#### RESEARCH

**Open Access** 

# Exogenous nano-silicon enhances the ability of intercropped faba bean to alleviate cadmium toxicity and resist *Fusarium* wilt



Chaosheng Luo<sup>1,2†</sup>, Ting Li<sup>1†</sup>, You Huang<sup>1</sup>, Taiqin Liu<sup>1</sup> and Yan Dong<sup>1\*</sup>

#### Abstract

Excessive soil cadmium (Cd) and the accumulation of pathogens pose serious threats to legume growth. However, it remains unclear whether intercropping (IFcd) and its combined treatment with silicon nanoparticles (Si-NPs) (IFcd + Si) can alleviate these challenges under Cd stress, as well as the underlying mechanisms involved. This study systematically elucidated the mechanism of faba bean-wheat intercropping and Si-NPs regulating faba bean growth under Cd stress using rhizosphere metabolomics and 16 S rRNA microbiome analysis. The results showed that IFcd and IFcd + Si treatments significantly reduced Cd accumulation by 17.3% and 56.2%, and Fusarium wilt incidence by 11.1% and 33.3%, respectively, compared with monoculture faba bean (MFcd) while promoting root and plant growth. These treatments reduced oxidative stress markers, including  $H_2O_2$ , MDA, and  $O_2^-$ , and increased the activity of defense enzymes, such as SOD, APX, and POD in plants. Furthermore, they increased  $NH_4^+$ -N and available potassium levels in rhizosphere soils. Interestingly, the NH<sub>4</sub><sup>+</sup>-N content increased and was significantly positively correlated with urease (URE) activity and negatively correlated with Cd. Beneficial bacteria and functional metabolites were enriched in the rhizosphere of faba bean. Joint analysis revealed increased relative abundances of Sphingomonas, Intrasporangium, and Streptomyces, which were positively correlated with antibacterial metabolites, such as sordarin, lactucin, and 15-methylpalmate. This explains the reduced Cd accumulation and Fusarium wilt in plants. These findings provide mechanistic insights into how intercropping with Si-NPs mitigates Cd stress and controls soil-borne diseases by regulating rhizosphere metabolites, bacterial communities, and plant resistance.

<sup>†</sup>Chaosheng Luo and Ting Li contributed equally to this work.

\*Correspondence: Yan Dong dongyanyx@163.com

Full list of author information is available at the end of the article



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.



#### Introduction

Soil is a critical foundation for supporting crop health and for ensuring the production of reliable agricultural products. However, cadmium (Cd) pollution in farmland and soil-borne pathogens are common abiotic and biotic stresses that affect crops in southern China [1, 2]. Authoritative data indicate that 7% of China's land area is affected by varying degrees of Cd pollution [3]. The primary sources of Cd in farmland soil include atmospheric deposition, sewage irrigation, excessive use of phosphate fertilizers, and improper application of animal manure [4]. Soil is considered Cd-contaminated when its Cd concentration exceeds 1 ppm [5]. Even at lower concentrations of Cd, plants can readily absorb and accumulate Cd through their roots, causing adverse effects on crop growth and posing risks to agricultural food safety [6]. Additionally, Fusarium can easily accumulate in continuous cropping soil, leading to frequent outbreaks of soil-borne diseases in various crops such as cucumber, tomato, and faba bean [7]. As China's population grows and urbanization advances, the availability of cultivated land has become increasingly limited, leading to largescale intensive planting [1]. Therefore, to address these challenges, sustainable agricultural practices, such as intercropping, have gained significant attention owing to their potential to enhance soil health and crop resilience.

Faba bean (*Vicia faba* L.) is a crucial cold-season legume crop that provides dietary protein as a vegetable, feed, and grain in many developing countries [8]. Faba

bean cultivation in Yunnan, China has made significant contributions to both national and global legume production. However, studies have shown that local faba bean cultivation in Yunnan is increasingly challenged by Cd pollution risks and frequent outbreaks of faba bean Fusarium wilt disease [9]. The wheat-faba bean intercropping system, which can maximize spatial and temporal differentiation, soil moisture, nutrients, and light resources while managing soil-borne diseases and other stresses, is an indispensable planting practice in the entire southwest faba bean cultivation [10]. Research has demonstrated that intercropping faba bean with the hyperaccumulating plant Sonchus asper (L.) Hill can effectively reduce the available cadmium (Cd) content of faba bean plants [9]. Intercropping of *Lolium perenne* and Medicago sativa can significantly reduce Cd levels in alfalfa plants and inhibit effects of Cd on plant growth [11]. Although faba bean is not a typical hyperaccumulator of Cd, heavy metal stress (such as Cd) can severely hinder its growth, product quality, and safety [12]. Our previous research has suggested that wheat-faba bean intercropping can effectively control Fusarium wilt in faba bean and increase crop yield [7]. Additionally, strategies including beneficial elements such as silicon may play a role in alleviating this pressure. However, there are no reports on whether intercropping wheat-faba bean under Cd stress can reduce Cd toxicity and control faba bean wilt disease to enhance growth.

Silicon (Si) is the second most abundant chemical element in the Earth's crust after oxygen. Although Si is not considered an essential nutrient for plants, it is recognized as a beneficial element in most plant species [13, 14]. Silicon stress resistance in plants is evident not only in their ability to mitigate abiotic stresses such as Cd toxicity [15, 16] but also in their unique silicification protective layer and strong adhesion properties. These features help crops resist pests and diseases, enhance disease resistance, and reduce pesticide residues [17]. In soil, Si mainly exists in the form of silicates and oxides, which plants cannot absorb directly. The absorbable form of Si in plants is primarily monosilicic acid  $(Si(OH)_4)$  [16]. Compared with conventional silicon products, silicon nanoparticles (Si-NPs) have been introduced in agriculture owing to their small size, large surface area, stability, and biocompatibility [18]. Studies have demonstrated that Si-NPs not only promote plant growth and flowering [17, 13] but also enhance seed vitality, increase enzyme activity, improve root growth, boost water and fertilizer utilization efficiency, accelerate metabolism, and strengthen resistance to diseases and pests, ultimately improving crop yield and quality [19-21]. These findings suggest that Si-NPs are viable alternatives to alleviate Cd toxicity and promote plant growth. Given that plants under biotic and abiotic stresses often exhibit stunted growth and reduced systemic resistance, it is crucial to explore how Si-NPs can mitigate these effects, particularly in the context of Cd toxicity and Fusarium wilt. Nonetheless, the potential of Si-NPs to concurrently modulate the rhizosphere microorganisms and metabolic pathways in faba bean, thereby mitigating Cd toxicity and enhancing resistance to Fusarium wilt, has yet to be fully elucidated.

Plants subjected to Cd and Fusarium stress often exhibit stunted growth, poor root development, reduced systemic resistance, and diminished yield and quality [7]. When Cd infiltrates plants, it binds to proteins and DNA, causing denaturation and damage, leading to metabolic disorders, inhibited photosynthesis and respiration, increased production of reactive oxygen species (ROS), exacerbated membrane lipid peroxidation, and damage to plant cell structure, ultimately inhibiting plant growth [16, 22]. Intercropping target crops with hyperaccumulating plants can utilize interactions between plants and associated microorganisms. This practice promotes the secretion of organic acids and enrichment of degrading bacteria, which affects the bioavailability of HMs in the crop rhizosphere [23, 24]. Si can reduce Cd absorption and toxicity by regulating antioxidant enzyme activities and the expression of Cd transport genes in plants [25, 26] while enhancing systemic resistance by influencing the microbial communities in the rhizosphere and altering the secretion of citric acid and other substances [24, 27]. It is currently uncertain whether exogenous Si-NPs have the ability to enhance interspecific root interactions in intercropped faba bean and thereby promote its growth. Furthermore, the mechanisms by which rhizosphere microbial-metabolite interactions might be involved in these processes are also not yet clear.

This study aimed to elucidate the effects of intercropping and the combination Si-NPs on Cd toxicity and resistance to Fusarium wilt in faba bean under Cd and Fusarium stress, as well as the interaction mechanism between rhizosphere microorganisms and metabolites. A pot experiment with the exogenous addition of Si-NPs was conducted to verify that (1) intercropping and Si-NPs superposition alleviated Cd toxicity and Fusarium wilt occurrence in faba bean; (2) Si-NPs and intercropping enhanced the antioxidant activity and systemic defense ability of faba bean plants; (3) Si-NPs and intercropping increased the NH4+-N content and related enzyme activity in the faba bean rhizosphere; and (4) the rhizosphere interaction effect of microbiota-metabolomics of faba bean explains the prominent effects of intercropping and Si-NPs superposition. These findings provide a theoretical basis for using intercropping and Si-NPs in agriculture to improve soil health, control disease, and increase crop yield.

#### **Materials and methods**

#### Test location and test materials

A soil pot experiment was conducted in a greenhouse at Yunnan Agricultural University (25°7'N, 102°44'E, altitude: 1950 m) using natural day-night lighting (daylight, 11 h), with temperatures ranging from 20 °C to 30 °C and relative humidity between 50% and 60%. The experiment was conducted from early October 2023 to mid January 2024, using artificial paddy soil in Efeng Village, Yuxi City, Yunnan Province as the test soil. The region has a tradition of continuous faba bean cultivation, which has led to a significant accumulation of the soil-borne Fusarium pathogen in the soil, causing faba bean Fusarium wilt. The basic physical and chemical properties of the sample soil were: organic matter 22.4 g kg<sup>-1</sup>, total nitrogen 1.5 g kg<sup>-1</sup>, total phosphorus 0.8 g kg<sup>-1</sup>, total potassium 12.6 g kg<sup>-1</sup>, alkali nitrogen 102.0 mg kg<sup>-1</sup>, Olsen phosphorus 16.9 mg kg $^{-1}$ , available potassium 116.5 mg kg<sup>-1</sup>, Cd<sup>2+</sup> 0.075 mg kg<sup>-1</sup>, and pH 7.1. The utilized fertilizers were urea (N 46.0%), ordinary superphosphate  $(P_2O_5 16.0\%)$ , and potassium sulfate (K<sub>2</sub>O 50.0%).

