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RESEARCH ARTICLE

The combined application of γ-PGA-producing bacteria and biochar reduced the content of heavy metals and improved the quality of tomato (*Solanum lycopersicum* L.)

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Abstract

Plant growth-promoting bacteria and biochar have been widely used as immobilizers to remediate heavy metal contaminated soil. However, few studies have unraveled the effect and synergistic mechanism of combined application of plant growth-promoting bacteria and biochar on in situ heavy metal contaminated soil remediation and plant yield and quality improvement under heavy metal pollution stress. In this study, the effects of biochar, γ-PGA-producing bacteria (*Bacillus amyloliquefaciens* strain W25) and their combined application on Cd and Pb immobilization, γ-PGA production in soil filtrate, the bacterial community in rhizosphere soil, physicochemical properties of soil, heavy metal uptake, and quality and yield of tomato in heavy metal-contaminated soil were investigated. The application of W25, biochar, and their combinations significantly reduced Cd content in mature tomato fruits by 22–60%, increased the single fruit weight and lycopene content by 7–21% and 23–48%, respectively, and the combination of biochar and W25 had the best effect. All the treatments significantly reduced DTPA-Cd and DTPA-Pb contents in rhizosphere soil (42–53% and 6.5–35%), increased the pH value and the activities of urease-alkaline phosphatase of soil, but did not affect the expression of heavy metal transporter gene *LeNRAMP1* in tomato roots. Biochar + W25 increased the relative abundance of plant growth-promoting bacteria such as *Bacillus* and *Streptomyces*. Biochar-enhanced plant growth-promoting bacteria to settle and colonize in soil significantly improved the ability of strain W25 to produce γ-PGA, and immobilized Cd in soil filtrate. The combination of biochar and plant growth-promoting bacteria ensures safe crop production in heavy metal-contaminated soil.

Keywords Biochar · γ-PGA-producing bacteria · Cd/Pb immobilization · Tomato (*Solanum lycopersicum* L.)

Introduction

Heavy metals are widely distributed in various environments due to atmospheric deposition, excessive chemical use (fertilizers and pesticides), and sewage irrigation (Harindintwali et al. 2020; Shao et al. 2013). In 2014, the survey released by Ministry of Environmental Protection and the Ministry of Land and Resources of China showed that 19% of farmland was polluted by cadmium and lead (Qin et al. 2021). Heavy metal contamination reduces crop yield and quality and threatens human health through the food chain (Afonne and Ifediba 2020; Gong et al. 2021). Effective technologies are urgently needed to remediate soils contaminated with heavy metals.

Extraction and immobilization are the main strategies for remediating heavy metals in polluted soils (Tu et al. 2020). Extraction remediation involves soil washing, flushing, and phytoremediation to remove heavy metals from soil (Liu et al. 2018), but these methods are time-consuming, costly, and environmentally unfriendly. In contrast, in situ stabilization of heavy metals is an economically feasible method for environmental remediation. Soil immobilization agents reduce the mobility and bioavailability of heavy metals, decrease the ecological and health risks of polluted soils, and foster the safe crop production of lightly and moderately polluted farmland (Bolan et al. 2014; Shen et al. 2019). Applying inorganic and organic chemicals

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such as lime, zeolite, and calcium magnesium phosphate is the most common remediation practice in heavy metal-contaminated soils. These immobilizers are highly effective in the remediation of heavy metals on the field scale, but they will have an adverse impact on soil structure and physicochemical properties. Soil microbial stabilizers, including fungi, mycorrhizal, and plant growth-promoting bacteria, stabilize heavy metals by biosorption, biotransformation, and biomineralization. The stabilizers are environmentally friendly and conducive to sustainable agricultural development (Kumar and Dubey 2020; Wang et al. 2020a, b). Plant growth-promoting bacteria secrete extracellular polymers and increase soil pH by adsorbing and precipitating heavy metals. The adsorption and precipitation inhibit the transfer of heavy metals from the soil to plants, thus, reducing the content of heavy metals in crops (Asad et al. 2019). *Burkholderia* sp. D54 and *Burkholderia* sp. CBMB40 reduced Cd bioavailability in the soil and decreased the Cd content of tomato fruit (Madhaiyan et al. 2007; Wei et al. 2018). *Pseudomonas taiwanensis* WRS8 colonized in wheat rhizosphere and reduced the available Cd content of rhizosphere soil, resulting in the decreased Cd uptake of wheat (Cheng et al. 2021). *Neorhizobium huautlense* T1-17 reduced water-soluble Cd and Pb content of rhizosphere soil, so as to reduce the contents of Cd and Pb in pepper fruit (Chen et al. 2016). Nevertheless, factors such as pH, nutrients, temperature, and local bacterial community competition can easily affect the colonization of exogenous soil microorganisms. Therefore, it is difficult to form the functional microbial groups, which will inhibit the microbial remediation efficiency (Kumar and Dubey 2020). Biochar has a distinct porous surface and rich nutrition, providing habitat for microorganisms to survive and grow (Hill et al. 2019). As a carbon-rich material, biochar is widely applied for remediating heavy metals in soil due to its porous structure, large surface area, and variable surface compositions (Wu et al. 2019; Yi et al. 2020). Biochar prepared from corn straw increased the yield, improved the quality, and reduced the heavy metal contents in tomato fruit (Almaroai and Eissa 2020). Biochar also increased soil pH and reduced the bioavailability of Pb, Cu, and Ni in soil and tobacco fruit (Zhang et al. 2021). However, the amount of biochar usage, the mixing depth with soil, the physical and chemical properties, and the microbial community of sample soil will affect the remediation effect of biochar (O'Connor et al. 2018).

In recent years, combining biochar and plant growth-promoting bacteria is considered as a novel, efficient, and feasible method for remediating heavy metal-contaminated soil (Harindintwali et al. 2020; Wang et al. 2017). It was found that biochar could promote the growth of metal-immobilizing bacteria *Serratia liquefaciens* CL-1, reduce Cd and Pb availability in wheat rhizosphere soil, and decrease Cd and Pb accumulation in wheat (Cheng et al. 2020). Moreover, combining *Pseudomonas* sp. NT-2 and biochar reduced the mobility and bioavailability of Cd and Cu in heavy metal-contaminated soil (Tu et al. 2020). *Neorhizobium huautlense* T1-17 combined with biochar reduced the available Cd and Pb content by increasing small and medium-sized aggregates in soil, thus decreasing the heavy metal contents in vegetables (Wang et al. 2016). In rice, combining biochar and *Delftia* sp. B9 reduced the accumulation of Cd (Liu et al. 2020). However, these studies mostly focused on the remediation effects of soils contaminated with heavy metals. There are few studies on the mechanism of its synergistic effects, such as the impact on plant rhizosphere microbial community, heavy metal transfer gene expression, microbial metabolites, and plant physiological and biochemical changes.

