Title: Influences of potassium solubilizing bacteria and K-feldspar on enzyme activities and metabolic activities of the bacterial communities in kiwifruit planting soil

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Abstract

A pot experiment was conducted with kiwifruit planting soil to evaluate the impacts of potassium solubilizing bacteria (KSB) and K-feldspar on the soil nutrient levels, enzyme activities, and microecological environment. The effects were investigated of three inoculation treatments (T1: K-feldspar, T2: KSB, and T3: KSB with K-feldspar) and a non-inoculation treatment (CK) on the enzyme activities and the metabolic activities of the bacterial communities in kiwifruit rhizosphere soil. The results showed that the total nitrogen, available phosphorus, available potassium, and organic matter contents in T3 were 18.19%, 45.22%, 15.06%, and 4.17% higher, respectively, than those in CK at the end of the experiment (90 days). Compared with CK, T3 significantly increased the invertase, urease, acid phosphatase, and polyphenol oxidase activities. T3 had a higher kiwifruit root activity, but there were no significant differences among the four treatments ($P > 0.05$). T3 significantly altered the bacterial community diversity, increased the utilization of phenolic compounds and polymers, and decreased the utilization of amino acids. Redundancy analysis indicated that soil nutrients (total nitrogen, available phosphorus, and available potassium) and enzyme activities (urease and acid phosphatase) had more important effects on the metabolic activities of the bacterial communities. Co-inoculation enhanced the soil nutrients, enzyme activities, and bacterial community diversity. KSB co-inoculated with K-feldspar has the potential to improve the soil fertility, microbial metabolic activity and plant growth.

Keywords Bacterial community; Enzyme activities; Potassium solubilizing bacteria; Rhizosphere soil

1. Introductions
Potassium (K) is one of the major macronutrients necessary for plant growth and crop productivity. K not only participates in nutrient transportation and uptake, but also confers resistance to abiotic and biotic stresses, leading to enhanced crop sustainability (Shabala and Pottosin, 2014). However, with the development of the intensive agricultural practices, soil K deficiency has become increasingly severe owing to crop uptake, water runoff and soil erosion (Sarikhani et al., 2018). K deficiency results in slow plant growth, incomplete root development and decreased biomass yield (Gupta et al., 2008). The application of K-containing fertilizer is one approach for mitigating the effects of K deficiency. However, the excessive use of K-containing fertilizers can increase costs, decrease the efficiency of K fertilization, destroy the soil structure and aggravate environmental pollution (Bakhshandeh et al., 2015; Basak and Biswas, 2009). Therefore, identifying an alternative and eco-friendly K resource is essential for the sustainable development of agriculture.

Most of the K in soil (90%-98%) is in the form of insoluble minerals, such as mica, illite and K-feldspar, which are unavailable or only slowly available to plants (Anon, 1998). K-feldspar is the largest and the most widely distributed reservoir of K resources in China. Compared with applying K-containing fertilizer, it is more economically viable to transform fixed slow-release sources of K (such as K-feldspar) into available K that can be absorbed by plants.

A variety of soil microorganisms can solubilize silicate minerals by releasing organic acids, which can facilitate the dissolution of K-bearing minerals and the chelation of silicon ions to introduce K into solution in the soil (Meena et al., 2005; Zarjani et al., 2013). These microbes are generally known as K solubilizing bacteria (KSB). Studies have shown that *Bacillus mucilaginosus*, which is one of the most common bacteria (Liu et al., 2006), can solubilize K compounds by secreting
polysaccharides and carboxylic acids (Ehrlich et al., 2010; Sheng, 2002). Singh et al. (2010) reported that inoculation with *B. mucilaginosus* resulted in much higher K mobilization than did *Azotobacter chroococcum* or *Rhizobium* inoculation in a maize and wheat hydroponics experiment. Zhang and Kong (2014) showed that following combined KSB and K-feldspar powder addition, tobacco dry weight and the uptake of both K and nitrogen (N) by tobacco seedlings increased significantly. A variety of studies have indicated that *B. mucilaginosus*, as an efficient plant growth promoting rhizobacterium, can increase the concentration of K in soil, the biomass and quality of crops, and soil nutrient contents. Therefore, the use of KSB holds promise as an approach for increasing the concentration of available K ions in the soil and may alleviate K deficiency, thereby improving the productivity of agricultural soils (Nath et al., 2017).

