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Influence of aged silicon-rich biochars (Si-chars) on leaf Cd accumulation in pakchoi (*Brassica rapa subsp. chinensis*): a pyrolysis temperature-dependent response

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Abstract

Silicon-rich biochars (Si-chars) have demonstrated effectiveness in heavy metal remediation. However, the evolution of their functionality during environmental aging remains poorly understood. Here, we investigated the effects of artificial aging on rice husk-derived Si-chars pyrolysis at 300 °C, 500 °C, and 700 °C, further evaluating their efficacy in mitigating cadmium (Cd) toxicity in soil-pakchoi systems. Aging induced a temperature-dependent response, which reduced the performance of high-temperature Si-chars but acted as an activation process for low-temperature variants. Notably, aged 300 °C Si-char exhibited the strongest suppression of Cd accumulation in pakchoi leaves, reducing concentrations by 27.1% and 15.6% compared to the control and non-aged 300 °C Si-char, respectively. This effect was attributed to the aging-induced release of bioavailable Si (ASi) and high-molecular-weight dissolved organic matter (DOM), both of which could interact with Cd to reduce its bioavailability in the amended soils. Meanwhile, ASi and DOM promoted the activities and enrichment of Cd-immobilizing bacteria. Furthermore, Si-char amendment enhanced Si deposition in pakchoi leaves, triggering a molecular defense network characterized by the down-regulation of Cd transporter genes and the up-regulation of stress-responsive pathways. Our findings establish a novel framework in which feedstock Si concentration and initial pyrolysis temperature jointly drive the functional evolution of biochar in the environment.

Highlights

- Aging enhances Cd remediation by adding Si-chars though the effect depends on pyrolysis temperature.
- Aged Si-chars, particularly A-RH300, effectively reduce leaf Cd accumulation via releasing ASi and DOM.
- NAged Si-chars enrich soil Cd-immobilizing bacteria and activate leaf molecular defense via enhanced Si deposition.

Keywords Biochar aging, Cd remediation, Bioavailable Si, Plant molecular defense, Soil microbiome

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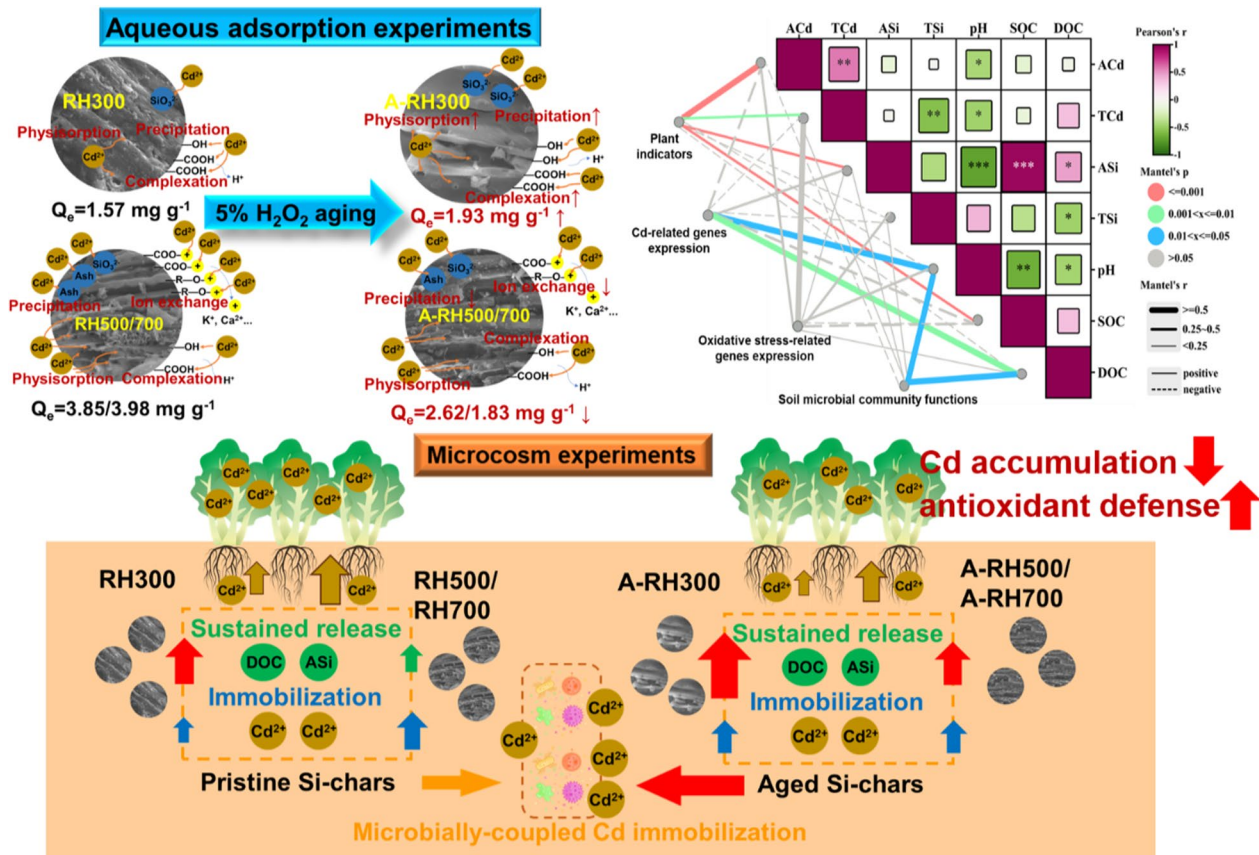
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Graphical Abstract



1 Introduction

Cadmium (Cd) contamination in agricultural soils poses a significant global threat to environmental safety and public health (Qi et al. 2025). Due to its high persistence and bioavailability, Cd disrupts nutrient uptake, inhibits plant growth, and induces oxidative stress, thereby compromising crop yield and quality (Peera Sheikh Kulsum et al. 2023; Zulfiqar et al. 2022). Therefore, developing effective strategies for Cd immobilization in soils is crucial for sustainable agriculture. Biochar, a carbon-rich material derived via biomass pyrolysis, has attracted considerable attention as a soil amendment for heavy metal (HM) remediation, owing to its high specific surface area, porous structure, abundant functional groups, and alkalinity (Fang et al. 2021). These physicochemical properties facilitate the effective immobilization of HMs, including Cd (Lian And Xing 2017), highlighting biochar as a promising tool for contaminated soil management.

However, once applied to soils, biochar inevitably undergoes aging processes driven by oxidation,

acidification, and microbial activity, which substantially alter its physicochemical properties and its capacity to immobilize HMs (Ke et al. 2023). As illustrated by previous studies, HM bioavailability following biochar application is highly dependent on aging, which itself is modulated by feedstock type and pyrolysis conditions. For instance, after six months of aging, sugarcane straw biochar pyrolyzed at 750 °C decreases sedimentary arsenic (As) bioavailability by 21%, whereas biochar produced at 550 °C increases it by 17%, likely due to differences in soluble metal-complexing organic compounds (Soares et al. 2022). Similarly, following a 180-day incubation, cattle manure biochar outperformed grain husk biochar in Zinc (Zn) immobilization through enhanced formation of organo-mineral coatings that facilitate metal retention (Kumar et al. 2018).

Silicon-rich biochars (Si-chars) have recently gained attention for their multifunctional role in remediating HM-contaminated soils. Silicon (Si) contributes to Cd immobilization via Si–O complexation and silicate

mineral precipitation (Huang et al. 2020; Wang et al. 2020b), while plant Si uptake promotes plant resilience to abiotic stress (Sui et al. 2020; Wang et al. 2019). Consequently, Si dissolution serves as a key indicator of Si-char remediation potential (Li And Delvaux 2019; Xiao et al. 2014). Previous studies have demonstrated that as pyrolysis temperature increases from 250 to 700 °C, the progressive decomposition of carbon matrix leads to greater exposure of siliceous structures, thereby enhancing Si release (Wang et al. 2019; Xiao et al. 2014), indicating that the C-Si architecture and degree of carbonization govern Si solubility. We propose that environmental aging may further amplify the efficacy of Si-chars in reducing Cd bioavailability and plant uptake, which is attributed to the mechanisms such as carbon matrix degradation, physical fragmentation, and increased exposure of reactive sites and soluble Si. Nevertheless, critical knowledge gaps exist in understanding how pyrolysis temperature and aging interact to regulate Si release, and how bioavailable Si influences Cd translocation and molecular responses in soil–plant systems.

To address these questions, this study systematically investigated the effects of artificial aging on rice husk-derived Si-chars produced at 300, 500, and 700 °C. We tested three hypotheses: (i) aging-induced alterations in Si-char properties and Cd adsorption capacity are pyrolysis temperature-dependent; (ii) aging enhances the release of bioavailable Si (ASi) and dissolved organic matter (DOM), thereby reducing Cd bioavailability in soil–plant systems; and (iii) released Si and DOM alleviate Cd phytotoxicity via activating molecular defense pathways in plant leaves. Integrating batch adsorption experiments, pot trials, soil microbial community profiling, and leaf transcriptomics, we elucidate the dynamic interactions between aged Si-chars and Cd behavior across the soil–plant systems. Our findings provide some novel mechanistic insights into the aging-driven functional evolution of Si-chars, supporting their optimized and sustainable deployment in Cd-contaminated agroecosystems.

