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# Supplying silicon reduces cadmium accumulation in pak choi by decreasing soil Cd bioavailability and altering the microbial community†

Rui Jing,‡[ac] Yao Yu,‡[b] Xuerong Di,[a] Xu Qin,[a] Lijie Zhao,[a] Xuefeng Liang,[a] Yuebing Sun[a] and Qingqing Huang *[a]

Silicon-containing materials have been widely used in Cd-contaminated soil remediation. However, the immobilization effects of sodium silicate on Cd migration and transformation in an acidic soil–vegetable system have not been thoroughly studied. Herein, a pot experiment was performed to investigate the effects of sodium silicate application on pak choi growth, oxidative status, Cd uptake and accumulation in pak choi, soil Cd bioavailability and fractions, and soil bacterial communities. The results showed that sodium silicate application significantly increased soil pH (0.29–1.61 units) and induced the transformation of the Cd fraction from an exchangeable fraction (Exc-Cd) into an iron and manganese oxide-bound fraction (OX-Cd) and organic matter-bound fraction (OM-Cd), decreasing Cd bioavailability by 13.7–20.8% in Cd-contaminated acidic soil. As a result, sodium silicate application significantly alleviated Cd toxicity, enhanced pak choi growth, and reduced Cd concentration in roots by 23.5–89.0% and in shoots by 58.5–81.0%, with Cd concentration in the edible part at a Si application rate equal to or greater than 0.4 g Si per kg soil falling below the safety limits for Cd as defined in China's food safety standard (GB 2762-2022). In addition, sodium silicate application significantly increased soil bacterial richness (Ace index and Chao1) and diversity (Shannon and Simpson index) and altered the soil microbial structure. These findings suggested that sodium silicate has great potential as an environmentally friendly amendment to immobilize Cd-contaminated acidic soil and reduce Cd accumulation in vegetables.

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**Environmental significance**

Soil contamination with Cd has become a major environmental issue for food security and sustainable agriculture in China. Recently, silicon-containing materials have been widely used to remediate Cd-contaminated soil. Pak choi (*Brassica rapa* ssp. *chinensis*) is one of the most consumed leafy vegetables in China, and pak choi generally accumulates more Cd than other types of vegetables, making it a significant contributor to dietary Cd intake. However, up to now, the mechanisms regarding how Si affects Cd migration and transformation in the soil–pak choi system are still poorly understood.

## 1 Introduction

Cadmium (Cd) is one of the most toxic and widespread pollutants in agricultural soil, and as a non-essential element, it compromises various biological functions in humans and plants.[1] Soil Cd can readily be absorbed by plant roots and accumulate in edible parts, eventually entering the food chain and posing risks to human health.[2] Vegetables, as a primary agricultural product, are an essential component of the human diet worldwide, providing essential nutrients like dietary fiber, protein, minerals, and vitamins.[3] However, vegetables also absorb and accumulate various nonessential elements such as heavy metals, and substantial evidence has indicate that vegetables can accumulate Cd over a wide range of concentrations and consumption of such Cd-contaminated vegetables is a significant health risk to humans.[4–6] Currently, Cd pollution in acidic soil in some areas of southern China poses a serious threat to vegetable production.[7] Thus, to ensure food safety and protect human health, effective strategies need to be developed to remediate Cd-contaminated agricultural soil.

Functional metal antagonist fertilizers like silicon (Si) fertilizer and selenium (Se) fertilizer have recently attracted attention as effective Cd mitigation strategies for crops.[8,9] Among these, Si is considered a "multitalented quasi-essential"

[a]*Key Laboratory of Original Agro-Environmental Pollution Prevention and Control, Agro-Environmental Protection Institute, Ministry of Agriculture and Rural Affairs, Tianjin 300191, China. E-mail: huangqingqing@caas.cn*

[b]*School of Resources and Environmental Engineering, Hefei University of Technology, Hefei 230009, China*

[c]*College of Resources and Environment, Northeast Agricultural University, Harbin 150030, China*

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‡ These authors contributed equally to this work.

element for plant growth and has received a lot of attention for its ability to reduce Cd toxicity and accumulation in numerous plant species, including wheat,[10] rice,[11,12] and soybean.[13] In soil, Si can immobilize Cd by increasing soil pH and creating silicate complexes with Cd, thereby reducing soil Cd bioavailability.[14–16] Furthermore, once absorbed by plant root systems from soil, Si can improve plant tolerance to Cd stresses by activating their antioxidative system.[10] In addition, Si in plants can increase Cd retention in roots by enhancing cell wall Cd adsorption capacity to trap more Cd in root cell walls and sequestering more Cd into the vacuole, finally reducing root-to-shoot Cd translocation.[10] Moreover, Si can decrease Cd accumulation in plants by reducing the expression level of genes involved in Cd absorption and transportation.[17] So far, numerous studies have been conducted to study the effect of Si on the alleviation of Cd stress in crops, as well as the mechanism underlying this effect. However, the majority of these studies focused on grain crops such as rice and wheat, while the effects of Si on Cd accumulation in plants differ based on species, with few studies on vegetables. Pak choi (*Brassica rapa* ssp. *chinensis*), one of the most popular leafy vegetables consumed in China, generally accumulates more Cd than other types of vegetables, making it an important source of Cd in the diet.[6,18] However, the mechanisms regarding how Si affects Cd migration, transformation, and accumulation in the soil–pak choi system remain poorly understood. Furthermore, the majority of these studies that have assessed Si as a tool to mitigate plant uptake of Cd have used soils either amended with Cd salts or soils with very high Cd concentrations, which have limited relevance to mild and moderate Cd-contaminated soil. Additionally, soil microorganisms are sensitive to environmental changes and can be used as bio-indicators to assess soil health.[19] Moreover, on the one hand, soil Cd can damage microbial activity by interfering with the function and structure of the microbial community, whereas soil microorganisms can detoxify heavy metals by immobilizing or transforming them.[20] However, the impact of Si on Cd migration and transformation, and the soil microbial community in the soil–vegetable system is currently poorly understood and needs to be investigated further.

In this study, we chose pak choi as our research material to determine whether Si fertilizer applied to soil can reduce Cd concentrations in pak choi grown in a moderate Cd-contaminated soil. The main aims of the study were the following: (1) to investigate the effects of exogenous Si application on pak choi growth, oxidative status, Cd uptake and accumulation in pak choi, Cd bioavailability and fractions in soil, and the soil bacterial community, and (2) to explore the underlying mechanisms involving Si for Cd migration and transformation in the soil–vegetable system. This study will provide theoretical and technological guidelines for safe pak choi production in Cd-contaminated soil.

# 2 Materials and methods

## 2.1 Soil characterization

The soil used in the current study was surface tillage soil (0–20 cm) collected from a moderate Cd-contaminated paddy field in Chenzhou (25°49′36″ N, 112°35′10″ E), Hunan Province, that was polluted from historical smelting and mining. The following were the chemical properties of the tested soil as determined by the methodology of Bao (2000):[21] the soil pH, 6.13; organic matter, 20.1 g $kg^{-1}$; cation exchange capacity, 25.3 cmol $kg^{-1}$; total nitrogen, 1.25 g $kg^{-1}$; available phosphorus, 9.34 mg $kg^{-1}$; available potassium, 183 mg $kg^{-1}$; total Cd, 1.83 mg $kg^{-1}$. Text S1† presents the detailed soil physicochemical analysis. Based on the Soil Environmental Quality Standards of China (GB 15618-2018), the concentration of Cd in the tested soil was 4.57 times greater than the threshold value of 0.4 mg $kg^{-1}$ ($5.5 < pH < 6.5$). After the visible plant residues and stones were removed, the collected soils were air-dried for 14 days, ground thoroughly, and then filtered through a 2 mm sieve for further use.