Kiwifruit is recognized as “the king of fruits”. Kiwifruit is not only rich in vitamin C but is also a good source of other nutrients, such as folate, K, amino acids and dietary fiber (Hong et al., 2016; Yang et al., 2013). Kiwifruit has a high medicinal value, and it can help reduce the risk of cardiovascular disease, cancer, and other degenerative disorders (Mikulic-Petkovsek et al., 2012; Yong et al., 2013). Shaanxi Province is the largest kiwifruit producing area in China, with its kiwifruit plantations distributed mainly in Zhouzhi County. Kiwifruit growth depends largely on the availability of K in the soil. K is not only a crucial nutrient for fruit yield and quality but is also important for prolonging the duration for which mature kiwifruit can be stored (Tong et al., 2009). K deficiency in kiwifruits is one of the most important problems in kiwifruit crop production.

Previous reports have shown that KSB inoculation can enhance the concentration of available K in the soil and K uptake by crops such as maize (Abou-El-Seoud and
Abdel-Megeed, 2012), wheat (Singh et al., 2010), tobacco (Zhang and Kong, 2014), and tea (Pramanik et al., 2019). However, little information is available regarding the effects of KSB, K-feldspar, or their combined application on the kiwifruit rhizosphere, soil enzyme activities and metabolic activities of the bacterial communities. The goals of this research were to study the influences of KSB and K-feldspar on the nutrient levels, enzyme activities, and metabolisms by the microbial community in kiwifruit planting soil.

2. Materials and methods

2.1 Experimental materials

Soil samples were collected from the kiwifruit planting base in Zhouzhi County, Shaanxi, China. The roots and pebbles in the soil were removed by hand, and the soil was homogenized before use. The soil characteristics were as follows: pH, 6.45; total N, 0.06%; organic matter, 1.18%; available K, 116.53 mg·kg\(^{-1}\) and available P, 41.57 mg·kg\(^{-1}\).

K-feldspar powder was purchased from Yantai Huawei Mineral Products Co., Ltd. It was passed through a 100-mesh sieve. The sieved powder was submerged in sterilized water for 3 days to eliminate soluble K.

KSB were isolated from the soil of the kiwifruit planting base in Zhouzhi County by our team. The KSB were identified as *Paenibacillus mucilaginosus* by standard biochemical characteristic and 16S rRNA gene sequence analysis and was named JK3. The GenBank accession number for JK3 is MK226445. The preparation of the liquid JK3 inoculum is described in the supplementary materials (S1).

2.2 Experimental setup
The pot experiment was performed in a greenhouse at the kiwifruit planting base of Zhouzhi County from September to December, 2018. The pot experiment included the following 4 treatments: CK, control (no inoculation); T1, K-feldspar at 1000 mg·kg⁻¹ soil; T2, JK3 at 50 mL·kg⁻¹ soil; and T3, K-feldspar at 1000 mg·kg⁻¹ soil mixed with JK3 at 50 mL·kg⁻¹ soil. The soil, K-feldspar powder and/or JK3 were mixed thoroughly and then placed in pots (30×30 cm, each containing 10 kg of soil), and each treatment included 20 replicates. A fully randomized design was used. Two uniform, virus-free, tissue-cultured kiwifruit plantlets (‘Hayward’) were transplanted into each pot. Soil moisture content was adjusted to approximately 60% of the soil water holding capacity during the experiment.

2.3 Sample collection
Rhizosphere soil samples from each treatment were collected from three random pots and then mixed thoroughly to obtain a representative sample. Samples were collected at 0, 30, 60, and 90 days. Each sample was stored at 4 °C for soil nutrients, enzyme activity and Biolog analysis.

2.4 Soil chemical property and enzyme activity analysis
Soil pH was measured in a 2.5:1 (water:soil) suspension using a pH meter. Total N was estimated using the Kjeldahl digestion method as described by Zhang et al. (2011). Organic matter was assayed by the dichromate oxidation method. Available P and available K were measured with the molybdenum antimony colorimetric method and the ammonium acetate-flame photometer method, respectively. The urease, invertase, acid phosphatase, and polyphenol oxidase activities in soil from all treatments were measured by spectrophotometry as described by Guan et al. (1986),
2.5 Root activity of kiwifruit plantlets

At the end of the pot experiment (at 90 days), the roots of the kiwifruit plantlets were collected and washed with distilled water. Root activity was determined using an assay measuring the red triphenyl formazan formed through triphenyl tetrazolium chloride reduction (Batish et al., 2007).

2.6 Community-level physiological profiles

Community-level physiological profiles were estimated by the Biolog Eco-microplate (Biolog Co., Hayward, CA, USA) as described by Guo et al. (2012). The microplate contained 31 carbon (C) sources and a control well without a C source, and three replications were prepared. Soil from the final sampling period (5 g dry weight) was dissolved in 45 mL of sterile 0.85% NaCl and shaken for 30 min at 150 rpm. The supernatant was stepwise diluted to $10^{-3}$ using sterile 0.85% NaCl, and 150 µL of diluted supernatant was inoculated into each well of the ECO-microplates. The Biolog EcoPlate™ microplates were incubated at 28°C for 240 h, and the absorbance at 590 nm were read every 24 h.