2 Materials and methods

2.1 Preparation and characterization of pristine and aged Si-chars

Rice husk, a naturally abundant Si-rich biomass, was pyrolyzed at 300, 500, and 700 °C to produce Si-chars with varying degrees of carbonization and Si speciation. Pyrolysis was conducted in a muffle furnace (SX-G07102, Zhonghuan Furnace Co., Ltd., China) under oxygen-limited conditions at a heating rate of 5 °C min⁻¹ with a 2-h holding time. The resulting Si-chars were repeatedly washed with deionized water until the filtrate pH stabilized, oven-dried at 65 °C, and ground to pass through a 100-mesh sieve. A subset of each initial Si-char was

subjected to artificial aging to simulate oxidative weathering (Jing et al. 2018; Mia et al. 2017). Briefly, biochar samples were mixed with 5% hydrogen peroxide (H₂O₂) at a solid-to-liquid ratio of 1:30 (w/v) and continuously stirred for 72 h at 80 °C. The aged materials were then thoroughly rinsed with deionized water to remove residual H₂O₂, dried at 65 °C to constant weight, and sieved through a 100-mesh screen. The initial and aged samples were designated as RH300, RH500, RH700 and A-RH300, A-RH500, A-RH700, respectively. Background Cd concentrations in all biochar samples were below the detection limit of inductively coupled plasma mass spectrometry (ICP-MS, Elan DRC-e, Perkin Elmer, USA), following digestion with aqua regia-perchloric acid.

Comprehensive physicochemical characterization was performed on both initial and aged Si-chars. Surface morphology and elemental composition were examined using field-emission scanning electron microscopy (SEM, JSM-7800F, JEOL, Japan) equipped with energy-dispersive spectroscopy (EDS). Specific surface area and pore structure were determined via the multipoint Brunauer-Emmett-Teller (BET) method using a surface area and pore size analyzer (ASAP 2460, Micromeritics, USA). Surface functional groups were identified by Fourier-transform infrared spectroscopy (FTIR, TENSOR 37, Bruker, Germany). Chemical states of surface elements before and after Cd adsorption were analyzed by X-ray photoelectron spectroscopy (XPS, Escalab 250Xi, Thermo Scientific, USA). Bulk elemental composition (C, N, H, O) was measured using an elemental analyzer (Lee-man EA3000, Italy). Total Si concentration was determined by the molybdenum blue colorimetric method, with absorbance measured at 700 nm using a UV–vis spectrophotometer (T6 New Century, Persee, Beijing, China) (Liu et al. 2022). Biochar pH was measured in a 1:20 (w/v) suspension using a calibrated pH meter.

2.2 Batch adsorption experiments for Cd(II) removal

Batch experiments were conducted to evaluate the Cd(II) adsorption capacity of initial and aged Si-chars. A stock Cd(II) solution (1000 mg L⁻¹) was prepared by dissolving analytical-grade Cd(NO₃)₂·4H₂O in deionized water. For adsorption isotherms, 0.1 g of Si-char was added to 20 mL of Cd(II) solutions with initial concentrations ranging from 0 to 50.0 mg L⁻¹ in centrifuge tubes. Suspensions were shaken at 200 rpm for 2 h at 25 ± 1 °C. After equilibration, samples were centrifuged and filtered through 0.22-μm membranes. For kinetic studies, an initial Cd(II) concentration of 20 mg L⁻¹ was used, with contact times varying from 5 to 720 min under identical conditions. All treatments were performed in triplicate. Residual Cd(II) concentrations in the supernatants were quantified by ICP-MS. Adsorption capacity and model

fitting parameters were derived accordingly (see Table S1 for details).

2.3 Pot experiments

A greenhouse pot experiment was conducted to assess the effects of initial and aged Si-chars on Cd dynamics in planted and unplanted soils. Agricultural loam soil was collected from Xiqing District, Tianjin, China (116°54′14″E, 39°4′6″N), air-dried, and sieved (<2 mm). The native total Cd concentration was $0.17 \pm 0.02 \text{ mg kg}^{-1}$ (determined by aqua regia-perchloric acid digestion and ICP-MS). The soil was spiked with $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ to achieve a targeted Cd concentration of 5 mg kg^{-1} and pre-incubated for two weeks to allow equilibration. Seven treatments were established in triplicate: (i) unamended control (CK), (ii–iv) three initial Si-chars (RH300, RH500, RH700), and (v–vii) their aged counterparts (A-RH300, A-RH500, A-RH700). Each Si-char was homogeneously incorporated into 200 g of Cd-contaminated soil at a rate of 1% (w/w) and transferred to plastic pots (6 cm diameter, 8 cm height). For the planted treatment, six pakchoi (*Brassica rapa subsp. chinensis*) seeds were sown per pot immediately after soil amendment. Seedlings were thinned to three uniform plants per pot one week after germination. Pots were maintained in a controlled greenhouse environment (22 °C, 12-h light/12-h dark photoperiod) for 45 days. Soil moisture was maintained at 65% of water holding capacity (WHC) through daily watering with deionized water. Basal nutrients were supplied uniformly at rates of 100 mg N kg^{-1} , 80 mg P kg^{-1} , and 100 mg K kg^{-1} using a standard nutrient solution.

2.4 Sampling and analytical methods

2.4.1 Plant sampling and analysis

One day before harvest, a subset of fresh pakchoi leaves was collected for physiological and transcriptomic analyses. Malondialdehyde (MDA) concentration, catalase (CAT) and peroxidase (POD) activities were measured to evaluate oxidative stress and antioxidant responses. Leaf tissues were homogenized in ice-cold phosphate buffer (1:10 w/v) and centrifuged at 8000 rpm for 10 min at 4 °C. Supernatants were analyzed using commercial assay kits (Beijing Solarbio Science & Technology Co., Ltd.) following the manufacturer's instructions. For transcriptomic profiling, additional leaf samples were flash-frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Total RNA was isolated and sequenced on an Illumina NovaSeq platform (Majorbio, Shanghai, China). Plants grown in unamended Cd-contaminated soil served as the control group. Comparative analyses between initial and aged Si-chars treatments were conducted to assess the effect of aging on transcriptomic responses. At harvest,

whole plants were carefully uprooted, separated into shoots and roots, rinsed with deionized water, and oven-dried to constant weight. Dry biomass was recorded for each tissue. Dried samples were ground, and leaf powders were digested with aqua regia-perchloric acid. Total Cd and Si concentrations in the digestates were quantified by ICP-MS.

2.4.2 Soil chemical analysis

Post-harvest soil samples were air-dried, sieved (<2 mm), and analyzed for key chemical properties. Soil pH was measured in a 1:2.5 (w/v) soil-to-water suspension. Total Cd and Si concentrations were determined after aqua regia digestion. Bioavailable Cd and Si were extracted using 0.01 M CaCl_2 solution (1:30 w/v) and quantified by ICP-MS and the molybdenum blue method, respectively (Huang et al. 2020; Liu et al. 2024b). Soil organic carbon (SOC) was analyzed via the dichromate-sulfuric acid oxidation method (Liu et al. 2024b). Dissolved organic carbon (DOC) and dissolved organic matter (DOM) were extracted at a 1:4 (w/v) soil–water ratio. DOC was quantified using a TOC analyzer (Multi N/C3100, Analytik Jena AG, Germany). DOM composition was characterized by excitation-emission matrix (EEM) fluorescence spectroscopy (HITACHI, F-7000, Japan).

2.4.3 Soil microbial community analysis

Fresh soil samples were collected at harvest and stored at −80 °C for microbial DNA extraction, with unamended Cd-contaminated soil as the control. The V3-V4 hyper-variable region of the bacterial 16S rRNA gene was amplified and sequenced on an Illumina platform. DNA extraction, PCR amplification, and sequencing followed established protocols (Liu et al. 2023b; Zhang et al. 2022).

2.5 Statistical analysis

All data are presented as mean $\pm$ standard deviation (SD). Significant differences among treatments were assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) using SPSS 17.0. EEM fluorescence data were visualized using OriginPro 2021, and fluorescence intensities across five characteristic DOM regions were calculated using MATLAB R2018b (Liu et al. 2024b). Redundancy analysis (RDA) was performed in R (v4.0.5) using the vegan package to investigate the linkages between soil properties and microbial community composition. Microbial functional potential was predicted from 16S rRNA gene sequences using the FAPROTAX database. Leaf transcriptomic data were processed on the Majorbio Cloud Platform (www.majorbio.com). Differentially expressed genes (DEGs) were identified using thresholds of adjusted p -value < 0.001 and $|\log_2\text{FC}| > 2$. Gene Ontology (GO)

and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed on DEG sets. Heatmaps illustrating microbial functional profiles and gene expression patterns were generated using OriginPro 2021. Pearson correlation analysis was used to evaluate relationships between soil physicochemical properties and plant physiological parameters.

