The Proposed Mechanism Underlying the Synergistic Effects of *Bacillus velezensis* and Coal Gangue (CG) on Wheat Growth

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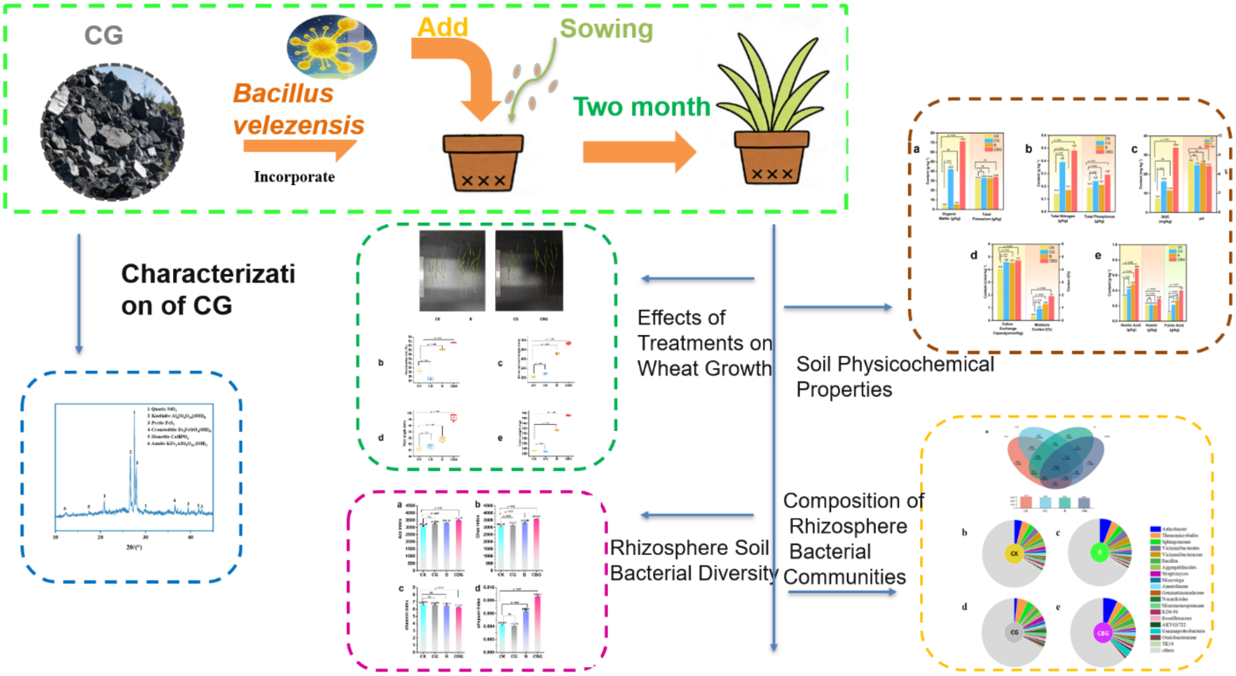
GRAPHICAL ABSTRACT



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The synergistic application of coal gangue (CG) and microorganisms has the potential to promote plant growth, yet the underlying mechanisms remain inadequately understood. This study examined the effects of co-applying *Bacillus velezensis* and CG on wheat growth through pot experiments. The growth parameters of wheat, the physicochemical properties of the soil, enzyme activity, and the microbial community composition were assessed. The combined treatment led to a significant enhancement in wheat growth, with plant height and root length increasing by 32.1% and 35.4%, respectively. Soil nutrient status was markedly improved, with increases in total nitrogen, total phosphorus, total potassium, organic matter, and humic acid. Key soil enzyme activities were also elevated. Microbial community analysis revealed an increase in soil microbial richness and significant enhancements in the generation of phytohormone IAA and ACC deaminase, which were 1.59 and 1.89 times higher than in the control respectively. In conclusion, the combined application of *B. velezensis* and CG promoted wheat growth by synergistically improving soil fertility, enhancing enzyme activities, and enriching beneficial microbial communities. This study provides a theoretical foundation and a practical strategy for the agricultural utilization of coal gangue and the remediation of infertile soils.

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Keywords: Coal gangue; Microorganism; Soil improvement; Soil enzymes

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INTRODUCTION

Coal gangue (CG) is a heterogeneous rock mixture generated during coal mining and processing. It represents a major solid waste that typically accounts for 10 to 20% of raw coal production (Dong *et al.* 2024). Recent estimates suggest that global CG stockpiles will exceed 9 billion metric tons by 2025, with China contributing more than 60% of this volume. Open-air stockpiling exposes CG to long-term weathering and leaching, which facilitates the mobilization of heavy metals into soils, leading to soil degradation and erosion, thereby posing substantial environmental burdens (Han *et al.* 2018, Yang *et al.* 2023). Notably, the release of heavy metals and other toxic substances from weathered and leached CG can directly alter the soil microbial habitat, thereby disrupting the diversity, composition, and metabolic activities of soil microbial communities (Li *et al.* 2014). For instance, elevated concentrations of heavy metals can inhibit the growth and activity of functional microorganisms involved in nutrient cycling (*e.g*., nitrogen-fixing and phosphorus-solubilizing bacteria) and organic matter decomposition. This, in turn, further exacerbates soil degradation by reducing microbially driven soil fertility improvement processes. Conventional landfilling methods are insufficient for mitigating this pollution and can even introduce additional hazards, such as spontaneous combustion (Zhang *et al.* 2022). Therefore, the scientific treatment and resource utilization of CG have become critical research priorities. In recent years, applications for CG have expanded to include energy recovery, building material production, soil amendment, and the synthesis of high-value chemical products (Gao *et al.* 2024). For instance, composite materials incorporating CG, fly ash, and trace triethanolamine composite material have been used to produce cost-effective, high-performance concrete, offering a viable pathway for solid waste utilization (Wang *et al.* 2025). The use of CG in land reclamation is particularly promising, as it simultaneously addresses waste disposal and ecological restoration (Gao *et al.* 2001). In such applications, CG can act as a soil conditioner to ameliorate soil structure, permeability, water retention, and fertility, thereby promoting plant growth (Tang *et al.* 2024). One study has shown that composite technosols formulated with CG, biochar, and microbial agents can significantly enhance maize growth (Liu *et al.* 2024). Furthermore, CG can modify the soil environment and shape distinct microbial communities involved in beneficial processes including nitrogen fixation, phosphorus solubilization, and heavy metal immobilization (Lu *et al.* 2024). For example, variations in polycyclic aromatic hydrocarbon concentrations within CG have been shown to drive the ecological succession of bacterial consortia, selectively enriching specialized *Mycobacterium* species while suppressing others like *Arthrobacter* (Wu *et al.* 2023).