In previous studies, we found that a poly-γ-glutamic acid (γ-PGA)-producing bacterium *Bacillus amyloliquefaciens* W25 reduced the bioavailability of heavy metals in soil and the content of Cd and Pb in edible tissues of lettuce (Wang et al. 2020a, b). In order to further improve the effect of *Bacillus amyloliquefaciens* strain W25, which was found and applied for the first time in the remediation of heavy metal-contaminated soil and promoting plant growth and improving quality, the impacts of biochar, strain W25, and their joint application on tomato growth, fruit quality, and Cd and Pb accumulation in heavy metal-contaminated soils were investigated in this study. The purpose of this study is to further evaluate the potential value of the combined application of biochar and plant growth–promoting bacteria in the in situ remediation of heavy metal-contaminated soil and to explore the effective technical strategy of plant safety production in heavy metal-contaminated soils.

Materials and methods

Soil sample collection, plant growth-promoting bacteria isolation, and biochar production

Soil samples were collected from 0 to 20 cm deep of cultivated land layer in the greenhouse of Qilu University of Technology. The basic properties of soil were pH, 6.83 ± 0.2; organic matter, 11.01 g kg^{-1}; NH_4^+-N, 12.65 mg kg^{-1}; NO_3-N, 50.65 mg kg^{-1}; available P, 81.26 mg kg^{-1}; available K, 189 mg kg^{-1}; and CEC, 15.4 cmol kg^{-1}.

Bacillus amyloliquefaciens W25 (NCBI accession number: MN894001) was isolated from farmland polluted by heavy metals in Shandong Province, China.

The biochar used in this study was prepared from rice straw, dried and crushed, sifted it through a 2.5-mm sieve, placed in a tubular furnace, and decomposed at 500 °C for 2 h under anaerobic conditions. Biochar was crushed through a 2-mm sieve before application. The basic properties of biochar were as follows: fixed carbon, 650 g kg^{-1}; specific

surface area, 9 m^2 g^{-1}; available phosphorus, 10.2 g kg^{-1}; available potassium, 55.65 g k^{-1}; unit weight, 0.19 g cm^{-3}; cation exchange capacity, 60.8 cmoL kg^{-1}; pH, 10.24.

Pot experiment and treatments

The above soils were air-dried and sieved through a 2-mm diameter stainless steel screen. Cadmium (2 mg kg^{-1}) and 500 mg kg^{-1} Pb ($CdSO_4 + PbNO_3$) were mixed evenly and incubated under dark conditions for 50 days (Han et al. 2018). Totally, five groups of pot experiments were set up, which were CK (soil without heavy metal pollutions), HM (soils contaminated with heavy metals), BC (soils contaminated with heavy metals + 3% biochar), W25 (soils contaminated with heavy metals + W25 bacterial suspension), and BC-W25 (soils contaminated with heavy metals + biochar + W25 bacterial suspension). Each pot experiment used a plastic pot with a diameter of 25 cm above, a diameter of 18 cm below, and a height of 20 cm. Each plastic basin contained 5 kg of soil, and each treatment had five pots.

After germination, the surface-sterilized tomato seeds (*Solanum lycopersicum* L. Fenguan, purchased from Shouhe Quality Seeds Co. Ltd., Shandong, China) were transplanted to the plot. Strain W25 was cultured in Luria Bertani (LB) medium at 37 °C for 20 h, centrifuged, washed, and suspended in deionized water to 1×10^8 cells mL^{-1}. After planting the tomato seeds, bacterial suspension (100 mL/pot) was added to the surrounding ditch (1–2 cm deep) (Wang et al. 2018). The other groups received the same amount of sterile water as the control. These pots were placed in the greenhouse (25 ± 3 °C, 50% with relative humidity, average photoperiod was 12 h per day) of Qilu University of Technology for growth examination.

Determination of water-soluble Cd and γ-PGA concentration, pH in soil filtrate under the combined application of biochar, and Bacillus amyloliquefaciens W25

Ten liters deionized water was added to 2.5 kg soils, shaken at 180 rpm for 48 h, and centrifuged at 5000 rpm for 15 min. The supernatant was collected, filtered through a Millipore filter (0.45 um pore size), and mixed with sterile basic fermentation medium (4:1). Strain W25 was cultured in LB medium, harvested, washed, and resuspended in sterile water to 1×10^8 cfu mL^{-1}. Different concentrations of Cd^{2+} were added into a 100-mL sterile mixture and inoculated with 0.5% bacterial suspension. The flask was shaken at 180 rpm at 37 °C. Bacterial growth was monitored by plate coating at 0, 12, 24, 48, 72, and 96 h, and the pH was measured by a pH meter (PB-10, Sartorius, Germany). The medium was centrifuged at 12,000 rpm for 10 min. The supernatant (10 mL) was used to determine the content of water-soluble Cd by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 2100dv, Perkin Elmer, USA). Another 10 mL of the supernatant was mixed with ice-cold ethanol overnight. The precipitate was collected by centrifugation and dissolved in deionized water. The content of γ-PGA was quantitatively analyzed at the wavelength of 216 nm (Zeng et al. 2012).

Determination of MDA content in tomato leaves

Malondialdehyde (MDA) content was determined following the method of Banerjee et al. (2016). After 15 days of tomato colonization, 0.1-g fresh leaf samples were collected, ground, and extracted with 1 mL 5% trichloroacetic acid solution (TCA). The supernatant (0.1 mL) was mixed with 0.3 mL 0.5% thiobarbituric acid (TBA), centrifuged at 10,000 rpm at 4 °C for 10 min, and kept at 95 °C for 30 min before cooling to room temperature. After centrifugation, the optical density of the supernatant was measured at 532 nm and 600 nm using a microplate reader (BioTek, USA).

Determination of tomato fruit quality and Cd–Pb accumulation in tomato tissues

Tomato roots, stems, and fruits were soaked in 0.01 M EDTA-2Na solution and washed with deionized water to remove surface-bound Cd and Pb. Samples were deactivated at 105 °C for 30 min and dried to a constant weight at 65 °C. A total of 200 mg of oven-dried tissues were ground, filtered through a 20-mesh sieve, and digested with a mixed acid (HNO_3:$HClO_4$ = 4:1, v/v) (Huang et al. 2021; Wang et al. 2020a, b). Cd and Pb concentrations were then determined by ICP-OES (Optima 2100dv, Perkin Elmer, USA).

