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Title: Interactive effect of biochar and potassium-solubilizing bacteria on soil depth-dependent potassium dynamics, enzymatic activities and bacterial community in acidic soil under irrigation practices

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Highlights

1. Potassium (K) availability in acidic soil decreased due to poor solubility and high leaching.
2. Co-application of biochar and K solubilizing bacteria (KSB) increased K accessibility.
3. Biochar exhibited a strong potential to retain K in acidic soil via its surface properties.
4. K retention facilitated beneficial soil bacterial growth (e.g., Actinomycetota, Bacteriodota).

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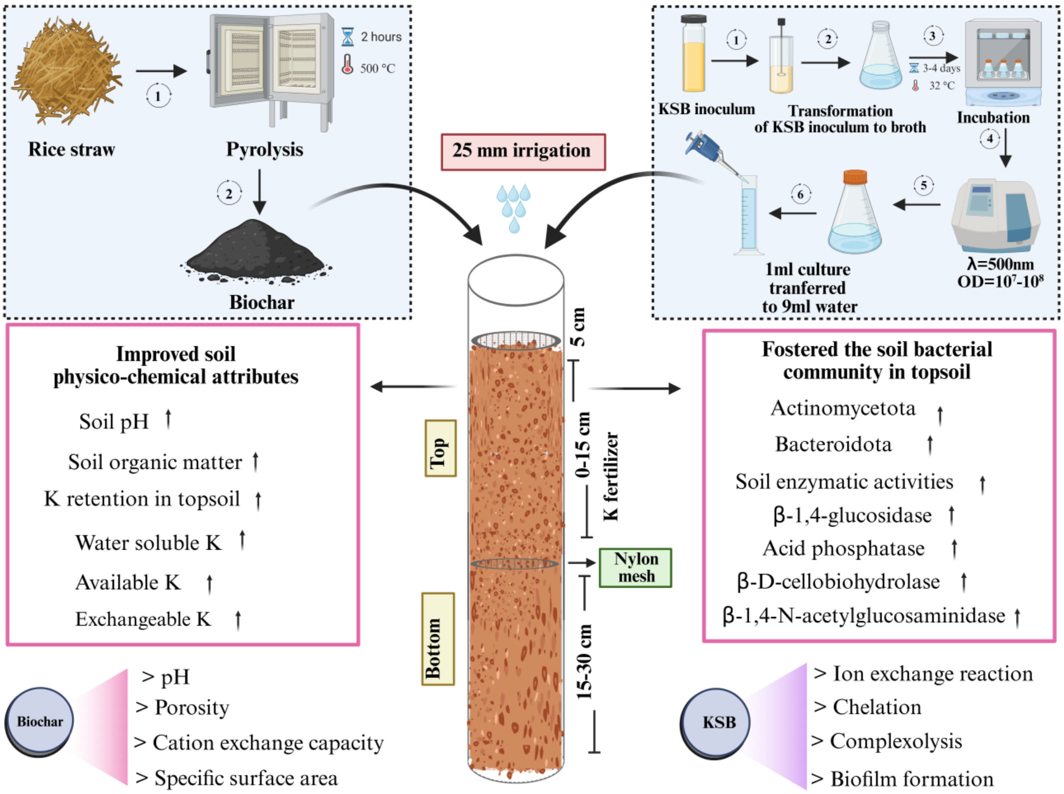
Graphical Abstract



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Abstract

Potassium (K), an essential macronutrient, its availability is often limited in acidic soils due to its fixation or low solubility. Meanwhile, the solubilized K fraction becomes leached under irrigation, owing to reduced K adsorption capacity of acidic soil, thus affecting soil fertility. Previous studies have demonstrated that biochar exhibits a strong potential to retain nutrients in soil. However, their effect on enhancing the K solubility and its retention capacity, especially under combined application of rice straw biochar (BC) with KSB (potassium solubilizing bacteria), remains uncleared. Therefore, an incubation study was carried out, in which a soil column containing 850-gram soil was divided into topsoil (0-15 cm) and subsoil (15-30 cm) portions. The treatment plan was as CK (without K fertilizer), K (K fertilizer), K+ BC (1% w/w), K+ KSB inoculum (CFU 10^7 to 10^8), and K+ KSB inoculum (CFU 10^7 to 10^8) + BC (1% w/w) with quadruplicate. K fertilizer was added only to the topsoil to assess the K retention effect. Results depicted that under integrated application of BC with KSB, soil pH and organic matter (SOM) contents in topsoil enhanced by 13.5% and 53.9% compared to K-treated topsoil. Meanwhile, water-soluble K and available K contents increased by 52.5% and 49.8% respectively. Such improvement in soil properties greatly enhanced the soil enzymatic activity and soil bacterial community (Actinomycetota and Bacteroidota) in topsoil. Thus, the overall result depicted that integrated application of BC with KSB notably increased the K bioavailability in topsoil by facilitating the KSB activity to solubilize K. Meanwhile, BC improved the K retention capacity through its enlarged surface area, porous structure, and high cation exchange capacity, thereby reduced the K leaching and enhanced the nutrient availability in acidic soil.

Keywords: Bacterial community, Bioavailability, Enzymatic activity, Leaching, Soil column

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Introduction

Potassium (K) is one of most important primary macronutrient for plant growth (Sustr et al., 2024), which participates in numerous plant physiological and biochemical processes, e.g., photosynthesis, ATP (adenosine triphosphate) production, protein synthesis, enzyme activation, and osmoregulation (Yuan et al., 2024; Bhardwaj et al., 2025). The global population is steadily increasing and expected to reach 8.90 -10.6 billion by 2050, which poses a significant challenge to farmers to meet their growing food demand (Roy et al., 2024). Farmers have extensively applied the K fertilizer to address this, but a significant proportion of K is lost through leaching or fixation (Zhu et al., 2024). This problem is more prevalent in acidic soil of China (Brownlie et al., 2024), where the soil has low pH, reduced soil organic matter (SOM), and poor cation exchange capacity (CEC) exacerbate the challenge of maintaining adequate K levels for optimal crop growth (Han et al., 2023; Chen et al., 2024). In addition, nutrient loss via leaching further enhances the soil degradation process, causes groundwater contamination, and disrupts the whole ecosystem, which poses a serious threat to sustainable agriculture. To address these challenges, there is an urgent need for sustainable approaches that could enhance K retention and its availability for plant uptake while improving overall soil health.

Biochar, a carbon-rich material obtained by pyrolysis of natural biomass under oxygen-limited conditions (Sun et al., 2024a), has been identified as promising amendment for improving soil fertility by facilitating the release of essential nutrients while enhancing their retention (Tan, 2023; Luo et al., 2024). Biochar, with its porous structure, enlarged surface area, high charge density and abundant functional groups, provides an effective medium for adsorbing and retaining essential nutrients such as K in acidic soil (He et al., 2024; Yan et al., 2025a,b). Additionally, Bao et al. (2024) stated that over 51-58% of the total K in biochar, retained during pyrolysis, is available for

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direct plant uptake. Furthermore, with high CEC, biochar acts as a 'protection net' for K fertilizers, reducing K leaching by up to 36% (Xia et al., 2024). In general, soil K exists in different forms, such as water-soluble K (WSK), exchangeable K (EK), and non-exchangeable (Non-EK) forms (Babar et al., 2024). The biochar addition, regulates this soil K dynamics by facilitating the conversion of slowly available K into more accessible WSK and EK forms (Bilias et al., 2023). Such accessible forms of K are prone to leaching due to high irrigation or irregular rainfall patterns, reducing its accessibility for plant assimilation (Yahaya et al., 2023; Wang et al., 2024a). In such conditions, biochar application enhanced the K retention through its high CEC, and enlarged surface area, which adsorb and retain K ions, reducing the K leaching (Xia et al., 2022). In addition, it improves soil aggregation, decreases water percolation, and increases the soil pH, which prevents the K from fixation (Ghorbani and Amirahmadi, 2024). Biochar also forms a stable nutrient matrix, improving K availability and retention in acidic soils (Marcińczyk and Oleszczuk, 2022). Thus, biochar functions as a dual-purpose amendment, supplying K for crop growth and boosting soil K reserves (Wan et al., 2024).

Furthermore, certain soil microorganisms that are key drivers in nutrient cycling process also participate in K release from clay minerals, enhancing their bioavailability (Bao et al., 2024). A broad range of potassium-solubilizing bacteria (KSB) has been identified (Soumare et al., 2023), such as *Bacillus edaphicus*, *Bacillus cereus, Bacillus mucilaginosus*, *Bacillus subtilis, Bacillus circulans*, *Paenibacillus* spp., *Pseudomonas*, *Burkholderia* and *Acidithiobacillus ferrooxidans* that release K form from K-bearing minerals (illite, biotite, feldspar, muscovite) in soils (Babar et al., 2024; Zhang et al., 2025a). Among these KSBs, *Paenibacillus mucilaginosus* is widely known for its effectiveness in solubilizing the K from mineral sources by adopting the diverse mechanisms such as ion exchange reaction, acidolysis, complexolysis, chelation, and exopolysaccharide

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production, in turn increasing its accessibility to plants (Chen et al., 2020; Zhang et al., 2024a). As in a previous study, application of *Paenibacillus mucilaginosus* significantly improved saffron cormels growth and their nutritional status, e.g. crocin, picrocrocin and safranal accumulation in stigmas by facilitating the K uptake from soil solution (Zhou et al., 2024a,b).