Bacillus velezensis (*B. velezensis*) is a Gram-positive, aerobic, and endospore-forming bacterium that has attracted significant research interest, primarily due to its considerable potential in biocontrol and plant growth promotion, as well as its adaptability to the CG-modified soil environment, which is a core focus of this study (Fan *et al.* 2018, Keshmirshekan *et al.* 2024). This species exhibits a broad distribution, inhabiting a variety of ecosystems, including soil, aquatic environments, and the rhizosphere (Kenfaoui *et al.* 2024). Many studies have demonstrated its multifunctional potential. For example, *B. velezensis* strain A-27 effectively neutralizes nematode larvae and enriches beneficial rhizobacteria, thereby controlling *Meloidogyne incognita* infestations and promoting celery growth. Similarly, this growth-promoting effect is applicable to wheat, the crop utilized in the present study (Yao *et al.* 2025). Furthermore, isolates from pear tree rhizospheres have been confirmed to produce indole-3-acetic acid, significantly enhancing barley root development (Elsoud *et al.* 2023). Genomic analysis of the potato-derived strain *B. velezensis* DMW1 revealed 12 secondary metabolite gene clusters encoding at least seven antagonistic compounds, demonstrating dual functionality against phytopathogens such as *Phytophthora sojae* while promoting the growth of tomato and soybean seedlings (Yu *et al.* 2023). Similarly, genomic sequencing of *B. velezensis* strains AOA1 and AKS2, obtained from maize rhizospheres, revealed conserved gene clusters responsible for synthesizing plant growth–promoting and antimicrobial metabolites, providing critical insights into their molecular mechanisms (Babalola *et al.* 2024). Given that coal gangue contains substantial amounts of insoluble nutrients and that *B. velezensis* possesses well-documented abilities to solubilize such minerals, produce phytohormones, and enhance soil enzyme activities, it is hypothesized in this work that co-applying *B. velezensis* with coal gangue would synergistically improve soil fertility and promote crop growth. Therefore, the present study aimed to investigate this synergy using wheat as a model crop, focusing on the mechanisms by which *B. velezensis* enhances the soil amendment potential of coal gangue.

Concurrently, microbial agents are increasingly recognized for their cost-effectiveness and low environmental impact in treating complex solid wastes (Shahrajabian *et al.* 2023). Emerging evidence indicates that certain microbial consortia not only can degrade organic pollutants in CG, but they also can facilitate their conversion into humic acid, thereby enhancing soil organic matter (Liu *et al.* 2023). Notably, a microbial activation strategy employing *Stenotrophomonas maltophilia* YZ1 has been shown to effectively solubilize key nutrients, including phosphorus, potassium, and silicon, from CG matrices (Zhu *et al.* 2022). Similarly, a study from the authors' research group demonstrated that *B. velezensis* facilitates the solubilization of available potassium and phosphorus through organic acid secretion, thereby significantly enhancing alfalfa growth in CG-amended soil matrices (Wang *et al.* 2024). Despite these promising findings, the synergy between *B. velezensis* and CG, particularly in promoting gangue decomposition, nutrient release, and rhizosphere microbiome restructuring, has remained unexplored. This work provides a comprehensive evaluation of co-treatment effects on soil fertility, enzymatic activity, phytohormone production, and microbiome assembly. To the authors' knowledge, this is the first study to systematically examine their synergistic mechanisms by integrating plant growth, soil physicochemical properties, enzyme activities, and rhizosphere microbial dynamics within a single system.

The current study systematically investigated the synergistic mechanisms through which the combined application of *B. velezensis* and CG promotes wheat growth. Specifically, alterations in soil physicochemical characteristics, nutrient content, enzymatic activities, and the diversity as well as structural composition of root-associated bacterial communities were examined. The objectives of this study were as follows: (1) to assess the synergistic effects on soil nutrient status and wheat growth; (2) to investigate the influence on soil enzymatic functions and the synthesis of plant growth–enhancing compounds; and (3) to examine the regulatory impact on the composition and potential functions of the wheat rhizosphere microbiome. This comprehensive study aimed to establish a theoretical basis and offer technical guidance for the practical implementation of the integrated *B. velezensis*–CG strategy in sustainable agriculture and soil remediation.

EXPERIMENTAL

Origins of Coal Gangue, Soil, and Bacterial Strain

The coal gangue (CG) employed for this investigation was sourced from an open-air dumping area at the Baojucai Coal Mine (37.856°N, 109.483°E), located in Yulin City, within China's Shaanxi Province. Adjacent farmland in the same region provided the surface soil samples, which were taken from a depth of 0 to 20 cm. The initial physicochemical properties of the soil were determined prior to the experiment, and the data are provided in the supplementary material. Following collection, samples were immediately placed into sterilized polyethylene containers and conveyed to the laboratory under controlled environmental conditions. Prior to utilization, the coal gangue underwent air-drying, mechanical crushing, and sieving through a 2 mm mesh. Soil samples were also air-dried at ambient temperature. Visible organic debris and gravel were manually extracted from these samples before the soil was passed through a 2 mm sieve for subsequent use in pot trials. This study utilized the bacterial strain *Bacillus velezensis*, which was isolated and characterized in the authors' laboratory prior to this research. The 16S rRNA gene sequence of this strain has been deposited in the GenBank database under accession number PQ277052.

Pot Experiments

The pot experiments were performed using standardized plastic pots, each filled with 500 cm^3 of soil substrate. Four treatments were established: CK (Control, soil only), CG (Soil amended with coal gangue), B (Soil inoculated with *B. velezensis*), and CBG (Soil with both coal gangue and *B. velezensis*). Each treatment was replicated three times, with three independent pots per treatment (a total of 12 pots). After overnight cultivation of *B. velezensis* in Lysogeny Broth (LB) liquid medium at 30 °C with shaking at 180 rpm for 18 h to reach the logarithmic growth phase, a 5 mL aliquot of the bacterial suspension (adjusted to 1.0×10^8 CFU/mL with sterile deionized water) was introduced into each pot in the B and CBG treatments. To eliminate potential interference from residual components of the culture medium, an equal quantity of sterilized LB broth was administered to the control groups (CK and CG). All pots were placed outdoors, exposed to natural temperature and light conditions under ambient conditions (average daytime temperature 22 ± 3 °C, natural photoperiod). Forty wheat seeds were sown uniformly in each pot, and the soil moisture was maintained at approximately 60% of field water capacity by regular watering throughout the growth period. Germination and growth of wheat were observed and recorded regularly from the start of sowing. After two months of cultivation, the wheat plants were harvested, and growth parameters such as germination rate, root length, plant height, and fresh weight were recorded respectively.

Soil Physicochemical Property Analysis

Soil samples were obtained from the wheat rhizosphere of each pot using a five-point sampling method. After thorough homogenization, the composite samples were transferred to sterile, sealed bags and stored at 4 °C in the dark. Visible gravel and plant debris were manually removed prior to subsequent processing. A portion of the fresh soil was promptly utilized for microbial diversity analysis, while the remaining sample was air-dried, ground, and sieved through a 2-mm mesh for physicochemical analysis. The soil's physicochemical properties were determined according to standard protocols previously described (Lu 2000), which are consistent with the following ISO and Chinese national standards. Soil pH was determined potentiometrically using a glass electrode (ISO 10390:2005), and organic matter content was quantified using the potassium dichromate titration method, in accordance with GB/T 7857-2014. Available nitrogen was determined by the alkaline hydrolysis diffusion method (ISO 14255:1998), and total nitrogen was analyzed using the semi-micro Kjeldahl method (ISO 11261:1995). For phosphorus analysis, total phosphorus was measured after digestion with $HClO_4$–H_2SO_4 (ISO 14869-2:2002), and available phosphorus was assessed *via* extraction with sodium bicarbonate solution (ISO 11263:1994), followed by measurement through the molybdenum-antimony spectrophotometric method. Both total and available potassium were determined by flame photometry, with total potassium analyzed following NaOH fusion (ISO 14869-2:2002), and available potassium extracted with ammonium acetate (ISO 23470:2018).