After 120 days of potted tomato planting, when the first fruit of all treatments was fully mature, fruits were collected from each plant per treatment in three replicates. Fruit weight and diameter were measured. The fruits were washed with deionized water, mixed, and pulverized using a blender. A part was filtered by gauze, and then the soluble solid (TSS) was determined by the digital refractometer. The total acidity (TA) was calibrated by 0.01 M NaOH, and the TA content was expressed as g citric acid/100 g fresh weight. The lycopene content of tomatoes was measured as described previously (Ravelo-Pérez et al. 2008). Homogenized tomato juice (100 uL) was extracted by 8 mL mixed solution of hexane:acetone:ethanol in the ratio of 2:1:1. After light incubation, add 1 mL H_2O and let stand for 10 min to separate the phases. The optical density (OD) of the upper layers was measured at 503 nm. The content of vitamin C was determined by Vc Elisa Kit (Jing-Mei, Jiangsu, China).

Determination of DTPA-Cd and Pb content, pH, and enzyme activity in tomato rhizosphere soil

Four rhizosphere soil samples closed to tomato roots were collected after 120 days growth of potted tomatoes. The pH value (soil:water = 1:2.5) was determined by a PB-10 pH meter (Sartorius, Göttingen, Germany). Meanwhile, available Cd and Pb contents in rhizosphere soils were determined as described previously (Ravelo-Pérez et al. 2008). Briefly, 5 g rhizosphere soil was added to 10 mL 0.005 M DTPA solution, shaken at 200 rpm for 2 h, and centrifuged at 5000 rpm for 10 min. The concentrations of Cd and Pb in the supernatant were determined by ICP-OES. Additionally, one part of rhizosphere soil sample was stored at − 4 °C to determine the activities of soil invertase, catalase, alkaline phosphorylase, and urease by Solid-Sucrase Kit (SSC-1-Y), Solid-Catalase Kit (SCAT-1-Y), Solid-AKP/ALP Kit (SAKP-1-W), and Solid-Urease Kit (SUE-1-Y) (Comin, Jiangsu, China), respectively, following the manufacturer's instructions. The other part was stored at − 80 °C for bacterial DNA extraction.

Bacterial community analysis of tomato rhizosphere soil

The total bacterial DNA from tomato rhizosphere soil was extracted by OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. The quantity and quality of the extracted DNA were determined by a spectrophotometer (NC 2000, Thermo Scientific, USA) and gel electrophoresis. The DNA was amplified with primer 338 F (5′- ACTCCTA CGGGAGGCA GCA -3) and 806 R (5′- GGACTACHVGGGTWTCTAAT-3′), which targeted the V3–V4 region of bacterial 16S rRNA. Sample-specific 7–10 bp barcodes were incorporated into the primers for multiplex sequencing. DNA sequencing was performed using Illumina Hiseq 2000 (Illumina Inc., CA, USA), while species annotation and abundance analysis were carried out by Personalbio GeneCloud Analysis Platform (https://www.genescloud.cn/home) (Personalbio Tech. Co. Ltd., Shanghai, China). α-diversity, β-diversity, and significant species difference analysis were used to study the differences of the bacterial community in the tomato rhizosphere under different treatments. Nonmetric multidimensional scaling (NMDS) analysis on Bray–Curtis distance was conducted to display the difference in microbial community composition through a two-dimensional sequence diagram. Linear discriminant analysis effect size (LEfSe) was performed to detect differentially abundant taxa across groups using the default parameters (Segata et al. 2011). Further details of bioinformatic analysis of 16S rRNA gene sequences are included in the Supplementary Materials.

LeNRAMP1 gene expression analysis in tomato

At the mature stage of tomato, root tissues were collected as materials to study the expression of *LeNRAMP1* gene. RNA was extracted using Takara Minibest Plant RNA Extraction Kit (Takara, Kusatsu-Shiga, Japan). RNA concentration and purity were determined by ultramicro microplate reader (DS-11, Denovix, USA). Primescript™ RT Reagent Kit (Takara, Japan) was used to reverse-transcribe RNA into cDNA. cDNA samples were diluted to 50 ug uL^{-1} with sterile water. TB Green Premix Ex Taq II (Takara, Japan) was used for real-time qRT-PCR reactions on the Quantum Studio 3 (Applied Biosystems, MA, USA). The following thermal cycling conditions were used: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, finally, 60 °C for 30 s. The relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ (Tian et al. 2021).

Statistical analysis

Statistical analysis was performed by SPSS version 22.0. One-way ANOVA analysis was performed while Tukey's test ($p < 0.05$) separated treatment means. Graphs were drawn by Origin Software v.12.0, Microsoft PowerPoint v.2019 and Adobe Photoshop CS4.

Results

Effect of biochar on the growth, γ-PGA production, Cd immobilization, and pH value of W25 in soil filtrate

Biochar significantly promoted the growth of *Bacillus amyloliquefaciens* W25, and increased γ-PGA production and Cd immobilization rate in soil filtrate (Figs. 1 and S1). The highest content of γ-PGA in W25 and BC-W25 treatment was 0.78 mg L^{-1} and 1.10 mg L^{-1} (Fig. 1A). In the presence of heavy metals, the production of γ-PGA in the W25 treatment decreased significantly, while the content of γ-PGA in the BC-W25 treatment increased (Fig. 1A). These results showed that the addition of biochar helps *Bacillus amyloliquefaciens* W25 produce γ-PGA both in normal and Cd-contaminated soil. The possible reason was that the addition of biochar promoted the survival and growth of W25, thus promoting W25 to produce more metabolites (Figs. 1A, S1).

Biochar also promoted the immobilization of Cd by strain W25, and the Cd concentration in soil filtrate decreased with the extension of treatment time. When the Cd content in soil filtrate was 3 mg L^{-1}, the Cd immobilization rates of W25 and BC-W25 were 77% and 96%, respectively (Fig. 1B). At 6 mg L^{-1} Cd concentration, the