Moreover, biochar addition further promotes the growth of KSBs by providing an ideal habitat for their proliferation via its porous structure (Xia et al., 2024), which further accelerates the K mineralization rate from biochar and clay minerals (Zhang et al., 2022; He et al., 2024). Thus, the beneficial role of biochar in increasing K availability and promoting plant growth has been widely documented in previous studies (Li et al., 2024a; Yan et al., 2025b). However, a knowledge gap still exists regarding the integrated effect of biochar with KSB on enhancing K availability and their retention under natural rainfall conditions or excessive irrigation practices. In addition, their influence on soil bacterial communities, enzymatic activities, and soil nutrient dynamics across soil depths in response to leaching requires in-depth investigation. A comprehensive understanding of associated variations in soil chemical and biological properties in response to the integrated application of biochar with KSB is fundamental for developing nutrient management practices.

In current study, a two-month incubation experiment was conducted to examine the effect of sole and combined applications of biochar and KSB on K availability and its retention under leaching conditions, with a focus on elucidating the underlying mechanisms. The main purpose of current study was to (1) enumerate the sole and integrated effect of biochar with KSB on soil chemical properties and enzymatic activities under artificially induced rainfall-driven leaching conditions; (2) explore the difference in soil K fractions and its retention at different soil depths in response to applied amendment; (3) investigate the changes in soil bacterial community composition in response to alteration in soil chemical properties with applied amendment. It was

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assumed that integrated application of biochar with KSB would improve the soil properties, especially enhance K bioavailability and retain it in topsoil. Meanwhile, it could promote soil enzymatic activity and beneficial soil bacterial community composition under artificially induced rainfall conditions.

2. Material and methods

2.1 *Potassium solubilizing bacterium (KSB) isolate*

A purified strain of potassium-solubilizing bacterium, *Paenibacillus mucilaginous* (AB 2021090) was obtained from China Center for Type Culture Collection in Wuhan. This bacterial strain was cultured in an autoclaved Aleksandrov medium with the following composition: 5 g glucose, 2 g Na_2HPO_4. $2H_2O$, 0.005 g $FeCl_3$,0.5g $MgSO_4.7H_2O$, 0.1 g $CaCO_3$ and 1.0 g feldspar (K source) in 1 L deionized water (DI), with pH adjusted to 7.0 (Han et al., 2024). The culture was incubated at 32°C on an orbital shaker at 180 rpm for 3 days to ensure optimal bacterial growth. Before its application in experimental soil, the K solubilizing potential of *Paenibacillus mucilaginous* (AB 2021090) strain was reconfirmed by transferring it to Pikovskaya's agar plates, which contained bromothymol blue (BTB) as a colour indicator. After 2 days of incubation, this KSB strain changed its colour from blue to yellow, indicating its K solubilization potential by forming the halo zones of 2-2.5 cm in diameter around the bacterial colony. In addition, colonies were round in shape and sticky in texture. Following this confirmatory test, a bacterial suspension of *Paenibacillus mucilaginous* strain was prepared with a cell density of 10^8–10^9 CFU/mL, standardized by an optical density (OD) of 0.5 at a wavelength of 600 nm. This suspension was subsequently applied in experimental treatments according to the specified plan.

2.2 *Soil and biochar characterization*

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The soil used in current study was sampled from 0-25 cm soil horizon of Xianning County (30°02′N, 114°37′E), Hubei province, China, situated in a subtropical monsoon climatic region. This soil originated from Quaternary red clay and shale, having high kaolinite clay contents and poor OM (Xia et al., 2024). Based on USDA (United States Department of Agriculture) soil taxonomic system, the studied soil falls under Ultisols order (Xia et al., 2024). After collection, the soil was air-dried, plant debris and stones were removed and then sieved and stored in the laboratory for experimental use. While biochar utilized in the current study was obtained from Hubei Academy of Agricultural Sciences, produced from rice straw that was pyrolyzed at 500 °C for 2 h, having a specific surface area (SSA) of 24.01 m^2/g and average pore size is 4.50 nm. The fundamental physicochemical characteristics of soil and BC are shown in Table S1. Furthermore, the characterization of BC via SEM (scanning electron microscope), FTIR (Fourier transform infrared spectroscopy) and XRD (X-ray diffraction) analysis have been mentioned in our prior research studies (Babar et al., 2025a; Wang et al., 2025).

2.3 *Incubation experimental design*

Plastic pots, with dimensions of 35 × 5.6 cm, were used in current study, as shown in Fig.1. Each pot was divided into two parts: topsoil (0-15 cm) and subsoil (15-30 cm), containing 850 grams of soil. These parts were separated using nylon mesh, whose pore size was about 50 µm, to allow better movement of water-soluble soil constituents and microorganisms across the soil zones. Meanwhile, 5 cm of space was left in each pot for watering. This setup enabled us to assess the variation in soil profile across two zones under leaching conditions. The experiment consisted of five treatments with quadruplicate, namely as CK (no K fertilizer), K (recommended K fertilizer), K+ BC (Biochar 1% w/w), K+ KSB inoculum (CFU 10^7 to 10^8), and K+ KSB inoculum (CFU 10^7 to 10^8) + BC (Biochar 1% w/w). Before incubation, the soil was thoroughly mixed with N and P

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fertilizer as urea (0.55 N g N per pot) and calcium superphosphate (0.15 P_2O_5 per pot), respectively. K fertilizer was added only in top soil (0-15 cm) as potassium chloride (0.52 K_20), while biochar was added throughout the whole soil according to the treatment plan. Afterwards, the soil was saturated with distilled water to achieve 60% of water-holding capacity. In addition, KSB inoculum was also applied to the soil surface of the corresponding treatment pot by aseptically mixing 1.0 ml of bacterial culture with CFU is 10^7 to 10^8 into 9.0 ml sterile water. This practice was repeated two times, after which the whole pots were left in a dark room for 10 days.

In order to simulate and identify the leaching effect of 25 mm precipitation on soil profile across two zones, an artificial watering was carried out on the 1st, 6th, 11th, 16th, 21st, 26th, and 31st day (at intervals of 5 days). In addition, a sterile filter paper was carefully laid over soil surface to preserve the soil integrity during irrigation. Following the final irrigation, the pots were incubated for 15 days at room temperature. Afterwards, soil sampling was carried out for analysis. This study was conducted at Huazhong Agriculture University, Wuhan, China, in December 2024 and lasted for 55 days.

2.4 *Soil collection and its chemical analysis*

After incubation, soil sampling was carried out from each pot by cutting the plastic pots with an electric knife. During sampling, soil was collected from two distinct zones within each pot using a sample destructive approach and ensured that the portion of soil contacted by an electric knife was excluded. One portion of the fresh soil was transferred to sterilized tubes and bags, then stored at -80°C for enzyme analysis and DNA extraction, while another portion was air-dried, sieved, and used for soil chemical analysis. Soil physicochemical properties were determined by daily routine methods, as proposed by Bao (2000). Soil pH was determined using pH meter (FE20, Mettler Toledo, Shanghai) with water to soil ratio 2.5:1. SOM contents were quantified via

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potassium dichromate ($K_2Cr_2O_7$) volumetric method. The concentration of available nitrogen (AN) and phosphorus (AP) in soil was measured via alkaline based hydrozable method and UV-visible light spectrophotometry (TU-1810, Beijing), respectively. Meanwhile, different forms of soil K content, e.g. water-soluble K (WK), available K (AK), and acid-soluble K (Acid-K) were determined using distilled water extraction (soil-water ratio, 1:5), ammonium acetate extraction (soil-solution ratio, 1:10) and acid extraction (soil-acid ratio, 1:10) methods, respectively. Meanwhile, K concentration in final extract were determined using a flame photometer (M410, Beijing). The concentration of exchangeable K (EK)and non-exchangeable K (Non-EK) was computed via substitution method (Xia et al. 2024). Additionally, available calcium (A-Ca), available magnesium (A-Mg), and available sodium (A-Na) content were determined as a sum of water-soluble and exchangeable fractions, extracted by distilled water and ammonium acetate extraction method, quantified using flame photometer (FP6450, Shanghai) and atomic absorption spectrometry (Agilent 204DUO, Shanghai) respectively.

Moreover, soil extracellular enzymatic activity was quantified by following the protocol prescribed by Liu et al. (2022). In short, 2.0 g of fresh soil was added into a sterilized conical flask having 200 mL of sterile dionized water and agitated at 180 rpm for 30 minutes to blend the soil suspension. This soil suspension was then transferred to a 96-well plate with a targeted fluorescent reagent and incubated at 30°C for 4 hours. The fluorescence intensity of each sample was assessed using a multifunctional microplate reader (Spark 10M, PerkinElmer Co., U.S.), with 365 nm excitation and 450 nm emission wavelength.

2.5 *DNA extraction and Illumina MiSeq sequencing*

Soil samples collected from the top layer were subjected to DNA extraction using the E.Z.N.A.® DNA kit. The purity and concentration of DNA were assessed using 1% agarose-based gel electrophoresis and a NanoDrop® ND-2000 spectrophotometer. Afterwards, DNA was kept at -80°C for subsequent analysis. V3-V4 regions of bacterial 16S rRNA genes were amplified via primer pairs 338F/806R, following the PCR system and cycling conditions outlined by Zhang et al. (2020). PCR products were extracted and purified by electrophoresis on a 2% agarose gel followed by the AxyPrep DNA gel extraction Kit. Quantification of PCR products was performed with the Quantus™ Fluorometer (Promega, USA). Raw sequence reads were uploaded to the NCBI database and the purified amplicons underwent paired-end sequencing on an Illumina MiSeq PE300 platform (Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China) after quality filtration and integration through Fastp and FLASH softwares (Chen et al., 2018). Following this, sequences were clustered into OTUs in accordance with 97% similarity using UPARSE 7.1 and subsampled to the lowest sequence count. Bacterial taxonomy was assigned through RDP classifier according to Silva database using 70% confidence threshold.