Determination of Soil Plant Hormones and ACC Deaminase Activity

IAA concentration in soil was determined following the Salkowski colorimetric assay (Glickmann and Dessaux 1995). For the extraction, 1.0 g of fresh soil was combined with 80 mL of sterile deionized water and agitated vigorously on a vortex mixer for 30 min. Following centrifugation at 12,000 × g for 10 min, the supernatant was collected and passed through a 0.22 µm filter membrane to yield a clarified soil extract. Next, 100 µL of this filtrate was combined with an equivalent volume of Salkowski reagent (composed of $FeCl_3$ and $HClO_4$) within a light-protected centrifuge tube. This mixture was then incubated at room temperature, shielded from light, for a duration of 30 min. Absorbance of the final colored solution was read at 530 nm using a spectrophotometer.

The activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase was determined with a colorimetric method (Penrose and Glick 2003). The soil supernatant (1 mL) was treated with 800 µL of a 0.56 mol/L hydrochloric acid solution, followed by 300 µL of 2,4-dinitrophenylhydrazine (DNPH) solution (2 g/L). The above solution was incubated in the dark at 30 °C for 30 min in a water bath to allow conversion of α-ketobutyrate to its phenylhydrazone derivative. Next, 2 mL of 2 mol/L NaOH was introduced, and the solution was vortexed and allowed to stand for 5 min, resulting in a color change from yellow to reddish-brown. Absorbance was measured at 540 nm using a UV-Vis spectrophotometer. The protein content in the soil samples was determined using the Coomassie Brilliant Blue G-250 method (Bradford 1976). The specific activity of ACC deaminase (U/mg) was calculated by dividing the enzyme activity (U) by the total protein mass (mg).

Soil Enzyme Activity Measurement

The analysis of soil enzyme activities was conducted to evaluate the impact of microbial treatments and coal gangue additions on soil health. Protease activity was analyzed using the Folin-Ciocalteu method (Ladd and Butler 1972). Briefly, a soil extract was incubated with a casein substrate at 40 °C for 20 min. After stopping the reaction, Folin-Ciocalteu reagent was added, and the released tyrosine was quantified by determining the optical absorbance at a wavelength of 660 nm. Amylase activity was measured using the iodometric method (Schinner *et al.* 2012). In brief, soil extract was incubated with a starch substrate at 40 °C for 30 min. After stopping the reaction, iodine solution was added to react with residual starch, forming a blue complex. Absorbance was recorded at 660 nm. The activity of phosphatase was determined employing the disodium phenyl phosphate assay (Eivazi and Tabatabai 1977). Soil samples were incubated with disodium phenyl phosphate substrate at 37 °C for 2 h.

The released phenol was quantified by measuring absorbance at 510 nm after reaction with a colorimetric reagent. The measurement of urease activity was conducted *via* the phenol-sodium hypochlorite colorimetric method (Kandeler and Gerber 1988). Soil samples were incubated with a urea substrate at 25 °C for 12 h. The ammonium nitrogen released was measured at 578 nm after reaction with phenol and sodium hypochlorite to form a blue indophenol complex. Laccase activity was quantified following the established 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method (Bourbonnais *et al.* 1995), utilizing ABTS as the substrate. The enzymatic reaction proceeded for 3 min at 25 °C within a pH 4.5 buffer. The resulting oxidation was tracked by recording the absorbance at a wavelength of 420 nm.

Analysis of Soil Microbial Community

A commercial soil DNA extraction kit was used to extract soil DNA, following the protocol provided by the manufacturer. The resulting DNA served as the template for polymerase chain reaction (PCR) amplification of the bacterial 16S rRNA gene V3–V4 region using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR was conducted under the following thermal cycling conditions: initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min and a final hold at 10 °C. The amplicons were then examined by 2% agarose gel electrophoresis, purified with a gel extraction kit, and quantified. Purified amplicon libraries were sequenced on an Illumina NextSeq 2000 platform. Raw sequencing reads were subsequently processed using the QIIME2 pipeline.

Data Analysis

Results are reported as the mean ± standard deviation for all measurements, including soil physicochemical properties, enzyme activities, and wheat growth parameters. Data analysis was conducted with SPSS Statistics, version 26.0. Differences among the four treatment groups were evaluated *via* one-way analysis of variance (ANOVA), considering results significant at $P<0.05$. A Student's t-test was employed to determine significant differences between bacterial treatments and their corresponding controls. All graphical representations were created using Origin (2018) software.

RESULTS AND DISCUSSION

Characterization of Coal Gangue

Table 1 displays the chemical composition of CG. The main constituents of CG included silica (47.6%) and alumina (19.9%), with smaller amounts of iron oxide (1.97%), potassium oxide (2.21%), and other elements. This composition confirms that CG is rich in silicon and contains essential plant nutrients, including potassium, phosphorus, iron, calcium, and magnesium, which aligns with previous findings (Wen *et al.* 2013; Liu *et al.* 2025). However, the potassium (K) and phosphorus (P) in CG are mainly present in insoluble forms, such as in annite and monetite (Fig. 1), rendering them largely unavailable for direct plant uptake. To overcome this limitation, this study investigated the use of *B. velezensis* to solubilize these fixed nutrients through its metabolic activity, thereby enhancing the agricultural utility of coal gangue.

Table 1. The Main Chemical Elements of Raw CG

Component	Content (wt%)
SiO_2	47.6
Al_2O_3	19.9
Fe_2O_3	1.97
CaO	1.86
K_2O	2.21
SO_3	0.77
MgO	1.23
TiO_2	0.84

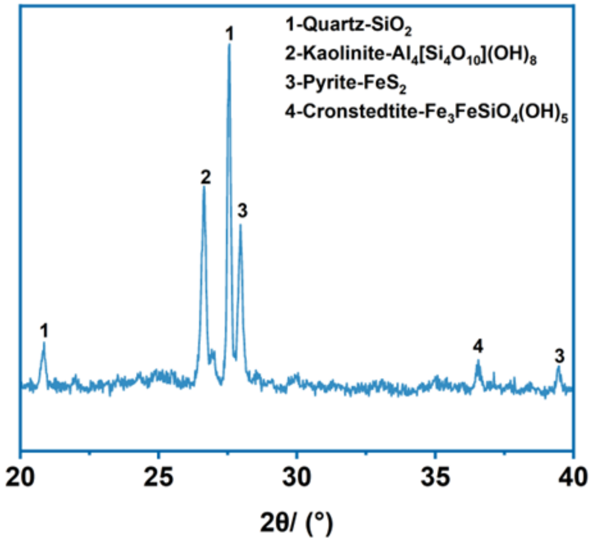

Fig. 1. XRD pattern of raw coal gangue

Effects of Treatments on Wheat Growth

The growth-promoting effects of *B. velezensis* and coal gangue were evaluated through a pot experiment with four treatments: soil only (CK), soil amended with CG only (CG), soil inoculated with *B. velezensis* only (B), and the combined application of CG and *B. velezensis* (CBG). Phenotypic analysis revealed that the CBG treatment resulted in superior wheat growth compared to all other groups (Fig. 2a). The germination rate was lowest in the CG group (54.89±0.81%) and highest in the CBG group (71.42±0.35%), representing a 22.4% increase over the CK group (58.33±1.26%) (Fig. 2b). The *B. velezensis* -only treatment (B) also improved germination (68.12±0.35%) compared to the control. Significant differences in plant height and root length were observed across treatments (Fig. 2c, Fig. 2d).