## 2.2 Experimental design

A pot experiment was conducted in a greenhouse situated in the Nankai District of Tianjin, China. Exogenous Si was applied in the form of solid sodium silicate ($Na_2SiO_3 \cdot 9H_2O$, analytically pure, Macklin Inc., Shanghai, China), with five different doses of Si added into soil including 0.00 g Si per kg soil (no addition of Si, $Si_{CK}$), 0.20 g Si per kg soil ($Si_{0.2}$), 0.40 g Si per kg soil ($Si_{0.4}$), 0.80 g Si per kg soil ($Si_{0.8}$), and 1.0 g Si per kg soil ($Si_{1.0}$). Each treatment was repeated four times, with each pot containing 2 kg of soil. The basic information of sodium silicate were as follows: the soil pH, 12.53; total Cd, 0.05 mg $kg^{-1}$; total Cr, 2.7 mg $kg^{-1}$; total Pb, 0.8 mg $kg^{-1}$; total As, 0.29 mg $kg^{-1}$; and total Hg, 0.003 mg $kg^{-1}$. Based on the conventional vegetable fertilization practices, 0.15 g $P_2O_5$ per kg soil for phosphorus (P) in the form of $CaH_2PO_4 \cdot H_2O$ (analytically pure, Macklin Inc., Shanghai, China), 0.2 g $K_2O$ per kg soil for potassium (K) in the form of KCl (analytically pure, Macklin Inc., Shanghai, China), and 0.2 g N per kg soil for nitrogen(N) in the form of $CO(NH_2)_2$ (analytically pure, Macklin Inc., Shanghai, China) were added as basal fertilizers in the pot experiment. Both exogenous Si and base fertilizers were thoroughly mixed with the tested soil in each pot 14 d prior to pak choi sowing.

Pak choi (*Brassica rapa* subsp. *chinensis*) seeds were immersed in 30% $H_2O_2$ for 30 min before rinsing with deionized water. Then, twenty seeds were initially scattered on the soil surface of each pot, and the seedlings were reduced to five when they reached 5 cm in height. In the period of pak choi growth, deionized water was added to maintain soil moisture at an approximate 75% of its water holding capacity. The growing conditions of pak choi were as follows: a 14 h natural sunlight period per day supplemented with sodium vapor lamps to maintain light intensity >350 μmol $m^{-2}$ $s^{-1}$; 28 °C and 20 °C daytime and nighttime temperatures, respectively; and 60–70% relative humidity. Furthermore, to account for possible spatial variations in temperature and light, the pots were randomly placed in a greenhouse.

## 2.3 Plant sampling and analysis

Pak choi was harvested 45 days after sowing, split into roots and shoots (edible part), rinsed with deionized water, and

weighed (fresh weight). Fresh shoots and roots were then frozen in liquid nitrogen, ground, and stored at −80 °C for further analysis.

**2.3.1 Malondialdehyde, $H_2O_2$ and antioxidants in plant tissues.** The activities of malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GSH-PX), glutathione (GSH), and ascorbic acid (AsA) in plant tissues were determined using the corresponding determination kits (Nanjing Jiancheng Bioengineering Institute, China). The frozen plant samples (0.1 g) were extracted with 0.9 mL of potassium phosphate buffer (pH 7.0) and centrifuged at 2500 rpm for 10 min to obtain the supernatant for determining all substances except ascorbate peroxidase (APX). To assess the activity of APX, 0.1 g frozen samples were subjected to maceration in 1.0 mL potassium phosphate and the resulting homogenate was subsequently centrifuged at 13 000 rpm for 10 min. The whole procedure was performed at 4 °C. The contents of MDA and $H_2O_2$ in plant tissues were expressed as nanomoles per gram of fresh weight (nmol $g^{-1}$ FW) and micromoles per gram of fresh weight (μmol $g^{-1}$ FW), respectively. The antioxidant activities were denoted as per milligram protein (U $mg^{-1}$ protein) or per gram protein (U $g^{-1}$ protein).

**2.3.2 Cd in plant tissues.** Fresh shoot and root samples (0.2500 g) were digested in a DigiBlock digestion system (ED54; LabTech, PRC) with 8 mL of $HNO_3$ (65% v/v, GR), and the concentration of Cd in the digested solution was measured by inductively coupled plasma mass spectrometry (ICP-MS, iCAP Q, Thermo Scientific, USA), with more details provided in Text S1.† For quality assurance purposes, blank samples and a certified reference material (SRM 1570a, Spinach leaves; National Institute of Standards and Technology, NIST) were incorporated. The recovery rate for SRM 1570a was 85–105%.

### 2.4 Soil sampling and analysis

Following the pak choi harvest, rhizosphere soil samples were collected from each pot.[22] The rhizosphere soil samples were separated into two parts: one was frozen in liquid nitrogen and stored at −80 °C for microbial analysis, while the other was air-dried at room temperature and sieved to determine soil pH, available Cd, and sequential Cd extraction.[23]

**2.4.1 Soil properties.** A pH meter (PB-10; Sartorius, Germany) was used to determine the soil pH at a soil-to-water ratio of 1 : 2.5.[24] Soil Cd fractionation was classified into five fractions: the exchangeable fraction (Exc-Cd; extracted with 1.0 M of $MgCl_2$, pH = 7), carbonate-bound fraction (CB–Cd; extracted by 1.0 M $CH_3COONa$, pH = 5), iron and manganese oxide-bound fraction (OX-Cd; extracted by 0.04 M $NH_2OH{\cdot}HCl$ in 25% $CH_3COOH$ solution), organic matter-bound fraction (OM-Cd; extracted by 0.02 M $HNO_3$ and 30% $H_2O_2$), and residual fraction (Res-Cd; digested with $HNO_3$–HF–$HClO_4$). ICP-MS was used to determine the total Cd content as well as that of each fraction. Furthermore, the mobility factor (MF) was determined as a ratio of Cd concentrations in Exc-Cd and CB-Cd to the sum of all fractions to assess Cd mobility in soil. The details of the above analysis methods are described in Text S1.†

**2.4.2 Soil bacterial community analysis.** Soil genomic DNA was isolated using a soil DNA kit (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's protocols, and DNA quality and concentration were evaluated using a NanoDrop2000 and 1% (w/v) agarose gel electrophoresis. For bacterial identification, the 16S rRNA gen hypervariable V3–V4 region was amplified with 515F-806R primers (Forward: 5′-ACTCCTACGGGAGGCAGCAG-3′, Forward: 5′-GGAC-TACHVGGGTWTCTAAT-3′). The TransGen AP221-02 kit, Axy-PrepDNA gel recovery kit, and Agencourt AMPure XP purification kit were used for PCR amplification, product recovery, and purification, respectively. The HiSeq2500 PE250 platform was used to construct and sequence the Miseq library.