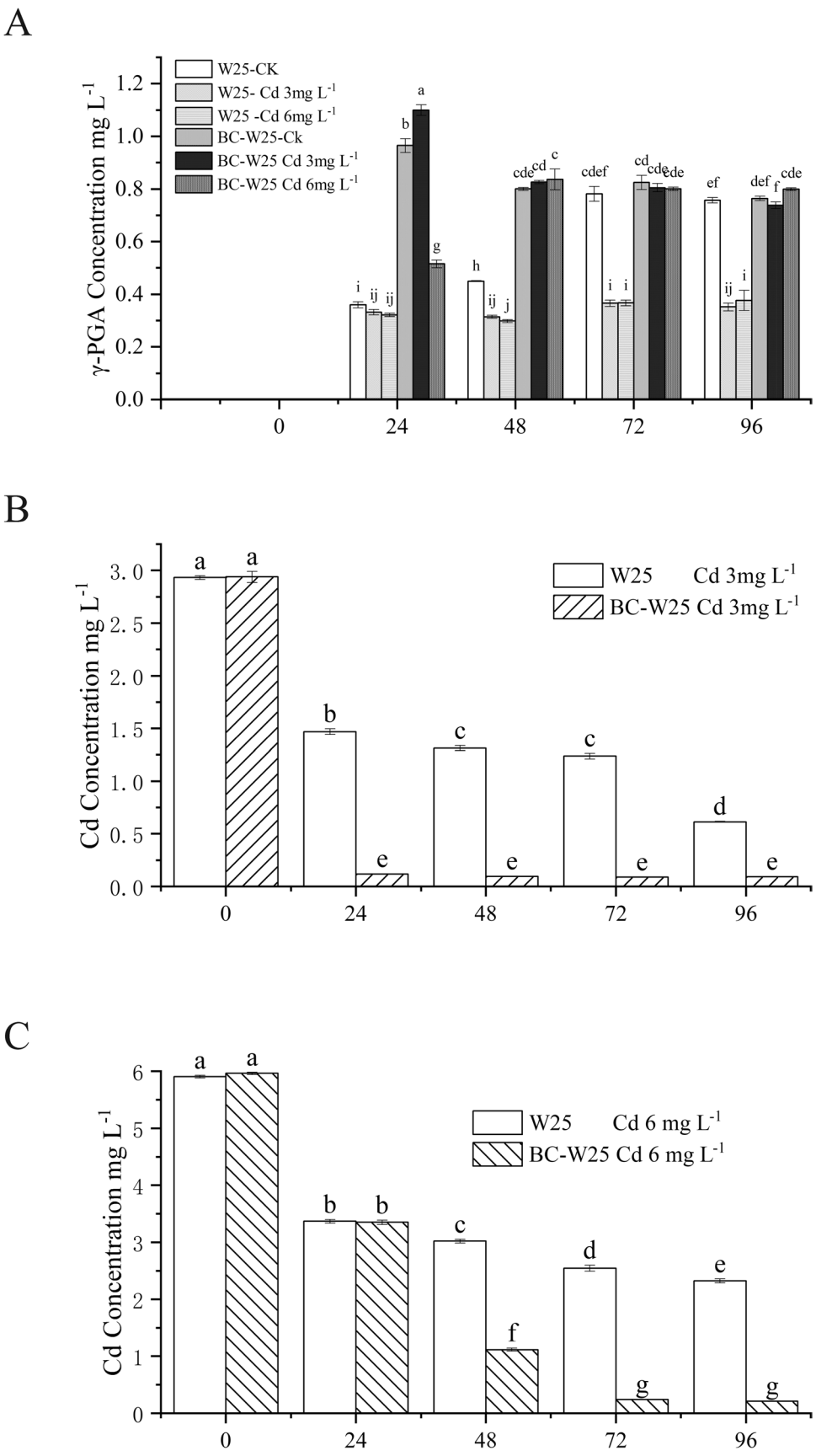

Fig. 1 γ-PGA (**A**) and Cd concentrations (**B** and **C**) in the culture solution of different Cd concentrations under different treatments. Error bars are ± standard error ($n = 3$). Bars indicated by the same letter were not significantly different ($p > 0.05$) according to Tukey's test

Cd concentration in soil filtrates treated with W25 and BC-W25 had no significant difference ($p > 0.05$) in 24 h. After that, the Cd content of the W25 treatment decreased slowly, with the final immobilization rate at 61%. After 24 h of BC-W25 treatment, the Cd concentration decreased rapidly, reaching the lowest concentration at 72 h, and the immobilization rate was 96% (Fig. 1C).

In addition, the pH value of soil filtrate increased with the extension of culture time. The pH values of W25 and BC-W25 treatment under different Cd concentrations were 7.51–8.41 and 8.26–9.19, respectively (Fig. S1).

Effects of different treatments on heavy metal accumulation in tomato

Application of biochar, strain W25, and biochar + strain W25 reduced the accumulation of Cd and Pb in tomato fruit; the decrease of Cd content was 49%, 22%, and 60%, respectively (Fig. 2A, B). The BC-W25 treatment had the best effect. When tomato was grown in Pb- and Cd-contaminated soil, high concentrations of Cd and Pb accumulated in roots and stems; the content of heavy metals in roots was higher than that in stems. Compared with HM treatment, BC, W25, and BC-W25 treatment significantly reduced Cd and Pb contents in tomato roots (28.8–37.3%; 7.0–52.1%) and stems (8.8–56.1%; 62.0–81.0%) (Fig. 2A, B), and the Cd and Pb contents in tomato roots and stems treated with biochar and strain W25 were the lowest. In addition, our study found that when tomato was planted in Cd- and Pb-contaminated soil, Cd accumulated in roots, stems, and fruits, while Pb accumulated only in roots and stems and not in fruits.

Applying biochar, strain W25, and biochar + strain W25 reduced DTPA-Cd and Pb contents by 42–53% and 6.5–35% in tomato rhizosphere soil (Fig. 2C), while BC-W25 treatment resulted in the lowest DTPA-Cd and Pb contents in the rhizosphere soil. The results showed that the combined application of biochar and W25 was more effective in reducing heavy metals than biochar or W25 singly.

Effects of different treatments on fruit quality and single fruit weight of tomato

Applying biochar, strain W25 and biochar + strain W25 in heavy metal-contaminated soil significantly improved tomato quality and yield (Table 1, Fig. S3). Compared with HM, biochar, strain W25, and biochar + strain W25 increased the diameter and weight of tomato fruit by 23–40% and 7–21% separately. Meanwhile, biochar, strain W25, and their combinations significantly improved the quality of tomato, and the vitamin C content increased by 13.6%, 16.3%, and 45.0%, respectively. Compared with the control, the lycopene content in HM treatment decreased significantly by 38.0%, while the lycopene content in BC, W25, and BC-W25 treatment increased by 23.2%, 25.5%, and 48%, respectively. It should be noted that after BC-W25 treatment, there was no significant difference in lycopene content between tomato fruits planted in heavy metal-polluted soil and tomatoes grown in normal soil.

Effects of different treatments on MDA content and LeNRAMP1 gene expression in tomato

HM treatment significantly increased the MDA content in leaves by 40.8% when compared with the control. The application of biochar, strain W25, and biochar + strain W25 reduced considerably ($p < 0.05$) the MDA content in tomato leaves (Fig. 3A) by 25.7%, 27.3%, and 33.3%, respectively, comparing with HM treatment. The above results showed that the combined application of biochar and strain W25 could more effectively ameliorate the inhibitory effect of heavy metals on tomato growth than biochar or strain W25 alone.