3 Results and discussion

3.1 Aging influence on the physicochemical properties of Si-chars

The physicochemical properties of Si-chars before and after aging are summarized in Table S2. All initial Si-chars exhibited alkaline pH values, ranging from 8.5 (RH300) to 11.0 (RH700). The increase in alkalinity with pyrolysis temperature (≥ 500 °C) is attributed to elevated ash concentrations and the thermal decomposition of acidic functional groups during pyrolysis (Zhao et al. 2018). Aging induced a significant pH decrease in all Si-char materials, with the RH300 exhibiting the most pronounced shift, from mildly alkaline (pH 8.45) to distinctly acidic (pH 5.06). This acidification primarily results from the generation of oxygen-containing acidic functional groups (e.g., $-\text{COOH}$, $-\text{OH}$) via H_2O_2 oxidation and the leaching of alkaline minerals such as carbonates (Liao et al. 2023). Elemental analysis reveals that

higher pyrolysis temperatures yielded Si-chars (RH500 and RH700) with increased C and Si concentrations but decreased O and H concentrations. Correspondingly lower O/C and H/C ratios indicate a greater aromaticity and thermal stability in these high-temperature Si-chars compared to RH300 (Huang et al. 2020). Following aging, a slight increase in H concentration was observed in all biochars (Table S2), which is likely due to the incorporation of H atoms during oxidation. Notably, Si concentrations remained largely unchanged by aging, indicating a strong resistance of siliceous-components to oxidative degradation. This observation was also supported by SEM–EDS analysis, which revealed a notable increase in Si signal intensity after the aging, particularly for A-RH300 (Fig. 1). The chemical stability of these Si-based phases enables them to resist environmental degradation, therefore reinforcing their potential as durable sites for HM immobilization.

Pore structure was significantly influenced by pyrolysis temperature and aging (Table S2). Among the initial Si-chars, RH500 and RH700 possessed substantially larger specific surface areas (SSAs) and microporosity than RH300, providing more accessible binding sites for Cd(II). The aging process profoundly altered the porosity of the low-temperature Si-char, as illustrated by our observation that the SSA of RH300 increased

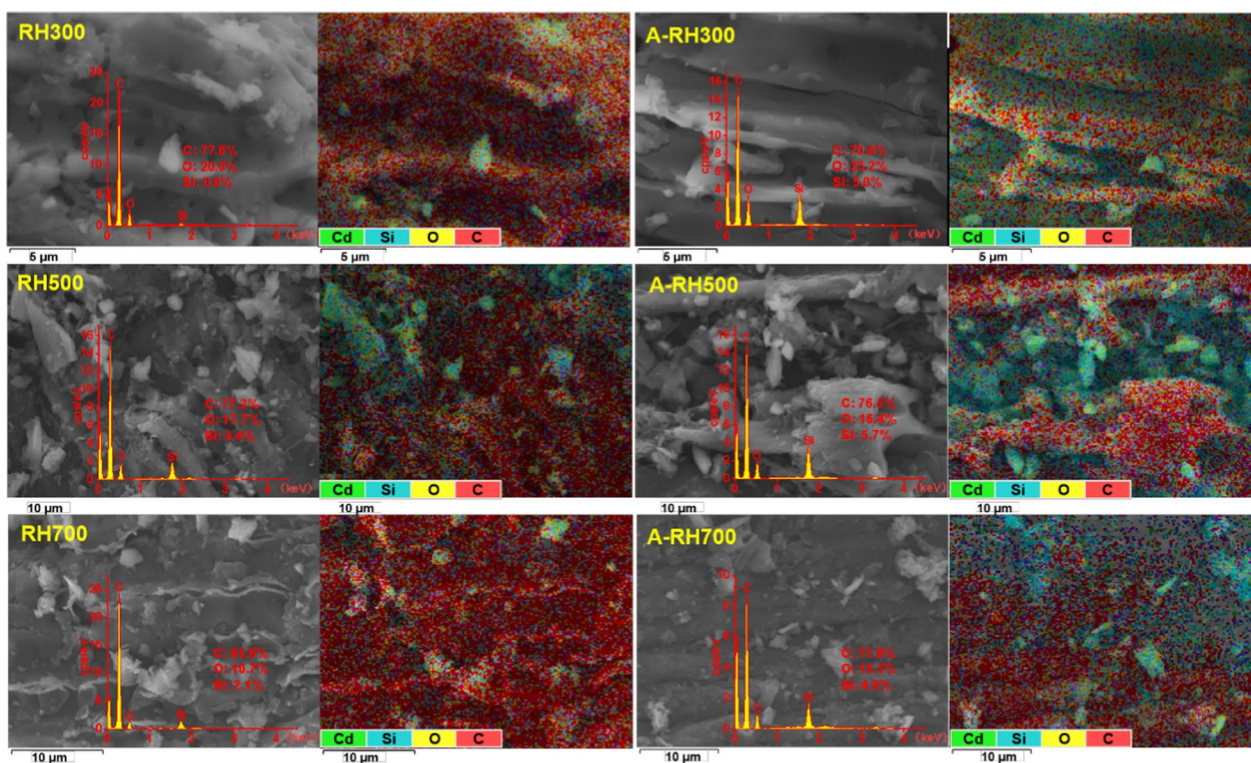


Fig. 1 SEM images and EDS elemental mapping of initial (RH300, RH500, RH700) and aged (A-RH300, A-RH500, A-RH700) Si-chars

approximately 15-fold (from 4.45 to 69.96 $\text{m}^2 \text{g}^{-1}$). This enhancement is likely due to the oxidative decomposition of unstable organic components that were formed at low pyrolysis temperature, leading to pore expansion and surface restructuring (Guo and Chen 2014; Xiao et al. 2014). SEM imaging confirmed these morphological changes. For instance, the surface of RH300 transformed into a more porous, flake-like architecture after the aging (Fig. 1), suggesting that oxidative aging can activate the surface of low-temperature Si-chars (Wang et al. 2020a).

3.2 Aging impact on aqueous Cd(II) adsorption capacity

The Cd(II) adsorption capacities of Si-chars were strongly influenced by both pyrolysis temperature and aging status (Fig. 2). Among the initial materials, high-temperature Si-chars (RH500 and RH700) exhibited significantly greater adsorption capacities (9.28 and 9.73 mg g^{-1} , respectively) than the RH300 (1.34 mg g^{-1}). However, aging induced a temperature-dependent contrasting effect, as it markedly reduced the adsorption

performance of RH500 and RH700 by 81.0% and 89.6%, respectively, while doubling the capacity of RH300. This divergence underscores the importance of initial pyrolysis temperature in determining the aging response of Si-chars. To elucidate the underlying mechanisms, kinetic and isotherm models were applied. Adsorption kinetics were best described by the pseudo-2nd-order model, as it provided an excellent fit ($R^2 > 0.90$) for RH700 and A-RH700 and a comparatively better fit than the pseudo-1st-order model for all other Si-chars (Table 1). This indicates that the chemisorption mediated by surface functional groups and active sites likely governs Cd(II) adsorption (Yu et al. 2021). Predicted equilibrium capacity (Q_e) from this model also agrees well with experimental values at 720 min (Table 1). Isotherm analysis showed that the Langmuir model offered the best fit for most samples, suggesting monolayer adsorption onto relatively homogeneous surfaces (Li et al. 2022). Consistent with experimental trends, the aging significantly reduced the theoretical maximum adsorption capacities