The sequencing reads were preprocessed with Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) software, which included quality filtering and merging. The sequences were then divided into Operational Taxonomic Units (OTUs) based on a 97% similarity level using Upares (version 1.8.0). Uchime was used to identify and delete chimeric sequences. The OTU sequences were then analyzed using the Mothur, QIIME, and R programs for alpha, taxonomy, and beta diversity.

### 2.5 Statistical analysis

All data for this research were analyzed and exhibited in the form of mean value ± standard deviation (SD; $n = 4$) utilizing Microsoft Excel 2010. The statistical analysis was performed utilizing SAS v. 9.4 (SAS Institute Inc., USA) with the least significant difference test applied to multiple comparisons when significant differences between treatments were identified ($p < 0.05$). The principal component analysis, correlation analysis, and figure plotting were all performed with Origin 2021b. Moreover, the direct and indirect effects of related parameters (Si application, soil pH, and Cd in the first four fractions (Exc-Cd, CB-Cd, OX-Cd, and OM-Cd)) on Cd concentrations in pak choi tissues based on hypothesized relationships were examined using a structural equation model (SEM), which was carried out using the Lavaan Package (version 0.6-9) in R (version 4.1.1, R Core Team, Austria 2015). Correlation analysis was used to analyze the relationships of microbial diversity indices and the richness index with soil properties. Furthermore, to examine the association between the soil bacterial community and soil parameters, a redundancy analysis (RDA) was conducted using Canoco 5.0.

## 3 Results

### 3.1 Plant biomass and Cd accumulation in pak choi tissues

The biomass of pak choi roots and shoots significantly increased with increasing Si dosage (when less than 0.8 g $kg^{-1}$); nevertheless, the fresh weights of both parts significantly declined by 23.0% and 21.6% at the highest Si dose compared to those with 0.8 g $kg^{-1}$ of Si (Fig. S1†).

As indicated in Fig. 1, Cd concentration in pak choi tissues greatly decreased with the increasing Si dosage with the reductions being more pronounced in the roots (Fig. 1). In comparison to the no Si control, the addition of sodium silicate

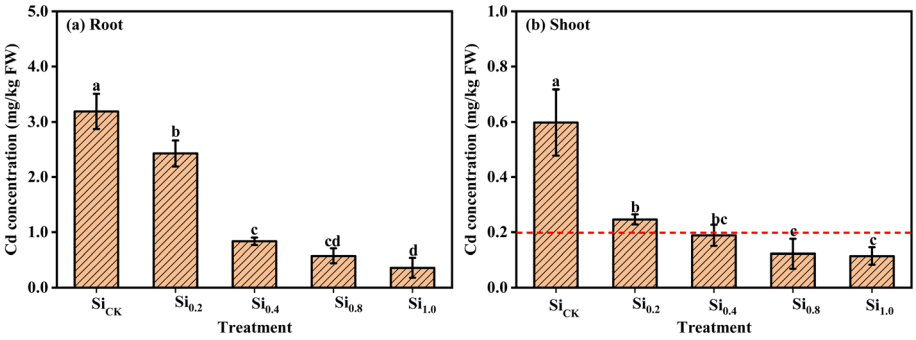


Fig. 1 The concentration of Cd in pak choi roots (a) and (b) shoots under five treatments. $Si_{CK}$, $Si_{0.2}$, $Si_{0.4}$, $Si_{0.8}$, and $Si_{1.0}$ represent treatments where no sodium silicate was added, and sodium silicate was added at a dosage of 0.20, 0.40, 0.80, and 1.00 g Si per kg soil, respectively. The dashed line in the figure represents the statutory Cd safety limits (0.2 mg $kg^{-1}$ fresh weight (FW)) in leafy vegetables as defined in China's food safety standard (GB 2762-2022). The data are presented as means ± SD ($n = 4$). According to the LSD test, different letters in the figure indicate significant differences according to the LSD test ($p < 0.05$).

decreased Cd concentration in roots by 23.5–89.0% and in shoots by 58.5–81.0%. The $Si_{1.0}$ treatment resulted in the lowest Cd concentration in both roots and shoots (0.36 mg $kg^{-1}$ fresh weight (FW) for roots and 0.11 mg $kg^{-1}$ FW for shoots) were both observed. Moreover, the application of Si at 0.4 g Si per kg soil effectively reduced the concentration of Cd in the edible part to the statutory safety limits of 0.2 mg $kg^{-1}$ (GB 2762-2022).

### 3.2 Oxidative stress and antioxidative activity in pak choi

In general, soil sodium silicate application significantly reduced the concentration of MDA and $H_2O_2$ in roots and shoots as compared to the no Si control (Fig. S2†), and further reducing effects were observed when higher doses of Si were applied. The lowest MDA and $H_2O_2$ concentrations in roots and shoots were observed under the $Si_{0.8}$ treatment; MDA and $H_2O_2$ concentrations in the $Si_{0.8}$ treatment were 36.9% and 17.7% in roots and 47.8% and 51.0% in shoots, respectively, lower than those of the control, but they were increased by the highest Si treatment ($Si_{1.0}$) as compared to the $Si_{0.8}$ treatment, for which they remained below the control levels.

Except for APX activity in pak choi shoots, soil treatment of sodium silicate had a significant influence on the activity of all the tested antioxidative enzymes as well as the concentration of GSH and AsA, The application of Si significantly enhanced SOD activity by 14.0–33.1% in roots and by 17.4–36.0% in shoots as compared to the no Si control (Fig. 2a). The addition of Si significantly increased CAT in roots, with the exception of the highest Si treatment ($Si_{1.0}$) as compared to the no Si control. The $Si_{0.8}$ treatment exhibited the greatest CAT activity in roots, approximately 1.80-fold higher than that of the no Si control (Fig. 2b). Similar to SOD activity, the application of Si significantly increased shoot CAT activity by 78.2–120% relative to the no Si control, but the Si application rate has no significant effects (Fig. 2b). The GR activity greatly increased with increasing Si application rates from 0.0 to 0.8 g $kg^{-1}$, and it reached the maximum under the $Si_{0.8}$ treatment (67.4% in roots and 101% in shoots higher than those of the no Si control (Fig. 2c)). The variation in APX activity was very minor; the only significant difference was observed in shoots at 0.8 g $kg^{-1}$ of Si compared to the control (Fig. 2d). At the same time, adding Si significantly increased the GSH and AsA concentration in pak choi at Si application rates less than 0.8 g $kg^{-1}$; the highest GSH and AsA concentrations in pak choi observed after the $Si_{0.8}$ treatment were 38.5% and 155% in roots and 41.0% and 16.1% in shoots, respectively, higher than that of the no Si control (Fig. 2e and f). However, the concentration of GSH and AsA in pak choi decreased after the highest Si treatment ($Si_{1.0}$) as compared to the $Si_{0.8}$ treatment.

### 3.3 Soil pH, Cd fractions and mobility

Soil application of sodium silicate significantly increased soil pH by approximately 0.29–1.61 units relative to the no Si control treatment, and soil pH increased as the Si application rate increased (Fig. 3a). In terms of Cd fractions in soil, Cd in the exchangeable fraction (Exc-Cd) was the most enriched Cd fraction, followed by Res-Cd (residual fraction), OX-Cd (manganese oxide-bound fraction), CB-Cd (carbonate-bound fraction), and finally OM-Cd (organic matter-bound fraction), regardless of Si application (Fig. 3b). The application of Si had a significant impact on soil Cd fractions, with the exception of Res-Cd. The Exc-Cd fraction had a notably reduction of 5.2–12%, whereas the CB-Cd fraction, OX-Cd fraction, and OM fraction showed a rise of 9.5–21.0%, 5.3–66%, and 6.2–52%, respectively. Additionally, soil Si treatment resulted in a 13.7–20.8% decrease in soil Cd mobility relative to the no Si control (Fig. 3c).