A schematic representation of the soil column, experimental treatments, and corresponding soil analysis employed in current study has been shown in Fig. 1.

2.6 *Statistical analysis*

The statistical analysis of soil physiochemical properties and soil enzymatic activities were carried out in IBM SPSS Statistics 25.0, using two-way analysis of variance (ANOVA). Duncan's multiple range test was used for statistical comparison ($p < 0.05$) among treatments. Origin Pro and GraphPad Prism 9.5 were used to create graphs that were combined in Adobe Illustrator 2021. Soil microbial data were analyzed through R-software. Meanwhile Principal Coordinate Analysis (PCoA) was conducted to determine the difference in soil bacterial community composition under different treatments. Alpha (α)-diversity and beta-diversity indices were plotted with the "vegan" package (Yu et al., 2021). In addition, differences in OTU (Operational Taxonomic Units)

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abundance were analyzed with the "DESeq2" package. Linear Discriminant Analysis Effect Size (LEfSe) analysis was conducted to determine the distinct taxa (phylum to genus level) across treatments (Segata et al., 2011). Two-group and multi group comparison-based analyses were performed to recognize the most influential biomarkers across treatments at varying soil depths. Moreover, Mantel test correlation analysis was performed at the phylum and genus classification levels to unveil the interaction between soil bacterial community composition, enzyme activity and soil physiochemical properties via "ggcorrplot" package (Liu et al., 2022).

3. Results

3.1 *Variation in soil properties, including soil K fractions along soil depth under irrigation practices*

The sole and integrated application of 1% BC with KSB greatly altered the soil pH, SOM content, and nutrient availability under irrigation practices at two different soil depths. The results of soil analysis demonstrated that under sole application of K fertilization and K+KSB application, soil pH, SOM, AN, AP, A-Ca and A-Mg contents were relatively higher in subsoil than in topsoil (Fig. 2a-f). In contrast, except A-Ca and A-Mg contents, these soil parameters were significantly higher in topsoil of K + 1% BC and K + 1% BC+ KSB treatment. In topsoil of K + 1% BC treatments, soil pH, SOM, AN, and AP improved by 1.20 folds, 2.11 folds, 1.48 folds, and 3.23 folds, respectively, whereas in K + 1% BC+ KSB treatment enhanced by 1.30 folds, 2.85 folds, 2.54 folds and 4.28 folds respectively, relative to the topsoil of CK (Fig. 2a-d). Even though K + 1% BC+ KSB remarkably improved soil properties in topsoil. Nevertheless, no significant difference was observed in SOM, AN, A-Ca and A-Mg contents with K + 1% BC application. In addition, compared to subsoil in K + 1% BC+ KSB treatment, soil pH, SOM, AN, and AP contents in topsoil enhanced by 8%, 24.6%, 15.2%, and 51.9%, respectively. Meanwhile, A-Ca and A-Mg

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contents in topsoil elevated by 9.30% and 11.05%, respectively, compared to subsoil in K + 1% BC+ KSB treatment (Fig 2e-f). In contrast, under sole application of K fertilizer soil pH, SOM, AN and AP contents were reduced by 4.0%, 13.0%, 12.7%, and 37.8 % in topsoil compared to subsoil. Notably, compared to other treatments, CK exhibited a lower soil pH, SOM contents, and available nutrients in both topsoil and subsoil. Besides this, two-way ANOVA also depicted that soil pH, SOM, and soil available nutrients were significantly affected by treatment, soil depth, and the interaction between treatment and soil depths under irrigation practices.

Moreover, the soil K fractions were remarkably affected by soil depth, treatments, and the interaction between them (Fig. 3a-d). Soil analysis showed that WSK, AK, EK, and Non-EK contents were significantly higher in topsoil of K + 1% BC+ KSB treatment, preceded by K + 1% BC treatment relative to subsoil of other treatments. Under K fertilizer treatment, WSK, AK, EK and Non-EK content in topsoil was reduced by 33.8%, 37.1%, 39.2%, and 45.2%, respectively, compared to the subsoil. In contrast, these WSK, AK, EK and Non-EK contents in topsoil of K + 1% BC+ KSB treatment were significantly enhanced by 51.5%, 40.3%, 34.4%, and 70.8%, respectively, compared to the subsoil. Similarly, in K + 1% BC treatment, the contents of WSK, AK, EK, and Non-EK significantly improved in topsoil compared to subsoil. While in K+KSB treatment, topsoil exhibited a lower concentration of K fractions than subsoil; however, it was significantly higher than CK in both topsoil and subsoil. As in K+KSB treatment, WSK, AK, EK, and Non-EK contents increased in topsoil by 3.5 folds, 4.8 folds, 5.6 folds, and 3.1folds respectively, while in subsoil enhanced by 3.10 folds, 3.5 folds, 3.7 folds, and 1.7 folds respectively, relative to CK (Fig. 3a-d). Furthermore, the results of two-way ANOVA revealed that the soil K fractions were incredibly varied in response to the interaction between treatment and soil depth.

3.2 *Variation in soil enzymatic activity along soil depth under irrigation*

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Soil enzymatic activities, a key determinant of soil health, were significantly influenced by sole and integrated application of 1% BC with KSB across soil depth (Fig. 4). The activity of β-glucosidase (βG), β-xylosidase (βX), β-cellobiohydrolase (CBH), N-acetyl-glucosaminidase (NAG) and acid phosphatase (AP) activity was greatly enhanced in topsoil of K+1% BC + KSB treatment by 74.4%, 73.5%, 60.4%, 39.3%, and 54.1% respectively, compared to sole K treated topsoil ($P < 0.05$, Fig. 4a-d). Afterwards, K +1% BC treatment showed a better effect on improving the soil enzymatic activities. Nevertheless, CBH and AP activity did not show any significant variation between K+1% BC + KSB and K +1% BC treated topsoil. In contrast, among subsoil of all treatments, the highest enzymatic activity of βG, CBH, NAG, and AP was reported in K-treated soil, increased by 9.6%, 11.3%, 13.2%, and 34.2%, respectively, compared to CK. In addition, with sole and integrated application of 1% BC with KSB, C: N and C: P were remarkably enhanced (Fig. 4f-g). The highest C: N and C:P ratios were observed in topsoil of K + 1% BC+ KSB treatment, increased by 42.08% and 65.3%, respectively, compared to sole K-treated soil. Whereas the N:P ratio was greatly improved under subsoil of K+KSB and sole K treated soil, enhanced by 57.5% and 51.6%, respectively, relative to CK (Fig. 4h). Furthermore, our results also indicate that the interaction between treatment and soil depth significantly influenced soil enzymatic activities.

3.3. *Alteration in soil bacterial community composition*

In total, 1023165 sequences were identified from 15 soil samples collected from the topsoil layer where most of the plant root zones exist to acquire nutrients. Notably, PCoA analysis based on OTU level depicted a distinct differentiation in soil bacterial community composition among different treatments, which accounts for 62.32% of total variation ($P < 0.05$) (Fig. 5a). While 37.68% of the variation remain unexplained, possibly due to fluctuation in soil physical and chemical properties with irrigation practices or climatic conditions. Similarly, NMDS (Non-metric

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multidimensional scaling) analysis at OTU-level also indicated a significant divergence (stress=0.079; R=0.93, p=0.001) in microbial community composition across treatments (Fig. S1). In addition, α-diversity assessment of inter-group variation indicated a notable difference in Chao, Simpson, and coverage indices with sole and integrated application of 1% BC with KSB (Fig. 5b-d). A notable enhancement in Chao index (species richness) was observed under K + 1% BC treatment relative to sole and integrated of KSB with 1% BC (Fig. 5b). In contrast, Simpson index (species evenness) was significantly enhanced under K + 1% BC+ KSB treatment, followed by K + 1% BC treatment (Fig. 5c). Interestingly, in coverage index, a significant difference between K and K + 1% BC treatment was observed (Fig. 5d). Thus, α-diversity was markedly improved with sole and integrated application of 1% BC with KSB.

Furthermore, a notable discrepancy in relative abundance of soil bacterial phyla was observed across the treatments. Within top 10 phyla, the most dominant phyla across different treatments were Pseudomonadota, Actinobacteriota, Bacillota, and Bacteroidota (Fig. 5e). However, the relative abundance of Pseudomonadota decreased among all treatments than CK. Interestingly, the relative abundance of Actinomycetota and Bacteroidetes have greatly improved under K + 1% BC+ KSB treatment, which was increased by 64.6% and 24.0%, respectively, relative to CK. In contrast, the highest relative abundance of Bacillota was observed in K+ KSB treatment, enhanced by 81.5% compared to CK. Additionally, key genera at genus level were *Pseudarthrobacter*, *Massilia*, *Dyella*, and *unclassified_f__Oxalobacteraceae* (Fig. 5f). The relative abundance of *Pseudarthrobacter* and *Mucilaginibacter* was markedly enhanced under K + 1% BC+ KSB treatment as increased by 67.9% and 30.0% compared to CK. Meanwhile, *unclassified_f__Oxalobacteraceae* increased by 55.0% in K-treated soil compared to CK.