While the CG group showed only minor, non-significant improvements over CK (plant height: 228.46±10.76 mm *vs*. 222.38±8.82 mm; root length: 72.88±3.04 mm *vs*. 71.32±3.53 mm, the B and CBG treatments induced substantial growth. Plant height and root length in the B group (271.42±2.68 mm and 78.81±2.22 mm, respectively) were significantly greater than in CK. The CBG combination yielded the most pronounced effect, producing the tallest plants (293.74±3.44 mm) and longest roots (96.58±2.66 mm) of any treatment.

A similar trend was observed for biomass accumulation (Fig. 2e). The fresh weight of the CG group (228.42±2.52 g/plant,) was significantly lower than that of CK (231.33±2.58 g/plant, $p < 0.001$), indicating that CG alone inhibited growth. In contrast, both the B (313.48±3.70 g/plant) and CBG (371.56±3.71 g/plant) treatments significantly enhanced biomass relative to CK ($p < 0.001$). The CBG treatment was the most effective, resulting in a 35% increase in biomass. In summary, comprehensive analysis of germination rate, plant height, root length, and biomass confirms that the synergistic application of *B. velezensis* and coal gangue (CBG) most effectively promotes wheat growth and development.

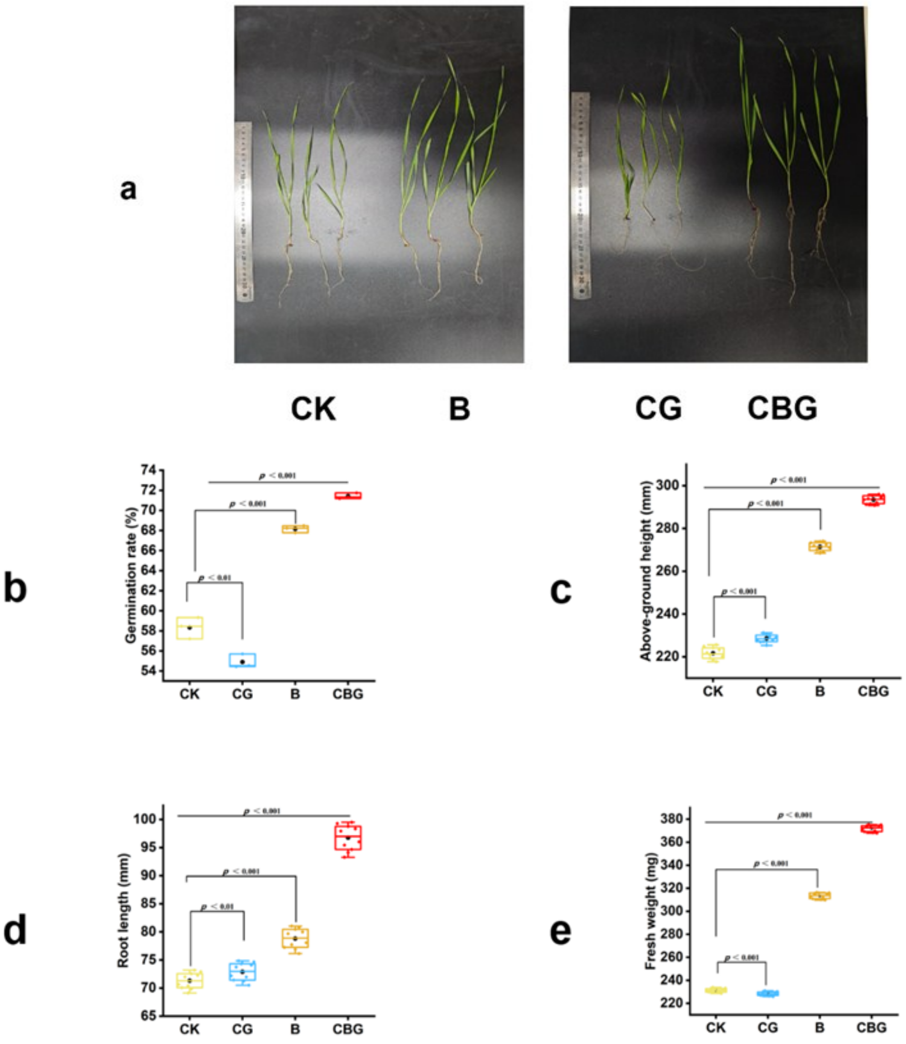

Fig. 2. Wheat growth parameters after two months of cultivation. (a) Representative photographs showing phenotypic differences among the four treatment groups. (b) Seed germination percent (%). (c) Shoot height (mm). (d) Root length (mm). (e) Fresh weight per plant (g). CK (Control, soil only), CG (Soil amended with coal gangue), B (Soil inoculated with *B. velezensis*), and CBG (Soil with both coal gangue and *B. velezensis*). Statistical significance (p-values) is shown above the brackets indicating the compared groups.

Soil Physicochemical Properties

Key physicochemical properties of soils from the four treatment groups were systematically measured (Fig. 3). Soils amended solely with coal gangue (CG) or *B. velezensis* (B) showed improved water retention, with moisture contents of 0.91±0.06% and 1.28±0.08%, respectively. Notably, the combined application of CG and strain *B. velezensis* (CBG) resulted in the highest moisture content (1.9±0.11%), representing a 5.76-fold increase over the control (CK, 0.33±0.03%) ($p < 0.01$). Soil pH values were lower in the CG (7.49±0.05), B (7.88±0.06), and CBG (7.33±0.04) treatments compared to CK (8.03±0.07), indicating that both microbial inoculation and CG amendment can shift alkaline soils toward a more neutral pH, which is more conducive to wheat growth (Ping *et al.* 2020). Nutrient analysis revealed a consistent trend across treatments: CBG > CG >

B > CK. The CBG treatment produced the highest concentrations of total potassium (TK: 33.96±0.72 g/kg), total phosphorus (TP: 0.29±0.02 g/kg), and total nitrogen (TN: 0.48± 0.03 g/kg). The CG (TK: 32.89±0.58 g/kg, TP: 0.24±0.02 g/kg, TN: 0.39±0.03 g/kg,) and B (TK: 32.56±0.65 g/kg, TP: 0.21±0.02 g/kg; TN: 0.17±0.02 g/kg) treatments showed moderate improvements, while the CK group had the lowest nutrient levels (TK: 31.42±0.49 g/kg, TP: 0.19±0.01 g/kg, TN: 0.14±0.01 g/kg). Soil organic matter (SOM) and dissolved organic carbon (DOC) were also greatest under the CBG treatment (SOM: 71.09±3.26 g/kg; DOC: 33.82±1.84 mg/L), dramatically exceeding the levels in CK (SOM: 3.62±0.21 g/kg; DOC: 7.21±0.34 mg/L). Analysis of humic substances showed that the CBG treatment resulted in the highest contents of humic acid (HA, 0.69±0.04 g/kg) and fulvic acid (FA, 0.4±0.03 g/kg), with a HA/FA ratio of 1.73, indicating enhanced humification. The humin content was also highest in the CBG group (0.29±0.02 g/kg). Consequently, the cation exchange capacity (CEC) followed the same pattern, with the CBG treatment achieving the highest value (4.71±0.19 cmol/kg), compared to 4.03±0.18 cmol/kg in CK.