### 3.4 Correlation analyses of plant biomass, Cd concentration in pak choi, and soil properties

As illustrated in Fig. 4, Cd concentrations in roots and shoots were correlated positively with Exc-Cd, but negatively with soil pH, CB-Cd, OX-Cd, and OM-Cd (Fig. 4a). Additionally, the relationship between plant biomass and Cd concentration in plants, as well as soil properties, revealed that root fresh weight was positively correlated with root-Cd, root SOD, root GR, root MDA, soil pH, and soil Exc-Cd, but negatively correlated with soil pH, root MDA, and root GR (Fig. S3a†), and the shoot findings were consistent with the root results (Fig. S3b†). Furthermore, the

 

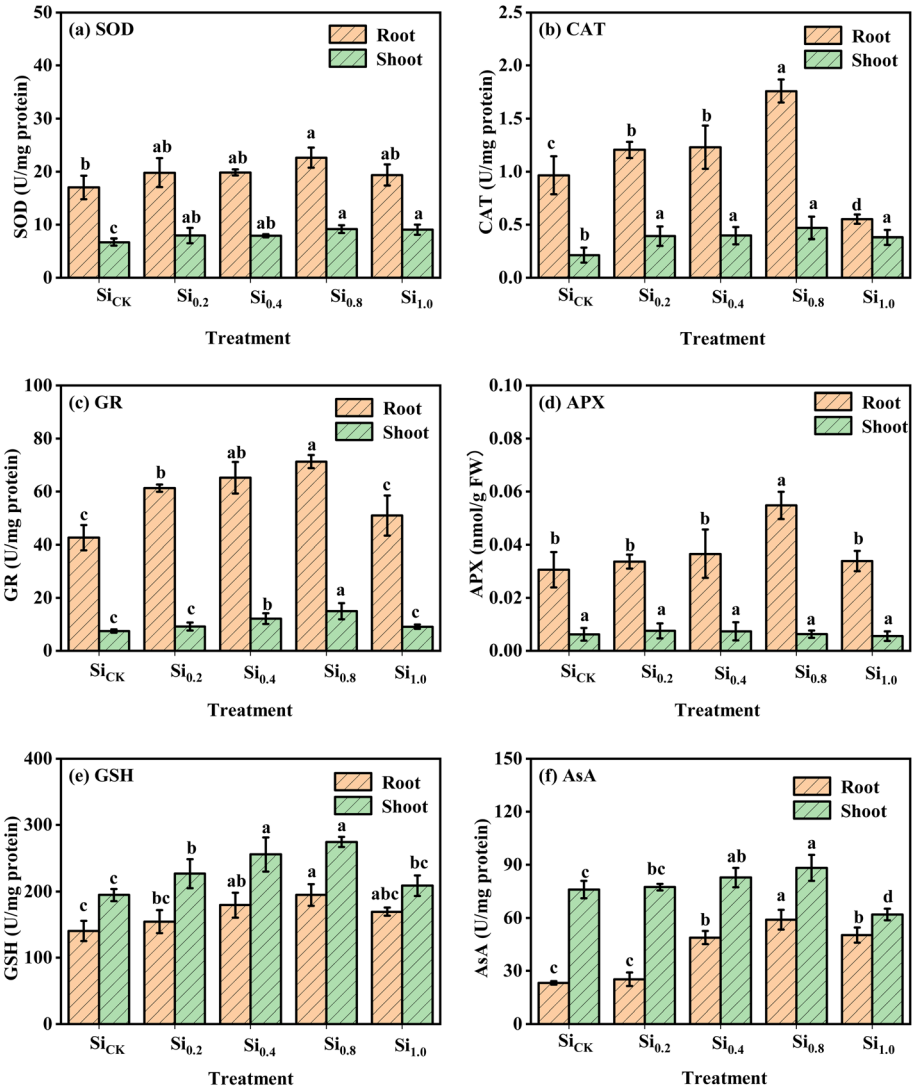


Fig. 2 The concentration of SOD (a), CAT(b), GR(c), APX (d), GSH (e), and AsA (f) in pak choi tissues under five treatments. $Si_{CK}$, $Si_{0.2}$, $Si_{0.4}$, $Si_{0.8}$, and $Si_{1.0}$ represent treatments where no sodium silicate was added, and sodium silicate was added at a dosage of 0.20, 0.40, 0.80, and 1.00 g Si per kg soil, respectively. The data are presented as means ± SD ($n$ = 4). According to the LSD test, different letters in the figure indicate significant differences according to the LSD test ($p$ < 0.05).

principal component analysis revealed that the first principal component accounted for 64.8% of the total variance and the second for 13.2%. Soil pH, CB-Cd, OX-Cd and OM-Cd all showed positive loadings on PC1, whereas Cd in pak choi tissues and Exc-Cd had relatively higher negative loadings (Fig. 4b). Furthermore, the eigen values for each factor were as follows: shoot-Cd 5.46, root-Cd 1.36, soil pH 0.811, Exc-Cd 0.164, CB-Cd 0.092, OX-Cd 0.060, OM-Cd 0.032, and Res-Cd 0.0128, respectively. Moreover, as compared to the no Si control, treatments with higher doses of Si were found to be more displaced to the right along PC1, with the $Si_{1.0}$ treatments having the highest score, indicating that soil Si application altered soil Cd availability by increasing soil pH and redistributing soil Cd speciation.

Additionally, a structural equation model (SEM) was constructed to analyze the pathways that explain the effects of soil Si application on Cd concentration in pak choi. The SEM built in this study was well-fitted ($\chi^2 = 12.226$, df = 13, $p = 0.509$, CFI = 1.000, and GFI = 0.999), and the fitted models explained 99.9% of the variance in pak choi Cd concentration (Fig. 5). According to the path coefficient, soil Si application showed no significant direct effects on Cd accumulation in pak choi, but it did have an indirect impact by significantly affecting soil pH and Cd fractions. As shown in Fig. 5a, soil Si application had significantly positive and direct impacts on the soil pH, CB-Cd, OX-Cd, and OM-Cd, but had negative and direct effects on Exc-Cd. Furthermore, soil pH and CB-Cd had a negative and direct influence on root Cd concentration, but Exc-Cd and OX-Cd had a positive effect. Overall, Si application was the most effective in suppressing Cd accumulation in pak choi, with the highest standardized total effects (direct plus indirect effects reached −0.933), followed by soil pH (direct effects reached −0.676), OX-Cd, CB-Cd, and Exc-Cd (Fig. 7B).

