies, ridge cultivation, the use of organic and inorganic soil amendments, and planting crops or varieties that absorb lower levels of As and Cd, can help ensure safe agricultural production on co-contaminated soils. We recommend that policymakers adopt a comprehensive approach of "adjusting measures based on agricultural conditions." This approach should not only consider the As and Cd content and pH in the target soil but also take into account the agricultural planting systems in the target region and the accumulation capacity of the target crops for As and Cd. The preparation process and application of soil amendments need to be screened and optimized to suit specific conditions, achieving the best remediation results. Remediation strategies should evolve and be updated continuously as new research findings emerge.

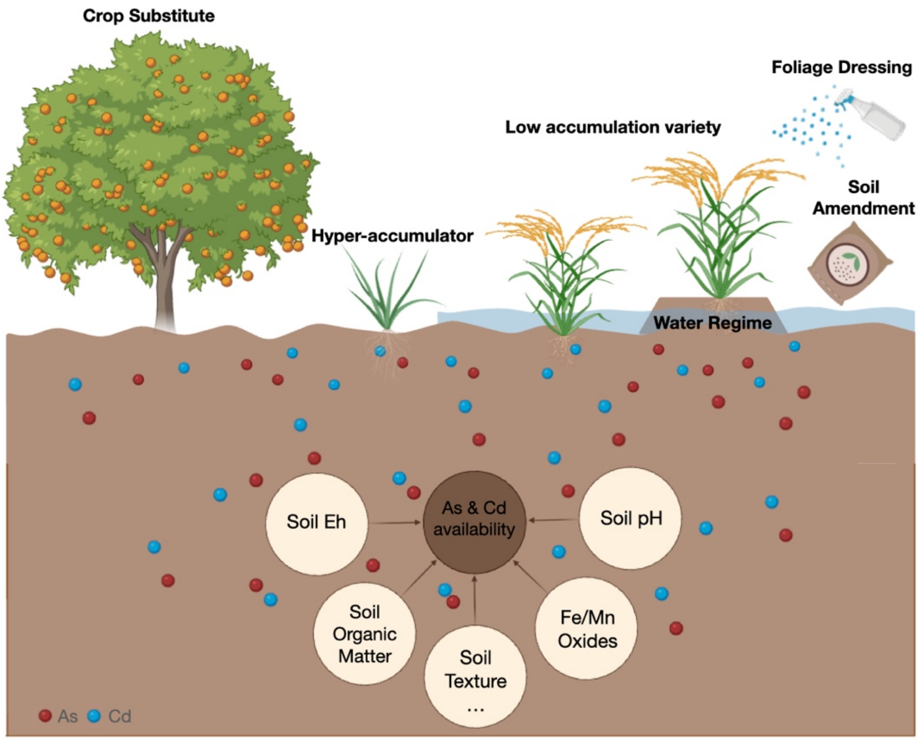


Figure 5-4. Current mitigation strategies for As and Cd in soil and their influencing factors.

This study aims to clarify the complexity of the co-contamination of As and Cd in agricultural soils and provide potential solutions, offering a framework for the future development path of agricultural soil remediation. Overall, ensuring the safe utilization of polluted farmland in the future will require significant effort, and this is worthy of further investigation. Firstly, more attention should be paid to the mechanisms and applications of multiple technologies. Given the regional differences in farmland pollution, it is necessary to classify farmland into different units based on the severity of contamination, adopting targeted technologies accordingly. Therefore, the application scenarios and effectiveness boundaries of different technologies are worthy of further study. Secondly, historical research has mainly focused on decreasing As and Cd bioavalability, often neglecting broader soil quality and health considerations. Remediation strategies should encompass soil improvement and health enhancement, while also reducing heavy metals and promoting improvements in soil quality, health, and carbon sequestration. Thirdly, as the main target audience and implementers of remediation strategies are farmers, there is a need to further optimize and simplify existing technologies to reduce costs or develop more economical new technologies to facilitate their practical application.