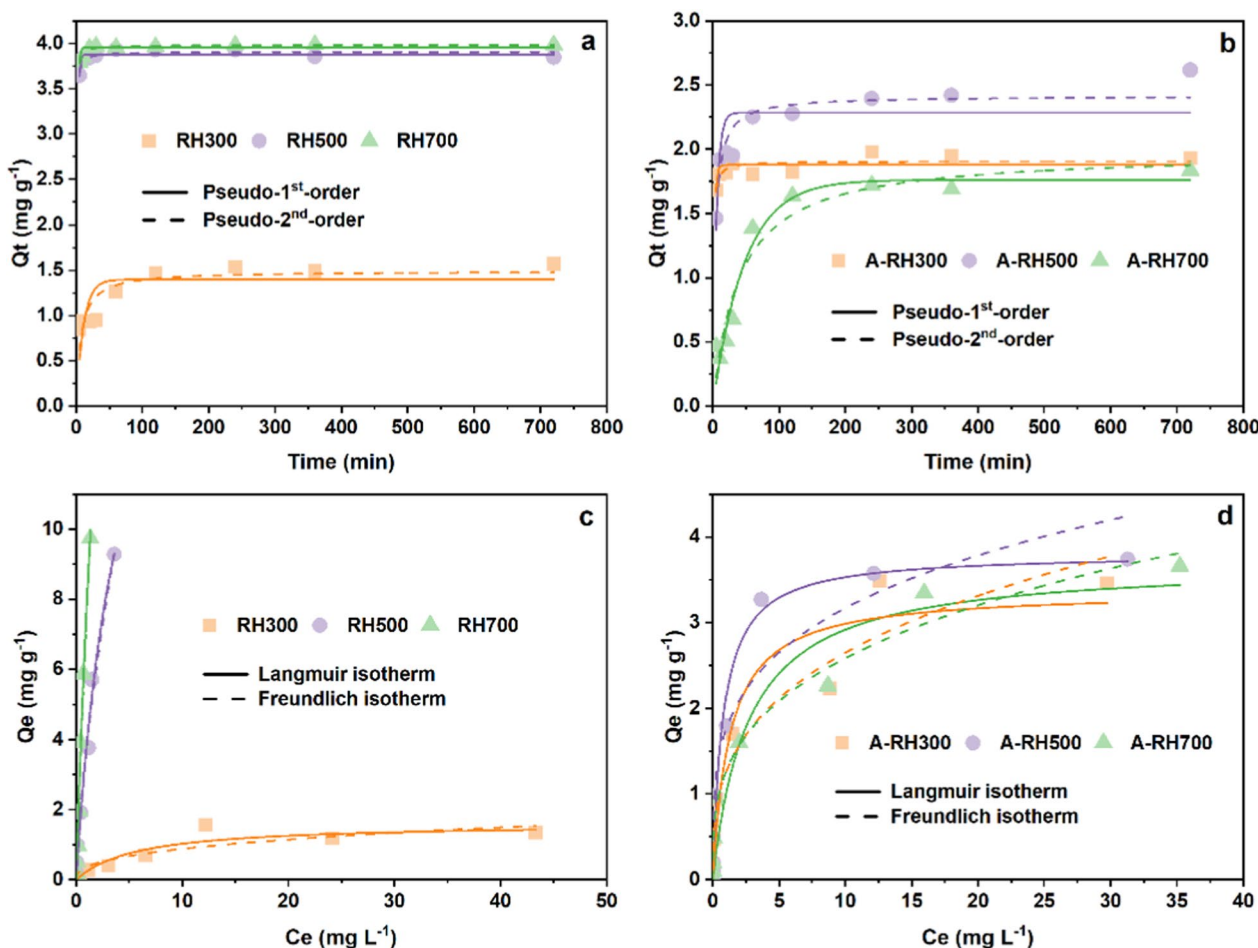


Fig. 2 Kinetics and isotherms of Cd(II) adsorption onto initial and aged Si-chars: **a–b** adsorption kinetics over 720 min, and **c–d** equilibrium isotherms across initial Cd(II) concentrations of 0.5–50 mg L^{-1}

Table 1 Kinetic and isotherm parameters for Cd(II) adsorption by initial (RH) and aged (A-RH) Si- chars achieved from 300 °C, 500 °C and 700 °C pyrolysis

Biochar	Pseudo-1st-order			Pseudo-2nd-order		
	Q_e (mg g ⁻¹)	k_1 (min ⁻¹)	R^2	Q_e (mg g ⁻¹)	k_2 (g mg ⁻¹ min ⁻¹)	R^2
RH300	1.40	0.09	0.39	1.49	0.10	0.72
RH500	3.88	0.56	0.77	3.91	0.80	0.79
RH700	3.96	0.62	0.61	3.98	0.92	0.93
A-RH300	1.88	0.44	0.45	1.91	0.77	0.64
A-RH500	2.29	c	0.63	2.42	0.12	0.86
A-RH700	1.76	0.02	0.94	1.98	0.01	0.94
Biochar	Langmuir isotherm			Freundlich isotherm		
	Q_m (mg g ⁻¹)	K_L (min ⁻¹)	R^2	K_F	n	R^2
RH300	1.61	0.17	0.88	0.37	2.63	0.81
RH500	20.04	0.24	0.99	3.88	1.45	0.98
RH700	35.53	0.29	0.99	7.81	1.17	0.98
A-RH300	3.37	0.78	0.94	1.26	3.10	0.94
A-RH500	3.81	1.27	0.97	1.76	3.90	0.92
A-RH700	3.70	0.37	0.93	1.27	3.23	0.98

(Q_m) of RH500 (from 20.04 to 3.81 mg g⁻¹) and RH700 (from 35.53 to 3.70 mg g⁻¹), which aligns with the reported susceptibility of high-temperature biochars to oxidative degradation (De la Rosa et al. 2018; Wang et al. 2020a). Conversely, Q_m for RH300 increased from 1.61 to 3.37 mg g⁻¹, corroborating the beneficial effect of aging on its specific surface area.

3.3 Effects of Si-char aging on Cd accumulation and physiological responses in pakchoi leaves

Building on batch adsorption results, we further investigated the performance of Si-chars in a soil–plant system to account for complex interactions with soil minerals, organic matter, and microbiota. Compared to the unamended control, most Si-char treatments significantly reduced Cd accumulation in pakchoi leaves, with the exception of RH500 (Fig. 3a). Aged Si-chars consistently outperformed their initial counterparts, reducing leaf Cd concentrations by 15.6% (A-RH300), 19.9% (A-RH500), and 14.1% (A-RH700) compared to their respective initial treatments. Particularly, low-temperature Si-chars demonstrated superior performance. The initial RH300 reduced leaf Cd by 13.6%, while its aged counterpart (A-RH300) achieved the highest reduction among all treatments at 27.1% relative to the control. This reduction in leaf Cd concentrations was accompanied by the improved plant growth, as evidenced by the significantly increased leaf and root biomass across multiple treatments (Fig. 3c). Si-char amendments also enhanced leaf Si accumulation, with the highest levels observed in

RH300 and A-RH300 treatments, exceeding the control by 63.2% and 46.7%, respectively (Fig. 3b). The strong correlation between reduced Cd accumulation and elevated bioavailable Si release for low-temperature Si-chars supports the hypothesis that Si inhibits Cd translocation via root cell wall fortification (Guo et al. 2018; Liu et al. 2024a) and competition for shared uptake pathways (Wang et al. 2020b).

Si-char amendments also mitigated Cd-induced oxidative stress. Aged Si-chars exhibited greater efficacy in alleviating Cd toxicity than their initial counterparts. Except for RH500, all Si-char additions significantly decreased MDA concentration in leaves. Specifically, A-RH300 and A-RH500 reduced MDA by 22.9% and 25.8%, respectively, compared to their initial counterparts (Fig. 3d), indicating the reduced membrane lipid peroxidation. Additionally, the activities of CAT and POD, which are key enzymes in reactive oxygen species scavenging, were significantly elevated (Fig. 3e, f). In particular, the aged Si-chars induced a substantial increase, as both A-RH300 and A-RH700 increased CAT activity by 42.9% and 52.1%, respectively, compared to their initial treatments. Similarly, A-RH300, A-RH500, and A-RH700 enhanced POD activity by 28.2%, 102.6%, and 53.7% compared to the initial materials. These findings underscore that the aged Si-chars, especially those derived from low-temperature pyrolysis, enhance plant resistance to Cd stress, primarily through the sustained release of bioavailable Si, which plays a more pivotal role in Cd mitigation than surface adsorption alone.