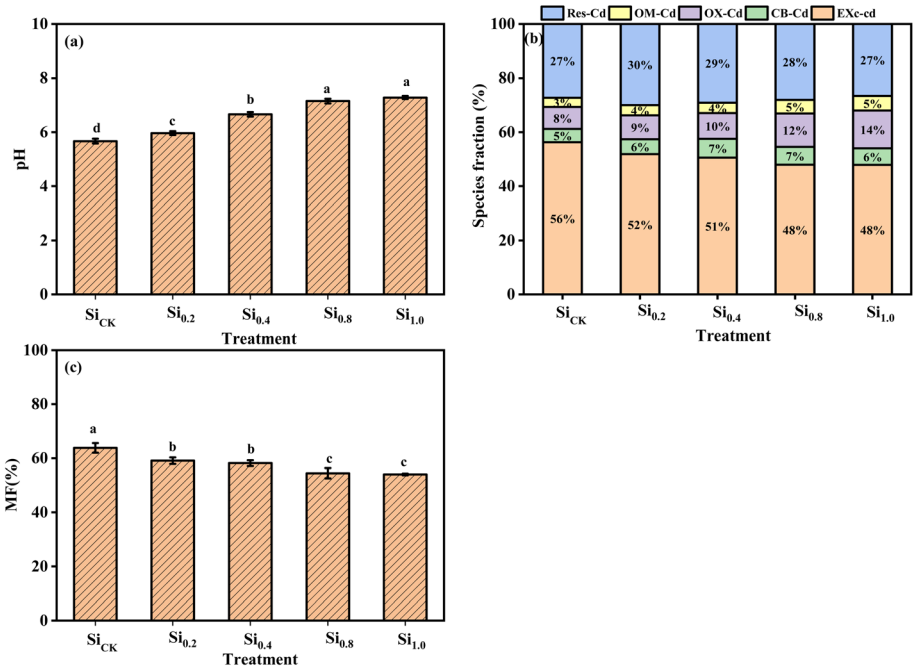


Fig. 3 Soil pH (a), Cd fraction distributions (b), and the Cd mobility factor (MF) (c) under five treatments. $Si_{CK}$, $Si_{0.2}$, $Si_{0.4}$, $Si_{0.8}$, and $Si_{1.0}$ represent treatments where no sodium silicate was added, and sodium silicate was added at a dosage of 0.20, 0.40, 0.80, and 1.00 g Si per kg soil, respectively. The data are presented as means ± SD ($n = 4$). According to the LSD test, different letters in the figure indicate significant differences according to the LSD test ($p < 0.05$).

### 3.5 Soil bacterial community and composition

**3.5.1 Richness and diversity of the bacterial community.** 16S rRNA gene sequencing was carried out to investigate the influence of sodium silicate application on the soil bacterial community. A total of 1 604 800 high-quality sequence reads were obtained and clustered into OTUs at a threshold of 97% sequence identity. All samples exhibited coverage indices more than 0.994, indicating that the majority of bacterial communities were detected with high data reliability, and that further sequencing would no longer create new OTUs (Table S1†). Soil Si application significantly increased the richness (expressed by Chao1 and Ace indices) and diversity indices (expressed by Shannon and Simpson indices), but the Si application dose has no significant effects (Table S1†).

**3.5.2 Composition of bacterial communities.** The dominant bacteria in the tested soils were *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, accounting for 44.39–49.04%, 14.10–21.19%, and 9.44–19.20% of the total bacterial abundance,

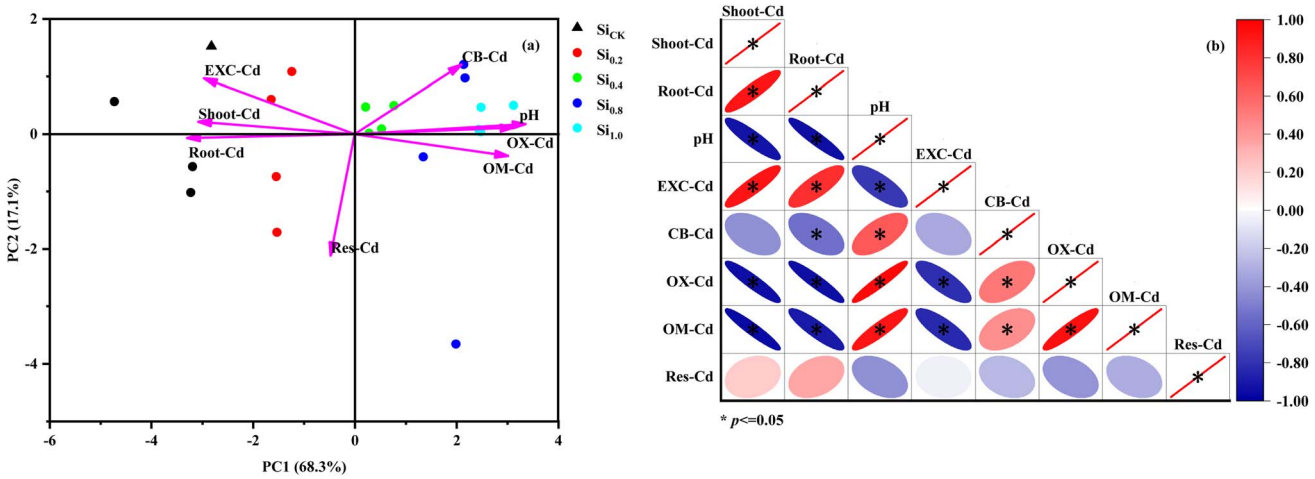


Fig. 4 Correlation analyses of Cd concentration in pak choi tissues and soil properties using principal component analysis (a) and correlation analysis (b). The soil qualities are represented by the pink line arrows (a). The *r* values are plotted in various colors, with the color range and associated *r* values shown on the right side of the legend (b). $Si_{CK}$, $Si_{0.2}$, $Si_{0.4}$, $Si_{0.8}$, and $Si_{1.0}$ represent treatments where no sodium silicate was added, and sodium silicate was added at a dosage of 0.20, 0.40, 0.80, and 1.00 g Si per kg soil, respectively.

 