Cd was added using CdCl<sub>2</sub>·2H<sub>2</sub>O (CAS: 10108-64-2,  $\geq$  99.0%), purchased from Beijing Huawei Ruike Chemical Technology Co., Ltd. (Beijing, China). Peptide-coupled fluorescent silicon nanoparticles (Si-NPs) (CAS: 7440-21-3,  $\geq$  99.0%) prepared by microwave irradiation were selected as nano-silicon materials. This material, which has high stability and dispersibility and is highly absorbable by plants, was obtained from Xi'an Qiyue Biotechnology Co., Ltd. (Shaanxi, China).

The tested varieties of faba bean and wheat were *Vicia faba* L. cv. Yuxidalidou and *Triticum aestivum* L. cv. Yunmai-112, purchased from the Grain and Crop Research Institute of Yunnan Academy of Agricultural Sciences.

#### Experimental design and crop management

The experiment utilized a single-factor design with three soil pot treatments: (a) a monoculture of faba bean under Cd stress (MFcd), with three rows per pot, three faba bean per row, plant spacing of 10 cm, and row spacing of 8 cm; (b) faba bean-wheat intercropping under Cd stress (IFcd); and (c) faba bean-wheat intercropping under Cd stress with the addition of Si-NPs (IFcd + Si). In the intercropping treatments, the planting arrangement consisted of one row of faba bean (three plants/row), followed by three rows of wheat (six plants/row), and another row of faba bean (three plants/row). Each treatment contained three biological replicates, resulting in a total of nine pots. The plastic pots were 22 cm in height, 34 cm in upper diameter, and 19 cm in lower diameter.

The topsoil (0–20 cm) collected from the field was airdried in a greenhouse for one week. Afterward, debris were removed, and the soil was sieved through a 2 mm sieve. Each pot was filled with 9.0 kg of soil. To prepare Cd-contaminated soil (2 mg kg<sup>-1</sup> Cd<sup>2+</sup>), 36.61 mg CdCl<sub>2</sub>·2H<sub>2</sub>O was mixed with 9.0 kg of experimental soil [14]. The soil was then left to equilibrate for four weeks, with soil moisture maintained at 60% of the field capacity to ensure consistent Cd concentrations across different soil layers, simulating typical Cd-polluted natural soil. Si-NPs were thoroughly mixed with distilled water and then uniformly mixed with the experimental soil at a dosage ratio of 250 mg kg<sup>-1</sup> [14] before being transferred into the pots.

To eliminate uncertainties related to nutrients, seed emergence rate, and light exposure, fertilizers were uniformly applied at the time of potting, with nitrogen, phosphorus, and potassium added at equal rates (N: 100 mg kg<sup>-1</sup>,  $P_2O_5$ : 100 mg kg<sup>-1</sup>,  $K_2O$ : 100 mg kg<sup>-1</sup>) for all treatments. Before starting the pot experiment, wheat and faba bean seeds were carefully selected to ensure that each seed had a uniform color, size, and plumpness. The seeds were surface sterilized with 10% (v/v)  $H_2O_2$  for 30 min, thoroughly rinsed, and germinated in the dark for three days [7]. The greenhouse was maintained at temperatures between 20 °C and 30 °C under natural light and a relative humidity of 50%. The pots were randomly placed and repositioned every three days. Watering was performed every two days with 200-250 mL of distilled water per pot, maintaining the soil moisture at 60-70% of the maximum field capacity. Other daily management practices were consistent with typical field management.

#### Plant and soil sample collection

After three months of co-cultivation, two plants were selected for destructive sampling. The entire faba bean plant was carefully removed from the pot, and agronomic parameters of the aboveground parts were quickly recorded, along with the investigation of faba bean Fusarium wilt and biomass measurement (dried at 105°C for 15 min and then at 65 °C to constant weight). Rhizosphere soil was collected using the "shaking soil method" [28]. When collecting plant roots, they were first rinsed with distilled water, and the plants were divided into different organs (roots and aboveground parts) using scissors. Special care was taken to maintain the integrity of root structures. The roots were then soaked in 20 mM Na<sub>2</sub>-EDTA for 15 min to remove Cd and Si ions attached to the surface, followed by thorough rinsing with distilled water [29]. The roots were then prepared for subsequent root scanning analysis and determination of defense substances and enzyme activity parameters.

The collected rhizosphere soil samples were sieved through a 2 mm aperture and divided into two parts. One part was used to determine the conventional physicochemical properties and Cd content of the rhizosphere soil, while the other part was stored in a sterile tube at -80  $^{\circ}$ C for bacterial and metabolomic analyses. The collection process strictly followed the sterile operating procedures to prevent contamination.

#### Indicator determination and methods Investigation of faba bean Fusarium wilt

Wilt disease investigation was conducted on all faba bean plants upon detachment of their aboveground parts. The classification of faba bean wilt disease followed a 5-level classification standard [7]. After investigation, the wilt disease index was calculated using the following formula:undefined

$$Disease index = \frac{\sum (Number of diseased plants at each level \times level value)}{The highest level \times total number of plants studied} \times 100$$

#### Determination of rhizosphere soil physicochemical properties

The collected soil samples were sieved and analyzed using the following methods: the soil pH was measured with a pH meter in a soil/water suspension (1:2.5); the organic matter was determined using the  $K_2Cr_2O_7$ - $H_2SO_4$  oxidation method; and ammonium nitrogen and nitrate nitrogen were measured via the indigo phenol blue colorimetric method and ultraviolet spectrophotometry, respectively. Available phosphorus was extracted with NaHCO<sub>3</sub> and measured using the molybdenum-antimony colorimetric method, and total phosphorus was determined using the HClO<sub>4</sub>- $H_2SO_4$  digestion method. The flame photometer method was used to determine the available and total potassium contents. All of the above physicochemical parameters were determined according to the method described by Bao [30]. The determination of rhizosphere soil enzyme activity was carried out using air-dried soil (5.000 g; 1 mm sieve screening) and placed in a 50mL centrifuge tube. The extraction solution and buffer solution were then added according to the manufacturer's instructions for constant-temperature cultivation. The activities of catalase (CAT), neutral phosphatase (NPase), dehydrogenase, urease (URE), cellulase (cell), and sucrase (SUC) in the rhizosphere soil were measured using soil enzyme activity assay kits purchased from Beijing Solaboo Technology Co., Ltd. (Beijing, China) according to the detailed steps and procedures provided in the manufacturer's instructions [31].

The bioavailable Cd content in the rhizosphere soil was extracted using diethylenetriaminepentaacetic acid (DTPA) [4], and the total Cd in the soil and plant leaves was analyzed using a mixed solution of  $HNO_3$ - $HClO_4$ -HF (5:1:1,v/v/v) and  $HNO_3$  digestion [32], followed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500a, USA). The standard test results were within the allowable error range with a recovery rate of 90–105%.

#### Scanning of root morphological characteristics

After washing the collected and separated roots with deionized water, they were placed on the root plate of a root scanner (EPSON Perfection 4990 Photo, China), injected with an appropriate amount of deionized water, and scanned using preset parameters. The scanned images were processed using image processing software (Adobe Photoshop CC 2019), and root morphology data, such as total root length, average diameter, perimeter, surface area, and root length-to-diameter ratio, were extracted using specialized root analysis software (Win-RHIZO Pro 2007d13).

# Determination of antioxidant capacity and defense enzyme activity of faba bean

Fresh third true leaf (0.1 g) was weighed, and 1 ml of extraction solution (containing buffer solution) was added to homogenize the mixture in an ice bath. After homogenization, the supernatant was centrifuged at  $4^{\circ}$ C (10000 ×*g*, 10 min), collected, and placed on ice to detect the contents of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), malondialdehyde (MDA), and superoxide anion (O<sub>2</sub><sup>-</sup>), and the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD). MDA, H<sub>2</sub>O<sub>2</sub>, and O<sub>2</sub><sup>-</sup> contents were determined using trichloroacetic acid-thiobarbituric acid (TCA-TBA), titanium sulfate, and hydroxylamine methods, respectively, following the procedures outlined by Yan et al. [14]. SOD activity was measured according to Giannopolis and Ries, APX

activity was measured according to Nakano and Asada [33], and POD activity was measured according to the guaiacol method described by Li et al. [34].

#### Determination of rhizosphere soil bacteria

Total DNA of the soil microbial genomes was extracted using the EZ.N.A° Soil DNA Kit (Omega Biotek, Norcross, GA, USA) from 0.5 g of freeze-dried soil sample. The AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) was used to purify the recovered product. A 2% agarose gel electrophoresis was employed to assess the quality of the extracted DNA, and NanoDrop2000 (Thermo Scientific, USA) was used to measure DNA concentration and purity. Genomic DNA was then sent to GENEWIZ for relative quantitative sequencing of the highly variable V3-V4 region of the 16S rRNA gene, which was selected for soil bacterial sequencing. The primers used were 338F (5'-ACTCCTACGGGAGGC AGCAG-3') and 806R (5'-GACTACHVGGGTWTC-TAAT-3') [35]. Paired-end sequencing (2 × 250 bp) was performed using an Illumina NovaSeq 6000 sequencing platform. The PCR system and procedure are described in the Supporting Information. Processing, sequencing, operational taxonomic unit (OTU) clustering, and bacterial species annotation of the PCR products were conducted on the cloud platform of Shanghai Meiji Biotechnology Co., Ltd. (https://cloud.majorbio.com) fol lowing the method described by Zhang et al. [36]. Raw sequence data were first stored in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) and then processed and analyzed using the Quantitative Insight Microbial Ecology (QIIME) software package version 1.8.0 [37]. The raw data provided in this study were submitted to NCBI under the accession number PRJNA1190895.