Moreover, to further investigate the statistical variation in soil bacterial community composition at genus level, two treatments CK and K set as control group (Fig. 6a-f). The relative abundance of Alicyclobacillus (98.5%) in K, *norak _f__Oxalobacteraceae* (82.4%) and *Paenibacillus* (38.1%) in K+1%BC, *Pseudarthrobacter* (54.6%), *Clostridium* (97.3%) and *Ectobacillus* (99.7%) in K+ KSB, *Pseudarthrobacter* (68.0%) and *Paenibacillus* (74.4%) in K + 1% BC+ KSB treatment were significantly improved, relative to CK (Fig. 6a-d).Whereas, the relative abundance of Massilia decreased among all treatments compared to CK. Notably, in comparison to K treatment, the relative abundance of *Paenibacillus* and *Massilia* were enhanced by 65.2% and 48.0% in K + 1% BC treatment, while, in K + 1% BC+ KSB treatment, raised by 37.8% and 84.7%, respectively (Fig. 6e-f). In addition, the relative abundance of *Pseudarthrobacter* and *Mucilaginibacter* was remarkably improved under K + 1% BC+ KSB treatment relative to K-treated soil. In addition, LEfSe based analysis was carried out to recognize the most dominant biomarkers for each group under the taxonomic composition of soil bacterial communities via LDA (Linear Discriminant Analysis) score > 3.5 (Fig. 6g, S2). LEfSe analysis indicated a substantial increase in number of biomarkers under both K + 1% BC+ KSB and K+ KSB treatment, with 19 and 17 biomarkers, respectively, relative to CK with 13 biomarkers. The primary contributing biomarkers were largely distributed among Pseudomonadota in CK, Chloroflexota in K, Alphaproteobacteria, and Hyphomicrobiales in K + 1% BC treatment. Meanwhile, in K+KSB treatment, Bacillota and Clostridium were dominant biomarkers, whereas, in K + 1% BC+ KSB, biomarkers were widely distributed among Actinobacteria, Micrococcaceae, and Pseudarthrobacter.

3.4 Co-occurrence network analysis and interactive Mantel test correlation

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Co-occurrence network analysis using one and two-factor correlations at the species level indicated a complicated association among soil bacterial community composition (Fig. 7a-h). One factor co-occurrence networks analysis with top 40 differential bacterial taxa depicted that the highest number of positive linkages were established under the treatment of K (62.5%), followed by K + KSB (54.6%) and K + 1% BC+ KSB (55.2%) compared to CK (50.1%) (Fig. 7a-e). Meanwhile, the network analysis identified that the core bacteria across different treatments were Pseudomonadota, Chloroflexota, Actinomycetota, Acidobacterioata, Bacterioidota, and Bacillota. In addition, a two-factor co-occurrence network analysis was also carried out with top 50 differential bacterial taxa in relation to environmental factors (Fig. 7f-h). Two-factor network analysis among soil bacterial community and different forms of soil K contents indicated that Pseudomonadota (28.0%), Actinomycetota (20.0%) and Bacillota (16.0%) were core bacterial taxa modulated by environmental variables (34.21%) (Fig. 7f). Whereas in relation to soil enzymatic activity, the most influential bacteria with environmental factors (12.82%) were Psuedomonadota (20.51%) and Chloroflexota (20.51%) (Fig. 7g). Meanwhile, with respect to the soil nutrients, Psuedomonadota (25.93%), Bacillota (20.51%) and Actinomycetota (14.81%) were prominent bacteria that were profoundly affected by environmental factors (22.22%) (Fig. 7h). In addition, two-factor bacterial networks exhibited more positive correlations than negative ones concerning the edaphic factors.

Furthermore, to evaluate the correlation between soil bacterial community and soil environmental variables, a Spearmen correlation and an interactive Mantel analysis were conducted both at phylum (broad) and genus (fine) taxonomic level (Fig. 8a-b, S3). Spearmen correlation analysis indicated that Actinomycetota, and Bacterioidota were positively correlated with soil environmental factors (Fig, S3). Meanwhile, the results of the Mantel test showed that α-

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diversity exhibited a strong positive correlation with WSK, AK, Non-EK, SOM, AP, and AMg (Mantel's P > 0.05) (Fig. 8a). At the same time, bacterial community composition showed a remarkable association with all soil nutrients (0.001 < Mantel's P < = 0.05). Additionally, the α-diversity indices and soil bacterial community were positively linked with overall soil enzymatic activities (Fig. 8b). However, a negative correlation of α-diversity index with C:P and N:P ratio was observed. Moreover, redundancy analysis (RDA) was further evidence of the formation of distinct clustering of bacterial communities in response to treatments. Among these, K + 1% BC+ KSB displayed a robust positive association with key soil nutrients and enzymatic activity (Fig. S4). Thus, soil environmental factors markedly influence the soil bacterial community composition.

Discussion

In current study, integrated application of BC with KSB greatly improved the soil properties both in topsoil and subsoil, even under irrigation practices. An increase in soil pH and SOM contents, especially in topsoil under K + 1% BC+ KSB treatment (Fig. 2), resulted from high nutrient retention potential of biochar (He et al., 2024; Taweengern et al., 2025). Biochar having porous structure, enlarged surface area (24.01 m^2/g) and high CEC acts as a nutrient reservoir by adsorbing and holding nutrient ions within its micropores and mesopores (Adhikari et al., 2024), preventing them from leaching to subsoil under irrigation (Luo et al., 2024). Such improvement in soil nutrient status, especially in topsoil, stimulates the microbial activity, which greatly contributes to the decomposition of organic matter OM); as a result, SOM contents become increased (Lee and Kwon, 2024). In addition, high CEC of biochar neutralizing the excessive protons, in turn, increased the soil pH (Zhang et al., 2025b). This pH buffering capacity is further improved when biochar is applied in conjugation with KSB, which solubilizes K minerals and enhances the K bioavailability (Babar et al., 2025b; He et al., 2024). Importantly, the interactive

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effect of biochar and KSB on accumulation of SOM is primarily driven by two mechanisms: (1) Sorption and sequestration of labile OM facilitated by biochar addition, which stimulate the microbial activity and contributes to subsequent mineralization of SOC (soil organic carbon), and (2) SOC immobilization through complexation with multivalent cations (e.g., Ca^{2+}, Al^{3+}) (Rasul et al., 2022; Bolan et al., 2024). Concurrently, KSB activity further elevated the SOM accumulation through nutrient mineralization and microbial polysaccharide production (Babar et al., 2024). In accordance with Li et al. (2024b), biochar facilitates SOM accumulation by adsorbing organic molecules and stabilizing them through surface-mediated catalytic processes.

Furthermore, biochar, a rich source of nutrients, significantly improved soil nutrient contents such as AK, AP AN, A-Ca, and A-Mg by gradually releasing them into soil solution (Karim et al., 2022; Sharma et al., 2025). Meanwhile, biochar enhanced their retention capacity in topsoil, where they fostered the microbial activity associated with nutrient cycling (Yan et al., 2025a). In current study, a different form of soil K (e.g., WSK, AK, EK) and other nutrients remained more concentrated in topsoil of K + 1% BC+ KSB treatment than in subsoil, despite multiple irrigations (Fig. 2,3). It was primarily due to the direct application of K fertilizer, BC, and KSB in topsoil, which established a nutrient-rich microenvironment (Xia et al., 2024). Biochar, with its porous structure, high surface area and enriched oxygen-containing functional groups, facilitate the K^+ ions retention in topsoil via electrostatic forces and ion exchange reactions (Wang et al., 2023). Thus, these negatively charged sites on biochar immobilized the nutrients and minimized their vertical movement, even under repeated irrigation (Zhao et al., 2023). Secondly, KSB in topsoil enhanced the solubilization of mineral-bound and non-exchangeable K, converting them into soluble and exchangeable forms (Babar et al., 2024). However, these newly released ions might be rapidly re-adsorbed onto biochar surfaces or soil colloids to create a dynamic equilibrium which

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favored K retention and other nutrients over leaching (Bilias et al., 2023; Luo et al., 2024). Thirdly, biochar addition might be improved the soil structure by lowering the bulk density, meanwhile increased the water-holding capacity, which slows down the water percolation during irrigation events (Rasul et al., 2022; Xia et al., 2024). Such delay in water movement minimized nutrient leaching by increasing the residence time of water and solutes in topsoil, allowing them greater interaction with adsorption sites (Cheng et al., 2025). In contrast, the subsoil, which only received biochar and no microbial input or direct source of K, might have low microbial activity and reduced nutrient cycling (Hou et al., 2021; Wang et al., 2024b,c) thereby limiting nutrients might be available for adsorption and stabilization (Chen et al., 2022). Meanwhile, a limited amount of nutrients reach the subsoil from the topsoil, which can shift the equilibrium toward meaningful nutrient accumulation (Sun et al., 2024b). These findings were in line with Li et al. (2024b) and Yang et al. (2024) study, who found that biochar exhibits a high nutrient retention capacity in topsoil via pH modification and sorption.

Moreover, the extracellular enzymatic activities, e.g. βG, βX, CBH, NAG and AP were greatly improved in topsoil compared to subsoil, with sole and integrated application of 1% BC with KSB (Fig. 4). It is probably due to nutrient enriched environment from the direct addition of K fertilizer and KSB, which provide the energy and substrates for microorganisms to enhance the nutrient cycling processes (Rasul et al., 2022; Mercer et al., 2025). Such increment in microbial activity promotes the secretion of enzymes involved in breakdown of OM and releasing the nutrients (Di et al., 2024; Yan et al., 2025a,b). In addition, biochar application might improve the soil structure, water retention, and nutrient adsorption capacity, that further supporting the topsoil enzymatic activity (Cheng et al., 2025). Conversely, the subsoil under sole K fertilizer treatment exhibited elevated enzymatic activity, potentially driven by the leaching of nutrients, dissolved organic

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compounds, and microbial by-products from the topsoil (Ahsan et al., 2023), which enriched the subsoil environment and stimulated microbial metabolism (Kayoumu et al., 2025).