2.7 Statistical analyses

The community-level physiological profiles data were analyzed following Qian et al. (2014) and Fu et al. (2015); the data included average well color development (AWCD) and soil microbial community functional diversity indices. All calculations were performed using the Biolog data at 120 h.

All data obtained in the pot experiments are reported as the means ± SD (n = 3).
Significant differences between treatments were evaluated by the least significant difference test at $P < 0.05$ using SPSS 19.0. Principal component analysis (PCA) was conducted with SPSS 19.0 according to the method described by Zhen et al. (2018). Redundancy analysis was performed using CANOCO 4.5.

3. Results

3.1 Effects of KSB and K-feldspar on soil nutrients

As shown in Table 1, the soil total N, available P, available K, and organic matter content in the four treatments first increased and then decreased over the course of the experiment, and the levels of soil nutrients were higher at day 90 than at day 0. The total N contents in CK during days 30–90 were 4.55%–8.00%, 13.64%–36.36%, and 18.18%–27.27% lower than those in T1, T2, and T3, respectively. Soil total N in T2 and T3 was significantly ($P < 0.05$) higher than that in CK, and there was no significant difference between T1 and CK ($P > 0.05$). This indicated that co-inoculation with KSB and K-feldspar significantly increased the total N content.

Organic matter contents in T1, T2, and T3 were significantly ($P < 0.05$) higher than CK after the first sampling time point. At 90 days, soil organic matter was highest in T2, but there was no significant difference among T2 and T3 ($P > 0.05$). Relative to the other treatments, the treatment with JK3 inoculation improved the levels of soil organic matter. Soil available P and K contents in the four treatments increased quickly and were highest at 60 days. At 60 days, the available P contents in CK, T1, T2, and T3 were 287.20%, 291.89%, 362.15%, and 372.35% higher, respectively, compared with the values at day 0. Available P in T1, T2 and T3 were markedly improved relative to that in CK, especially for T3, and there was no significant difference in soil available P between T2 and T3 ($P > 0.05$). The soil available K in
the four treatments showed patterns similar to those of soil available P. The available K content in T3 was 8.58%–31.64%, 5.60%–23.99% and 2.79%–3.81% higher than in CK, T1 and T2, respectively, after the first sampling time point.

After the pot experiment began, the pH exhibited a decreasing trend but it stabilized by 90 days. CK exhibited a slight decrease from 6.44–6.30 over the course of the pot experiment. T3 and T2, which involved inoculation with JK3, led to greater decreases in the soil pH.

3.2 Effects of KSB and K-feldspar on soil enzyme activity

Fig. 1A shows that invertase activity increased over the course of the experiment, with the extent of change decreasing in the order T3>T2>T1>CK, except for 30 days. The highest invertase activity was observed at 30 d in T2, however, there was no significant difference among T1, T2, and T3 ($P > 0.05$). Invertase activities in T3, T2, and T1 were 12.20%–27.83%, 12.14%–26.42%, and 5.68%–14.42% higher, respectively, than those in CK.

As shown in Fig. 1B and Fig. 1C, urease and acid phosphatase activities showed similar trends, increasing to their highest levels on 60 days and then decreasing. At 90 days, urease activities in T3, T2, and T1 were 69.33%, 66.67%, and 57.33% higher, respectively, than those in CK. Urease activity was significantly higher in T3 ($P < 0.05$) than in CK, and there were no significant differences among T3, T2 and T1 ($P > 0.05$). The highest acid phosphatase activities were observed in T3, although there were no differences among T1, T2, and T3 after the first sampling time point. At 60 days, the acid phosphatase activity in CK was 45.83%, 45.20%, and 47.77% lower than those in T1, T2, and T3, respectively. At 90 days, acid phosphatase activity in T3 was 52.35%, 15.23%, and 2.25% higher than that in CK, T1, and T2, respectively.
As shown in Fig. 1D, polyphenol oxidase activities increased over time and ranked in the order T3>T2>T1>CK. There were no differences in polyphenol oxidase activity among T1, T2, and T3 after the first sampling ($P > 0.05$). The activity in T3, T2, and T1 was 35.42%, 30.21%, and 29.17% higher, respectively, than that in CK. At 90 days, polyphenol oxidase activity differed between CK and T3.