#### Metabolomics analysis of rhizosphere soil

**Sample preparation** First,  $1000.0 \pm 5.0 \text{ mg}$  of the freezedried rhizosphere soil sample was accurately weighed into a 2 mL centrifuge tube and a 6 mm grinding bead was added. Subsequently,  $1000 \ \mu\text{L}$  of extraction solution (methanol: water = 4:1, v: v) containing 0.02 mg/mL of the internal standard (L-2-chloroalanine) was added for metabolite extraction. The sample solution was ground using a frozen tissue grinder for 6 min (-10 °C, 50 Hz), followed by low-temperature ultrasonic extraction for 30 min (5 °C, 40 kHz). The sample was then placed at -20 °C for 30 min, centrifuged for 15 min (4 °C, 13,000 × *g*), and the supernatant was transferred to an injection vial with an inner tube for analysis. Additionally, 20  $\mu$ L of supernatant from each sample was pooled to create a quality control sample. During the analysis, a QC sample was inserted every three samples to monitor the repeatability of the entire process.

LC-MS detection Detection was performed using a Thermo Fisher Scientific ultra-high-performance liquid chromatography-tandem Fourier transform mass spectrometry (UHPLC-Q Exactive HF-X) system. The chromatographic column was ACQUITY UPLC HSS T3 (100 mm  $\times$  2.1 mm i.d., 1.8 µm; Waters, Milford, USA). Mobile phase A consisted of 95% water and 5% acetonitrile with 0.1% formic acid, while mobile phase B contained 47.5% acetonitrile, 47.5% isopropanol, and 5% water with 0.1% formic acid. The injection volume was 3 µL, column temperature was set to 40 °C, and flow rate was 0.4 mL/min. The gradient elution program was as follows: 0-3.5 min, 0-24.5% B; 3.5-5 min, 24.5-65% B; 5-5.5 min, 65-100% B; 5.5-7.4 min, 100% B, 0.4-0.6 mL/ min; 7.4-7.6 min, 100-51.5% B; 7.6-7.8 min, 51.5-0% B, 0.6-0.5 mL/min; 7.8-9 min, 0% B, 0.5-0.4 mL/min; 9-10 min, 0% B, 0.4 mL/min. The mass spectrometry conditions included a spray ion source (ESI) temperature of 425 °C, mass spectrum voltages of 3500 V (positive mode) and – 3500 V (negative mode), and a mass scanning range of 70-1050 m/z. The sheath gas flow rate was 50 psi, auxiliary gas flow rate was 13 psi, ion transport tube temperature was 325 °C, and normalized collision energy was 20–40–60 V in the cyclic collision energy. The resolution of the primary mass spectrometry was set to 60,000, and that of the secondary mass spectrometry was 7,500, with the data collected in DDA mode.

Computer data processing and differential metabolite analysis The raw data were imported into the metabolomics processing software Progenesis QI v2.3 (Waters Corporation, Milford, USA) for analysis. The software was used for feature peak searching and library identification, matching MS and MS/MS spectrometry information with metabolic databases, with the MS error set to less than 10 ppm. Metabolites were identified based on secondary mass spectrometry-matching scores. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed on the metabolites using the ropls package in R, and model stability was evaluated using a 7-cycle interaction validation. Additionally, Student's t-test and fold-change analysis were performed. Differential metabolites were screened using variable weight values (VIP values > 1 and p < 0.05), and the top 30 significantly different metabolites in each group were intersected. The metabolic pathway enrichment analysis of the differential metabolites was conducted using the KEGG database (https://www.kegg.jp/ kegg), with the P-values < 0.05, indicating the significant pathway enrichment.

#### Statistics and analysis

Microsoft Excel 2010 and GraphPad Prism 8.0 were utilized to organize and plot the test data. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Duncan's multiple range test (SPSS 19.0, IBM, USA) to evaluate the differences in indicators, such as Fusarium wilt occurrence, root morphology characteristic parameters, growth parameters, soil enzyme activity, Cd content, and soil physicochemical factors among the MFcd, IFcd, and IFcd + Si treatments, with statistical significance set at P < 0.05. Alpha diversity of bacterial communities (Chao, Shannon, and Simpson indices) was evaluated using the Rogers's [38] method, and inter-group differences were assessed using the Wilcoxon rank sum test. Beta diversity analysis based on Bray-Curtis distance was used to compare the microbial community structure between treatments, which was verified and visualized through principal coordinate analysis (PCoA). Data in the tables and figures are presented as mean±standard deviation (SD) of three replicates. Correlation analysis between parameters was conducted using Spearman's method. Metabolomics of the rhizosphere soil, microbiota, and their association maps with differential metabolites were analyzed and generated using the free online bioinformatics cloud platform of Shanghai Meiji Biomedical Technology Co., Ltd. (https:/ /cloud.majorbio.com).

#### Results

### Exogenous Si-NPs and intercropping superposition alleviate cd toxicity and *Fusarium* wilt in faba bean

Compared with the monoculture of faba bean (MFcd) under Cd stress, both intercropping (IFcd) and intercropping with Si-NPs (IFcd + Si) improved faba bean growth (Fig. 1a and b). The IFcd and IFcd + Si treatments significantly increased the dry weight (both aboveground and underground) by 80.7% and 126.7%, respectively, and plant height by 28.3% and 57.8%, respectively, but also significantly reduced the incidence of *Fusarium* wilt by 11.1% and 33.3% and Cd accumulation in plant leaves by 17.3% and 56.3%, respectively (P < 0.05) (Fig. 1c–f). However, IFcd alone did not significantly reduce the incidence of *Fusarium* wilt compared to MFcd (P > 0.05). These results indicated that intercropping with Si-NPs could notably reduce Cd accumulation in plants and decrease the occurrence of soil-borne diseases.

Further analysis of the root morphology of faba bean under different treatments (Table S1) indicated that both the IFcd and IFcd + Si treatments increased the total root length, perimeter, root length diameter (rang1), and surface area diameter (rang 1 and rang 2) to varying degrees, while reducing the average root diameter and root surface area. The increases in total root length by 7.6% and 8.1% and surface area diameter (rang 1 and rang 2) by



**Fig. 1** Effects of intercropping (IFcd) and Si-NPs superposition (IFcd + Si) on faba bean growth and *Fusarium* wilt occurrence. Panels (**a**)–(**f**) show plant growth status, faba bean phenotype per plant, dry weight of aboveground and belowground tissues, plant height, *Fusarium* wilt incidence, and Cd content in plant leaves, respectively. Values are presented as the mean  $\pm$  SD (n=3). Different lowercase letters indicate significant differences between treatments (P < 0.05)

5.4%, 26.4%, 23.6%, and 18.2% were significant (P < 0.05). Compared with IFcd alone, IFcd + Si did not significantly increase the total root length, average diameter, circumference, or surface area, but significantly enhanced the root length diameter (rang 1) and surface area diameter (rang 1) of the main root parts (P < 0.05). This revealed that intercropping with Si-NPs could effectively increase the diameter and total root length of the main root parts, improving resistance to Cd toxicity and *Fusarium* pathogen stress. Additionally, Si-NPs can also promote faba bean growth to a certain extent.

#### Exogenous Si-NPs and intercropping superposition enhance antioxidant activity and systemic defense in faba bean plants

The contents of  $H_2O_2$ , MDA, and  $O_2^-$  in plant leaves reflect oxidative stress and membrane lipid peroxidation levels, which serve as key indicators of plant stress resistance. Compared with the MFcd treatment, the IFcd and IFcd + Si treatments showed a trend of reducing  $H_2O_2$ , MDA, and  $O_2^-$  levels, and the IFcd + Si treatment significantly lowered these levels by 37.5%, 74.1%, and 90.2%, respectively (P < 0.05) (Fig. 2a–c). Another analysis of the defense enzyme activity in faba bean leaves revealed that both the IFcd and IFcd + Si treatments significantly increased the activities of SOD (16.5% and 32.9%), APX (15.4% and 40.1%), and POD (80.0% and 209.9%) enzymes (P < 0.05). Additionally, IFcd + Si treatment resulted in a significant increase in the activities of these enzymes compared to IFcd alone (Fig. 2d–f). These results indicated that intercropping with Si-NPs, both individually and in combination (IFcd + Si), could effectively enhance the antioxidant and systemic defense mechanisms of faba bean plants under Cd and soil-borne pathogen stress.