The effect of biochar, strain W25, and biochar + strain W25 on the expression of heavy metal transport gene *LeNRAMP1* in tomato roots was investigated, which showed that all treatments did not affect the expression of *LeNRAMP1* in tomato roots (Fig. 3B) indicating that biochar and strain W25 did not reduce the absorption of heavy metals in tomato by affecting the expression of heavy metal transfer genes.

Effects of different treatments on available heavy metal content and enzyme activity in tomato rhizosphere soil

In Cd- and Pb-contaminated soil, the pH value of rhizosphere soil (0.1–0.3 units) under different treatments was significantly higher than that of the control (Fig. S2). Compared with HM, the application of biochar, W25 and biochar + W25 significantly improved the activities of soil urease, sucrase, and alkaline phosphorylase by 18–36%, 9–20%, and 42–53%, respectively (Fig. 4). Strain W25 alone could improve soil urease activity, while biochar could effectively improve soil alkaline phosphorylase activity. The effect of the combined application of biochar and strain W25 on urease and alkaline phosphorylase activities was better than that of biochar alone or strain W25.

Effects of different treatments on bacterial community diversity and structure in tomato rhizosphere soil

A total of 1,816,851 sequences were obtained from 20 tomato rhizosphere soil bacterial samples by 16S rRNA high-throughput sequencing; among them, 925,456 were high-quality sequences. Each tomato rhizosphere soil sample

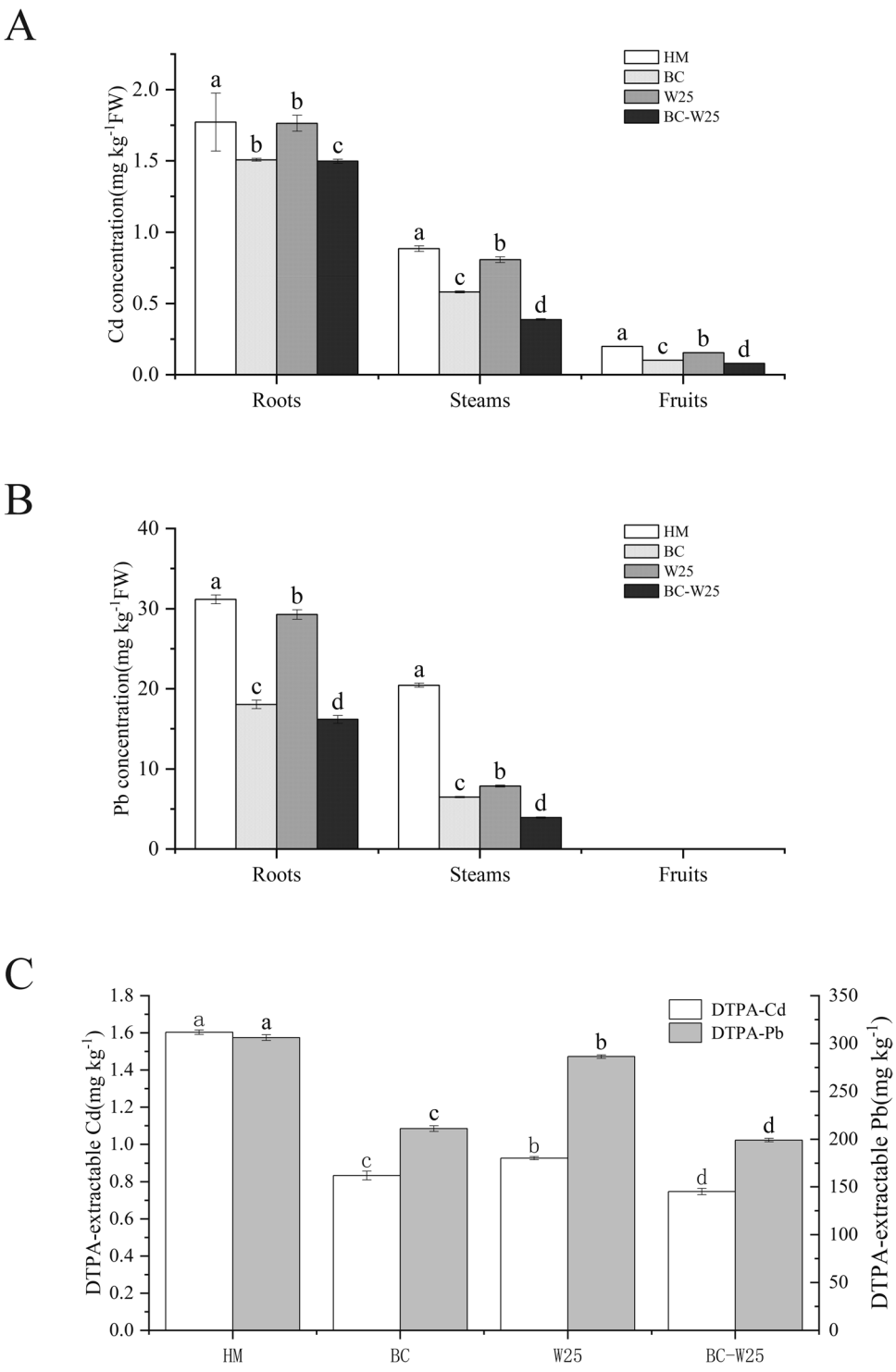

Fig. 2 Cd and Pb concentrations (**A** and **B**) in different tomato organs under different treatments. DTPA-extractable Cd and Pb contents in the rhizosphere soil of tomato under different treatments (**D**). Error bars are ± standard error ($n = 3$). Bars indicated by the same letter were not significantly different ($p > 0.05$) according to Tukey's test

produced 30,124 high-quality sequences with an average of 46,273 high-quality sequences. The Shannon index curve increased from high to flat, indicating that the sequencing results were sufficient to reflect the diversity contained in the current samples (Fig. S4). Non-metric multidimensional scaling plots (NMDS) of Bray–Curtis distance ordinations were conducted to visualize the shifts of bacterial community composition in rhizosphere soil (stress = 0.0657, Fig. 5A). The results showed that the application of biochar and strain W25 significantly changed the composition of the bacterial community in tomato rhizosphere soil polluted with heavy metals.