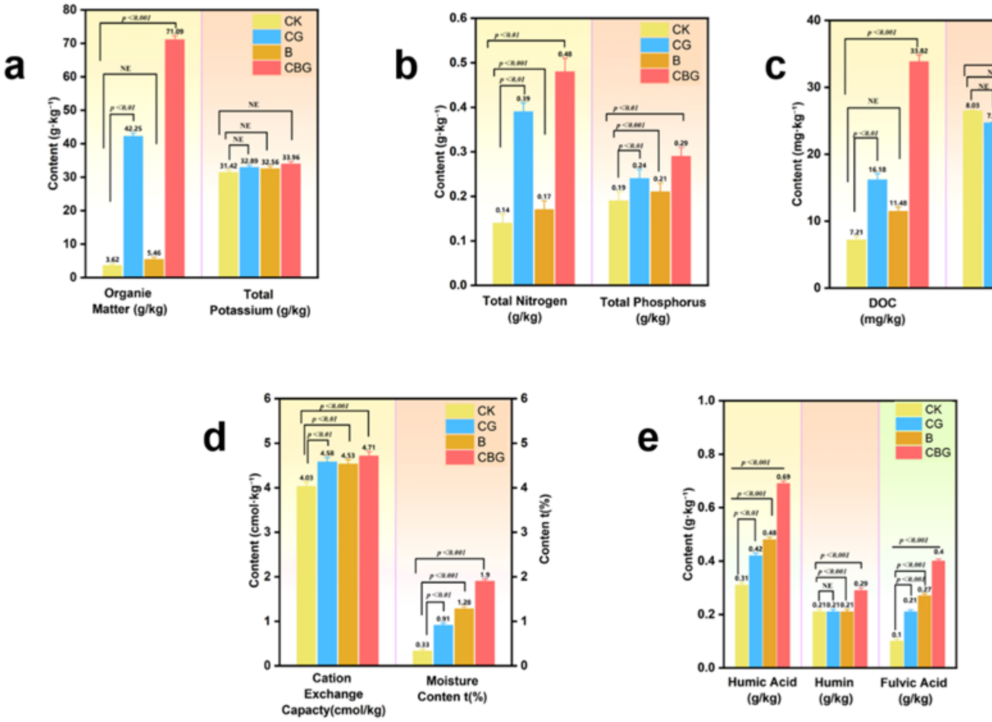

Fig. 3. Key physicochemical properties of soils from the four treatment groups. (a) Soil organic matter (OM) and total potassium (TK); (b) Total nitrogen (TN) and total phosphorus (TP); (c) dissolved organic carbon (DOC) and pH; (d) Cation exchange capacity (CEC) and moisture content; (e) Humic acid (HA), humin (HM), and fulvic acid (FA). Statistical significance (p-values) is shown above the brackets indicating the compared groups, and "NE" denotes no significant difference between groups.

Analysis of Soil Phytohormones and ACC Deaminase Activity

Auxin, particularly indole-3-acetic acid, is a key phytohormone that regulates plant growth and development, especially root and shoot formation (Lobo *et al.* 2022). As shown in (Fig. 4a), soil IAA content differed significantly among treatments, following the order CBG > B > CG > CK. The CBG and B treatments exhibited the highest IAA concentrations

(21.86±1.25 μg/mL and 20.99±1.18 μg/mL, respectively), representing 1.58-fold and 1.53-fold increases relative to CK (13.76±0.82 μg/mL). Although the CG treatment (15.31±1.05 μg/mL) also elevated IAA levels compared with CK, its effect was notably weaker than that of the *B. velezensis* -inoculated groups. These findings suggest that the introduction of strain *B. velezensis* was the primary factor enhancing soil IAA production, while coal gangue likely provided precursors that further facilitated the biosynthesis of IAA.

A similar trend was observed for ACC deaminase activity, a key enzyme that cleaves the ethylene precursor ACC to reduce plant stress and promote root development (Herpell *et al.* 2023). As shown in (Fig. 4b), ACC deaminase activity followed a trend consistent with IAA content (CBG > B > CG > CK). The CG treatment (1.84±0.13 U/mg) increased enzyme activity by 1.14-fold compared with CK (1.62±0.09 U/mg), indicating that coal gangue may improve the microenvironment for microbial metabolism. However, *B. velezensis* inoculation resulted in more pronounced effects, with activities of 2.84±0.16 U/mg (B) and 3.06±0.18 U/mg (CBG). Notably, The CBG group showed the highest activity, being 1.89-, 1.66-, and 1.08-fold greater than the CK, CG, and B groups, respectively. These results clearly demonstrate a synergistic effect between *B. velezensis* and coal gangue in enhancing soil ACC deaminase activity and, consequently, promoting wheat growth.

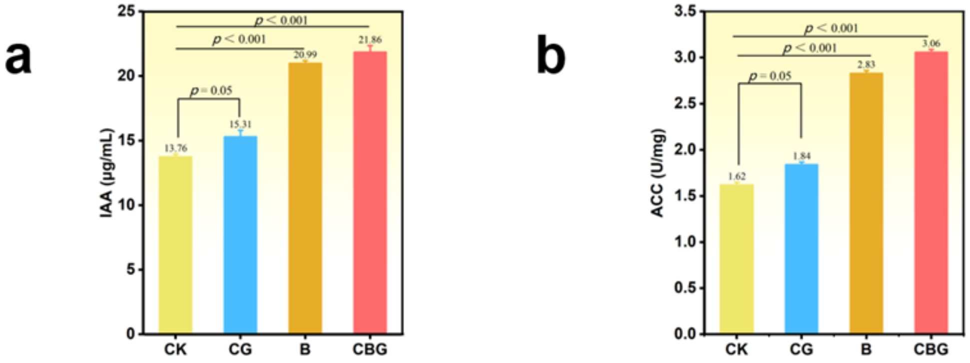

Fig. 4. Phytohormone content and ACC deaminase activity in soil. (a) IAA concentration. (b) ACC deaminase activity. Data are presented as the mean ± standard error (SE). Statistical significance (p-values) is shown above the brackets indicating the compared groups.