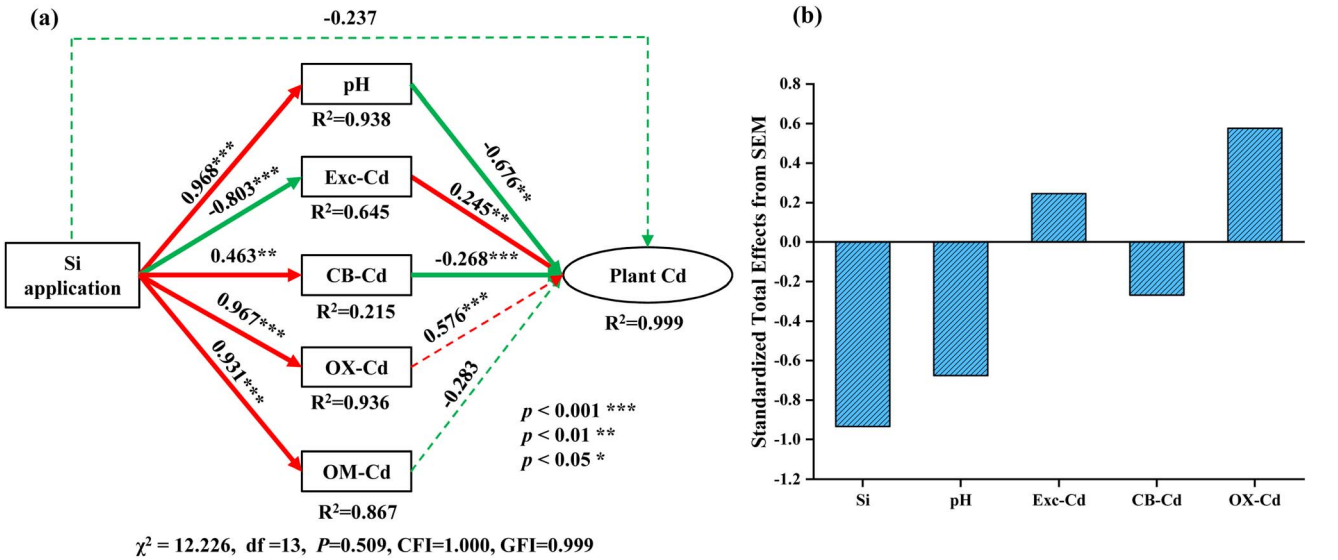


Fig. 5 The structural equation modeling (SEM) for pathway analysis of soil sodium silicate application effects on soil properties (soil pH and Cd fractions) and Cd accumulation in pak choi (a), and standardized total effects (direct plus indirect effects) derived from SEM (b). The latent variable "Plant Cd" represented the variations of Cd concentration in roots and shoots. Positive relationships are shown by red lines, while negative associations are represented by green lines. The solid lines indicate significant impacts between two variables ($p < 0.05$), while the dotted lines indicate non-significant effects ($p < 0.05$).

respectively, and the sum of the relative abundance of these three phyla across all treatments reached 79.37–80.57%. Soil application of sodium silicate significantly altered the abundance of soil bacteria. When soil Si was applied, the relative abundances of *Bacteroidetes*, *Gemmatimonadetes*, *Acidobacteria*, *Verrucomicrobia*, and *Planctomycetes* increased by 10.55–79.69%, 70.72–96.48%, 14.03–107.59%, 123.31–600.61%, and 42.65–115.07%, respectively, as compared to the no Si control treatments. Conversely, the application of Si reduced the relative abundances of *Actinobacteria*, *Firmicutes*, and *Thermomicrobia* by 25.85–32.35%, 32.50–84.05%, and 67.06–88.54%, respectively. Furthermore, the unweighted UniFrac NMDS, which relied on the relative abundance and OTUs, demonstrated distinct separation of the bacterial communities between treatments. Notably, the most pronounced variations in soil bacterial composition were observed between $Si_{1.0}$ and CK (Fig. S4†).

**3.5.3 Correlations of environmental characteristics with soil bacterial communities.** In the present study, to amply evaluate the relative effect of soil physicochemical variables (pH, Exc-Cd, CB-Cd, OX-Cd, OM-Cd, and Res-Cd) and sodium silicate on the abundance and diversity of bacterial communities in Cd

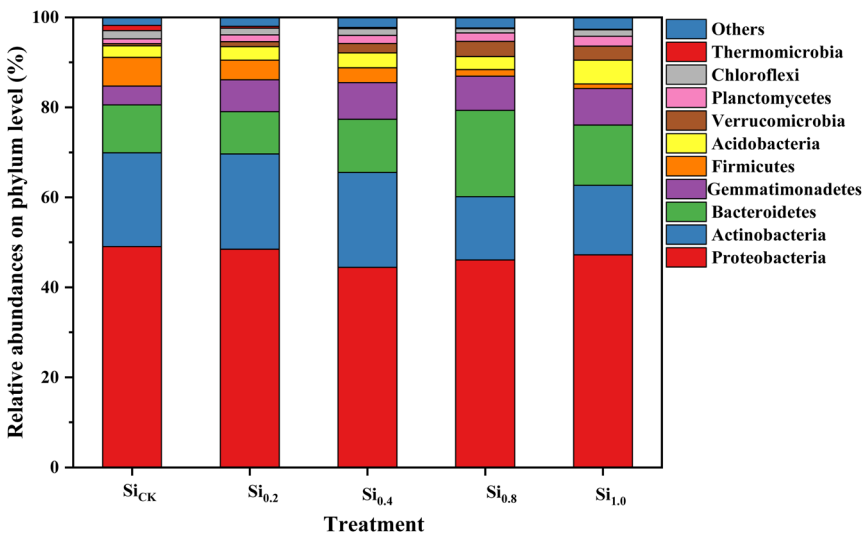


Fig. 6 Relative abundances of the ten most abundant bacterial phyla in tested soil. $Si_{CK}$, $Si_{0.2}$, $Si_{0.4}$, $Si_{0.8}$, and $Si_{1.0}$ represent treatments where no sodium silicate was added, and sodium silicate was added at a dosage of 0.20, 0.40, 0.80, and 1.00 g Si per kg soil, respectively.

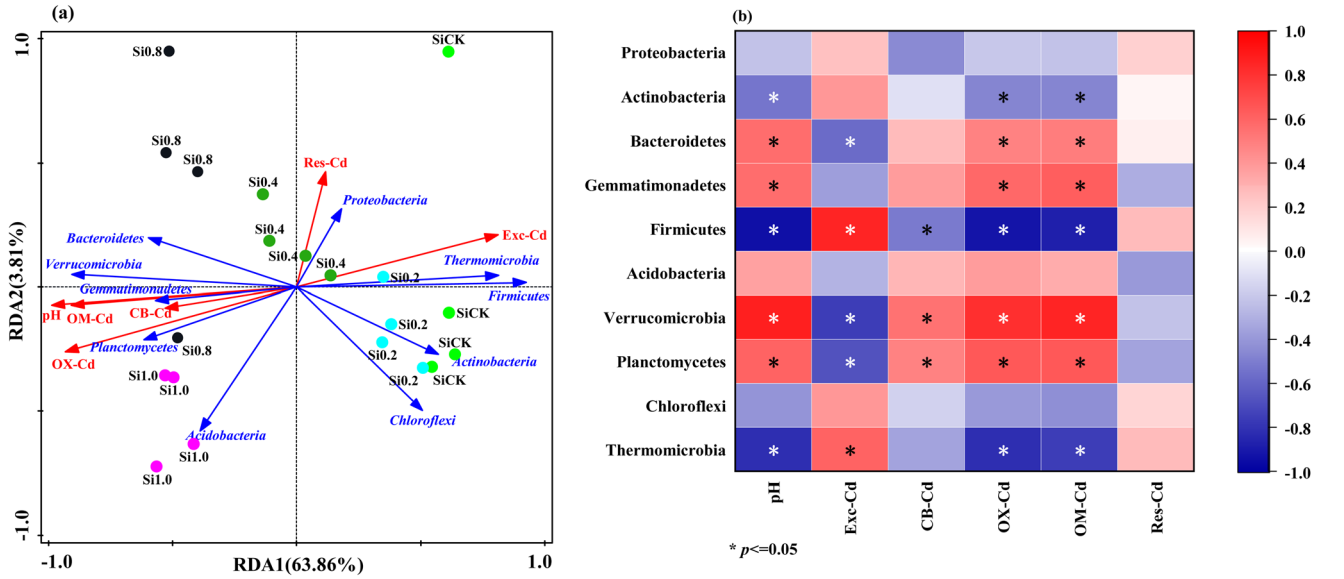


Fig. 7 (a) Redundancy analysis (RDA) demonstrating the relationships between environmental factors and the microbial community at the phylum level. The red and blue line arrows depict environmental factors and the main microbial phyla, respectively. $Si_{CK}$, $Si_{0.2}$, $Si_{0.4}$, $Si_{0.8}$, and $Si_{1.0}$ represent treatments where no sodium silicate was added, and sodium silicate was added at a dosage of 0.20, 0.40, 0.80, and 1.00 g Si per kg soil, respectively. (b) Pearson correlation heatmap of the top 10 phyla of the microbial communities and the environmental factors. The r values are plotted in various colors, with the color range and associated r values shown on the right side of the legend.