#### Effects of exogenous Si-NPs and intercropping superposition on rhizosphere soil physicochemical properties of faba bean

The available Cd content in the rhizosphere of faba bean could be a vital indicator for assessing the effectiveness of planting patterns and exogenous measures in treating Cd-contaminated soil. Compared to the MFcd treatment, the IFcd and IFcd + Si treatments increased the total Cd content to varying degrees while reducing the available Cd content. The IFcd + Si treatment significantly reduced both the available Cd content (32.2%) and its proportion in total Cd compared to MFcd alone (Fig. 3a and b). As an important indicator of soil ecosystem dynamics and health, soil enzyme activity was significantly increased by the IFcd and IFcd + Si treatments compared with the MFcd treatment. Notable increases included 24.1% and 27.0% for CAT, 47.5% and 101.1% for dehydrogenase,



**Fig. 2** Effects of intercropping (IFcd) and Si-NPs superposition (IFcd + Si) on antioxidant and defense ability of faba bean plant leaves. Panels (**a**)–(**c**) show the contents of  $H_2O_2$ , MDA, and  $O_2^-$  in plant leaves. Panels (**d**)–(**f**) show the enzyme activities of SOD, APX, and POD in plant leaves. Values are presented as the mean  $\pm$  SD (n = 3). Different lowercase letters indicate significant differences between treatments (P < 0.05)



**Fig. 3** Effects of intercropping (IFcd) and Si-NPs superposition (IFcd + Si) on rhizosphere soil physicochemical properties of faba bean. (**a**) and (**b**) show the total Cd and available Cd contents in the rhizosphere soil. (**c**)–(**h**) represent the activities of CAT, neutral phosphatase, dehydrogenase, urease, cellulase, and sucrase in the rhizosphere soil of faba bean, respectively. Values are presented as the mean  $\pm$  SD (n=3). Different lowercase letters indicate significant differences between treatments (P < 0.05)

104.8% and 78.0% for URE, 13.5% and 42.1% for Cell, and 23.2% and 42.4% for SUC enzymes (P < 0.05) (Fig. 3c–h). IFcd+Si treatment further increased the activities of NPase, dehydrogenase, Cell, and SUC enzymes compared with IFcd, whereas it did not significantly increase CAT and URE enzyme activities (P > 0.05).

Another analysis of the physicochemical properties of faba bean rhizosphere soil revealed that compared with the MFcd treatment, both the IFcd and IFcd+Si treatments exhibited an increasing trend in pH, OM, NH<sub>4</sub><sup>+</sup>-N, and AK content, whereas the NO3--N and Olsen-P contents decreased. The NH4+-N content significantly increased by 23.1% in the IFcd+Si treatment, whereas the NO3<sup>-</sup>-N content significantly decreased by 72.3% (P < 0.05). The IFcd + Si treatment reduced the NO<sub>3</sub><sup>-</sup>-N content by 64.6% compared with IFcd (P < 0.05), whereas the increase in other physicochemical factors was not significant (Table 1). Spearman correlation analysis (|r|>0.6 and P < 0.05) showed a significant positive correlation between NO3-N and nutrient cycling enzymes such as URE, whereas Cd was significantly negatively correlated with OM, A-K, H<sub>2</sub>O<sub>2</sub>, and URE (Figure S1). This suggests that intercropping and its reinforcement treatment (IFcd+Si) primarily affected the transformation of ammonium and nitrate nitrogen, leading to improvements in soil physicochemical properties.

# Effects of exogenous Si-NPs and intercropping superposition on the rhizosphere bacterial community of faba bean

The 16 S rRNA sequencing results of the rhizosphere soil demonstrated that compared with the MFcd treatment, both IFcd and IFcd + Si treatments showed an increasing trend in the richness (Chao index) and diversity (Shannon index) indices. Specifically, IFcd treatment significantly reduced the Simpson index, whereas IFcd + Si treatment significantly increased the Chao and Shannon indices (P < 0.05) (Fig. 4a), indicating an increase in bacterial community diversity and richness. Both the IFcd and IFcd + Si treatments reduced the number and proportion of unique OTUs in the bacterial community compared to the MFcd treatment. Analysis of community  $\beta$  diversity revealed a clear differentiation between the MFcd and the IFcd and IFcd + Si treatments but no significant

influence the rhizosphere bacterial community. Analysis of the rhizosphere bacterial composition revealed that compared to the MFcd treatment, the IFcd and IFcd + Si treatments increased the relative abundance of Actinobacteria, Proteobacteria, and Chloroflexi at the phylum level, while reducing the abundance of Acidobacteriota. At the genus level, the relative abundances of Sphingomonas, Intrasporangium, Marmoricola, and Streptomyces increased, whereas that of Gemmatimonas decreased. Further intergroup difference analysis revealed significant differences among Sphingomonas, Intrasporangium, and Nocardioids. Nocardioids were significantly positively correlated with URE, H<sub>2</sub>O<sub>2</sub> activity, and NO3<sup>-</sup>-N content, whereas Sphingomonas was positively correlated with NO<sub>3</sub><sup>-</sup>-N, cellulase, pH, H<sub>2</sub>O<sub>2</sub>, SUC, AP, and Si content. Intrasporangium was significantly positively correlated with URE (P < 0.05). These findings suggested that intercropping and Si-NPs treatment primarily recruited beneficial bacteria, thereby improving the rhizosphere bacterial community by influencing specific enzymes and soil physicochemical properties.

# Effects of exogenous Si-NPs and intercropping superposition on rhizosphere metabolites of faba bean

Metabolomic analysis was conducted on faba bean rhizosphere soil samples influenced by exogenous addition of Si-NPs and intercropping. PLS-DA results demonstrated a clear separation among the three treatments and strong clustering among the repeated samples (Fig. 5a). The model permutation test parameters  $R^2 Y \mbox{ and } Q^2 \mbox{ were}$ 0.9883 and -0.5532, respectively, indicating that the PLS-DA model performed well and supported further data analysis. Compared to MFcd treatment, IFcd treatment resulted in significant changes in 64 differential metabolites (DMs) (32 upregulated and 32 downregulated), whereas IFcd + Si treatment showed significant changes in 26 DMs (10 upregulated and 16 downregulated) compared to IFcd. Venn analysis of all DMs revealed 17 unique DMs for IFcd and six for IFcd + Si compared to MFcd. KEGG compound classification of these metabolites grouped them into seven major categories (Fig. 5f),

 Table 1
 Changes in physicochemical factors of rhizosphere soil of faba bean under different treatments

Treatments	рН	Organic matter (OM) (g kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> - <i>N</i> (mg kg <sup>-1</sup> )	NO <sub>3</sub> <sup></sup> N (mg kg <sup>-1</sup> )	Total phosphorus (TP) (g kg <sup>-1</sup> )	Olsen- <i>P</i> (AP) (mg kg <sup>-1</sup> )	Total potas- sium (TK) (g kg <sup>-1</sup> )	Available potassium (AK) (mg kg <sup>-1</sup> )
MFcd	7.04±0.07 <sup>a</sup>	27.43±1.32 <sup>a</sup>	27.48±3.02 <sup>b</sup>	2.67±0.28 <sup>a</sup>	0.53±0.07 <sup>a</sup>	63.72±3.71 <sup>a</sup>	0.17±0.00 <sup>a</sup>	176.00±1.22 <sup>b</sup>
IFcd	7.24±0.15 <sup>a</sup>	30.25±0.68 <sup>a</sup>	29.85±1.67 <sup>ab</sup>	2.09±0.26 <sup>a</sup>	0.69±0.02 <sup>a</sup>	47.06±3.56 <sup>b</sup>	0.18±0.01 <sup>a</sup>	209.50±4.16 <sup>a</sup>
IFcd + Si	7.21±0.25 <sup>a</sup>	30.22±1.10 <sup>a</sup>	33.83±1.75 <sup>a</sup>	0.74±0.04 <sup>b</sup>	0.45±0.06 <sup>a</sup>	53.52±2.59 <sup>b</sup>	0.17±0.00 <sup>a</sup>	203.00±4.49a

Note: Different lowercase letters in the same column indicate significant differences between treatments (P < 0.05). Data are presented as mean  $\pm$ SD (n = 3)



**Fig. 4** Effects of intercropping (IFcd) and Si-NPs superposition (IFcd + Si) on the rhizosphere bacterial community of faba bean. (**a**) Alpha diversity indices (Chao, Shannon, and Simpson) of bacterial community under different treatments (MFcd, IFcd, and IFcd + Si); (**b**) Venn diagram showing bacterial OTU overlap across different treatments; (**c**) Beta diversity analysis of the bacterial community (PCoA); (**d**) composition analysis of bacterial communities at the phylum and genus levels; (**e**) intergroup comparison of dominant bacterial genera (Top 15); (**f**) Spearman correlation analysis between dominant bacterial genera (Top 15) and environmental factors (Cd, Npase: neutral phosphatase, OM: organic matter, TP, TK, AK, URE: urease, NO<sub>3</sub><sup>-</sup>-N, Cell: cellulase, pH, H<sub>2</sub>O<sub>2</sub>, SUC: sucrase, AP, Si, NH<sub>4</sub><sup>+</sup>-N, Dehydrogenase). \*0.01 <  $p \le 0.05$ , \*\*0.001 <  $p \le 0.01$ , \*\*\* $p \le 0.001$ 



Fig. 5 Classification and analysis of differential metabolites in the rhizosphere of faba bean using intercropping (IFcd) and Si-NPs superposition (IFcd + Si). (a) Partial least squares discriminant analysis (PLS-DA) of metabolites between MFcd, IFcd, and IFcd + Si treatments; (b) PLS-DA model permutation test; (c) Venn diagram of differential metabolites across treatments; volcano plot of differential metabolites in (d) IFcd\_vs\_MFcd and (e) IFcd + Si\_vs\_IFcd comparisons; (f) KEGG compound classification and statistical chart

with flavonoids being the most representative, followed by terpenoids and phenylpropanoids.

Further analysis of the enrichment of dominant DMs revealed that compared to MFcd, the IFcd treatment increased the secretion of compounds such as scymnol, 5-deoxyribose-1-phosphate, and 4-hydroxy-5-phenyltetrahydro-1,3-oxazin-2-one, while reducing the secretion of compounds such as dehydrotropingosine and cis-p-coumaric acid. Compared to IFcd, IFcd+Si treatment increased the levels of metabolites, such as ectoine, isopentanol, and gemfibrozil, while reducing the levels of compounds, such as isorhamnetin and phthalic acid (Fig. 6a and b). The differential metabolites influenced by IFcd treatment were primarily enriched in certain pathways such as Fc gamma R-mediated phagocytosis, GnRH signaling, and carotenoid biosynthesis. In contrast, the differential metabolites affected by IFcd+Si treatment were mainly enriched in ABC transporters and cell cyclerelated metabolic pathways (Fig. 6c and d).