Analysis of Soil Enzyme Activities

The assessment of soil enzyme activities represents a key biological method for evaluating fertility, as these enzymes are responsible for breaking down exogenous organic matter and promoting nutrient cycling, thereby providing essential nutrients for plant growth (Dick and Tabatabai 1992). The present investigation quantified the activities of several important enzymes, such as urease and protease, amylase, phosphatase, and laccase, which were measured in soil samples. While the individual application of coal gangue (CG) or *B. velezensis* (B) led to modest, non-significant increases in urease and protease activities, the combined treatment (CBG) significantly enhanced the activities of most enzymes, particularly amylase, phosphatase, and laccase. The CBG treatment resulted in the highest observed activities. Protease activity was increased by 1.21-fold (12.81±0.74 U/mL to 15.46±0.89 U/mL) (Fig. 5a), and urease activity by 1.18-fold (0.68±0.05 mg/d/g to 0.80±0.06 mg/d/g) (Fig. 5b), indicating a more active conversion of organic nitrogen and an accelerated nitrogen cycle (Kuscu 2019). Amylase activity rose by 1.54-fold (2.76

± 0.19 U/mL to 4.26 ± 0.28 U/mL) (Fig. 5c), reflecting enhanced decomposition of carbohydrates and more active soil microbial metabolism (Damaris *et al.* 2019). Most notably, phosphatase activity increased by 4.25-fold (0.28 ± 0.02 mg/d/g to 1.19 ± 0.08 mg/d/g) (Fig. 5d), highlighting the increased secretion of phosphatase by soil microorganisms. This significant rise indicates enhanced conversion of organic phosphorus compounds to available phosphorus, thereby supporting plant phosphorus requirements (Guo *et al.* 2023). Furthermore, laccase activity increased by 1.99-fold (from 648.89±32.67 U/L to 1288.26±58.41 U/L) (Fig. 5e). As a key oxidoreductase, laccase plays a central role in soil carbon cycling, particularly by catalyzing the oxidative decomposition of recalcitrant aromatic compounds such as lignin. In summary, while both the *B. velezensis* microbial agent and coal gangue individually promoted soil enzyme activity, the synergistic application of *B. velezensis* and coal gangue (CBG) most effectively enhanced soil enzyme activities. The improved soil environment created by CG likely provided a favorable habitat for the inoculated bacteria, which in turn secreted higher levels of enzymes, driving a more efficient nutrient cycle including nitrogen cycling, carbohydrate decomposition, phosphorus availability, and the breakdown of recalcitrant organic matter.

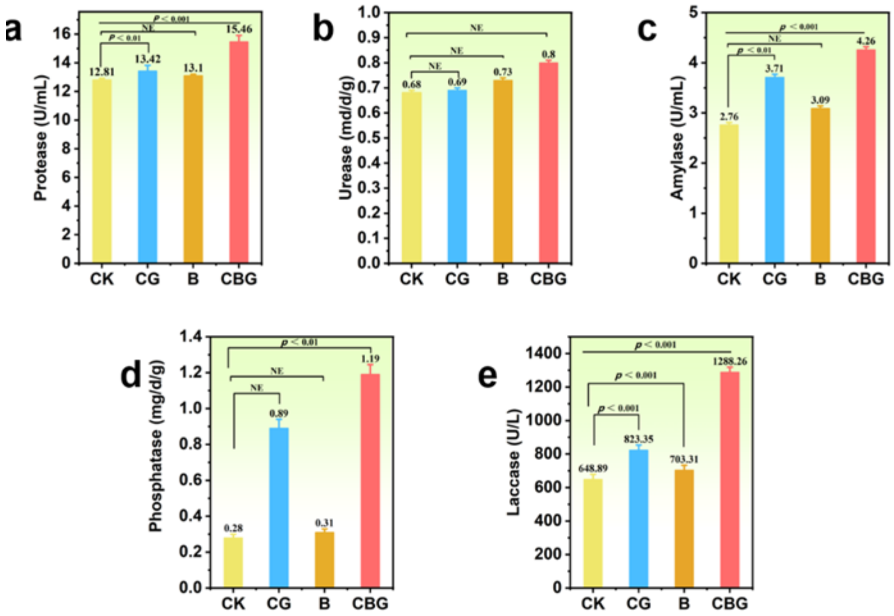

Fig. 5. Comparative analysis of soil enzyme activities. Activities of (a) protease, (b) urease, (c) amylase, (d) phosphatase, and (e) laccase are shown for the CK, CG, B, and CBG treatment groups. Statistical significance (p-values) is shown above the brackets indicating the compared groups, and "NE" denotes no significant difference between groups.

Rhizosphere Soil Bacterial Diversity

To evaluate the effects of combined application of coal gangue and *B. velezensis* on the diversity of bacterial communities in rhizosphere soil, this study conducted a diversity analysis of bacterial communities in rhizosphere soil across different treatment groups. Specifically, in terms of species richness (Fig. 6a, Fig. 6b), the combined treatment group of *B. velezensis* and coal gangue (CBG) exhibited the highest Ace index (3523.04,) and Chao1 index (3623.81), which were significantly greater than those of the CK group

(Ace: 3092.38; Chao1: 3081.24) ($p < 0.05$). The treatment groups with coal gangue alone (CG) and *B. velezensis* inoculation alone (B) also showed higher indices compared to the CK group (CG: Ace 3270.85, Chao1 3188.13; B: Ace 3340.28, Chao1 3342.85), although the increases were considerably lower than those observed in the CBG group. These results indicate that while the individual application of coal gangue or *B. velezensis* could enhance soil bacterial species richness to some extent, the combined treatment (CBG) demonstrated the most pronounced promotive effect on rhizosphere microbial diversity, suggesting a clear synergistic effect.

Differences in the alpha diversity indices of microbial communities were also observed among the four treatment groups (CK, CG, B, and CBG). The variation in the Shannon index (Fig. 6c) indicated that, compared with the control group (CK), species diversity exhibited a stepwise decline in the treatments with coal gangue addition alone (CG), *B. velezensis* inoculation alone (B), and the combined treatment (CBG). The decline in the Shannon index, despite increases in species richness (Ace and Chao1) and evenness (Simpson), can be attributed to the greater sensitivity of the Shannon index to changes in rare or low-abundance taxa. The combined CBG treatment likely reduced the proportion of rare, transient, or functionally redundant species while selectively enriching specific beneficial genera. This shift toward a more simplified and functionally optimized community structure, dominated by a few well-adapted taxa, may lead to a lower Shannon index even when overall richness and evenness increase. The Simpson index (Fig. 6d) showed that, consistent with the previously observed increases in species richness (Ace and Chao1 indices), the combined CBG treatment (0.00867) exerted the strongest promotive effect on enhancing the evenness of the rhizosphere bacterial community relative to the CK group. When *B. velezensis* was applied alone (B, 0.00641), it also led to an improvement in community evenness, although this effect was less pronounced compared to the combined treatment. In contrast, the CG treatment (0.00411) did not significantly enhance community evenness and even showed a slight negative effect. Taken together, the combined treatment not only significantly increased species richness but also promoted a more balanced species distribution, collectively shaping a rhizosphere microbial community with greater structural complexity, potentially higher functional stability, and enhanced resilience.