contaminated acid soil, redundancy analysis (RDA) was conducted (Fig. 7). The first two axes, RDA1 and RDA2, explained 63.86% and 3.81%, respectively, of the overall variance of the soil bacterial community compositions. The soil pH and Cd in the first four fractions (Exc-Cd, CB-Cd, OX-Cd, and OM-Cd) had a substantial influence on the soil bacterial community. According to the results of the correlation analysis, there exists a significant positive correlation between the relative abundances of *Bacteroidetes*, *Gemmatimonadetes*, *Verrucomicrobia*, and *Planctomycetes* and soil pH, OX-Cd, and OM-Cd. Conversely, the abundances of *Actinobacteria*, *Firmicutes*, and *Thermomicrobia* are significantly negatively correlated with the aforementioned variables. The abundance of *Firmicutes* and *Thermomicrobia* was positively correlated with Exc-Cd, whereas it was negatively correlated with *Bacteroidetes*, *Verrucomicrobia*, and *Planctomycetes*. There was a positive correlation observed between CB-Cd and the abundance of *Verrucomicrobia* and *Planctomycetes* while a negative correlation was noted with the abundance of *Firmicutes*. Furthermore, Pearson correlation analysis also indicated that microbial diversity (Shannon and Simpson indices) and microbial abundance (Chao1 index) had a positive correlation with soil pH OX-Cd, and OM-Cd, but were negatively correlated with Exc-Cd (Fig. S5†).

# 4 Discussion

The objective of this study was to investigate the effects of Si fertilizer on Cd migration, transformation, and accumulation in the soil–pak choi system, as well as to explore the underlying mechanisms. At the same time, the effects of silicon fertilizer addition on soil environmental quality were discussed from the perspective of microorganisms. The findings of the present study demonstrated that Si application could greatly immobilize Cd, decrease its bioavailability, and subsequently reduce Cd accumulation in pak choi tissues. Furthermore, the unique mechanisms of exogenous Si on soil Cd migration and transformation in the soil–vegetable system were discussed and primarily elucidated in the following sections.

## 4.1 Si redistributed Cd fractions and reduced its bioavailability in Cd-contaminated acidic soil

Heavy metal fractions in soil exhibit different mobility and bioavailability characteristics. It has been demonstrated that the transformation of heavy metals from labile to stable fractions is a critical step in immobilization remediation.[25,26] In the current study, soil sodium silicate application significantly reduced the proportions of Exc-Cd (Fig. 3b and c), which is easily absorbed by plants, while increasing the proportions of OM-Cd and OX-Cd, which are difficultly absorbed by plants. Consistent with these findings, Ma *et al.* discovered that Si-rich materials significantly reduced the mobility of Cd,[27] indicating that Si application effectively immobilized Cd and hence reduced Cd concentrations in the rice tissues. The primary mechanisms of exogenous Si changing the fraction and bioavailability of soil Cd are as following: first, Si application altered the soil properties (mainly pH), which subsequently altered the soil Cd fraction and the bioavailability. In this study, exogenous Si in the form of sodium silicate was alkaline (pH value of 12.53), with the ability to neutralize soil acidity and enhance soil pH (Fig. 3a). Furthermore, following application, the dissolution of sodium silicate releases $SiO_4^{2-}$ ions, which can react with water molecules to generate silicic acid ($H_4SiO_4$) and four $OH^-$ ions, neutralizing $H^+$ ions and increasing soil pH.[28] Similarly, numerous research studies have reported that Si-rich materials application increased soil pH.[8] It is well

 

established that soil pH is the most critical factor influencing the mobility and bioavailability of heavy metals in polluted soil.[29] An elevation in soil pH can result in an increase in negatively charged surface sorption sites, the generation of hydroxyl species of metal cations, and the precipitation of $Cd^{2+}$ as $Cd(OH)_2$ or $CdCO_3$, ultimately leading to lower Cd mobility and bioavailability in soils.[30,31]

In addition, it has been reported that a high pH led to co-precipitation between iron oxides with heavy metals, which promoted the heavy metal adsorption on soil and reduced heavy metal bioavailability.[3] This is supported by the present results that soil sodium silicate application significantly increased the fractions of OX-Cd (Fig. 3b). Furthermore, the application of Si caused the redistribution of Cd fractions in soil by forming Si–Cd complexes, which facilitated the transformation of Cd from easily exchangeable fractions to more stable fractions. Xiao[15] discovered that Si application promotes the transformation of soil Cd and Pb from acid soluble and reducible fractions to oxidizable and residual fractions, decreasing their bioavailability and rice absorption. Previous research recorded that $Cd^{2+}$ in soil could react with silicic acid to form a Si–Cd complex, which the plant root cannot absorb directly.[16,19] Moreover, researchers have used different spectroscopic techniques to confirm the formation of the Si–Cd complex in soil with Si application.[16] Additionally, silicate in soil could polymerize and link to iron oxide surfaces, then complex with ferrosilicon to produce a large number of negatively charged functional groups, such as silanol, reducing the bioavailability of $Cd^{2+}$ by providing many adsorption sites.[32] However, the micro-mechanism of Cd transfer and transformation in soil by sodium silicate application is not well established and requires further investigation. Based on the findings and discussion presented above, the following conclusions could be drawn: in Cd-contaminated acidic soil, sodium silicate application reduced soil Cd bioavailability by increasing soil pH and redistributing the Cd fraction from exchangeable fractions to more stable fractions, resulting in a lower Cd availability to pak choi roots. However, due to the complex conditions of wild fields, the actual performance of soil amendments on the field scale may be inconsistent with their effects in pot experiments, and thus more investigation is needed to evaluate the performance of sodium silicate in the field-scale actual environment.

### 4.2 Si reduced Cd accumulation and alleviated Cd toxicity in pak choi tissues

Research has been extensively performed to minimize Cd uptake by plants through Si application. In this study, soil sodium silicate application significantly reduced Cd accumulation in pak choi (Fig. 1). Similar findings have been reported in wheat[10] and rice.[12] A possible explanation for these findings is that sodium silicate application reduced soil Cd bioavailability by increasing soil pH and redistributing Cd fractions from exchangeable fractions to more stable fractions. This was verified by the SEM results, which demonstrated that Si application had an indirect impact on Cd concentration in pak choi *via* its strong effect on soil pH and Cd fractions (Fig. 5). Furthermore, standardized total effects derived from SEM further indicated that Cd concentration in pak choi was mainly driven by soil pH, followed by OX-Cd, CB-Cd, and Exc-Cd. The above findings indicate that the predominated mechanisms involving sodium silicate for Cd migration and transformation in the soil–pak choi system is by regulating the soil pH value. Moreover, the reduction in Cd concentrations in plants caused by Si application is not just related to its immobilization effects on soil Cd bioavailability, but could also result from its reducing effect on root uptake. Previous studies[33] discovered that applying silicates at a lower rate had no effect on soil pH but greatly reduced Cd uptake and transport in Cd-stressed maize. Furthermore, Si has been shown to suppress the expression of genes involved in Cd absorption and transportation (*OsHMA2* and *OsNramp5*).[17] Zhou *et al.* similarly observed that Si application inhibited Cd uptake in wheat growing in Cd-contaminated soils by upregulating the expression of efflux transporters (*TaTM20* and *TaHMA3*) and downregulating the expression of influx transporters (*TaNramp5*).[10]