### 3.3 Root activity of kiwifruit seedlings

Root activity is one of the most important indices reflecting the growth level of a plant. The root activities in CK, T1, T2, and T3 were $99.04 \pm 5.63$, $108.96 \pm 14.08$, $114.91 \pm 6.02$, and $120.91 \pm 6.93 \mu g \cdot (g \cdot h)^{-1}$, respectively (Fig. 2). The root activity in CK was 18.09%, 13.81%, and 9.10% lower than those in T3, T2, and T1, respectively. T3 had a higher root activity, but there were no significant differences among the four treatments ($P > 0.05$). The results indicated that co-inoculation with K-feldspar and KSB had little effect on the root activity in kiwifruit.

### 3.4 Effects of KSB and K-feldspar on community-level physiological profiles

AWCD directly reflects the overall C source-based metabolic activity of microbes (Garland and Mills, 1991). As shown in Fig. S1, there were marked differences in AWCD among the four treatment groups at 48 h, although the AWCD of T3 and T2 were similar after incubation for 144 h. The AWCD in T3 was significantly higher than that in CK after incubation for 48 h ($P < 0.05$) (Fig. S1). At 120 h, the values under T3, T2, and T1 were 27.55%, 16.15%, and 5.56% higher, respectively, than the value under CK (Fig. S2), and there were significant differences in AWCD among the CK and T3 treatments ($P < 0.05$). Thus, co-inoculation with KSB and K-feldspar enhanced the soil microbial metabolic activity.
The relative utilization ratios of carbohydrates and carboxylic acids in CK were slightly higher than those in the other three treatments (Fig. S3), although there were no significant differences among the four treatments ($P > 0.05$). The relative utilization ratio of phenolic compounds in T3, T2, and T1 was 80.70%, 85.14%, and 57.63% higher, respectively, than that in CK. T2 yielded the highest utilization of phenolic compounds, but there were no significant differences among T3, T2, and T1. The relative utilization ratio of polymers in CK was 21.06%, 23.46% and 24.45% lower than that in T3, T2, and T1, respectively, and significant differences were observed among treatments ($P < 0.05$). The relative utilization ratio of amino acids decreased in the order T3<T2<T1<CK. The ratio in CK was 41.40%, 37.32% and 11.76% higher than that in T3, T2 and T1, respectively. Compared to the CK, co-inoculation with KSB and K-feldspar increased the relative utilization ratio of phenolic compounds and polymers, and decreased the relative utilization ratio of amino acids.

Among the treatments, CK yielded the lowest Shannon index (H), Evenness index (E), Simpson index (D) and McIntosh index (U) values (Table 1). The H value in T3 was significantly higher than the other treatments, by 1.02–1.57 times ($P < 0.05$). There were no significant differences in D and U for among the four treatments. Compared with CK, co-inoculation increased the diversity of the soil bacterial community.

3.5 Principal component analysis based on the effects of KSB and K-feldspar on metabolism by microbial communities

Three principal components were identified that explained 47.45% of the total variance (Fig. 3). There was a significant difference in the spatial variation between
T3 and CK in the principal component analysis system. The results indicated that co-inoculation with KSB and K-feldspar had a greater influence on the bacterial community than did the other treatments.

Table S1 shows the C sources and types that were closely related to the three principal components. The main C sources that differentiated groups along PC1 were five carbohydrate carbon sources, one carboxylic acid source and one amino acid source. There were four types of C sources that contributed extensively to PC2: polymers and carboxylic acids, which contributed 33.33%, and carbohydrate and amino acids, which each accounted for 16.67%. These findings suggest that these sources were probably the main C sources differentiating groups along PC2. The main C sources that differentiated groups along PC3 were a polymer (Tween 80) and an amino acid (L-phenylalanine).