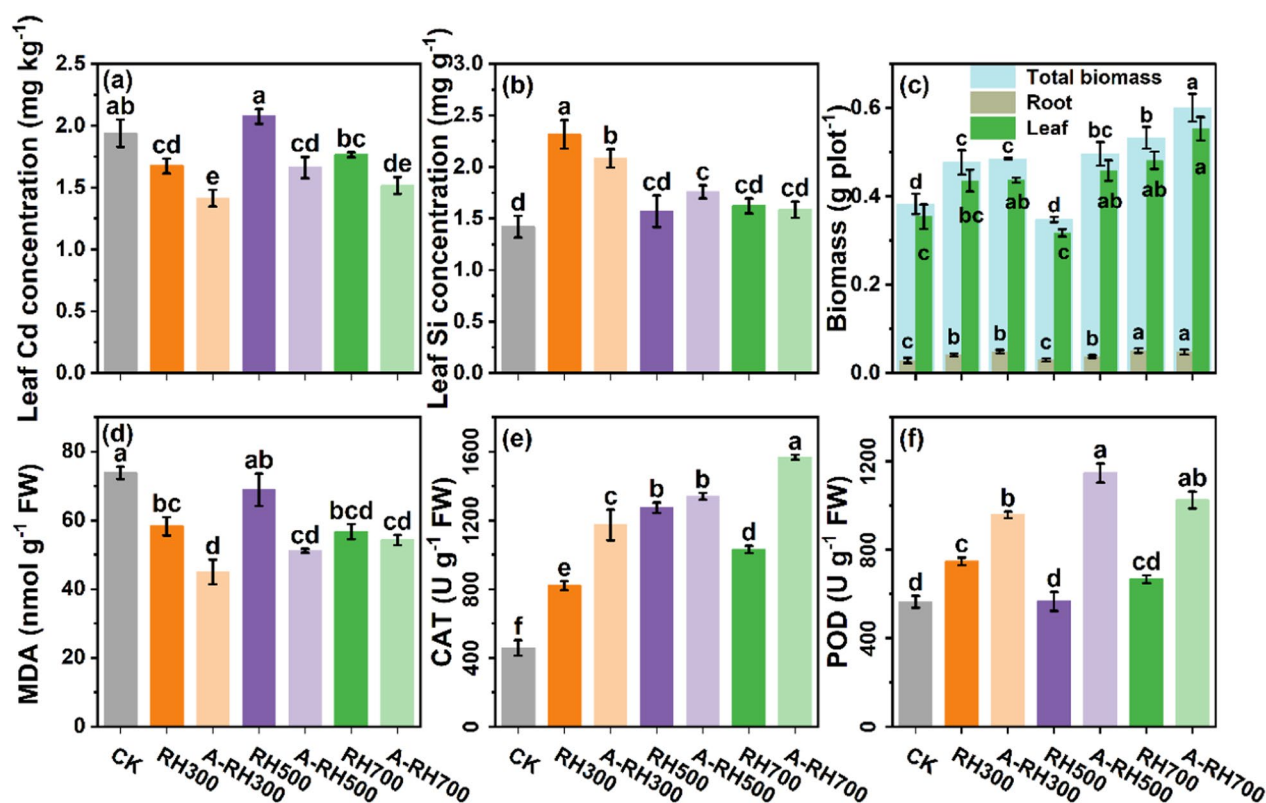


Fig. 3 **a** Leaf Cd concentration, **b** leaf Si concentration, **c** biomass, **d** leaf MDA concentration, **e** leaf CAT activity, and **f** leaf POD activity in pakchoi. Lowercase letters indicate significant differences among Si-char treatments within each planting condition ($p < 0.05$)

3.4 Influence of aging on Cd immobilization mechanisms

To elucidate the mechanisms underlying the observed Cd adsorption behaviors, FTIR and XPS analyses were conducted on initial and aged Si-chars before and after Cd adsorption in aqueous solution. Spectroscopic data revealed that the aging differentially reshaped Cd immobilization pathways in a pyrolysis temperature-dependent manner (Figs. 4 and S1). For low-temperature Si-chars, surface complexation emerged as a dominant mechanism. FTIR spectra of RH300 showed pronounced bands corresponding to hydroxyl ($-\text{OH}$, $\sim 3400 \text{ cm}^{-1}$) and carbonyl ($\text{C}=\text{O}$, $\sim 1697 \text{ cm}^{-1}$) groups, which were less abundant in high-temperature Si-chars due to thermal decomposition (Zhao et al. 2025). Aging intensified these oxygen-containing group signals across all samples, indicating successful surface oxidation (Qian and Chen 2014; Xu et al. 2018). The attenuation of these peaks following Cd adsorption, especially in A-RH300, provides direct evidence that carboxyl and hydroxyl groups serve as primary ligands for Cd complexation, which is a process markedly enhanced by the aging (Liao et al. 2023; Qian and Chen 2014).

Precipitation also contributes substantially to Cd retention. Post-adsorption XPS spectra revealed Cd 3d

peaks characteristic of $\text{Cd}(\text{OH})_2$ and CdCO_3 (Song et al. 2022). Concurrent increases in M-OH (O 1 s) and M-CO_3^{2-} (C 1 s) signals after Cd loading further support the formation of these precipitates (Cai et al. 2021; Liu et al. 2023a; Wu et al. 2024). Additionally, ion exchange plays a notable role in pristine high-temperature Si-chars, as evidenced by the disappearance of K 2p signals in RH500 and RH700 after Cd exposure through cation exchange between Cd^{2+} and K^+ (Selvakumar et al. 2018). However, this pathway is significantly reduced after aging due to leaching of exchangeable cations. Critically, Si-containing functional groups serve as persistent Cd immobilization sites. FTIR confirmed the presence of stable Si-O-Si bonds (~ 1097 , ~ 800 , $\sim 465 \text{ cm}^{-1}$) in all Si-chars (Qian and Chen 2014; Wang et al. 2019). XPS Si 2p and O 1 s spectra showed reduced intensities of C-Si-O and O-Si-O features after Cd adsorption, indicating inner-sphere complexation or silicate precipitation (e.g., CdSiO_3) (Cai et al. 2021). Importantly, these Si-based groups remained chemically intact and reactive even after aggressive oxidative aging, underscoring their potential for long-term HM remediation.

These results reveal that the aging reconfigures Cd immobilization mechanisms in a temperature-dependent

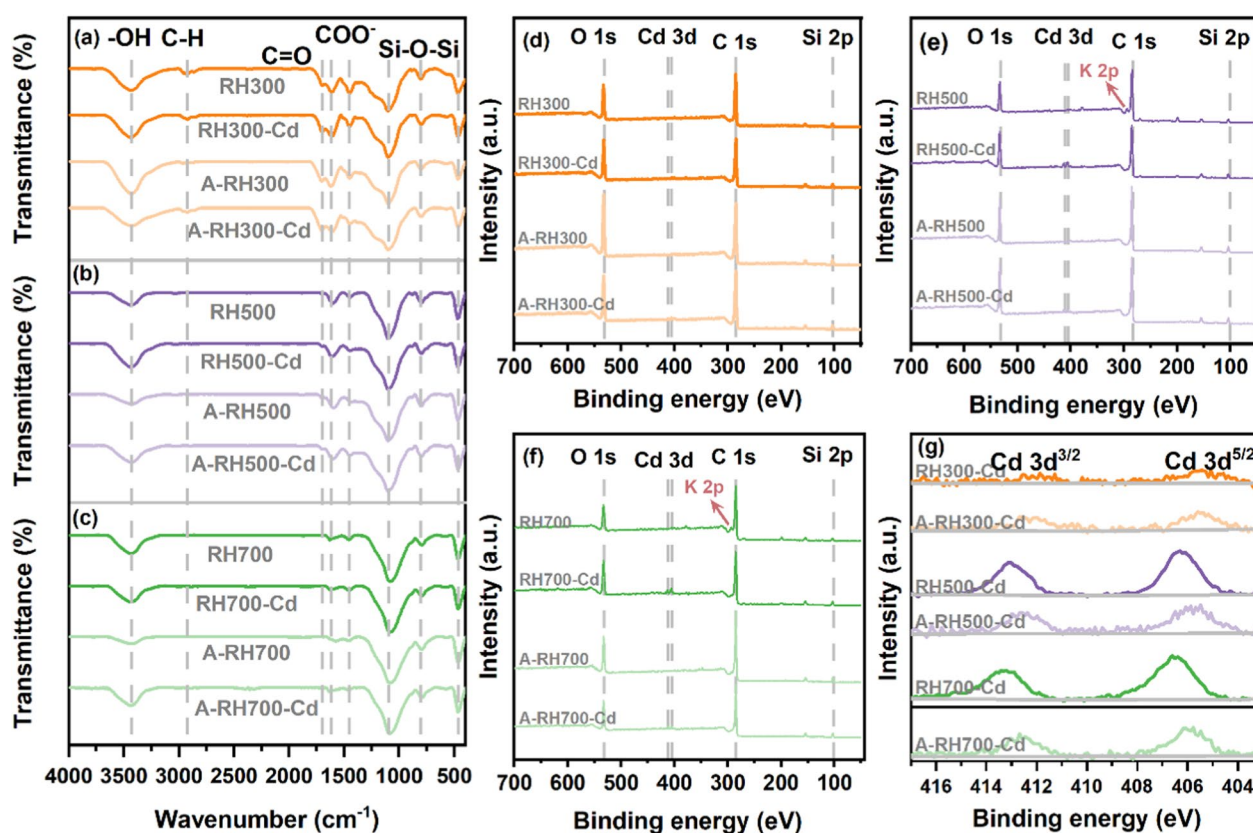


Fig. 4 FTIR spectra **a–c** and XPS survey scans **d–f** of initial and aged Si-chars produced at 300°C, 500°C, and 700°C before and after Cd²⁺ adsorption, and XPS spectra of Cd 3d (**g**) in Si-chars after adsorption reaction

manner. For low-temperature Si-chars, aging enhances Cd adsorption by increasing specific surface area and generating oxygen-containing functional groups, thereby promoting physisorption and complexation. Conversely, aging reduces the performance of high-temperature Si-chars by leaching mineral constituents and exchangeable cations (e.g., K⁺) (Ke et al. 2023). Although Si-based functional groups remain active, the loss of complementary mechanisms leads to an overall decline in adsorption capacity. This dual-response paradigm challenges the prevailing assumption that aging uniformly degrades biochar functionality, providing a refined framework for predicting the environmental behavior of Si-chars.