Interestingly, the soil bacterial composition was analyzed only from the topsoil, a region that is significantly affected by environmental variables such as temperature, rainfall, the intensity of sunlight, etc. (Xu et al., 2023). In addition, the plant roots are more abundant in this region (Hou et al., 2021), which affects the soil bacterial diversity and their activity (Liang et al., 2024). Thus, in this study, soil bacterial community composition was profoundly improved with both sole and integrated application of 1% BC with KSB. Our PCoA analysis of soil bacterial community was clear evidence of variation in soil bacterial community with applied amendments (Fig. 5). These results were align with findings of Babar et al. (2025b) study, which demonstrated that integrated application of biochar and microbial inoculum profoundly influenced soil microbial community.

Notably, at phylum level the relative abundance of beneficial soil bacterial taxa such as Actinomycetota and Bacteroidota were markedly improved under K + 1% BC+ KSB treatment (Fig. 5). Actinomycetota (previously known as Actinobacteria) are Gram-positive copiotroph bacteria (Han et al., 2025) that can exist either symbiotically or as free-living diazotrophs (Boubekri et al., 2022; Mondaca et al., 2024). They play a key role in carbon cycling by degrading the organic compounds via production of cellulase and chitinase enzymes (Behera and Das, 2023; Di et al., 2024). Additionally, their beneficial role in K solubilization and N fixation has been demonstrated in previous studies, especially under biochar amended soils (Kannan et al., 2021; Wang et al., 2024b). Importantly, Actinomycetota releases various plant growth-promoting substances (PGPR) that help in alleviation of abiotic stresses e.g., salinity, drought etc., and combating the antibiotic resistance (Mitra et al., 2022; Muhammad et al., 2024). Furthermore, they are also actively participate in the remediation of soils contaminated by heavy metals, especially

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cadmium (Song et al., 2024). While Bacteroidota are Gram-negative, copiotroph bacteria that actively participate in P cycling and the regulation of soil N dynamics via nosZ gene (Pan et al., 2023). These genes encode nitrous oxide reductase that is involved in reduction of nitrous oxide (N_2O), thereby enhancing the soil N_2O sink capacity (Duan et al., 2024). Additionally, Bacteroidetes serve as PGPR that improves soil health and plant growth through multiple mechanisms (Xu et al., 2021; Yan et al., 2025b). These results aligned with Xu et al. (2023), Shan et al. (2024) and Zhao et al. (2024), who reported a similar trend in the abundance of Actinomycetota and Bacteroidota under biochar application at different soil depths. Thus, these findings support our results that available soil nutrients contents such as AK, AN, and AP enhanced with the growth and activity of Actinomycetota and Bacteroidota under K + 1% BC+ KSB treatment.

Moreover, the relative abundance of *Pseudarthrobacter*, a Gram-positive, non-motile, facultatively aerobic bacterium that functions as PGPR (Di et al., 2024), was greatly enhanced under K + 1% BC+ KSB treatment at genus level (Fig. 6). Similar observations were made by Zhang et al. (2022) under 4-year biochar study in acidic soil that the relative abundance of *Pseudarthrobacter* was enhanced by 700 times in biochar-treated soil. In addition, *Pseudarthrobacter* promotes plant growth by releasing phytohormones like indole-3-acetic acid (IAA), which stimulate elongated root growth and enhance nutrient uptake (Zhang et al., 2025a,b). Besides this, it exhibited a strong potential to detoxify the organic pollutants and phenanthrene compounds in soil, thereby improving soil sustainability (Li et al., 2024a). According to Zhao et al. (2023), *Pseudarthrobacter* has a strong potential to solubilize the P by releasing numerous metabolic products and facilitating synergistic interactions with other native soil microorganisms under biochar application that contributes to nutrient cycling. Interestingly, the relative abundance

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of *Paenibacillus* was markedly improved under K + 1% BC+ KSB treatment due to addition of KSB inoculum (*Paenibacillus mucilaginosus*). Meanwhile, a nutrient-rich environment is provided by biochar for proliferation of *Paenibacillus*. Cheng et al. (2025) stated that biochar provides diverse and readily available organic substrates that function as carbon and energy resevoir for the proliferation of copiotrophic bacteria like *Bacillus spp*. Additionally, biochar improves soil structure, aeration, moisture retention, nutrient availability and thermal properties, which is more conducive to microbial colonization and metabolic activity (Bolan et al., 2023; Ighalo et al., 2025). According to Bisht et al. (2024), *Paenibacillus*, a facultative endospore-forming bacterium greatly contributes to the improvement in soil nutrient status and plant growth via K solubilization, N fixation, and phytohormone production.

Furthermore, in network analysis, the number of positive correlations under K + 1% BC+ KSB treatment was slightly lower than in K-treated soil (Fig. 7). It might be due to enhanced microbial diversity and functional redundancy, which promote the competitive and niche-differentiated interactions rather than cooperative associations (Deng et al., 2025). However, the number of positive correlations was higher than CK. Meanwhile, in two-factor network analysis, the most influential core bacteria with environmental factors were Pseudomonadota, Chloroflexota, and Bacillota. Pseudomonadota is a metabolically versatile, predominantly Gram-negative bacteria that is involved in various nutrient cycling processes (Zhang et al., 2025c) and catabolism of polycyclic aromatic hydrocarbons (Parasar and Agarwala, 2025). In contrast, Chloroflexota is filamentous, primarily anaerobic or facultative bacteria involved in decomposition of OM and C cycling under low-oxygen conditions (Zhang et al., 2024a,b). While, Bacillota is a Gram-positive, endospore-forming bacteria known for its resilience in harsh environments (Mondaca et al., 2024). They are involved in detoxification of heavy metals and toxic pollutants in soils and also act as

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biocontrol agents (Liu et al., 2024; Nghia et al., 2025). Beside this, these bacteria function as PGPR, thus inducing a positive effect on soil health and plant development, as noted in previous studies (Zhang et al., 2022; Xia et al., 2023; Wu et al., 2025).

Interestingly, our Mantel test correlation analysis also showed that α-diversity indices and the composition of soil bacterial community were positively linked with soil-available nutrients and enzymatic activity (Fig. 6). These findings were in line with Zhang et al. (2022); Xia et al. (2024) and Babar et al. (2025b) study, who found that biochar addition in acidic soil greatly enhanced the soil bacterial communities via improved soil properties and providing the C substrate for the proliferation of bacterial community that is associated with nutrient cycling processes. In addition, these positive linkages between soil bacterial community and enzymatic activities reflects the functional diversity, as distinct taxa producing the specific enzymes depending on available substrate and environmental cues (Wang et al., 2021; Zhou et al., 2024a,b), in turn, facilitate the breakdown of OM and increased the nutrient cycling processes (Kayoumu et al., 2025). Furthermore, the beneficial effects of biochar addition in acidic soil were further improved by KSB addition via synergistic microbial-mineral interactions (Han et al., 2024). Importantly, biochar ameliorates the soil acidity and offers a conducive habitat for KSB colonization (Bolan et al., 2024; Ighalo et al., 2025). These KSBs, in turn, secrete the various organic acids and exopolysaccharides (EPS) that promote mineral weathering and enhance the nutrient release such as K, N, and P in soil solution (Olaniyan et al., 2022), thereby improving soil fertility and nutrient cycling efficiency (Saravanan et al., 2023; Zhang et al., 2025a,b). Overall findings demonstrated that biochar application in conjugation with KSB greatly improved soil physiochemical properties by mineralizing the SOM, reducing nutrient leaching, and enhancing K availability in topsoil compared to subsoil. Meanwhile, it promoted the growth of multifaceted beneficial soil bacterial

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taxa that mediate the nutrient transformation processes, thus optimizing soil fertility and sustaining long-term soil functionality.

Conclusion

This study elucidated the beneficial impact of sole and co-applications of BC with KSB on improving soil properties, particularly on enhancing K bioavailability in acidic soil. Although K fertilizer was applied to all treatments except CK, a notable K leaching from topsoil to subsoil of sole K-treated soil was observed under irrigation. In contrast, the soil that received BC application either alone or in conjugation with KSB significantly increased the K retention in topsoil through improved soil structure and adsorption mechanisms. However, this effect was well pronounced under integrated application of BC with KSB in K-treated topsoil, where it greatly improved the soil pH and SOM contents. Meanwhile, soil available nutrients, especially AK and WSK, were enhanced by 52.5% and 49.8% in topsoil compared to sole K-treated soil, indicating better K retention and stabilization in topsoil matrix. Such improvement in topsoil properties remarkably enhanced soil enzymatic activity and bacterial community composition, e.g., at phylum level, the relative abundance of Actinomycetota (64.6%) and Bacteroidota (24.0%) increased. While, under sole K-treated soil, it became reduced due to leaching of essential nutrients. Overall, improvements in soil properties under integrated application of biochar with KSB stemmed from the alkaline nature of biochar, which mitigates the soil acidity; meanwhile, its high porosity and CEC facilitated K adsorption, resulting in reduced K losses via leaching. In addition, KSB might further modify this effect by secreting organic acids or EPS that solubilize K-bound minerals and boosting the microbial biomass by providing C substrates. Such multifaceted mechanisms significantly contribute to soil improvement by promoting the synergistic interaction of BC with KSB.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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Fig.1 A schematic diagram of the soil column, including the topsoil layer (0-15 cm depth) and subsoil layer (15-30 cm depth), accompanied by a brief experimental workflow.

Fig. 2 Variation in soil chemical properties under individual and co-application of 1 % BC (1 % rice straw biochar) with KSB (potassium solubilizing bacteria) inoculum. (a) soil pH; (b) SOM: contents of soil organic matter; (c) AN: available nitrogen; (d) AP: available phosphorus; (e) A-Ca: available calcium; and (f) A-Mg: available magnesium. Different lowercase letters indicated significant differences among topsoil and subsoil layers across treatments based on LSD test ($P < 0.05$). The statistical results of the two-way ANOVA are shown as F-values. *$P < 0.05$; **$P < 0.01$.