#### Joint analysis of exogenous Si-NPs and intercropping superposition on rhizosphere metabolome and microbiome interactions of faba bean

Understanding the relationship between metabolites and rhizosphere microorganisms is crucial for optimizing crop growth mechanisms through intercropping and Si-NPs treatment. Hence, we first analyzed the overall correlation between the rhizosphere bacterial community and metabolite expression. The results with a Monte Carlo p-value < 0.01 and  $M^2 > 0.5$  indicated a significant consistency in trends between microbial community structure and metabolite expression levels across different groups, demonstrating a strong correlation between the two datasets (Fig. 7a). Further analysis of the relationship between major differential metabolites and dominant bacteria (Top 15) revealed that Nocardioids, Sphingomonas, and Intrasporangium exhibited varying degrees of negative correlation with metabolites such as aflatoxin B2 and Cinobufagin, while presenting positive correlations with antibacterial compounds such as sordarin, lactucin, and 15-methylpalmate (Fig. 7b). These correlations highlight the fundamental reasons for the



Fig. 6 Cluster analysis of rhizosphere metabolites and KEGG enrichment analysis of differential metabolites in faba bean using intercropping (IFcd) and Si-NPs superposition (IFcd + Si). (a) Cluster heatmap analysis of the top 30 differential metabolites in IFcd\_vs\_MFcd and (b) IFcd + Si\_vs\_IFcd metabolome (based on Bray-Curtis distance); (c) KEGG pathway enrichment analysis of differential metabolites in IFcd\_vs\_MFcd and (d) IFcd + Si\_vs\_IFcd

changes in soil physicochemical properties and enhancement of crop growth due to the application of exogenous Si-NPs and intercropping.

#### Discussion

Cd and *Fusarium* are two major abiotic and biotic stresses affecting current faba bean production. The synergistic effect of exogenous Si-NPs and intercropping leverages the beneficial properties of Si-NPs and interspecific root interactions. This combination enhances the activity of rhizosphere soil enzymes and the transformation of soil physicochemical factors, diminishes the adverse effects of soil-borne pathogens and Cd on host plants, and improves the plant's stress resistance. The findings of this study support the use of exogenous Si-NPs to strengthen the ability of intercropped faba bean to mitigate Cd toxicity and *Fusarium* wilt and to elucidate the interaction mechanisms between root microbiota and metabolites.



**Fig. 7** Joint analysis of metabolome and microbiome interactions in the rhizosphere of faba bean. (a) Correlation analysis between rhizosphere metabolites and microbial communities using the Procrustes test; (b) Spearman correlation heatmap illustrating the relationship between key differential metabolites and dominant bacterial genera (Top 15). \*0.01  $, **0.001 <math>, ***<math>p \le 0.001$ 

# Exogenous Si-NPs and intercropping effectively reduce Cd toxicity in faba bean and control the *Fusarium* wilt

The toxicity of HM Cd can affect crop growth and product safety and threaten human health through the food chain [39]. This study revealed that compared to the monoculture faba bean under Cd stress (MFcd), the wheat-faba bean intercropping (IFcd) significantly reduced Cd accumulation in faba bean, promoted plant growth, and reduced soil-borne diseases, consistent with the findings of similar studies on Sonchus asper (L.) Hill intercropped with faba bean [9]. However, some studies have indicated that intercropping faba bean with rapeseed (Brassica napus L.), Sedum alfredii Hance, or Arabidopsis alpine can promote Cd enrichment in the aboveground parts of faba bean [12, 40, 41]. These differences may be attributed to intercropping plant types, soil physicochemical properties, and experimental conditions (e.g., hydroponics, soil culture, or field experiments) [1]. Additionally, the wheat-faba bean intercropping stimulates nitrogen fixation in faba bean through interspecific competition [10, 42], with nitrogen acting as a key detoxifying factor for Cd stress [1]. Nitrogen enhances the biosynthesis of cysteine and other compounds, alleviates Cd stress, increases nutrient absorption, and reduces forage alfalfa disease [11, 43]. Notably, this study also found that intercropping increased the effective Cd content in the faba bean rhizosphere, possibly because of the enhanced root interactions between wheat and faba bean, stimulating the secretion of organic acids, such as citric and malic acid, which may increase the effective Cd content in the rhizosphere [9]. Most current intercropping systems focus on using hyperaccumulating plants and crop intercropping to study the absorption and toxicity of Cd [1, 44]. However, few studies have addressed the toxic effects of crop-crop intercropping on target crops. This study confirms that intercropping faba bean with wheat can mitigate the toxic effects of Cd on faba bean.

This study proved that compared to the IFcd treatment, intercropping with Si-NPs (IFcd+Si) significantly reduced Cd accumulation in faba bean, decreased rhizosphere Cd availability, reduced the incidence of Fusar*ium* wilt, and improved plant growth conditions (Fig. 1). Si plays a key role in mitigating Cd toxicity in plants by compartmentalizing heavy metals [45] and inhibiting their transport to aboveground parts [46]. It also strengthens the plant cuticle and forms a silica layer in the cell wall, making pathogen penetration more difficult and reducing disease occurrence [47]. As a new type of nanomaterial, Si-NPs offer enhanced stress resistance in plants under both biotic and abiotic stresses [14]. Si-NPs have demonstrated greater potential for promoting plant growth and stress resistance than traditional silicon materials because they can be absorbed by plants without the need for silicon-related transport proteins [48, 49]. The excellent adsorption capacity of Si-NPs and their ability to reduce Cd flux in plants are key mechanisms for alleviating Cd toxicity [14]. The Si-NPs stacking with intercropping treatment exhibited stronger effects in reducing Cd toxicity and controlling faba bean Fusarium wilt than the MFcd treatment. This could be attributed to the precipitation of Si-NPs in plant epidermal tissues, forming silicified cells that could delay and hinder the

invasion of *Fusarium* pathogens, thereby enhancing their resistance to *Fusarium* wilt [17, 18]. Additionally, we first found that the IFcd + Si treatment significantly increased the total root length, root length diameter (range 1), and surface area diameter (range 1), which could provide a crucial foundation for nitrogen fixation, nutrient acquisition, and promotion of plant growth in faba bean root nodules.

# Enhancement of defense resistance and soil enzyme activity in faba bean plants through intercropping with exogenous Si-NPs

The toxic effects of Cd on plants are seen not only in inhibited growth but also in disruptions to physiological and biochemical processes, such as increased oxidative stress and altered resistance enzyme activity [50]. This study suggested that compared to the MFcd treatment, both the IFcd and IFcd+Si treatments reduced the levels of MDA and  $O_2^-$  and significantly increased the activity of antioxidant enzymes such as SOD, APX, and POD. The decrease in oxidation levels and Cd content may be related to interspecific root interactions that promote Cd-tolerant endophytic bacteria, which in turn enhances the activity of oxidoreductases in plants [51]. Plants respond to stress by overproducing reactive oxygen species (ROS), sulfur species (RSS), and nitrogen species (RNS). If not cleared in time, they can cause oxidative damage to cells and even lead to plant death [16]. Our experimental results indicate that the addition of Si-NPs and intercropping can enhance the antioxidant capacity and Fusarium wilt resistance of host plants, which is similar to the findings of Sun et al. [52] in cucumber. Notably, IFcd + Si treatment was more effective than IFcd alone in reducing oxidative stress and increasing resistance enzyme activity (Fig. 2), indicating that wheat-faba bean intercropping combined with Si-NPs synergistically enhanced plant antioxidant capacity and defense performance, making it an effective strategy for managing Cd and soil-borne pathogen stress. However, it is important to note that the Si-NPs effect observed in this study was based on Cd stress conditions, and caution should be exercised in determining whether Si-NPs can improve the antioxidant capacity and promote growth of faba bean in conventional soil.

Rhizosphere soil is most closely associated with plants and serves as a critical reference for assessing the bioavailability of HMs and overall plant health through changes in enzyme activity and physicochemical properties [53]. This study indicated that IFcd and IFcd + Si treatments significantly increased the activities of CAT, NPase, dehydrogenase, URE, Cell, and SUC enzymes in the soil, which are vital for nutrient cycling, plant growth, and reducing soil-borne diseases [25]. Moreover, the IFcd + Si treatment significantly increased the NH<sub>4</sub><sup>+</sup>-N and AK contents while reducing the NO<sub>3</sub><sup>-</sup>-N and Olsen-P levels (Table 1), unlike the IFcd treatment alone. This may be due to the enhanced activity of NH4+-N microorganisms facilitated by Si-NPs, which have a large specific surface area and strong adsorption capacity, thereby promoting phosphorus adsorption. As a result, this leads to a decrease in the contents of NO3<sup>-</sup>-N and Olsen-P in the rhizosphere soil. The significant negative correlation between Si and Cd, along with their positive correlation with most enzymes, further highlighted the beneficial effects of Si-NPs in regulating soil properties (Figure S1). These findings indicate that intercropping and Si-NPs synergistically enhanced soil enzyme activity and promoted nutrient cycling. Furthermore, URE can serve as an enzyme sensitive to HM pollution and is an important indicator for evaluating Cd toxicity [54]. When applying intercropping and Si-NPs in the field, attention should be paid to the potential adverse effects of other nutrients, such as managing soil TK content, in mitigating Cd toxicity.