3.5 Si-char aging modulates soil–plant interactions to reduce Cd accumulation

To elucidate mechanisms underlying the reduced Cd accumulation in pakchoi leaves, we analyzed soil properties after a 45-day pot experiment. Most Si-char treatments significantly decreased soil bioavailable Cd (ACd) compared to the unamended control, with aged Si-chars consistently exhibiting greater efficacy than their initial counterparts (Fig. 5a). Concurrently, all amendments

substantially increased bioavailable Si (ASi) (Fig. 5b), with RH300 and A-RH300 yielding the highest levels, consistent with previous reports that low-temperature Si-chars (<350 °C) retain more soluble and amorphous Si than the crystalline forms prevalent at higher temperatures (Li And Delvaux 2019; Wang et al. 2021; Xiao et al. 2014). Aging further promoted Si release from RH300, likely by exposing embedded siliceous phases through oxidative etching. Critically, low-temperature Si-char treatments yielding the highest ASi levels corresponded to the greatest leaf Si accumulation and lowest Cd concentrations (Fig. 3), underscoring the importance of Si-mediated Cd mitigation. By substantially releasing bioavailable Si, the aging process amplifies its biological Cd mitigation strategy.

Beyond elemental dynamics, Si-char amendments improve the targeted soil health. All treatments enhanced soil buffering capacity, as evidenced by greater pH increases in pakchoi-planted soils ($\Delta\text{pH}=0.40\text{--}0.53$) compared to the planted control ($\Delta\text{pH}=0.26$) (Table S3). This alkalization likely arises from the inherent alkalinity of Si-chars and possible silicate-aluminum co-precipitation (Huang et al. 2020), highlighting their

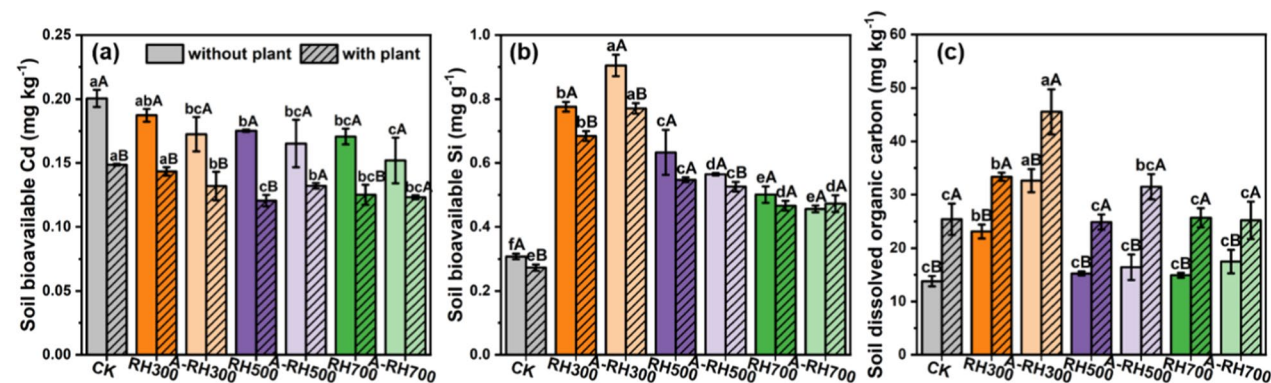


Fig. 5 **a** Bioavailable Cd, **b** bioavailable Si, and **c** dissolved organic carbon in soils with and without pakchoi planting under different Si-char treatments. Lowercase letters indicate significant differences among Si-char treatments within each planting condition ($p < 0.05$), and uppercase letters indicate significant differences between planting conditions under the same amendment ($p < 0.05$)

multifunctional role in stabilizing soil chemistry under plant growth. Furthermore, all treatments significantly increased SOC (Table S3), with low-temperature Si-chars also elevating DOC (Fig. 5c). Fluorescence spectroscopy revealed that low-temperature Si-chars released more DOM than high-temperature variants, while the aging further enhanced DOM release, particularly for the fulvic-like and

humic-like fractions (Figs. 6 and S2). These components are known to immobilize HMs via low-solubility complexation and adsorption onto soil or biochar surfaces (Zhang et al. 2019). Although plant root exudates may partially mobilize Cd through low-molecular-weight organics (Li et al. 2013; Lin et al. 2018), the net effect of Si-char amendments was a reduction in Cd bioavailability and leaf Cd accumulation.

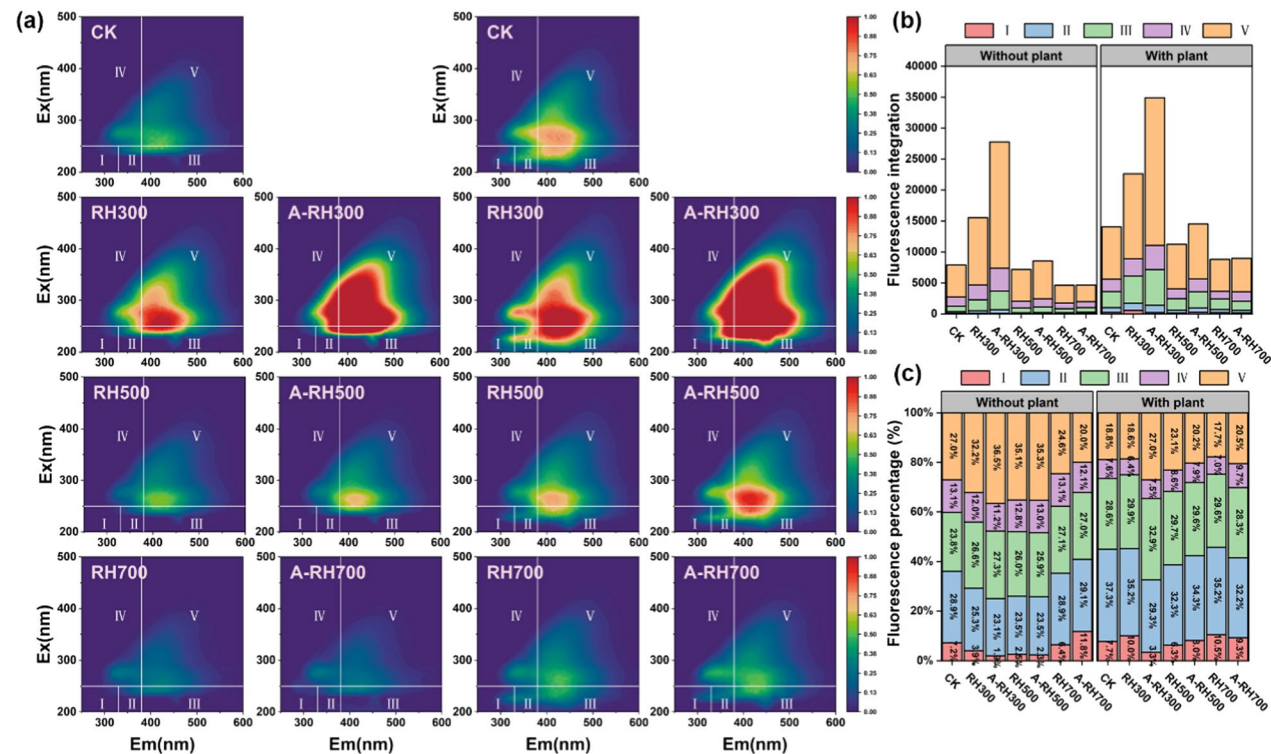


Fig. 6 **a** 3D-EEM fluorescence spectra, **b** fluorescence intensity integrals, and **c** relative contributions of five DOM components in soils with and without plant cultivation under initial and aged Si-char treatments

This indicates that the immobilizing capacity of Si-char-derived DOM overweighs any mobilizing influence of root exudates. Therefore, the aging-enhanced release of high-molecular-weight DOM represents a key mechanism for Cd fixation, even under the plant cultivation.

Linear regression analysis revealed strong negative correlations between leaf Cd accumulation and beneficial soil/plant parameters including ASi, SOC, DOC, and leaf Si concentration (Fig. 7), in agreement with established roles of Si in enhancing HM resistance and plant growth (Das et al. 2021; Lu et al. 2024). Importantly, our study demonstrates that artificial oxidative aging consistently enhances the Cd-mitigation efficacy of Si-chars across all pyrolysis temperatures, suggesting that natural aging processes in the field may further improve their remediation performance.

3.6 Si-char aging modulates soil microbial community structure and function

To evaluate the impacts of Si-char amendments on the soil microbiome, we analyzed bacterial communities in soils and its association with key environmental variables. Given the critical role of ASi in modulating Cd translocation and plant response, we selected initial and aged Si-chars produced at 300 °C and 700 °C for microbial analysis, with the unamended Cd-contaminated soil as the control. Redundancy analysis (RDA) identified ASi as the dominant driver of microbial community composition ($p < 0.05$, $R^2 = 0.994$) (Fig. 8a), with low-temperature Si-char treatments (RH300/A-RH300) strongly associated with elevated ASi.