Fig. 3 Variation in soil K (potassium) fraction between topsoil and subsoil layers under sole and combined application of 1 % BC (1 % rice straw biochar) with KSB (potassium solubilizing bacteria) inoculum. Different lowercase letters indicated significant differences among topsoil and subsoil layers across treatments based on LSD test ($P < 0.05$). The statistical results of the two-way ANOVA are shown as F-values. *$P < 0.05$; **$P < 0.01$.

Fig. 4 Extracellular soil enzymatic activity in topsoil and subsoil layer across different treatments (a) βG, β-D-glucosidase; (b) βX, β-1, 4-glucosidase;(c) CBH: cellobiohydrolase; (d) NAG, N-acetylglucosaminidase; (e); AP, alkaline phosphatase; (f) C:N, ratio of soil C:N enzyme activity as (CBH+ βG+ βX)/NAG (g): C:P, ratio of soil C:P enzyme activity as (CBH+ βG+ βX)/AP (h) N:P, ratio of soil N:P enzyme activity as NAG/AP. Different lowercase letters denoted significant differences among topsoil and subsoil layers across treatments based on LSD test (P < 0.05). The statistical results of the two-way ANOVA are shown as F-values. *$P < 0.05$; **$P < 0.01$.

Fig. 5 Principal Component Analysis (PCoA) of soil bacterial community at OTU level in topsoil across treatments(a); Alpha diversity test for inter-group differences in topsoil, (a) Chao index; (b)

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Simpson index; (c) coverage index. *P < 0.05; **P < 0.01. Composition of soil bacterial communities in topsoil at phylum (d) and genus (e) level, respectively.

Fig. 6 Comparison between two groups at genus level across treatments in topsoil (a–f). P value < 0.05 annotated as *. Linear discriminant analysis effect size (LEfSe) identified taxa exhibit a significant difference across treatments in topsoil (g). The dots of varying colours from center to outward represent the phylum, class, order, family, and genus levels.

Fig. 7 Single factor co-occurrence network analysis of soil bacterial communities based on genus level across treatments in topsoil (a-e); Only top 40 genera are shown based on the highest Spearman's correlation coefficients (Pearson,s r > 0.7, P < 0.01). Node size represents the relative abundance of each taxon, while red and green lines indicate positive and negative correlations. Two-factor network analysis of soil microbial community based on genus level with environmental factors including soil K fractions (f), soil nutrients (g), and enzymatic activities (h) across different treatments in topsoil. Only the top 50 genera are depicted based on significant Spearman's correlation (Pearson's r > 0.7, P < 0.01). Different colored nodes indicate each phylum, whereas node size indicates the relative abundance of that phylum. Meanwhile, the red and green lines show positive and negative correlations.

Fig. 8 Interactive Mantel test correlation analysis between α diversity index and bacterial community composition with soil nutrients (a) and enzymatic activity (b). ∗, ∗∗ and ∗∗∗ indicate significant difference at P < 0.05, P < 0.01 and P < 0.001, respectively. The solid lines represent a positive correlation, while the dashed line indicates a negative correlation.

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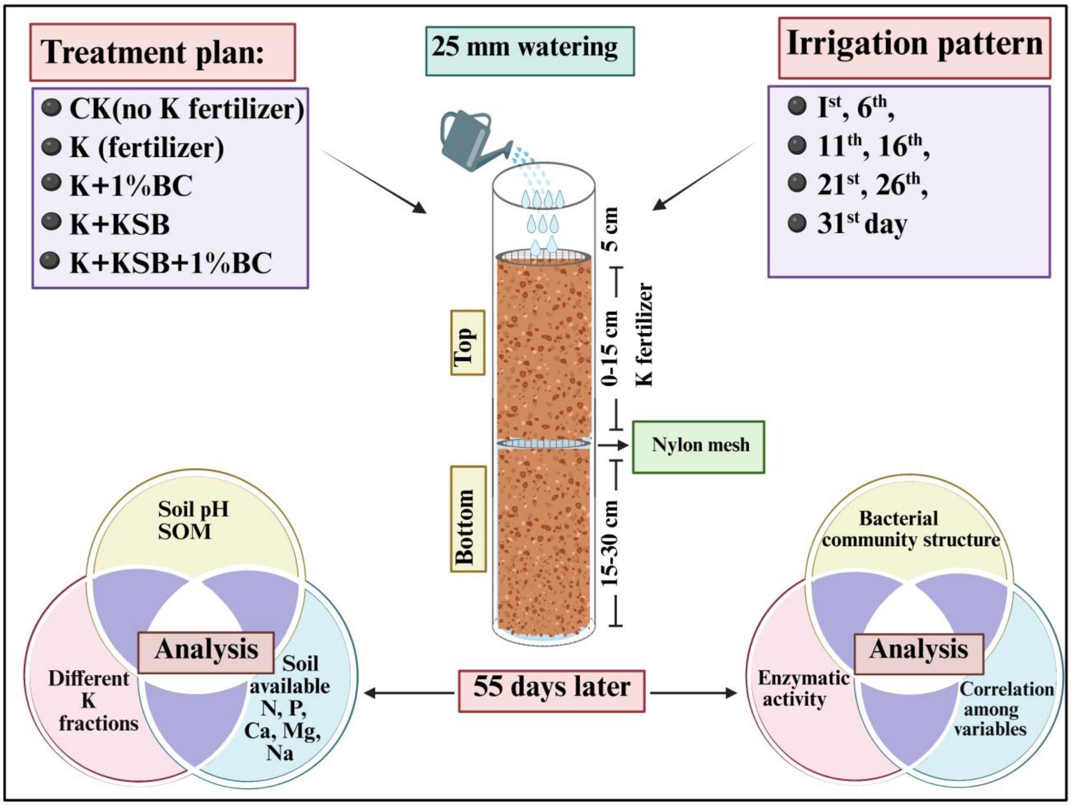

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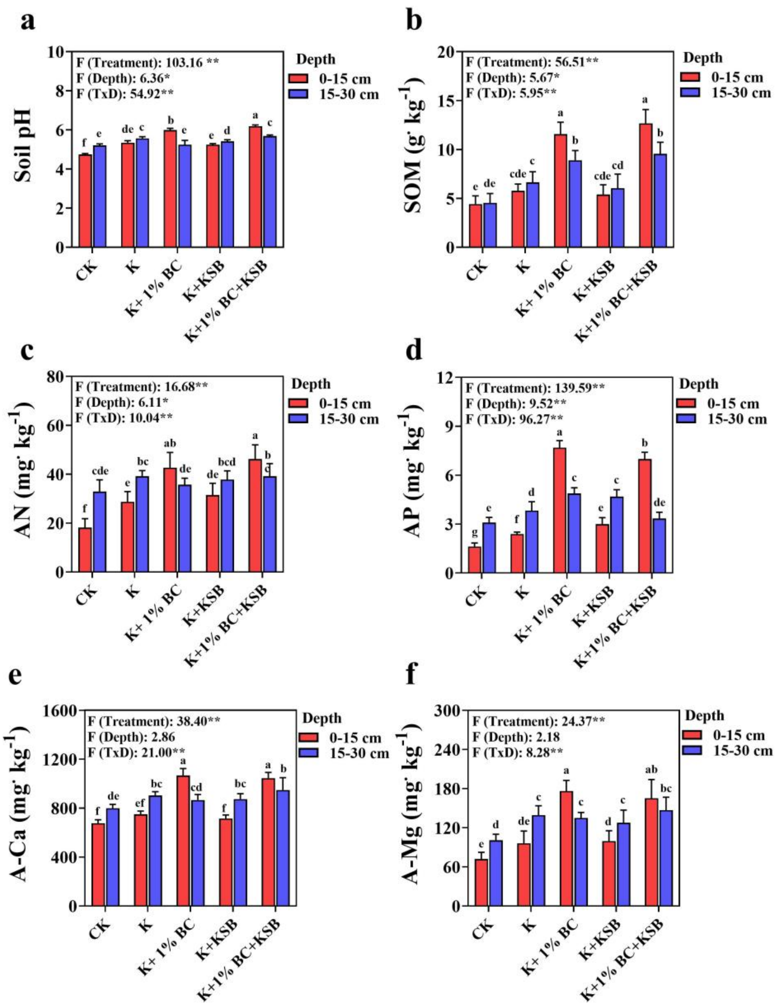