The ASV-level-dominant phylum was *Actinobacteria* (45.15–60.87%), followed by *Proteobacteria*

Table 1 Fruit quality and productivity of tomato under different treatments

	Fruit diameter (cm)	Fruit weight (g)	Vc (mg 100 g^{-1})	Total acidity (%)	Lycopene (mg kg^{-1} FW)	TSS (°Brix)
CK	59.93 ± 6.24 a	81.33 ± 4.35 a	153.84 ± 4.71 a	0.31 ± 0.01	79.69 ± 0.95 a	5.57 ± 0.01 c
HM	41.65 ± 1.93 b	65.46 ± 4.87 c	102.24 ± 5.82 c	0.37 ± 0.01	49.51 ± 0.94 d	5.05 ± 0.06 d
BC	51.30 ± 0.41 a	69.88 ± 1.55 b	116.10 ± 4.59 b	0.38 ± 0.03	61.01 ± 3.38 cd	6.03 ± 0.06 bc
W25	56.16 ± 7.94 a	76.35 ± 1.29 ab	118.91 ± 6.79 b	0.36 ± 0.01	62.12 ± 1.31 c	6.03 ± 0.06 b
BC-W25	58.17 ± 2.38 a	79.49 ± 1.64 a	148.22 ± 6.07 a	0.33 ± 0.03	73.37 ± 0.99 b	6.52 ± 0.09 a

Data followed by the same letters (a-d) within the same line are not significantly different ($p < 0.05$) according to Tukey's test

(16.71–32.92%), *Chloroflexi* (4.48–9.21%), *Acidobacteria* (2.31–7.69%), *Firmicutes* (0.56–7.97%), *Bacteroidetes* (1.21–3.35%), *Patescibacteria* (0.66–2.58%), and *Gemmatimonadetes* (1.41–2.81%) (Fig. 5B). The relative abundance of *Actinomycetes* in BC, W25, and BC-W25 treatment significantly increased than that of HM treatment, while the relative abundance of *Firmicutes* in BC-W25 treatment was higher than other treatments. However, the application of biochar and W25 did not change the dominant species of bacteria in the tomato rhizosphere soil bacterial community but changed their abundance (Fig. 5A, B).

LEfSe analysis was performed to analyze the biomarker in the tomato rhizosphere bacterial community after treatments. The results revealed 16 bacterial genera with an LDA level ≥ 3.6 (Fig. 5C). Genera *Subgroup_6*, *RB41*, *A4b*, *KD4_96*, and *Haliangium* were the biomarkers of the CK treatment; genera *Sphingomonas* and *Massilia* were the biomarkers of the HM treatment. In contrast, genera *Flavobacteriales* and *Microvirga* were the biomarkers of the BC treatment. The biomarkers in W25 treatment included genera *IMCC262256*, *Mycobacterium*, *Blastococcus*, and *Aeromicrobium*. In addition, *Streptomyces*, *Promicromonospora*, and *Bacillus* were associated with biomarkers of the BC-W25 treatment. LEfSe result showed that the application of biochar, strain W25, and biochar + strain W25 significantly shifted the bacterial community composition and structure in the tomato rhizosphere soil. BC-W25 treatment enriched genera *Bacillus* significantly (Figs. 5C and S5).

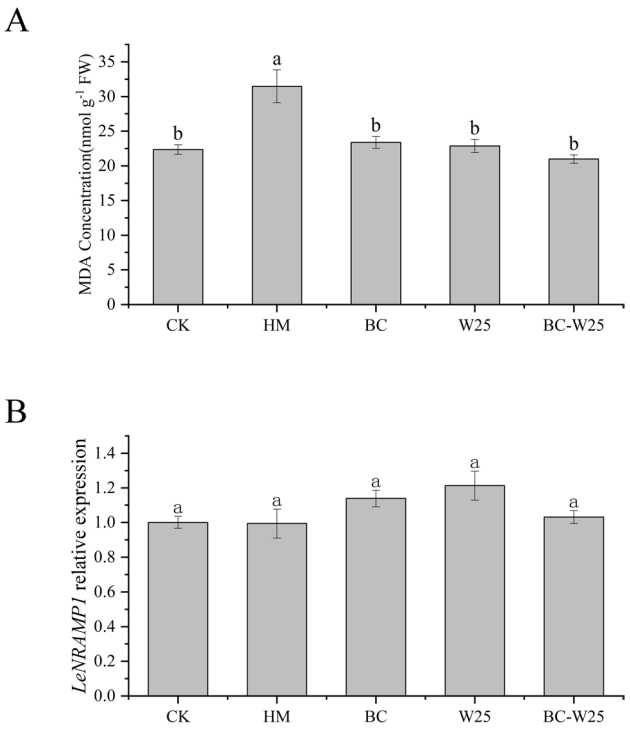

Fig. 3 The MDA concentrations of leaf (**A**) and *LeNRAMP1* relative expression of root (**B**) of tomato under different treatments. Error bars are ± standard error ($n = 3$). Bars indicated by the same letter were not significantly different ($p > 0.05$) according to Tukey's test

Discussion

In recent years, researchers have carried out projects on the combined application of biochar and plant growth-promoting bacteria for the remediation of heavy metal-contaminated soil (Harindintwali et al. 2020; Wang et al. 2017). These studies focused on the conditions of bacteria and biochar combined application and the remediation effect of soil heavy metals, and lack of researches on the synergistic mechanism of bacteria and biochar combined application.

Our previous work showed that poly-γ-glutamic acid (γ-PGA) produced by *Bacillus amyloliquefaciens* W25 reduced the bioavailability of heavy metals in soil, and improved the quality of lettuce and affecting the, physicochemical properties and microbial community of soil (Wang et al. 2020a, b). γ-PGA is a non-toxic, water-soluble, biodegradable, and environmentally friendly biopolymer composed of D/L-glutamate monomer and fermented by *Bacillus subtilis* (Luo et al. 2016), which is applicable in many fields such as food, medicine, cosmetics, and agriculture (Ogunleye et al. 2015). Recent studies showed that γ-PGA is important in plant growth and regulation as a water-retaining agent and soil conditioner (Guo et al. 2017). The exogenous application of γ-PGA significantly enhanced the stress resistance