At the genus level, aged Si-chars enriched several beneficial taxa (Fig. 8b). Compared to the initial counterparts, aged Si-chars promoted the proliferation of Cd-immobilizing genera such as *Arthrobacter*, *Lysobacter*, and *Bradyrhizobium*, known to sequester HM via the processes of biosorption and cell-wall binding (Bianucci et al.

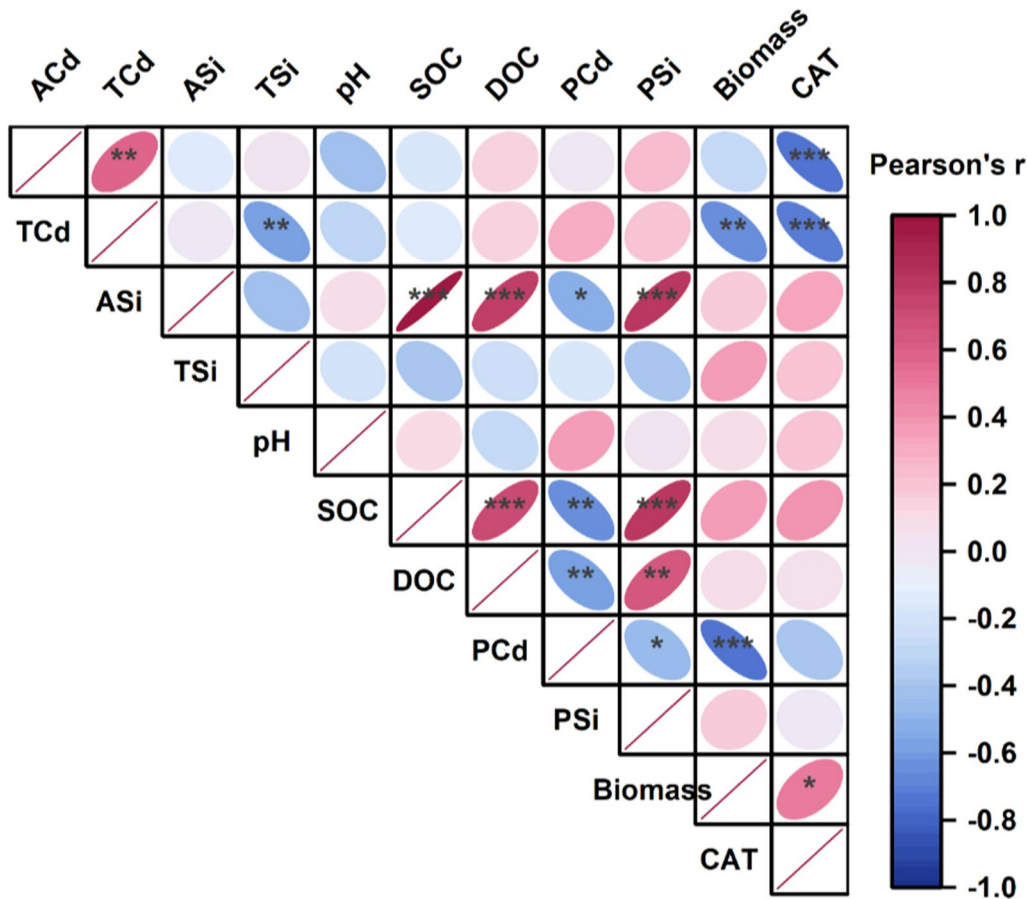


Fig. 7 Correlation heatmap with two-tailed *t*-test linking soil properties and plant physiological parameters (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). ACd, bioavailable Cd; TCd, total Cd; ASi, bioavailable Si; TSi, total silicon; SOC, total organic carbon; DOC, dissolved organic carbon; PCd, plant leaf Cd; PSi, plant leaf Si; CAT, catalase activity

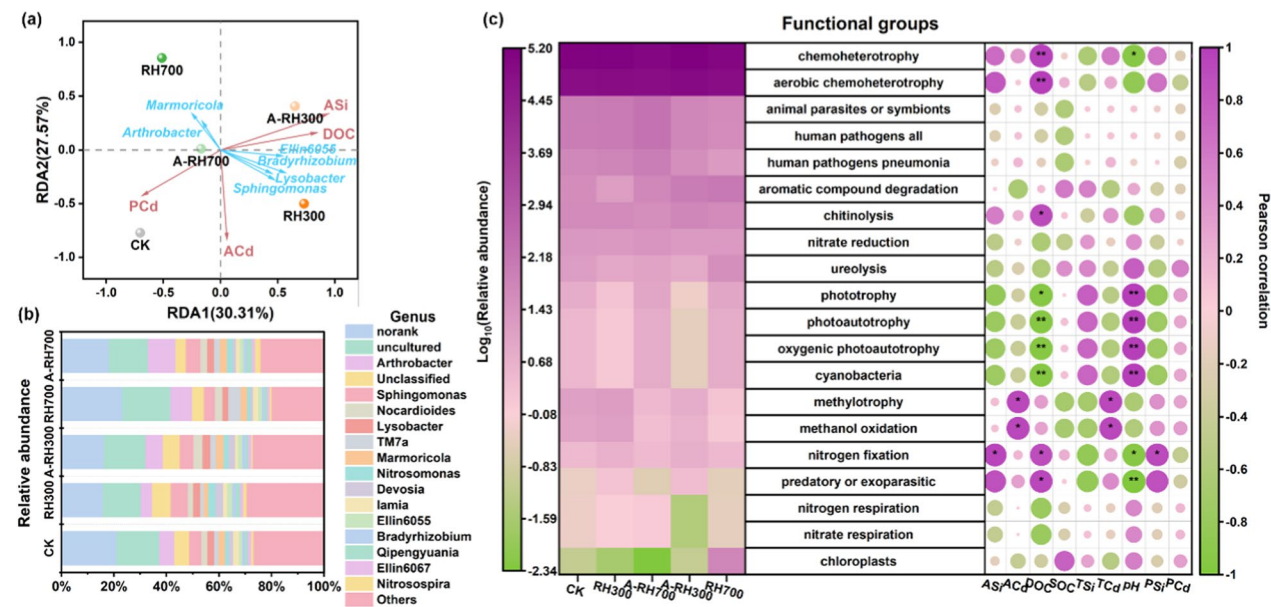


Fig. 8 **a** Genus-level relative abundance of soil bacteria; **b** RDA ordination linking microbial communities to soil/plant parameters; and **c** FAPROTAX-predicted functional profiles and their correlations with environmental variables

2012; Chen et al. 2019; Jin et al. 2023; Wang et al. 2023). Similarly, the amendments also increased the abundance of rhizosphere-colonizing taxa such as *Marmoricola* and *Ellin6055*, which inhibit Cd translocation from soil to plants (An et al. 2024; Sun et al. 2024a). Notably, aged Si-chars enhance the abundance of *Spingomonas*, a genus involved in silicate weathering to promote ASI release (Sun et al. 2024b). Redundancy analysis further confirmed that the alterations in soil properties induced by Si-chars were the key drivers of microbial community change. The abundances of *Arthrobacter* and *Marmoricola* were negatively correlated with ACd, indicating the reduced Cd toxicity and enhanced bacterial Cd accumulation in aged Si-char treatments. In contrast, *Lysobacter*, *Ellin6055*, and *Bradyrhizobium* were positively correlated with ASI and DOC, suggesting a stimulation induced by aged Si-chars.

Functional prediction via FAPROTAX indicated that these microbial shifts altered the key biogeochemical processes related to carbon and nitrogen cycling (Fig. 8c, d). Low-temperature Si-char treatments (RH300/A-RH300) stimulated chemoheterotrophy (C-degradation) but suppressed phototrophy (C-fixation) (Yao et al. 2025), which is strongly correlated with soil DOC dynamics. These treatments also enhanced the potential for nitrogen fixation, as it was positively correlated with both ASI and DOC.

Collectively, these findings demonstrate that aged Si-chars promote a functional microbial community, contributing to Cd mitigation via two synergistic pathways:

- (i) direct enrichment of Cd-immobilizing bacteria, and
- (ii) stimulation of soil nutrient-cycling processes reflective of improved soil ecological health. The enhanced release of ASI and DOC acts as an ecological filter, reshaping a microbiome conducive to plant growth and HM resistance.