## Exogenous Si-NPs and intercropping superposition regulate rhizosphere microbiota

Rhizosphere soil microorganisms, often referred to as the "second genome", can serve as vital indicators of crop health [36]. Under HMs stress, they typically experience certain changes, such as homogenization of species composition and reduced functional diversity [13]. This study discovered that intercropping, especially when combined with Si-NPs, increased bacterial community diversity and richness compared with MFcd treatment. Although the IFcd treatment exhibited an improvement trend, the IFcd+Si treatment significantly enhanced these microbial parameters (Fig. 4a-c), which is consistent with previous intercropping studies [29, 44]. Recent studies have suggested that Si can positively influence plant diversity and nutrient availability, including soil Si, while reducing Cd toxicity [4, 25, 55]. Both the IFcd and IFcd + Si treatments increased the available Cd content in the rhizosphere, but the IFcd + Si treatment reduced this content compared to the IFcd alone, indicating that the exogenous Si-NPs lowered the Cd bioavailability in the soil [56, 57]. These changes are closely linked to increased rhizosphere microbial diversity [25, 58]. Interestingly, this study first revealed that, under Cd stress, intercropping had a limited effect on enhancing rhizosphere bacterial diversity and richness. In contrast, intercropping combined with Si-NPs was found to be more effective in improving microbial diversity in the rhizosphere of faba bean.

Actinobacteria, Proteobacteria, and Chloroflexi were the dominant bacterial phyla in the intercropping systems and Si-NPs treatments. They are known for their roles in decomposing organic matter, increasing soil

enzyme activity, and promoting soil nutrient cycling [59]. Actinobacteria have been reported to produce indole-3-acetic acid and catechol acid iron carriers and form complexes with Cd to reduce Cd content. The presence of Cd can induce Pseudomonas aeruginosa to produce pyocyanin, thereby reducing the number of pathogenic fungi, such as Fusarium, thereby resisting soil-borne diseases and promoting plant growth [25, 60]. Further analysis of bacterial genus composition across different treatments revealed that the relative abundance of microbial genera in the IFcd and IFcd + Si treatments increased with improvements in soil chemical properties and plant growth parameters compared with the MFcd treatment. Among them, Sphingomonas, Intrasporangium, Marmoricola, Streptomyces, and Nocardioids genera significantly increased in the IFcd and IFcd + Si treatments. Sphingomonas not only promotes plant growth, inhibits pathogens, and participates in nitrogen cycling and antioxidation but also has the ability to fix soil heavy metals [2, 13]. Beneficial bacteria such as Streptomyces and Nocardioids play crucial roles in promoting plant growth and controlling soil-borne diseases in the rhizosphere [61]. Additionally, Nocardioides and Sphingomonas were identified as metabolically active bacteria in heavy metalcontaminated soils, which could enhance the tolerance of Dahlia pinata Cav to Cd [25]. These findings further explain the positive effects of intercropping under Cd stress, especially when combined with Si-NPs, on reshaping rhizosphere microbial communities and improving crop health. However, in this study, the addition of Si-NPs under Cd stress did not lead to significant enrichment of beneficial bacteria, such as Paenibacillus and Pseudomonas aeruginosa [2, 63]. This could be because of the presence of pathogenic bacteria and Cd stress in faba bean continuous cropping, where crops could recruit more complex functional microorganisms in the rhizosphere than relying on single stress-resistant or growthpromoting bacteria, reflecting a plant strategy to balance growth and stress resistance.

#### Regulation of rhizosphere metabolites by exogenous Si-NPs and intercropping superposition and their interaction mechanisms with microorganisms

Changes in plant rhizosphere metabolites can serve as key indicators of plant phenotypic responses to environmental stress [39, 64]. Rhizospheric metabolites can significantly influence crop growth and disease resistance [5, 39]. This study compared the root metabolites of monoculture, intercropping, and intercropping enhanced with Si-NPs and identified a substantial impact on the rhizosphere metabolites of faba bean. Flavonoids were the most abundant metabolites (Fig. 5f), playing a crucial role in regulating plant-environment interactions, recruiting beneficial microbial communities, reducing free radical damage, alleviating Cd toxicity, and resisting disease invasion [4, 65]. Terpenoids also contribute to plant adaptation to stress, pathogen inhibition, and induced resistance, which exert positive effects [66]. Analysis of differential metabolites revealed an increase in ribonucleotides related to DNA synthesis, along with metabolites including 5-Deoxyribose-1-phosphate and 4-Hydroxy-5-phenyltetrahydro-1,3- oxazin-2-one, which promoted plant growth and defense. These metabolites were enriched in KEGG pathways related to cell phagocytosis and hormone signal transduction. Si-NPs enhance the secretion of antioxidant secondary metabolites such as ectoine, which helps regulate plant cell permeability [16]. The main enriched KEGG pathways, including ABC transporters and the cell cycle, support plant resilience to salt and other stressors [67].

The plant rhizosphere is a crucial zone for soil-plantmicrobe interactions [23, 24], and the microbiome and metabolome within this area play key roles in understanding the mechanisms underlying plant oxidative stress responses, healthy growth, and disease resistance [18, 68]. Our association analysis between dominant bacteria and differential metabolites revealed a strong correlation between microbial and metabolomic datasets. Microorganisms linked to plant stress, including Nocardioides, Sphingomonas, and Intrasporangium, exhibited strong positive correlations with sordarin, lactucin, and 15-methylpalmate (Fig. 7), and these antibacterial compounds are connected to both plant rhizosphere defense mechanisms and overall plant defense capabilities [69, 70]. Previous studies have indicated that the absorption of Si-NPs by plants can enhance the metabolism of rhizosphere sugars, organic acids, and amino acids, improve microbial nutrition, and increase the colonization of Cd-tolerant bacteria, thereby reducing Cd toxicity in the rhizosphere [68, 71]. This study suggested a strong correlation between the recruited microbiota and metabolites under Cd stress, whether through intercropping or Si-NPs treatment. Notably, compounds such as sordarin, lactucin, and 15-methylpalmate were significantly enriched. In summary, our findings indicate that intercropping and Si-NPs treatment significantly enriched the differential metabolites and key microorganisms involved in faba bean root zone defense, offering first and important insights into enhancing the resilience of faba bean against environmental stress. These findings demonstrate the vital role of rhizosphere metabolites in shaping microbial communities and strengthening plant resistance to soil-borne diseases. The close association between microbes and metabolites explains how intercropping and Si-NPs could alleviate the dual stress of soil-borne pathogens and Cd by enhancing plant antioxidant capacity, boosting rhizosphere soil enzyme activity, reshaping the bacterial community structure, and

regulating key rhizosphere metabolites, ultimately promoting healthy crop growth.

#### Conclusion

Our results indicated that compared to the monoculture faba bean under Cd stress, IFcd and IFcd + Si reduced Cd accumulation in plants, decreased the incidence of faba bean Fusarium wilt, and increased the diameter and total root length of the main roots. These treatments also enhanced the plant antioxidant and systemic defense capabilities, increased the activity of root enzymes such as NPase, dehydrogenase, and SUC, improved the physicochemical properties of the soil, regulated flavonoid secretion, and promoted the enrichment of functional bacteria such as Nocardiides, Sphingomonas, and Intraporangium, along with secondary metabolites such as sordarin, lactucin, and 15-methylpalmate in the rhizosphere. The close interaction between microorganisms and their metabolites revealed the rhizosphere microecological mechanisms by which IFcd and IFcd + Si alleviated Cd toxicity and resisted Fusarium wilt. Although IFcd alone improved these factors to some extent, the regulatory effects of intercropping combined with Si-NPs were more pronounced. In summary, this study demonstrated the significant advantages of using intercropping and Si-NPs to alleviate Cd and pathogen stress in faba bean, with promising potential for large-scale agricultural practices in the future.

#### Supplementary Information

The online version contains supplementary material available at https://doi.or g/10.1186/s12951-025-03330-0.

Supplementary Material 1

#### Acknowledgements

This work was supported by the National Key Research and Development Program of China (2022YFD1901503-4) and thanks the MJEditor (www. mjeditor.com) for its linguistic assistance during the preparation of this manuscript.

#### Author contributions

CL and TL conceived the original screening, research plans, and designed the experiments. CL finished re-writing and revising this thesis. YD supervised the experiments and agreed to serve as the author responsible for contact and ensures communication. YH and TL provided the technical assistance and analyzed the data to CL and TL. All authors contributed to the article and approved the submitted version.

#### Funding

This work was supported by the National Key Research and Development Program of China (2022YFD1901503-4).

#### Data availability

The data presented in the study are deposited in the NCBI repository, accession number PRJNA1190895.

#### Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

#### **Competing interests**

The authors declare no competing interests.

#### Author details

<sup>1</sup>Key Laboratory for Improving Quality and Productivity of Arable Land of Yunnan Province, College of Resources and Environment, Yunnan Agricultural University, Kunming 650201, China <sup>2</sup>College of Plant Protection, Yunnan Agricultural University, Kunming 650201, China