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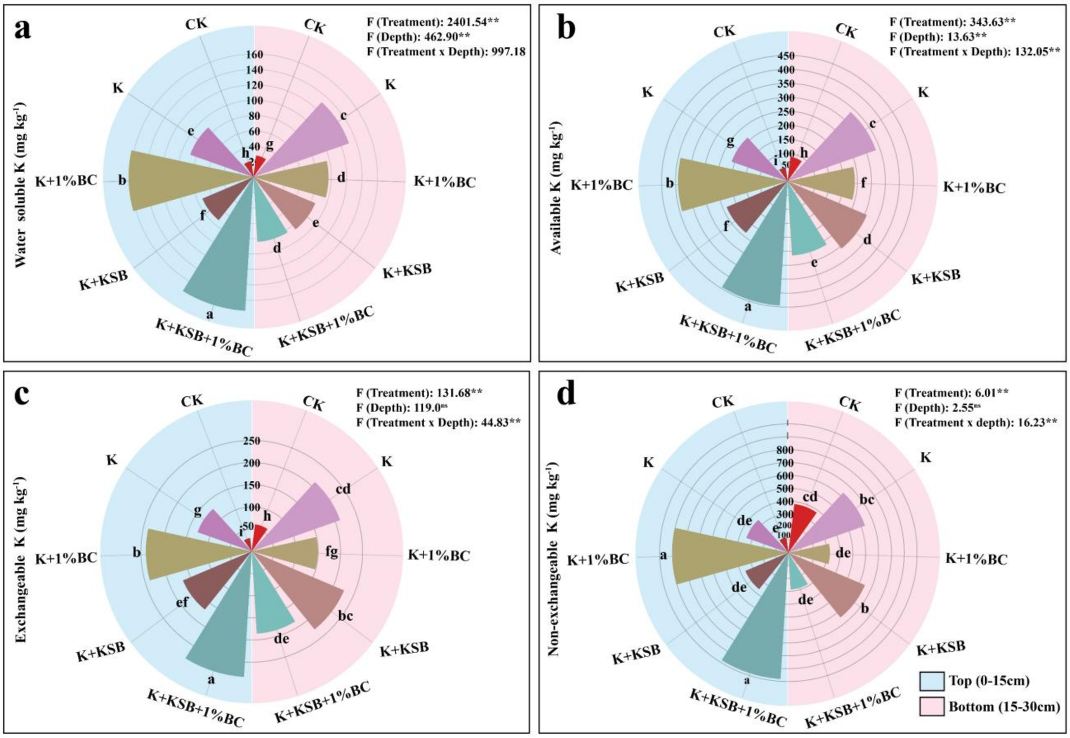

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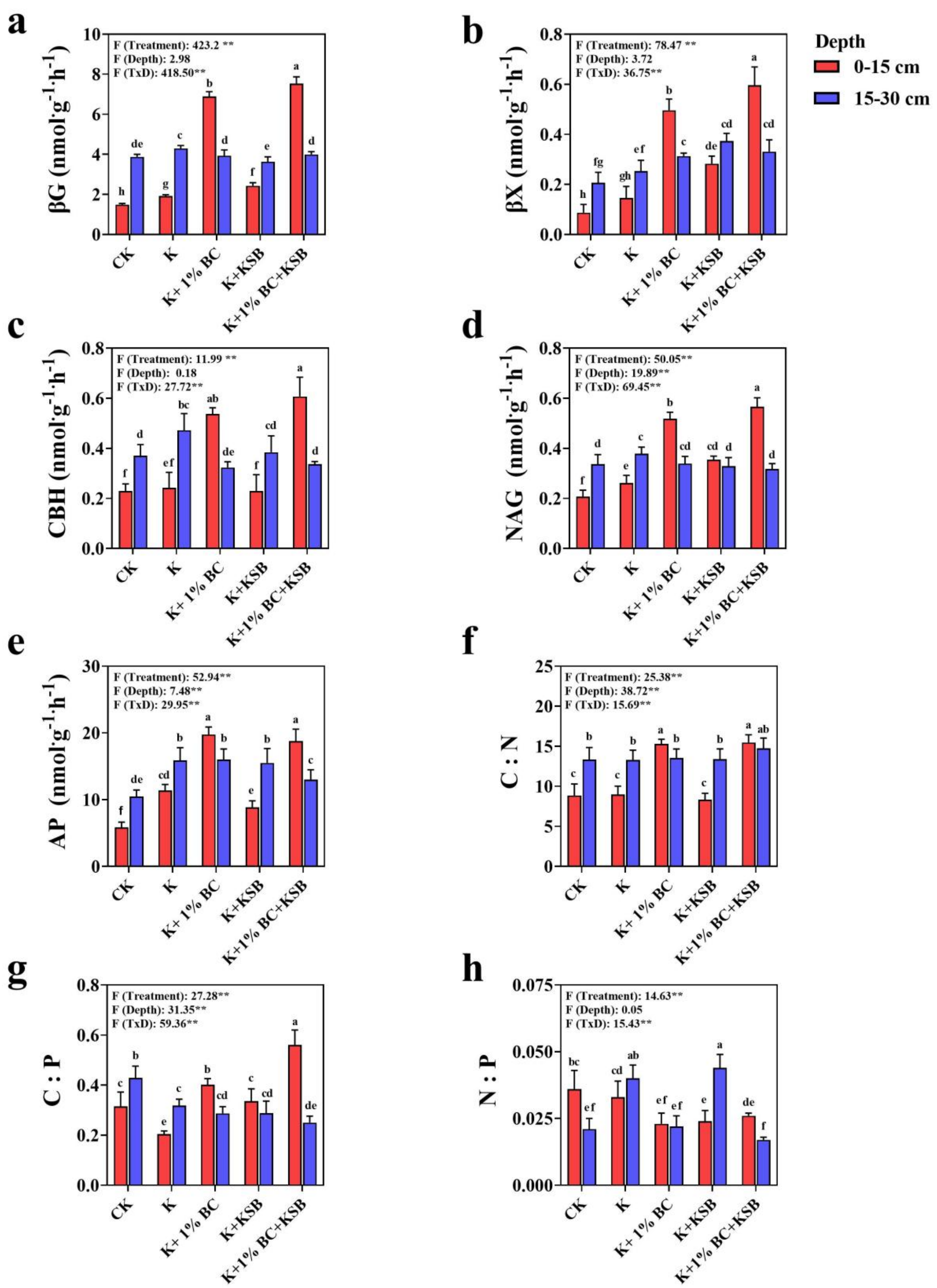

947 **Fig. 4** Extracellular soil enzymatic activity in topsoil and subsoil layer across different treatments

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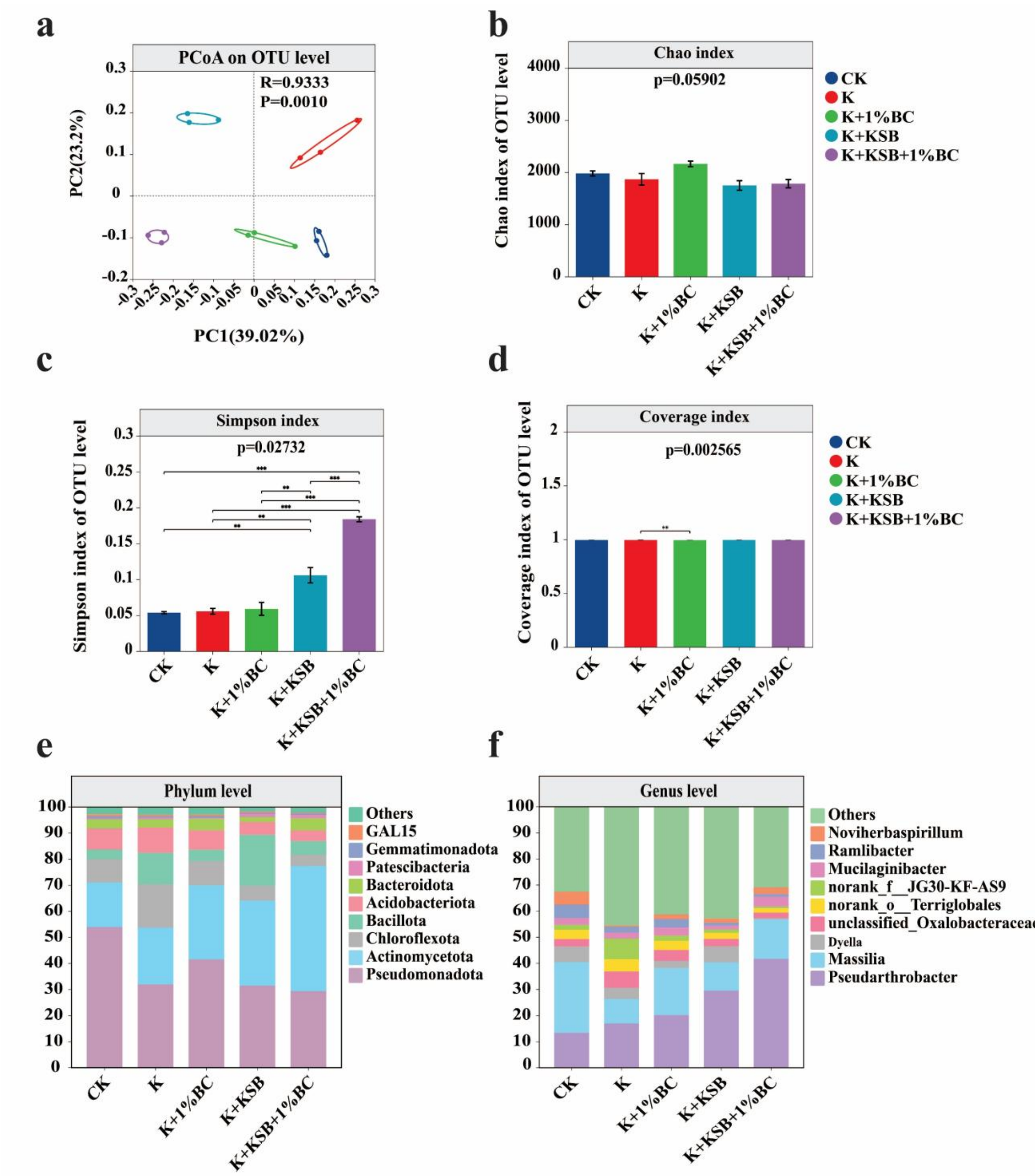