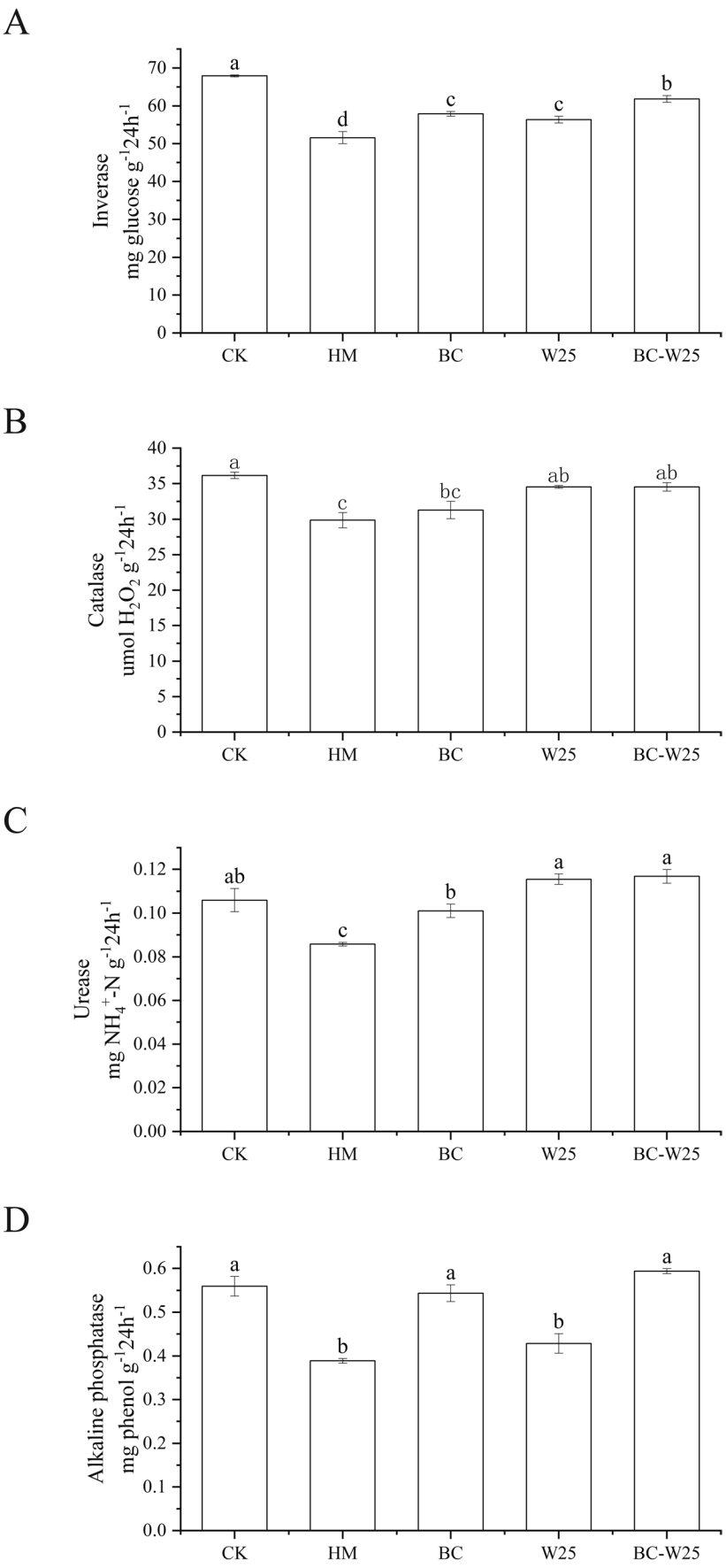

Fig. 4 The activity of invertase (**A**), catalase (**B**), urease (**C**), and alkaline phosphatase (**D**) in rhizosphere soil. Error bars are ± standard error ($n = 3$). Bars indicated by the same letter within each treatment are not significantly different ($p > 0.05$) according to Tukey's test

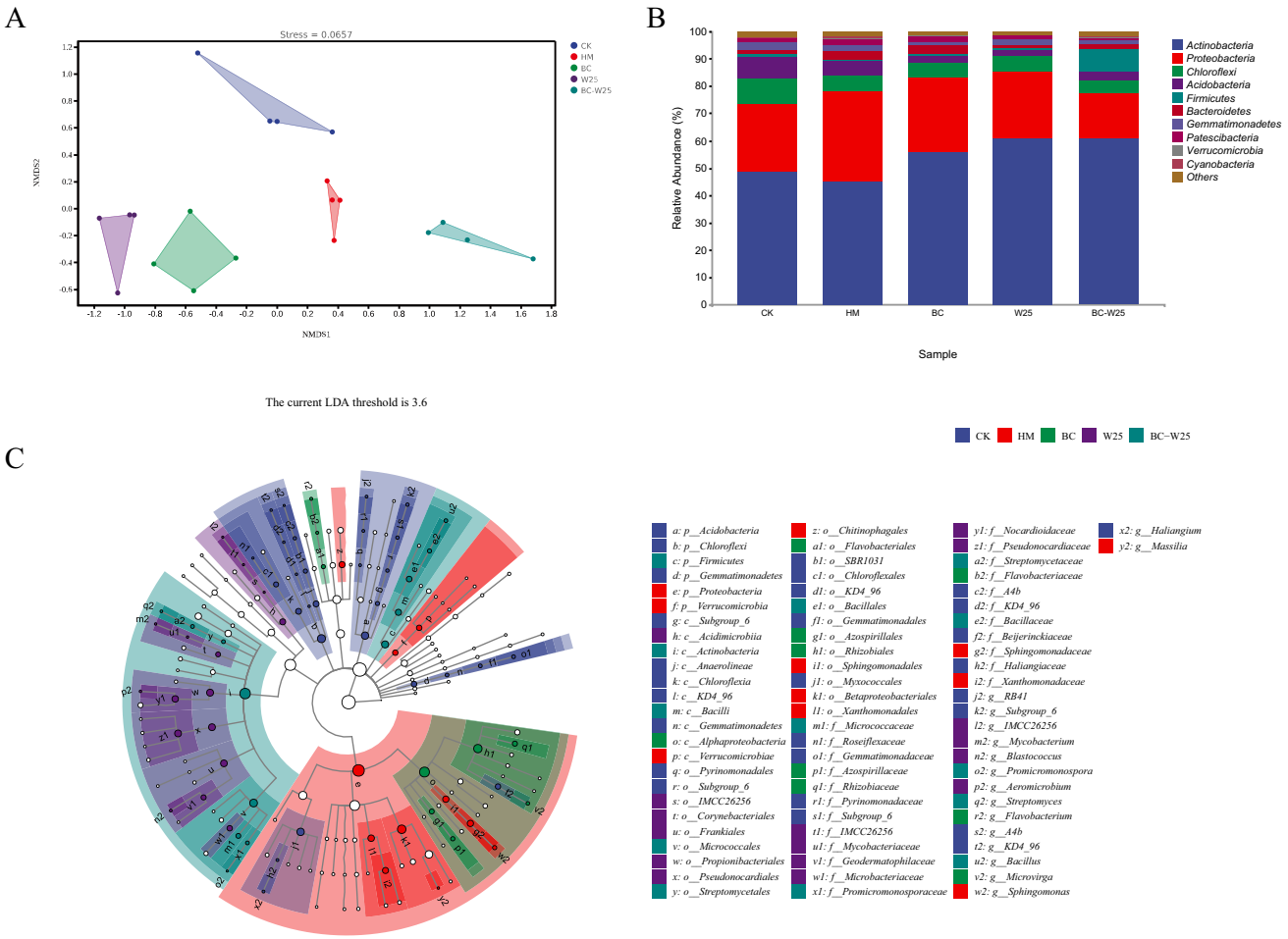

Fig. 5 The NMDS, relative abundance and LEfSe analysis. **A** Non-metric multidimensional scaling (NMDS) shows the grouping patterns under different treatments on weighted UniFrac distance of all community. Each colored dot represents a sample. **B** Relative abundance (%) of bacterial communities at phylum level in the rhizosphere soil of tomato under different treatments. **C** LEfSe analysis (LDA ≥ 3.6) showed the species with the most significant variation in the rhizosphere soil under different treatments