3.7 Transcriptomic insights into leaf responses to aged Si-char remediation

While the physiological effects of biochar on plants are well-documented, the molecular mechanisms, particularly transcriptomic responses to aged Si-chars, remain poorly understood. To address this gap, RNA sequencing was performed on pakchoi leaves cultivated in Cd-contaminated soil amended with both initial and aged Si-chars pyrolyzed at 300 °C, 500 °C, and 700 °C. Transcriptomic profiling reveals that Si-char amendments significantly altered gene-expression patterns in pakchoi leaves, with six comparisons conducted: RH300/CK, A-RH300/CK, RH500/CK, A-RH500/CK, RH700/CK, and A-RH700/CK (Fig. S3). GO enrichment analysis showed distinct functional signatures (Fig. S3). RH300/CK upregulated transferase activity and carbohydrate metabolism, whereas A-RH300/CK enriched terms related to gene-expression regulation, photosynthesis, and stress response. RH500/CK primarily affected protein synthesis, while A-RH500/CK shifted toward defense and metabolism. Both RH700/CK and A-RH700/CK enriched stress-response categories, with RH700/CK additionally affecting transcriptional regulation.

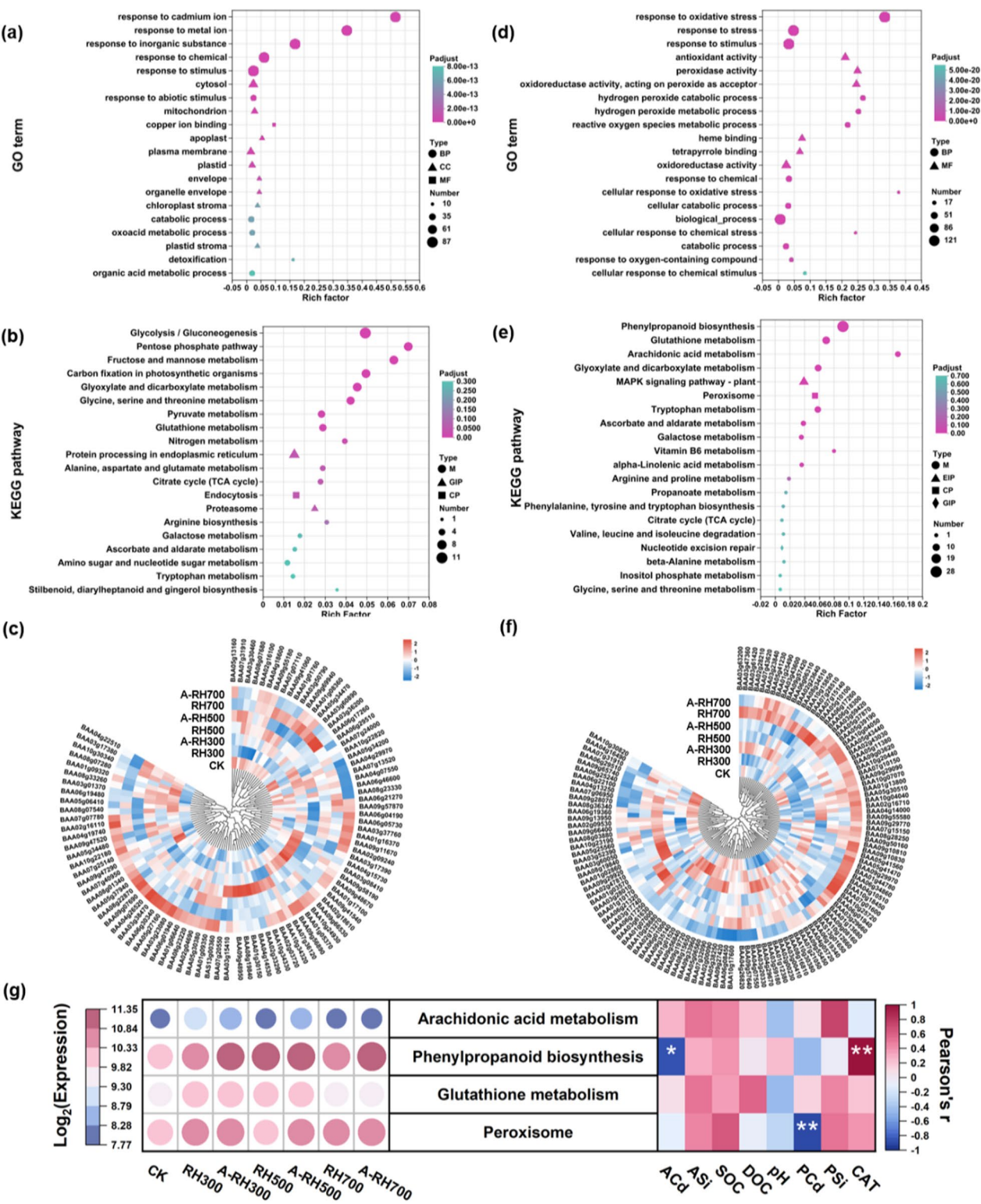


Fig. 9 a–c GO and KEGG enrichment of Cd-related DEGs; d–f oxidative stress-related pathways; and g heatmaps of DEG expression patterns and correlations with soil/plant parameters

To elucidate aging-specific effects, we analyzed three comparative groups including A-RH300/RH300, A-RH500/RH500, and A-RH700/RH700. Cd- and oxidative stress-related DEGs were identified and functionally annotated. Enrichment analyses showed significant enrichment of “response to Cd ion” (GO) and activation of key metabolic pathways including glycolysis/gluconeogenesis, glutathione metabolism, and ascorbate/aldarate metabolism (KEGG) (Fig. 9a, b). At the gene level, most Cd response-related DEGs were markedly up-regulated (Fig. 9c). Crucially, metal transporter genes including *HMA1* and *HMA2* were significantly down-regulated in aged Si-char treatments. This provides transcriptional evidence for restricted Cd translocation to leaf tissues (Guo et al. 2021), corroborating our physiological observations of reduced leaf Cd accumulation. Concurrently, transcriptomic profiling indicates that aged Si-chars enhanced oxidative stress tolerance in leaves. As revealed by functional enrichment analysis, there was a significant enrichment of GO terms associated with antioxidant activity and stress response, alongside activation of upstream KEGG pathways including MAPK signaling and arachidonic acid metabolism (Fig. 9d, e) (Feng et al. 2025; Guo et al. 2021). Expression profiling further elucidates these defense mechanisms (Fig. 9f and Table S4). Notably, DEGs involved in arachidonic acid metabolism were predominantly up-regulated in aged Si-char treatments, indicating reinforced stress signaling and lipid-mediated defense mechanisms. Similarly, most DEGs in the phenylpropanoid biosynthesis pathway exhibit an up-regulation. Given the critical role of this pathway in synthesizing antioxidant and defense-related secondary metabolites (Dong and Lin 2021), its activation can underscore a systemic enhancement of leaf resilience against Cd-induced oxidative stress.

To assess the regulatory role of aged Si-char amendments, we integrated these transcriptomic changes with soil/plant parameters. As illustrated by Fig. 9g, the activity of these key defense pathways exhibited strong positive correlations with beneficial factors including soil ASi, DOC, and plant Si concentration. These findings support the conclusion that the improved soil environment induced by aged Si-chars can directly activate molecular defense networks in leaves, mechanistically explaining their superior efficacy in Cd detoxification.

4 Conclusion

This study demonstrates that artificial aging significantly enhances the efficacy of Si-chars in mitigating Cd toxicity in soil–plant systems. Aged Si-char amendments consistently outperformed their initial counterparts in reducing Cd accumulation in pakchoi leaves. Aging acts as a beneficial activation process that promotes the release

of bioavailable Si and high-molecular-weight DOM, collectively enhancing Cd immobilization in soils. These bioactive substrates subsequently trigger a cascade of beneficial biological responses, including enrichment of Cd-immobilizing and nutrient-cycling microbes, alongside the activation of plant molecular defense networks.

Crucially, initial pyrolysis temperature governs the aging behavior and ultimate functionality of Si-chars. For low-temperature Si-chars, aging serves as a surface-activating process that significantly improving their performance. In contrast, high-temperature Si-chars undergo mineral leaching and functional degradation upon aging. These findings challenge the conventional paradigm that higher pyrolysis temperatures invariably yield superior HM-immobilizing amendments. Instead, we propose a revised framework in which the environmental performance of Si-chars is co-determined by feedstock Si concentration and pyrolysis temperature. While our controlled aging experiments provide valuable mechanistic insights, it should be noted that accelerated aging cannot fully replicate long-term natural weathering processes. Therefore, future validation through long-term field trials is essential to confirm the sustainability and real-world application of these Si-char-based remediation strategies.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s42773-025-00556-x>.

Supplementary Material 1.

Author contributions

Yuting Zhang: Writing—original draft, Investigation, Visualization. Linan Liu: Writing—review and editing, Methodology, Data curation. Yizi Hua: Investigation, Visualization. Zimin Li: Conceptualization, Revision, Writing—review and editing. Xin He: Writing—review and editing, Methodology. Jingmin Sun: Writing—review and editing, Investigation. Jingchun Tang: Writing—review and editing, Funding acquisition, Project administration, Resources. All authors read and approved the final manuscript.

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Data availability

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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