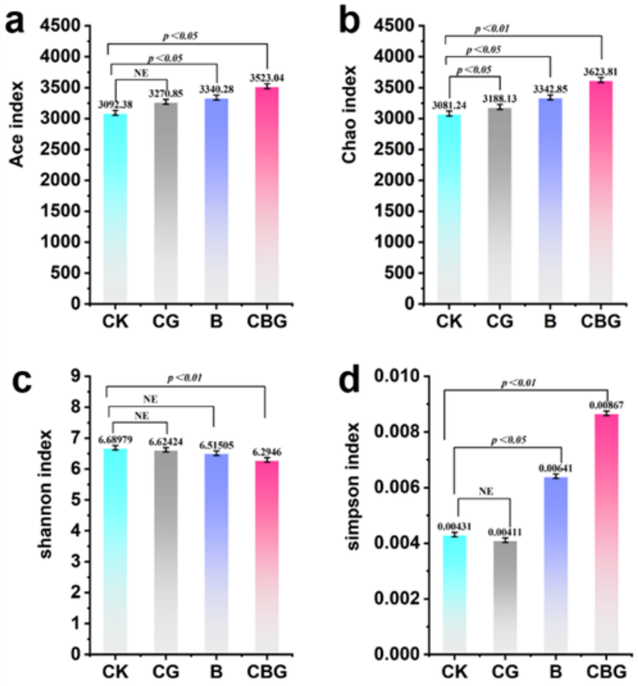

Fig. 6. Soil microbial alpha-diversity indices. The values of the (a) Ace index, (b) Chao index, (c) Shannon index, and (d) Simpson index are shown for the four treatment groups: CK, CG, B, and CBG. Statistical significance (p-values) is shown above the brackets indicating the compared groups, and "NE" denotes no significant difference between groups.

Composition of Rhizosphere Bacterial Communities

Through Venn diagram analysis, a total of 4,824 OTUs were identified across the four treatment groups (CBG, B, CG, and CK) (Fig. 7a). The number and proportion of unique OTUs specific to each treatment group were as follows: the control group (CK) contained the highest number of unique OTUs (3,216), accounting for 66.7% of the total OTUs; the coal gangue treatment group (CG) had 3,034 unique OTUs (62.9%); the *B. velezensis* inoculant treatment group (B) contained 2,927 unique OTUs (60.7%); while the combined treatment group (CBG) possessed the fewest unique OTUs (2,831), representing 59.7% of the total. Regarding shared OTUs, 860 OTUs (17.83%) were common to any two treatment groups, and 989 OTUs (20.50%) were shared among any three treatment groups. Notably, 1,504 OTUs (31.2% of the total) were consistently present across all four treatment groups. These results indicate that the different treatments led to varying degrees of reduction in OTU richness. Despite the distinct treatment conditions, a substantial portion of the microbial taxa exhibited a similar response, forming a stable core microbiota that underpins this soil ecosystem.

At the genus level, the treatments induced significant shifts in the abundance of key taxa including *Arthrobacter*, *Thermomicrobiales*, and *Sphingomonas* (Fig. 7b). *Arthrobacter* was the most abundant genus across all groups, with its relative abundance significantly enhanced in the CBG treatment (9.14%). This was 1.78-, 3.89-, and 1.20-fold higher than in the CK (5.13%), CG (2.35%), and B (7.63%) groups, respectively, demonstrating a clear synergistic effect between coal gangue and *B. velezensis* in enriching this genus. Given its known role in producing ACC deaminase and IAA, this enrichment likely contributed directly to the observed wheat growth promotion (Roy and Kumar 2020).

Thermomicrobiales is an order of thermophilic bacteria known for promoting organic matter degradation in high-temperature environments (Wu *et al.* 2009). The relative abundance of *Thermomicrobiales* was highest in the CG group (6.55%), suggesting coal gangue enriched these thermophilic decomposers. However, its abundance was lower in the CBG group (4.8%), indicating that *B. velezensis* inoculation mitigated this specific selective pressure. Similarly, the *Sphingomonas*, an α-proteobacterial genus with pollutant-degrading abilities (Luo *et al.* 2019), was most abundant in the CG group (4.20%) but decreased to 3.10% in the CBG group. This reduction suggests that the *B. velezensis* inoculant may have inhibited the over-enrichment of *Sphingomonas* by altering the microecological balance or competitive interactions within the soil. Furthermore, the combined treatment influenced taxa linked to plant growth and soil health. The abundance of *Oxalobacteraceae*, a family known to promote root development (Yu *et al.* 2021), was notably present in the CBG and CG groups. The response pattern of *Vicinamibacteraceae*, a key functional group in soil carbon cycling, serves as a sensitive indicator of soil ecological status (Francis *et al.* 2021). Its abundance was suppressed by CG alone but enriched in *B. velezensis*–inoculated treatments (B and CBG), suggesting that the inoculant promotes a more stable and healthy soil microbiome.

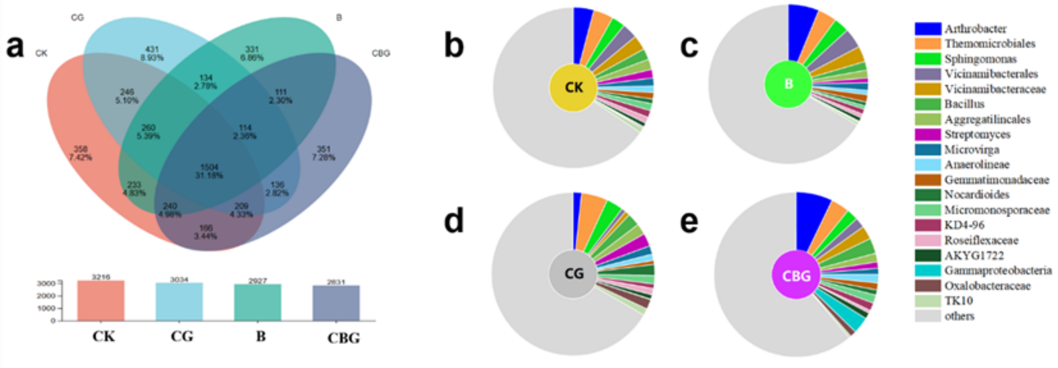

Fig. 7. Analysis of bacterial community composition. (a) Venn diagram illustrating shared and unique operational taxonomic units (OTUs) across the four treatment groups. Relative abundance of the dominant bacterial genera within the rhizosphere microbial community for each treatment: (b) CK, (c) B, (d) CG, and (e) CBG.

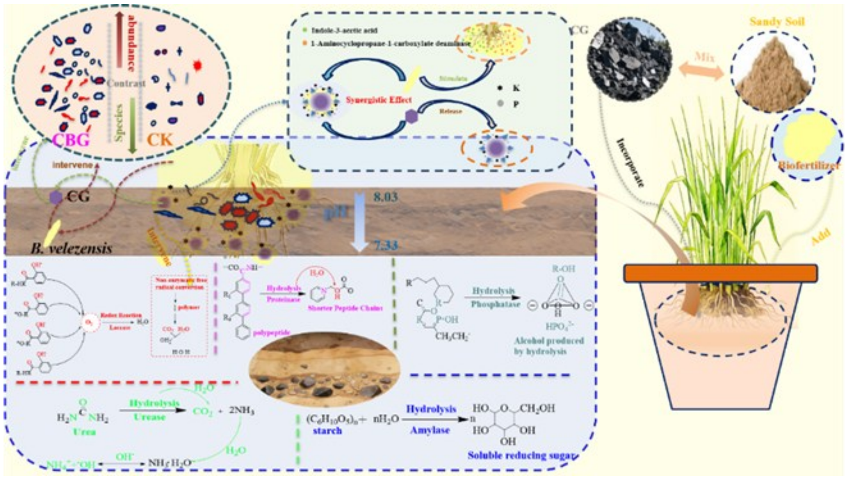

Fig. 8. Schematic illustration of the proposed mechanism underlying the synergistic effects of *B. velezensis* and CG on wheat growth

This study demonstrated a significant synergistic effect of *B. velezensis* and CG (CBG treatment) on promoting wheat growth (Fig. 8). The addition of CG improved soil structure and water retention, while *B. velezensis* produced IAA and ACC deaminase, promoting root development. Enhanced enzyme activities, including urease (converting urea to NH_4^+), amylase (degrading starch into reducing sugars), and phosphatase (releasing PO_4^{3-} from organic P), drove nutrient cycling, while soil pH decreased from 8.03 (CK) to 7.33 (CBG), improving nutrient availability. The CBG treatment enriched beneficial genera (*Arthrobacter*, *Oxalobacteraceae*) and suppressed stress indicators (*Thermomicrobiales*), thereby reshaping the rhizosphere microbiome. Together, these effects enhanced wheat growth, increasing germination, height, root length, and biomass.