# Chapter 6

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# Annexes

**Annexe** 1. Concentration of tested ions in soil solution.

| Day | Treatment | pH | pe | $Ca^{2+}$ ($mg\cdot L^{-1}$) | $Mg^{2+}$ ($mg\cdot L^{-1}$) | $Fe^{2+}$ ($mg\cdot L^{-1}$) | $Mn^{2+}$ ($mg\cdot L^{-1}$) | $Cd^{2+}$ ($\mu g\cdot L^{-1}$) | $SO_4^{2-}$ ($mg\cdot L^{-1}$) | $Cl^-$ ($mg\cdot L^{-1}$) | $CO_3^{2-}$ ($mg\cdot L^{-1}$) | $NO_3^-$ ($mg\cdot L^{-1}$) | DOM ($mg\cdot L^{-1}$) | $As^{III}$ ($\mu g\cdot L^{-1}$) | $As^V$ ($\mu g\cdot L^{-1}$) | As/Cd (mol/mol) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | mCK | 5.46±0.07c | 0.75±0.06a | 2.13±0.88a | 0.37±0.14a | 0.16±0.06a | 0.66±0.15a | 0.39±0.06a | 13.02±1.30b | 1.30±0.35b | 4.65±0.47a | 0.34±0.16b | 10.34±0.61a | 6.94±0.20a | 0.41±0.11a | 28.49 |
| | mR | 4.94±0.03d | 4.42±0.14b | 3.05±0.23a | 0.72±0.09a | 2.69±0.78b | 0.48±0.03a | 0.66±0.04b | 11.33±0.99a | 0.54±0.15a | 6.41±0.26a | 0.03±0.03a | 12.98±0.65b | 27.40±9.52b | 1.07±0.13ab | 64.76 |
| | mR+B | 7.31±0.03a | 0.26±0.83a | 33.58±1.08c | 2.71±0.02b | 0.86±0.28a | 0.15±0.00a | 0.55±0.1ab | 14.31±1.82b | 1.84±0.09b | 46.54±0.88c | 0.00±0.00a | 24.32±1.12d | 16.43±2.40ab | 38.93±4.98c | 150.47 |
| | mR+CMP | 6.83±0.03b | 2.57±0.17ab | 8.70±0.54b | 2.95±0.17b | 0.27±0.01a | 0.54±0.05a | 0.49±0.03ab | 15.78±1.22b | 1.22±0.13b | 22.77±1.18b | 0.51±0.08b | 16.06±0.64c | 65.79±2.21c | 8.96±0.66bc | 228.06 |
| 20 | mCK | 6.68±0.01a | -3.14±0.04a | 1.88±0.06c | 0.36±0.04a | 0.40±0.16a | 0.19±0.07b | 0.07±0.00a | 0.42±0.16a | 0.65±0.09a | 6.01±0.07bc | 0.53±0.03a | 28.06±2.96b | 1.26±0.39ab | 70.15±8.19bc | 1437.13 |
| | mR | 5.37±0.07b | 6.49±0.10d | 0.93±0.01a | 0.17±0.01a | 0.57±0.08a | 0.04±0.01ab | 0.11±0.00a | 6.99±0.28ab | 1.07±0.28ab | 0.02±0.02a | 0.97±0.19a | 27.08±1.50b | 0.72±0.12a | 0.90±0.18a | 22.96 |
| | mR+B | 6.68±0.06a | 3.69±0.07b | 1.53±0.08b | 0.83±0.36a | 0.73±0.19a | 0.02±0.00a | 0.13±0.00b | 11.46±0.22b | 1.88±0.12b | 7.90±0.43c | 15.87±0.27c | 19.16±1.28a | 1.30±0.37ab | 7.78±0.72b | 106.87 |
| | mR+CMP | 6.70±0.06a | 4.99±0.08c | 4.19±0.04d | 1.46±0.02a | 0.77±0.08a | 0.01±0.00a | 0.12±0.02a | 13.67±0.24a | 0.65±0.01a | 1.24±0.07ab | 8.38±0.15b | 24.25±0.89ab | 2.14±0.36b | 3.07±0.59ab | 56.31 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | mCK | 7.20±0.02a | -3.37±0.05a | 1.14±0.06a | 0.27±0.02ab | 1.04±0.15ab | 0.07±0.01b | 0.13±0.00a | 2.95±1.34a | 0.86±0.06a | 3.76±0.12a | 1.08±0.49a | 28.04±3.42c | 3.26±0.60a | 35.66±6.96b | 436.01 |
| | mR | 5.14±0.09c | 6.80±0.09b | 1.63±0.06b | 0.38±0.04b | 1.43±0.29b | 0.04±0.01b | 0.15±0.01a | 9.65±0.46a | 1.34±0.20a | 0.90±0.90a | 3.42±0.1a | 38.54±2.71b | 5.76±0.49b | 0.73±0.50a | 65.52 |
| | mR+B | 5.64±0.08b | 4.94±0.10c | 2.45±0.05c | 0.21±0.00a | 0.21±0.06a | 0.01±0.00a | 0.14±0.01a | 18.33±0.61a | 2.16±0.04a | 5.31±0.30b | 43.53±1.17c | 22.26±1.58a | 6.14±0.97b | 3.37±0.46ab | 104.08 |
| | mR+CMP | 5.56±0.03b | 6.75±0.21b | 1.49±0.18b | 0.51±0.07b | 0.13±0.01a | 0.01±0.00ab | 0.15±0.00a | 19.61±0.85a | 1.47±0.57a | 0.05±0.01a | 37.93±0.85b | 16.81±1.14a | 4.60±0.45ab | 2.20±0.60a | 68.67 |

Note: Data are mean ± SE (n = 3). Different letters represent significant differences ($P < 0.05$, LSD test or Kruskal-Wallis test) between the treatments and the control of the same rice variety.

# Appendix

# Appendix

## *List of Publications*

**Zhang, T.**, Jiku, M.A.S., Li, L., Ren, Y., Li, L., Zeng, X., Colinet, G., Sun, Y., Huo, L. and Su, S., 2023. Soil ridging combined with biochar or calcium-magnesium-phosphorus fertilizer application: Enhanced interaction with Ca, Fe and Mn in new soil habitat reduces uptake of As and Cd in rice. *Environmental Pollution*, *332*, p.121968.

**Zhang, T.**, Sun, Y., Parikh, S.J., Colinet, G., Garland, G., Huo, L., Zhang, N., Shan, H., Zeng, X. and Su, S., 2024. Water-fertilizer regulation drives microorganisms to promote iron, nitrogen and manganese cycling: A solution for arsenic and cadmium pollution in paddy soils. *Journal of Hazardous Materials*, *477*, p.135244.

Mabagala, F.S., **Zhang, T.**, Zeng, X., He, C., Shan, H., Qiu, C., Gao, X., Zhang, N. and Su, S., 2024. A review of amendments for simultaneously reducing Cd and As availability in paddy soils and rice grain based on meta-analysis. *Journal of Environmental Management*, *366*, p.121661.

Ren, Y.X., Geng, Z.X., Song, N.N., **Zhang, T.**, Zhang, N., Wu, C.X., Liu, W., Zeng, X.B. and Su, S.M., 2024. Screening for the Priority Crops in Over-standard Planting Region Based on the Difference in Arsenic and Cadmium Accumulated in Crops. *Huan jing ke xue= Huanjing kexue*, *45*(11), pp.6654-6664.