of plants (Lei et al. 2015; Xu et al. 2020). Meanwhile, biochar application changed the physicochemical properties of soil, such as ion exchange, water, and fertilizer retention capacity, and increased soil pH (Rodríguez-Vila et al. 2016; Tong et al. 2014). The increase of soil pH value enhanced the ability of soil to adsorb and chelate heavy metals and reduce their bioavailability (Bravo et al. 2017). In addition, biochar contains many nutrients (organic matter, N, P, K), which can be used as a slow-release fertilizer to provide long-term help for plant growth (Masiello et al. 2013).

In this study, it was found that biochar, W25, and their combined application blocked the transfer of heavy metals from soil to tomato to varying degrees, and improved the biomass, Vc, lycopene, and other quality indexes of tomato under heavy metal pollution stress. Among them, the combination of biochar and W25 had the best effect. We speculated that the main reason was that due to the porous structure and rich nutrition on the surface of biochar, it can create better conditions for microorganisms to survive and grow in soil (Hill et al. 2019; Zhu et al. 2017). Our experimental results confirmed that biochar in soil filtrate can promote the growth and reproduction of W25, improve the ability of W25 to produce γ-PGA to fix Cd in soil filtrate (Figs. 1 and S1), significantly reduce the contents of DTPA-Cd and Pb in tomato rhizosphere soil (Fig. 2C), and and reduce the toxicity of heavy metals to tomato fruit (Fig. 3). At the same time, the promotion of W25 growth and reproduction further improved the γ-PGA production capacity of W25 (Figs. 1 and S1), which affected soil physical and chemical properties, rhizosphere soil microbial community structure, and the abundance of beneficial bacteria, and created the best conditions for effectively blocking the transfer of heavy metals to tomato and promoting the growth and quality of tomato (Table 1). In addition, the application of biochar also increased soil nutrients, soil pH (Fig. S2), and improved the absorption of nutrients by plants.

Plants produce reactive oxygen species (ROS) under heavy metal stress, and malondialdehyde (MDA) is a toxic product of membrane lipid peroxidation (Rizwan et al. 2017). The results showed that biochar and W25 reduced the MDA content in tomato leaves (Fig. 3A), which was a potential factor leading to the improvement of tomato's ability to carry out normal metabolic activities under heavy metal stress. *LeNRAMP1* is a metal ion transport gene expressed in tomato roots. The application of biochar and strain W25 had no significant effect on the expression of *LeNRAMP1* in roots (Fig. 3B), indicating that the effect of biochar and W25 on reducing heavy metals in tomato fruits may not be affected by the expression of heavy metal transfer gene *LeNRAMP1*.

Soil enzyme activity has a sensitive and complex response to heavy metal pollution and plays an important role in the soil element cycle. Biochar provides habitat and nutrients for microbial growth and metabolism and promotes the secretion of active substances and enzymes by microorganisms (Wang et al. 2019). In this study, biochar and strain W25 increased soil enzyme activity (Figs. 4 and S2). Soil urease decomposes urea to produce NH_4^+, NH_3, and CO_3^{2-}, which improves soil pH and combines with heavy metal ions, reducing the availability of heavy metals in soil (Wang et al. 2020a, b). Soil alkaline phosphorylase converts soil organic phosphorus into available phosphorus and promotes absorption (Dick et al. 2000). The combination of biochar and strain W25 was more effective in improving the enzyme activity of tomato rhizosphere soil than biochar or strain W25 alone.

Soil microbiome community is closely related to soil quality and plant growth. Soil pollution will reduce the relative abundance of soil functional microorganisms (Yu et al., 2018). In this study, the application of biochar, strain W25, and biochar + strain W25 did not change the dominant phylum in tomato rhizosphere soil. However, it changed their relative abundance, such as increasing the relative abundance of *Actinomycetes* (Fig. 5), thus improving the composition of soil bacterial community. *Actinobacteria* are common plant growth-promoting and heavy metal immobilization bacteria, which help to release P element into rhizosphere soil and promote plant growth (Shariffah-Muzaimah et al. 2020; Soumare et al. 2021). After applying biochar + strain W25, *Bacillus* and *Firmicum* in tomato rhizosphere soil were significantly enriched (Fig. 5C), which helped to improve the physical and chemical properties of tomato rhizosphere soil, optimize the composition of bacterial community, promote the remediation of heavy metal contaminated soil, and finally improve the growth performance of tomato (Wang et al. 2018).

It was worth mentioning that this study found that the combined application of biochar and strain W25 significantly increased the contents of lycopene and Vc in tomato fruit, which were 48% and 45% higher than HM control, respectively, almost the same as those in tomatoes grown under normal soil conditions. Lycopene is one of the most important bioactive components in tomato which is related to immunoregulation and prevention of cardiovascular disease, prostate, and breast cancer (Ferreira-Santos et al. 2018; Liang et al. 2019). Although its mechanism was unclear, how exogenous biological and abiotic factors affect the synthesis of plant secondary metabolites is a research direction worthy of attention.

Conclusion

This study demonstrated that biochar promoted the survival and growth of γ-PGA-producing bacteria, *Bacillus amyloliquefaciens* W25 in soil filtrate, and promoted γ-PGA production and soil pH, which further increased the ability of W25 to immobilize Cd and Pb in soil. The combined application of biochar and plant-promoting strain W25 significantly increased soil enzyme activity and the relative abundance of metal-immobilizing bacteria such as *Streptomyces* and *Bacillus*. This improvement contributed to the decrease of DTPA-Cd and Pb contents in the soil, the accumulation of Cd in tomato fruit, the increase of tomato single fruit weight, and the improvement of lycopene and vitamin C contents in tomato fruit. The combined application of biochar and plant growth-promoting bacteria is encouraged in in situ remediation of heavy metals in farmland as it provides an economical and ecofriendly protection route for plant safety and high-quality crop production.

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Author contribution Xingwang Liu: conceptualization, methodology, investigation, data curation, writing—original draft. Xiaohan Wang: methodology, investigation. Tianyu Xu: methodology, investigation. Haizheng Ma methodology, investigation. Tao Xia: supervision, conceptualization, writing—review and editing, funding acquisition.

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Data availability All data are available upon request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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