Generally, plant biomass reflects plant growth conditions under abiotic stress, and it is also a significant indicator for accessing plant tolerance to Cd toxicity.[34] The present study found that soil sodium silicate application greatly increased the biomass of pak choi (Fig. S1†), indicating that sodium silicate application could alleviate Cd toxicity in pak choi. Comparable findings have been reported in Chinese cabbage,[35] wheat,[10] soybean,[13] and rice.[17] The underlying mechanisms could be described as follows. Firstly, exogenous addition of Si alleviated Cd toxicity in pak choi by reducing Cd accumulation through increasing soil pH and redistributing Cd fractions, as minimizing Cd uptake and accumulation is critical to decrease Cd toxicity in plants. Secondly, exogenous Si improved plant defense capacity against oxidative damage provoked by the heavy metal through enhancing the activity of the antioxidant system.[10,13] And in this research, the results of correlations among the biomass, MDA and $H_2O_2$ content, antioxidant enzyme activity, and antioxidant content confirmed the above notion (Fig. S3†). In general, Cd stress causes the generation of reactive oxygen species (ROS), which inhibits plant development and reduces biomass.[13] To scavenge excess ROS and decrease oxidative damage, plants have developed defense systems include enzymatic and non-enzymatic antioxidant mechanisms.[36] In general, Cd stress generates reactive oxygen species (ROS), inhibiting plant development and reducing biomass.[13] Plants have developed defense systems to scavenge excess ROS and reduce oxidative damage, including enzymatic and non-enzymatic antioxidant mechanisms. In the present study, soil sodium silicate application increased antioxidant enzyme activities (SOD, CAT, GR, and APX) as well as antioxidant concentration (GSH and AsA) to maintain a higher antioxidant capacity, thereby alleviating Cd-induced oxidative stress as evidenced by lower MDA and $H_2O_2$ contents in pak choi tissues (Fig. 2, S2 and S3†). These findings all showed that soil sodium silicate application could increase the antioxidant capacity of pak choi and alleviate Cd toxicity.

### 4.3 Effects of Si application on the soil environment

In the study, sodium silicate application effectively immobilized Cd significantly by increasing soil pH and redistributing the Cd fraction from labile fractions to stable fractions, and consequently reduced Cd accumulation in pak choi. However, as an additive to soil, the application of soil amendments may pose a risk of heavy metal accumulation in soil. For sodium silicate itself, heavy metal levels in sodium silicate are much lower than the ecological index of As, Cd, Pb, Cr, and Hg in fertilizers (GB/T 23349-2009). Furthermore, sodium silicate mainly composed of Na, Si, and a small amount of the Fe element, would not cause heavy metal pollution in soil. Therefore, possible harm from the sodium silicate application to the soil environment is very low. However, it is worth noting that although sodium silicate effectively immobilized Cd in contaminated soil, excessive application of sodium silicate may result in soil alkalization and soil hardness. Furthermore, even though the highest dose of sodium silicate application resulted in the lowest Cd concentration in pak choi tissues, Si application at 0.4 g $kg^{-1}$ ($Si_{0.4}$) was successful in reducing Cd concentration in the edible part to the safety limits for Cd. Moreover, the $Si_{0.8}$ treatment produced the highest fresh weights of pak choi, as well as the highest concentrations of MDA and $H_2O_2$ concentration in pak choi tissues, which were all recorded in the $Si_{0.8}$ treatment. Herein, the application dosage should be considered for practical application in the field, and further long-term trials are required to determine the optimal dosage.

Besides, it is well recognized that Cd contamination affects soil microbial biological activity; as indicators of soil environment quality, soil microbes are vital for material cycling, energy flow, and nutrient conversion. The microbial community in the current study differed noticeably between Si treatments and control samples; additionally, sodium silicate application significantly increased soil bacterial richness (Chao1 and Ace indices) and diversity (Shannon and Simpson indices). Rhizosphere-associated microorganisms are important in reducing Cd toxicity and promoting plant growth,[37] and the rhizosphere of plants cultivated in Cd-contaminated soils has been identified to contain the majority of the prevalent bacteria at the phylum level (*e.g.*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*).[38] According to a previous study,[39] *Proteobacteria* and *Actinobacteria* are the most active bacteria in soils contaminated with heavy metals, and the current study found that their relative abundances accounted for more than 60% (Fig. 6). Furthermore, sodium silicate application reduced *Actinobacteria* abundance while increasing *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, and *Planctomycetes* abundance. Previous research has shown that *Actinobacteria* are tolerant of Cd whereas *Proteobacteria*, *Bacteroidetes*, and *Verrucomicrobia* are susceptible to it.[40,41] Furthermore, soil pH and Cd in the first four fractions were the primary factors determining the variation of bacterial communities (Fig. 7). Similarly, previous studies[38] discovered that the environment index and Cd faction were largely related to the microbial community. Among soil properties, soil pH is a key factor influencing the microbial community and activity. Soil pH can affect the precipitation-dissolution balance and soil Cd bioavailability of Cd in soil, resulting in a shift in abundances of Cd-sensitive/tolerant bacteria, and consequently considerably influencing microbial abundance and diversity.[40] Furthermore, based on the current findings, more in-depth research in the future is needed to investigate the effect of sodium silicate on Cd migration and transformation in the soil–pak choi system *via* microbial activity using the soil microbiome and metabolomics technology. Taken together, these findings revealed that the fluctuations in the bacterial community were driven by variation of soil treatment with sodium silicate, and all of the above results indicate that soil immobilization with sodium silicate helped maintain soil functionality in Cd-contaminated acidic soil. Furthermore, long-term effectiveness and persistence of sodium silicate application for remediating Cd-contaminated acidic soil, as well as concerns about the effects on soil health and crop yields require evaluation on the field-scale.

## 5 Conclusions

The current investigation demonstrated that sodium silicate application significantly increased soil pH and induced the transformation of the Cd fraction from Exc-Cd to OM-Cd and OX-Cd, resulting in a lower Cd bioavailability in Cd-contaminated acidic soil. As a result, effective immobilization of Cd in polluted soil with sodium silicate application significantly reduced Cd toxicity and accumulation in pak choi. Meanwhile, soil bacterial richness and diversity were significantly increased by sodium silicate application, and the microbial community differed noticeably between Si treatments and no Si control. Furthermore, soil pH and Cd in the first four fractions were the primary factors determining the variation of bacterial communities, and soil pH was the most important factor controlling pak choi Cd accumulation after exogenous Si treatment, according to a structural equation model (SEM). These findings suggested that sodium silicate has great potential as an environmentally friendly amendment to immobilize Cd-contaminated acidic soil if applied at an appropriate dosage. Further research is required to evaluate the environmental safety and long-term stability of sodium silicate application in the field, thereby supporting large-scale soil remediation with sodium silicate.

## Data availability

Data will be made available on request.

## Conflicts of interest

There are no conflicts to declare.

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