#### Received: 25 October 2024 / Accepted: 14 March 2025 Published online: 01 April 2025

#### References

- Liu Y, Huang L, Wen Z, Fu Y, Liu Q, Xu S, et al. Effects of intercropping on safe agricultural production and phytoremediation of heavy metal-contaminated soils. Sci Total Environ. 2023;875:162700. https://doi.org/10.1016/j.scitotenv.20 23.162700.
- Wang X, Dong G, Xingwang L, Zhang S, Li C, et al. Poly-γ-glutamic acid-producing bacteria reduced cd uptake and affected the rhizosphere microbial communities of lettuce. J Hazard Mater. 2020;398:123146. https://doi.org/10. 1016/j.jhazmat.2020.12314.
- Wu M, Luo Q, Liu S, Zhao Y, Yue L, Pan Y. Screening ornamental plants to identify potential cd hyperaccumulators for bioremediation. Ecotoxicol Environ Saf. 2018;162:35–41. https://doi.org/10.1016/j.ecoenv.2018.06.049.
- Ahmed T, Shou L, Guo J, Muhammad N, Qi Y, Yao Y, et al. Modulation of rhizosphere microbial community and metabolites by bio-functionalized nanoscale silicon oxide alleviates cadmium-induced phytotoxicity in bayberry plants. Sci Total Environ. 2024;933:173068. https://doi.org/10.1016/j.scit otenv.2024.173068.
- Qiang L, Zhao N, Liao K, Sun X, Wang Q, Jin H. Metabolomics and transcriptomics reveal the toxic mechanism of cd and nano TiO<sub>2</sub> coexposure on rice (*Oryza sativa* L). J Hazard Mater. 2023;453:131411. https://doi.org/10.1016/j.jh azmat.2023.131411.
- Dong Q, Fei L, Wang C, Hu S, Wang Z. Cadmium excretion via leaf hydathodes in tall fescue and its phytoremediation potential. Environ Pollut. 2019;252:1406–11. https://doi.org/10.1016/j.envpol.2019.06.079.
- Lv J, Dong Y, Dong K, Qian Z, Yang Z, Ling C. Intercropping with wheat suppressed fusarium wilt in Faba bean and modulated the composition of root exudates. Plant Soil. 2020;448(1–2):153–64. https://doi.org/10.1007/s11104-0 19-04413-2.
- Etemadi F, Hashemi M, Barker AV, Zandvakili OR, Liu X. Agronomy, nutritional value, and medicinal application of Faba bean (*Vicia Faba* L). Hortic Plant J. 2019;5(4):170–82. https://doi.org/10.1016/j.hpj.2019.04.004.
- Zhan FD, Li Q, Guo XH, Jian BT, Liu NN, Zu YQ, et al. Cadmium and lead accumulation and low-molecular-weight organic acids secreted by roots in an intercropping of a cadmium accumulator *Sonchus Asper L.* with *Vicia Faba* L. RSC Adv. 2016;6(40):33240–8. https://doi.org/10.1039/c5ra26601g.
- Luo C, Ding H, Guo Z, Dong Y. Appropriate nitrogen supply and intercropping reduce epidemics, the compound disease of Faba bean, and yield and economic losses. Plant Soil. 2024;1–18. https://doi.org/10.1007/s11104-024-0 6705-8.
- Cui T, Fang L, Wang M, Mao J, Shen G. Intercropping of gramineous pasture ryegrass (Lolium Perenne L.) and leguminous forage alfalfa (Medicago sativa L.) increases the resistance of plants to heavy metals. J Chem. 2018;2018:1– 11. https://doi.org/10.1155/2018/7803408.
- Xiao Z, Zou DS, Zeng XY, Zhang LQ, Fen L, Wang AD, et al. Cadmium accumulation in oilseed rape is promoted by intercropping with Faba bean and ryegrass. Ecotoxicol Environ Saf. 2020;205111162. https://doi.org/10.1016/j.eco oenv.2020.111162.
- 13. Rajput VD, Minkina T, Feizi M, Kumari A, Khan M, Mandzhieva S, et al. Effects of silicon and silicon-based nanoparticles on rhizosphere microbiome, plant

stress, and growth. Biology. 2021;10(8):791. https://doi.org/10.3390/biology10 080791.

- 14. Yan GC, Jin H, Yin C, Hua Y, Huang Qy, Zhou GF, et al. Comparative effects of silicon and silicon nanoparticles on the antioxidant system and cadmium uptake in tomato under cadmium stress. Sci Total Environ. 2023;904:166819. https://doi.org/10.1016/j.scitotenv.2023.166819.
- Mir RA, Bhat BA, Yousuf H, Islam ST, Raza A, Rizvi MA, et al. Multidimensional role of silicon to activate resilient plant growth and to mitigate abiotic stress. Front Plant Sci. 2022;13819658. https://doi.org/10.3389/fpls.2022.819658.
- Tripathi DK, Vishwakarma K, Singh VP, Ved P, Sharma S, Muneer S, et al. Silicon crosstalk with reactive oxygen species, phytohormones and other signaling molecules. J Hazard Mater. 2021;408:124820. https://doi.org/10.1016/j.jhazma t.2020.124820.
- Mahawar L, Ramasamy KP, Mohammad S, Mohan PS, Živčák M, Brestic M, et al. Silicon nanoparticles: comprehensive review on biogenic synthesis and applications in agriculture. Environ Res. 2023;232:116292. https://doi.org/10.1 016/j.envres.2023.116292.
- Wang L, Ning CC, Pan TW, Cai KZ. Role of silica nanoparticles in abiotic and biotic stress tolerance in plants: A review. Int J Mol Sci. 2022;23(4):1947. https: //doi.org/10.3390/ijms23041947.
- Ahammed GJ, Yang Y. Mechanisms of silicon-induced fungal disease resistance in plants. Plant Physiol Biochem. 2021;165:200–6. https://doi.org/10.10 16/j.plaphy.2021.05.031.
- 20. Chhipa H. Applications of nanotechnology in agriculture. Nanotechnology. 2019;46:115–42. https://doi.org/10.1016/bs.mim.2019.01.002.
- Tripathi DK, Singh S, Singh VP, Prasad SM, Chauhan DK, Dubey NK. Silicon nanoparticles more efficiently alleviate arsenate toxicity than silicon in maize cultivars and hybrids differing in arsenate tolerance. Front Environ Sci. 2016;4:46. https://doi.org/10.3389/fenvs.2016.00046.
- Bokor B, Santos Carla S, Kostoláni D, Machado J, Nunes da Silva M, et al. Mitigation of climate change and environmental hazards in plants: potential role of the beneficial metalloid silicon. J Hazard Mater. 2021;416:126193. https ://doi.org/10.1016/j.jhazmat.2021.126193.
- Li X, Li B, Liu Y, Xu JC. Rhizospheric *Lactobacillus* spp. Contribute to the high Cd-accumulating characteristics of phytolacca spp. In acidic Cd-contaminated soil. Environ Res. 2023;238117270. https://doi.org/10.1016/j.envres.202 3.117270.
- Wan X, Zeng W, Cai W, Mei L, Xiaoyong L, Chen T. Progress and future prospects in co-planting with hyperaccumulators: application to the sustainable use of agricultural soil contaminated by arsenic, cadmium, and nickel. Crit Rev Environ Sci Technol. 2023;53(24):2112–31. https://doi.org/10.1080/10643 389.2023.2215684.
- Song A, Li Z, Wang EZ, Xu D, Wang S, Bi J, et al. Supplying silicon alters microbial community and reduces soil cadmium bioavailability to promote health wheat growth and yield. Sci Total Environ. 2021;796148797. https://doi.org/10 .1016/j.scitotenv.2021.148797.
- Thakral V, Sudhakaran S, Jadhav H, Mahakalka B, Sehra A, Dhar H, et al. Unveiling silicon-mediated cadmium tolerance mechanisms in Mungbean (*Vigna radiata* (L.) Wilczek): integrative insights from gene expression, antioxidant responses, and metabolomics. J Hazard Mater. 2024;474:134671. https://doi.o rg/10.1016/j.jhazmat.2024.134671.
- Harizanova AS. Silicon application unveiled: A review of insights into plant defense mechanisms under biotic challenges. Agricultural Sci. 2024;16(41):39–56. https://doi.org/10.22620/agrisci.2024.41.004.
- Liu CJ, Gong XW. Changes in rhizosphere soil nitrogen fractions associated with enzyme activities are linked to the microbial community following intercropping combined with nitrogen fertilization. Land Degrad Dev. 2022;33(7):1101–13. https://doi.org/10.1002/ldr.4239.
- Cao X, Luo J, Wang X, Chen Z, Liu G, Khan MB, et al. Responses of soil bacterial community and cd phytoextraction to a sedum alfredii-oilseed rape (*Brassica Napus* L. and *Brassica juncea* L.) intercropping system. Sci Total Environ. 2020;723138152. https://doi.org/10.1016/j.sci totenv.2020.138152.
- Bao SD. Methods of agrochemical analysis of soils. China Agricultural Science and Technology; 2000.
- Hu B, Zheng Y, Wang D, Guo Y, Dong Y. Intercropping wheat and appropriate nitrogen supply can alleviate Faba bean wilt disease by reshaping soil microbial community structure. Ind Crops Prod. 2024;222:119538. https://doi. org/10.1016/j.indcrop.2024.119538.
- 32. Jones JB. Laboratory guide for conducting soil tests and plant analysis. Boca Raton: CRC; 2001.

- Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 1981;22(5):867–80. http s://doi.org/10.1093/oxfordjournals.pcp.a076232.
- Li HS, Sun Q, Zhao SJ, et al. Principles and techniques of plant physiology and biochemistry experiments [M]. Beijing: Higher Education Press; 2000. pp. 167–9.
- Liu C, Zhao D, Ma W, Guo Y, Wang A, Wang Q et al. (2016). Denitrifying sulfide removal process on high-salinity wastewaters in the presence of halomonas sp. *Applied microbiology & Biotechnology*. 2016, 100(3): 1421–1426. https://doi. org/10.1007/s00253-015-7039-6
- 36. Zhang K, Rengel Z, Zhang F, White PJ, Shen J. Rhizosphere engineering for sustainable crop production: entropy-based insights. Trends Plant Sci. 2022a;28:390–8. https://doi.org/10.1016/j.tplants.2022.11.008
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335–6. https://doi.org/10.1038/nmeth.f.303.
- Rogers MB, Firek B, Shi M, et al. Disruption of the microbiota across multiple body sites in critically ill children. Microbiome. 2016;4:66. https://doi.org/10.1 186/s40168-016-0211-0.
- Yuan X, Wu D, Zhang D, He CY, Wang Z, Xu W, et al. Combining Microbiome and pseudotargeted metabolomics revealed the alleviative mechanism of *Cupriavidus* Sp. WS2 on the cadmium toxicity in *Vicia Unijuga* A.Br. Environ Pollut. 2024;342:123040. https://doi.org/10.1016/j.envpol.2023.123040.
- Li ZR, Wang JX, Li ZA, Tan JB, Zhan FD, Wu J, et al. Effect of root exudates of intercropping vicia Faba and arabis alpina on accumulation and sub-cellular distribution of lead and cadmium. Int J Phytoremediation. 2019;21(1):4–13. ht tps://doi.org/10.1080/15226514.2018.1523867.
- Tang L, Hamid Y, Zehra A, Sahito ZA, He Z, Beri WT, et al. Fava bean intercropping with sedum Alfredii inoculated with endophytes enhances phytoremediation of cadmium and lead co-contaminated field. Environ Pollut. 2020;265:114861. https://doi.org/10.1016/j.envpol.2020.114861.
- 42. Guo Z, Luo CS, Dong Y, Dong K, Zhu J, Ma L. Effect of nitrogen regulation on the epidemic characteristics of intercropping Faba bean rust disease primarily depends on the canopy microclimate and nitrogen nutrition. Field Crops Res. 2021;274:108339. https://doi.org/10.1016/j.fcr.2021.108339.
- Zhang F, Wan X, Zhong Y. Nitrogen as an important detoxification factor to cadmium stress in Poplar plants. J Plant Interact. 2014;9:249–58. https://doi.or g/10.1080/17429145.2013.819944.
- Xu Y, Feng J, Li H. How intercropping and mixed systems reduce cadmium concentration in rice grains and improve grain yields. J Hazard Mater. 2021;402:123762. https://doi.org/10.1016/j.jhazmat.2020.123762.
- Monica DC, Antonella M, Dalcorso G, Makarena R, Pietro DCG P. Endomembrane reorganization induced by heavy metals. Plants. 2020;9(4):482. https:// doi.org/10.3390/plants9040482.
- Lu H, Qin ST, Zhao J, Pan P, Wang F, Tang S, et al. Silicon inhibits the upward transport of cd in the first internode of different rice varieties in a cd stressed farmland. J Hazard Mater. 2023;458:131860. https://doi.org/10.1016/j.jhazmat. 2023.131860.
- Kim SG, Kim WK, Woo PE, Choi D. Silicon-induced cell wall fortification of rice leaves: A possible cellular mechanism of enhanced host resistance to blast. Phytopathology. 2002;92(10):1095–103. https://doi.org/10.1094/phyto.2002.9 2.10.1095.
- Cui J, Jin Q, Li F, Chen L. Silicon reduces the uptake of cadmium in hydroponically grown rice seedlings: why nanoscale silica is more effective than silicate. Environ Science:Nano. 2022;9(6):1961–73. https://doi.org/10.1039/d1en00973 g.
- Priyanka D, Nidhi K, Gaurav R, Ray P, Anupriya B, Srivastava A, et al. Silicon nanoforms in crop improvement and stress management. Chemosphere. 2022. https://doi.org/10.1016/j.chemosphere.2022.135165. 305,135165.
- Yang J, Hu R, Zhao C, Wang L, Mei L, Guo G, et al. Challenges and opportunities for improving the environmental quality of cadmium-contaminated soil in China. J Hazard Mater. 2023;445:130560. https://doi.org/10.1016/j.jhazmat. 2022.130560.
- Thanwisai L, Tran HTK, Siripornadulsil W, Siripornadulsil S. A cadmium-tolerant endophytic bacterium reduces oxidative stress and cd uptake in KDML105 rice seedlings by inducing glutathione reductase-related activity and increasing the proline content. Plant Physiol Biochem. 2022;192:72–86. https://doi.or g/10.1016/j.plaphy.2022.09.021.
- 52. Sun S, Yang Z, Song Z, Wang N, Guo N, Niu J, et al. Silicon enhances plant resistance to fusarium wilt by promoting antioxidant potential and photosynthetic capacity in cucumber (*Cucumis sativus* L). Front Plant Sci. 2022;13:1011859. https://doi.org/10.3389/fpls.2022.1011859.

- Liu J, Li X, Zhu Q, Zhou J, Shi L, Lu W, et al. Differences in the activities of six soil enzymes in response to cadmium contamination of paddy soils in high geological background areas. Environ Pollut. 2024;346123704. https://doi.org /10.1016/j.envpol.2024.123704.
- 54. Aponte H, Paula M, Butler B, Paolini J, Matus F, Merino C, et al. Metaanalysis of heavy metal effects on soil enzyme activities. Sci Total Environ. 2020;737139744. https://doi.org/10.1016/j.scitotenv.2020.139744.
- Tian LY, Shen JP, Sun GX, Wang B, Rong J, Zhao LJ. Foliar application of SiO<sub>2</sub> nanoparticles alters soil metabolite profiles and microbial community composition in the Pakchoi (*Brassica chinensis* L.) rhizosphere grown in contaminated mine soil. Environ Sci Technol. 2020;54(20):13137–46. https://d oi.org/10.1021/acs.est.0c03767.
- Liang Y, Wong JWC, Wei L. Silicon-mediated enhancement of cadmium tolerance in maize (*Zea Mays* L.) grown in cadmium contaminated soil. Chemosphere. 2005;58(4):475–83. https://doi.org/10.1016/j.chemosphere.20 04.09.034.
- Seyfferth AL, Douglas A, Limmer MA, Guilherme LRG. Combined impacts of Si-rich rice residues and flooding extent on grain as and cd in rice. Environ Int. 2019;128:301–9. https://doi.org/10.1016/j.envint.2019.04.060.
- Pastore G, Kernchen S, Spohn M. Microbial solubilization of silicon and phosphorus from bedrock in relation to abundance of phosphorus-solubilizing bacteria in temperate forest soils. Soil Biol Biochem. 2020;151:108050. https:// doi.org/10.1016/j.soilbio.2020.108050.
- Singh B. Myceliophthora thermophila syn. Sporotrichum thermophile: a thermophilic mould of biotechnological potential. Crit Rev Biotechnol. 2016;36(1):59–69. https://doi.org/10.3109/07388551.2014.923985.
- Rajkumar M, Ae N, Prasad MNV, Freitas H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol. 2010;28(3):142–9. https://doi.org/10.1016/j.tibtech.2009.12.002.
- Mowlick S, Hirota K, Takehara T, Kaku N, Ueki K, Ueki A. Development of anaerobic bacterial community consisted of diverse clostridial species during biological soil disinfestation amended with plant biomass. Soil Sci Plant Nutr. 2012;58(3):273–87. https://doi.org/10.1080/00380768.2012.682045.
- Li X, Li B, Tao J, Chen H, Zhao G, Xu J. Rhizospheric microbiomics integrated with plant transcriptomics provides insight into the cd response mechanisms of the newly identified cd accumulator dahlia pinnata. Front Plant Sci. 2022;131091056. https://doi.org/10.3389/fpls.2022.1091056.
- 63. Lin X, Mou R, Cao ZY, Ping X, Wu X, Zhu Z, et al. Characterization of cadmiumresistant bacteria and their potential for reducing accumulation of cadmium in rice grains. Sci Total Environ. 2016;569–70. https://doi.org/10.1016/j.scitote nv.2016.06.121.

- 64. Tong Y, Wang P, Sun J, Li X, Wang T, Zhou Q, et al. Metabolomics and molecular networking approaches reveal differential metabolites of radix scrophulariae from different geographical origins: correlations with Climatic factors and biochemical compounds in soil. Ind Crops Prod. 2021;174:114169. https://doi.org/10.1016/j.indcrop.2021.114169.
- Bag S, Mondal A, Majumder A, Mondal SK, Banik A. Flavonoid mediated selective cross-talk between plants and beneficial soil Microbiome. Phytochem Rev. 2022;21(5):1739–60. https://doi.org/10.1007/s11101-022-09806-3.
- 66. Heuberger AL, Robison FM, Lyons SMA, Broeckling CD, Prenni JE. Evaluating plant immunity using mass spectrometry-based metabolomics workflows. Front Plant Sci. 2014;5:291. https://doi.org/10.3389/fpls.2014.00291.
- Li S, Chang L, Sun R, Dong J, Zhong C, Gao Y, et al. Combined transcriptomic and metabolomic analysis reveals a role for adenosine triphosphate-binding cassette transporters and cell wall remodeling in response to salt stress in strawberry. Front Plant Sci. 2022;13:996765. https://doi.org/10.3389/fpls.2022. 996765.
- Cui Q, Beiyuan J, Chen Y, Li M, Qiu T, Zhao S, et al. Synergistic enhancement of plant growth and cadmium stress defense by azospirillum Brasilense and plant Heme: modulating the growth-defense relationship. Sci Total Environ. 2024;946:174503. https://doi.org/10.1016/j.scitotenv.2024.174503.
- Bischoff TA, Kelley CJ, Karchesy Y, Laurantos M, Nguyen-Dinh P, Arefi AG. Antimalarial activity of lactucin and lactucopicrin: sesquiterpene lactones isolated from *Cichorium intybus* L. J Ethnopharmacol. 2004;95(2–3):455–7. https://doi. org/10.1016/j.jep.2004.06.031.
- Shao Y, Molestak E, Su W, Stankevič M, Tchórzewski M. Sordarin—An anti-fungal antibiotic with a unique modus operandi. Br J Pharmacol. 2022;179(6):1125–45. https://doi.org/10.1111/bph.15724.
- Zhao J, Wang J, Cao K, Liu Y, Wang B, Wang X, et al. Foliar application of selenium and gibberellins reduce cadmium accumulation in soybean by regulating interplay among rhizosphere soil metabolites, bacteria community and cadmium speciation. J Hazard Mater. 2024;476134868. https://doi.org/10.101 6/j.jhazmat.2024.134868.

#### **Publisher's note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.