The results demonstrate that *B. velezensis* significantly enhanced nutrient cycling and enzymatic processes within the CG-soil system. The combined treatment (CBG) led to substantial increases in soil organic matter, total nitrogen, phosphorus, potassium, dissolved organic carbon, and humic acid, indicating a profound improvement in overall soil fertility. While CG alone contributed mineral elements and improves water retention, the addition of *B. velezensis* further amplified these benefits, particularly by increasing the availability of phosphorus and potassium. This enhancement is primarily attributed to its ability to solubilize fixed minerals (Wang *et al.* 2024), consistent with established evidence that *B. velezensis* secretes metabolites like organic acids and chelators to weather insoluble phosphate and silicate compounds in soil, thereby sustainably releasing plant-available nutrients such as phosphorus (Mani *et al.* 2024). Previous studies have similarly reported that *B. velezensis* Ag75 improves crop phosphorus absorption through efficient phosphorus solubilization, thereby reducing reliance on chemical fertilizers (Mosela *et al.* 2022). Concurrently, the *B. velezensis* inoculation markedly elevated key soil enzyme activities. The significant increase in phosphatase activity directly correlates with the enhanced phosphorus solubilization. Similarly, the promoted activities of protease and urease accelerated the mineralization of organic nitrogen, ensuring a more readily available nitrogen supply for the plant (Hayat *et al.* 2025; Zhang *et al.* 2025). The synergistic enhancement of these enzymes optimized the transformation and availability of both nitrogen and phosphorus. Furthermore, the observed increases in amylase and laccase activities indicate a reinforced soil carbon cycle. Elevated amylase activity suggests a heightened metabolism of labile organic carbon, while the boost in laccase, a key enzyme in lignin degradation, points to an enhanced capacity to break down recalcitrant organic matter, facilitating humus formation and nutrient cycling (Theuerl *et al.* 2010; Gougoulias *et al.* 2014).

The high-throughput sequencing results demonstrate that *B. velezensis*, in synergy with (CG), played a crucial role in regulating and reshaping the structure and function of the rhizosphere bacterial community. The addition of CG alone resulted in a reduction in soil bacterial diversity, as well as an increase in the relative abundance of drought-tolerant and thermophilic bacterial groups, such as *Thermomicrobiales*. This indicates the biological stress exerted on the soil microenvironment during the early stages of coal gangue addition (Zhu *et al.* 2023). However, the inoculation with *B. velezensis* effectively mitigated this stress, significantly reducing the relative abundance of *Thermomicrobiales* and fostering the enrichment of beneficial bacterial genera with specific ecological functions. Among the taxa that showed notable enhancement was *Sphingomonas*, which is involved in organic pollutant degradation (White *et al.* 1996). Others included *Streptomyces*, which is known for its biocontrol potential, and nutrient-cycling bacteria such as *Vicinamibacterales* and *Oxalobacteraceae*, which play key roles in carbon and phosphorus transformations (Barka *et al.* 2016). Notably, the effect of strain *B. velezensis* on the microbial community was not limited to a simple increase in taxonomic richness; rather, it promoted a more functionally efficient and streamlined community structure. Venn analysis revealed a lower total number of bacterial genera in the combined CBG (Soil with both coal gangue and *B. velezensis*) treatment (2,831) compared to the control CK (3,216), suggesting *B. velezensis* mediates a functional selection and structural optimization of the indigenous microbiota, likely through competitive exclusion and other ecological mechanisms (Song *et al.* 2021; Zhang *et al.* 2025). The authors propose that *B. velezensis* influences the soil microbial community through two primary mechanisms: (1) it accelerates the degradation of CG through its metabolic activity, releasing trace elements that improve the soil's chemical environment, and (2) directly modulating the growth of other microorganisms. In summary, the directed remodeling of the bacterial community structure in CG-amended soil by *B. velezensis* constitutes a key mechanism behind its soil improvement and plant growth-promoting effects.

While this study provides robust evidence for the synergistic benefits of *B. velezensis* and CG, several limitations should be acknowledged. First, the pot experiments were conducted under controlled outdoor conditions, which may not fully capture the complexity of field environments. Second, the long-term effects of repeated CBG application on soil health and heavy metal accumulation remain unknown, as coal gangue may contain trace metals that could leach over extended periods. Future research should therefore focus on: (i) field-scale trials across different soil types and climatic regions to assess practical applicability; (ii) long-term monitoring of soil metal dynamics and microbial stability. Addressing these aspects will further strengthen the *B. velezensis*–CG partnership as a sustainable strategy for waste management and agricultural reclamation.

CONCLUSIONS

1. This study demonstrated that the combined application of *B. velezensis* and coal gangue synergistically enhanced wheat growth by comprehensively improving soil properties and functions.
2. The combined application of *B. velezensis* and coal gangue (CBG treatment) exerted the most significant promotional effect on wheat growth. Compared with the control group, it effectively improved germination rate, plant height, root length, and fresh weight of wheat.
3. The addition of coal gangue alone had no significant effect on wheat growth and may even have caused slight inhibition during the early stage. In contrast, co-application with *B. velezensis* effectively alleviated the adverse effects of coal gangue, optimized the soil environment, and created favorable conditions for wheat growth.
4. The synergistic effect of *B. velezensis* and coal gangue not only improved the soil microenvironment, but it also promoted healthy wheat growth, thereby providing a practical technical pathway for the resource utilization of coal gangue and the high-quality production of wheat.

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Conflicts of Interest

The authors state that there are no competing financial interests or personal relationships which could have potentially affected this research.

Data Availability

The data presented in this study are available upon request from the corresponding author.

Use of Generative AI

During the preparation of this manuscript, the authors used the DeepSeek AI tool to assist with grammar checking and improving textual fluency. In the Discussion section, it also helped refine the logical flow of content concerning "CG improving soil physical structure" and "*Bacillus velezensis* shaping the soil microbial community." The authors have thoroughly reviewed and revised all AI-generated content and take full responsibility for the final manuscript.

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APPENDIX

Supplementary Information

Table S1. Physicochemical Properties of the Sandy Soil

Component	pH	Organic matter(g/kg)	Total nitrogen (g/kg)	Available phosphorus (g/kg)	Available potassium (g/kg)	Dissolved organic carbon (mg/kg)	Cation exchange capacity (cmol/kg)	Moisture content (%)
Content	8.24	3.77±0.32	0.14±0.03	0.18±0.02	30.24±3.2	7.16±0.12	3.74±0.02	0.14±0.02