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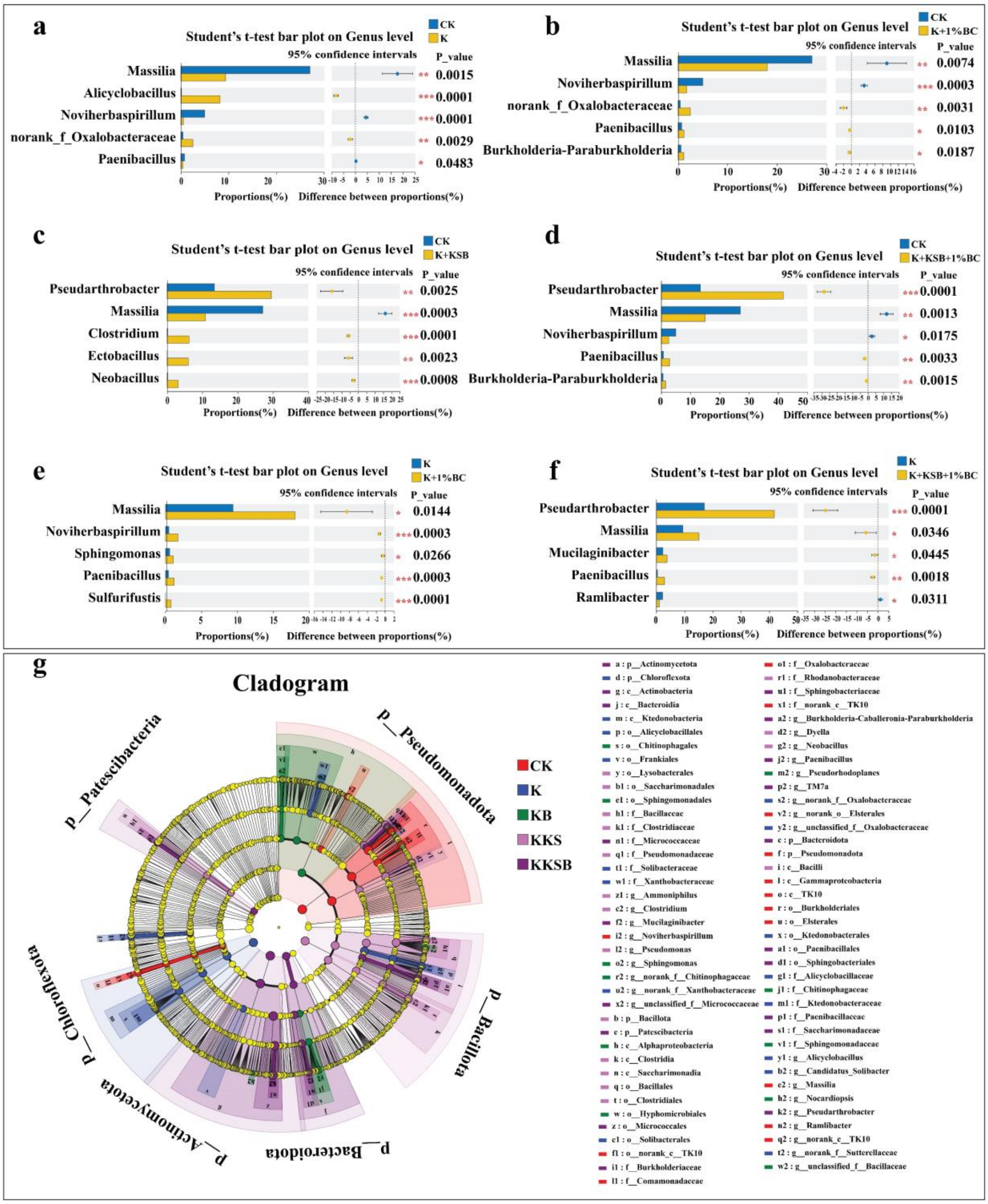

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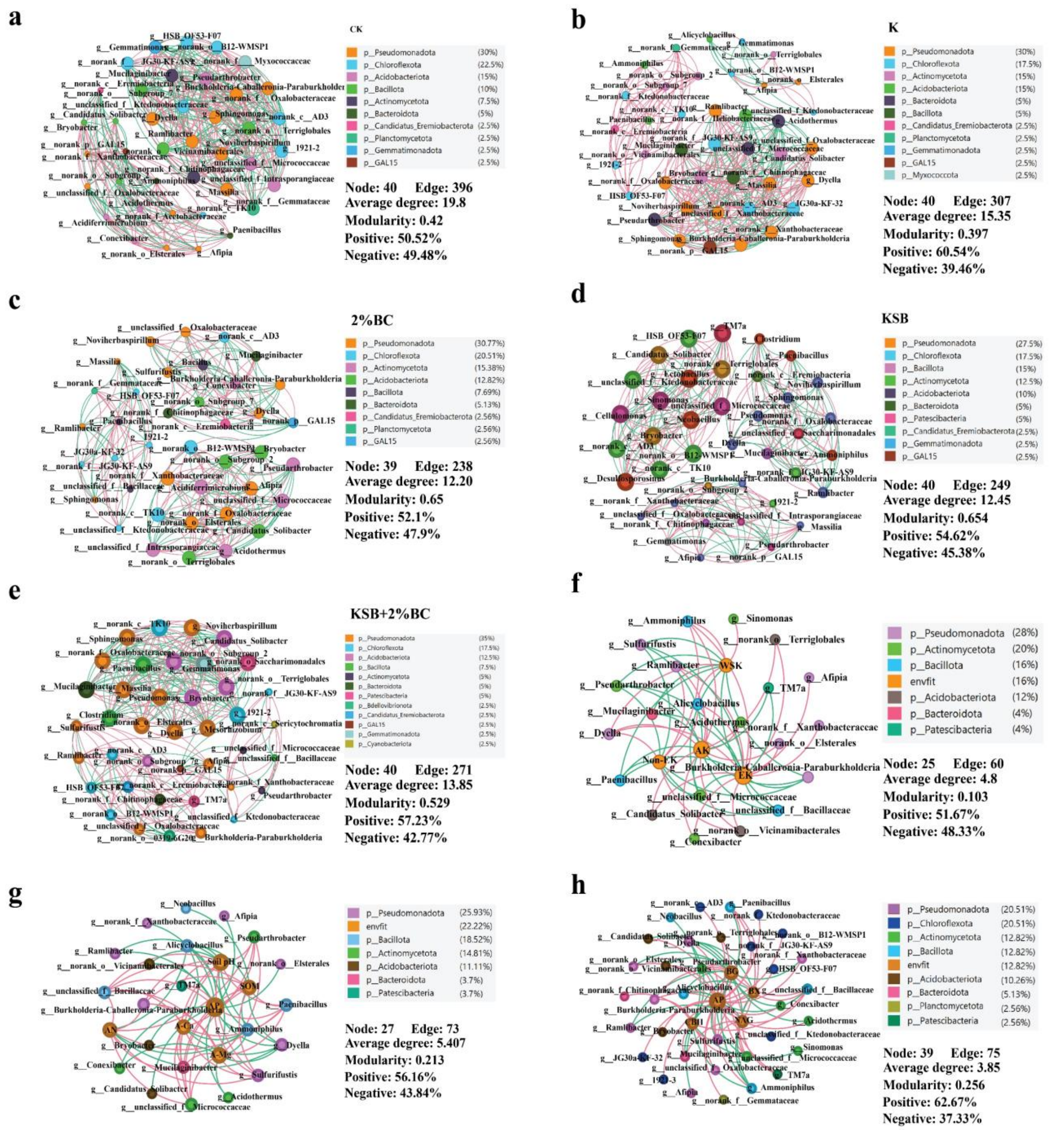

Fig. 7 Single factor co-occurrence network analysis of soil bacterial communities based on genus level across treatments in topsoil (a-e); Only top 40 genera are shown based on the highest Spearman's correlation coefficients (Pearson,s r > 0.7, *P* < 0.01). Node size represents the relative abundance of each taxon, while red and green lines indicate positive and negative correlations. Two-factor network analysis of soil microbial community based on genus level with environmental

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factors including soil K fractions (f), soil nutrients (g), and enzymatic activities (h) across different treatments in topsoil. Only the top 50 genera are depicted based on significant Spearman's correlation (Pearson's r > 0.7, *P* < 0.01). Different colored nodes indicate each phylum, whereas node size indicates the relative abundance of that phylum. Meanwhile, the red and green lines show positive and negative correlations.

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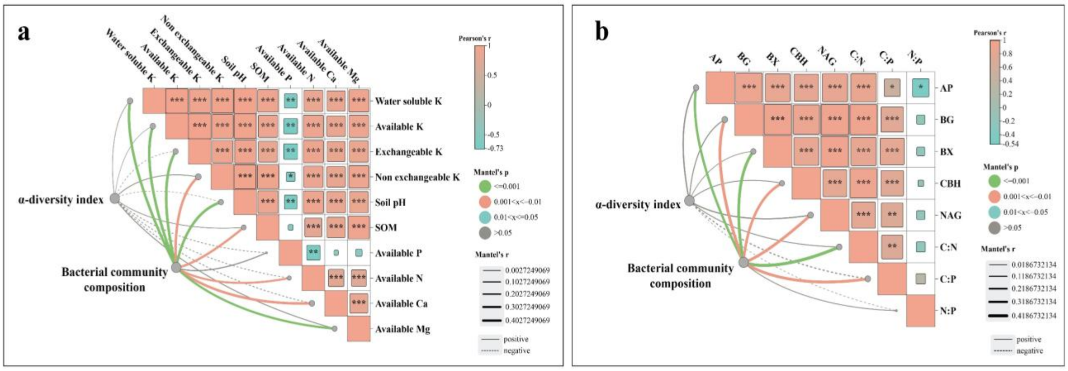

Fig. 8 Interactive Mantel test correlation analysis between α diversity index and bacterial community composition with soil nutrients (a) and enzymatic activity (b). ∗, ∗∗ and ∗∗∗ indicate significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. The solid lines represent a positive correlation, while the dashed line indicates a negative correlation.