4. Discussion

The total nutrient contents of the soil reflects the soil nutrient reserves, whereas available nutrient contents reflect the dynamic balance between mineralization of the soil and nutrient adsorption by plants (Qian et al., 2014). The application of KSB and K-feldspar biofertilizer is one of the main factors affecting the nutrient levels of orchard soils. In the present study, soil nutrient parameters (total N, available P, available K, and organic matter) first increased and then decreased over the course of the pot experiment. The reason for this pattern may be that the added KSB underwent an adjustment period and then began to adapt and gradually increase in abundance as the pot experiment progressed and KSB appeared to play a role in improving soil nutrient levels. At the end of the experiment, much of the soil nutrients had been taken up to support the growth of the kiwifruit plantlets, so the contents of soil
nutrient contents had decreased. At 90 days, the soil nutrients of T2 and T3 were significantly higher than those of CK. This result is consistent with Ehrlich et al. (2010). In addition, Paenibacillus is similar to Bacillus in its action as a PGPR, but its N-fixing abilities are superior to those of Bacillus (Wu et al., 2010). Therefore, the application of JK3 enhanced the total N content, increasing soil fertility. Subhashini (2015) showed that inoculation with KSB resulted in a significant increase in soil organic matter content relative to that of the control. Abou-El-Seoud and Abdel-Megeed (2012) investigated co-inoculation with KSB and K-containing mineral and found that this increased K availability, soil fertility, plant growth, and K uptake by maize. K-feldspar has a high K content, can slowly release available K into soil, and may be used by soil microbes that participate in soil N and P cycling. KSB can increase the content of available K in soil and may help to activate indigenous microorganisms. Therefore, co-inoculation with KSB and K-feldspar not only increases soil K content but also increases soil N and P contents and improves N and P absorption by plants. Among the treatments, inoculation with KSB led to greater decreases in the soil pH. This result is consistent with Zhang and Kong (2014). The reason for this result may be that KSB releases organic acids when dissolving insoluble minerals, thereby decreasing soil pH.

Soil enzymes are involved in biological cycling and the development of soil fertility, so they are effective indicators of soil biochemistry. Invertase, urease, and acid phosphatase are involved in the cycling of C, N, and P, and they play important roles in maintaining and increasing soil fertility (Burns et al., 2013). The changes in the activity levels of urease and acid phosphatase followed the same trends as the changes in total N and available P. Furthermore, the Pearson correlation analysis revealed positive correlations between each of total N, available P and urease, acid
phosphatase activities (Table S2), possibly because these two enzymes are readily
induced by these substrates. At the end of the experiments, the decreasing levels of
urease and acid phosphatase activities were lower in the T1, T2, and T3 than in CK.
Soil polyphenol oxidase can oxidize phenols and react with proteins, amino acids,
sugars, minerals, and other substances to form organic matter, which can slow down
the pollution of soil with phenols. Invertase and polyphenol oxidase activities had
positive correlations with soil organic matter, which might explain why these two
enzymes activities were highest and lowest in T3 and CK, respectively. In addition,
the concentration of K ions in the soil is positively associated with polyphenol
oxidase activity (Garrett, 1996). Co-inoculation with KSB and K-feldspar resulted in
the highest K ion concentrations in soil, which was due to the dissolving of insoluble
K in minerals and in the soil. Relative to the control treatment, co-inoculation with
KSB and K-feldspar improved soil enzyme activities.

The levels of root activity and nutrient absorption by the root system directly
impact the biomass of the above ground parts of a plant and the yield and quality of
fruits (Mi et al., 2013). In this study, co-inoculation with KSB and K-feldspar led to
higher root activities in kiwifruit seedlings. This result is consistent with Basak and
Biswas (2009), who found that biomass yield increased when mica was applied along
with a bacterial inoculant. These observations suggest that co-inoculation with KSB
and K-feldspar enhanced root activity by stimulating the production of plant growth
hormones, thereby increasing biomass yield. However, significant differences in root
activity were not found among the four treatments. This result may have been
obtained because “Hayward” kiwifruit seedlings have high tolerance to root zone
variation (Mi et al., 2013) such that marked changes in root activity did not occur in
over the short experimental period.
Biolog assays measure culturable, heterotrophic organisms and reflect functional C source utilization by microbial communities (Buyer et al., 2002). AWCD reflects the oxidative capacity of soil microorganisms that develop in the Biolog system and it may be used as an indicator of microbial metabolic activity (Gomez et al., 2006). The metabolic activity of the microbial community is higher when the AWCD value is greater. The results showed that co-inoculation with KSB and K-feldspar enhanced the soil microbial metabolic activity and increased the diversity of the soil bacterial community. In addition, the relative utilization ratios of phenolic compounds and polymers were increased by co-inoculation with KSB and K-feldspar, whereas the relative utilization ratio of amino acids decreased under this treatment. Amino acids have positive effects on soil organic matter, stimulating the decomposition of organic matter and decreasing the soil organic matter content (Sun et al., 2014). The application of KSB and K-feldspar can decrease the microbial metabolic activities of amino acids and reduce the decomposition of soil organic matter. Wang et al. (2015) found that the accumulation of phenolic compounds was one of the most important factors causing continuous cropping obstacles. Therefore, using KSB and K-feldspar as a bio-organic fertilizer has the potential to reduce continuous cropping obstacles by decreasing the content of phenolic compounds in soil.

Redundancy analysis was conducted to investigate the relative contributions of all the factors that might have affected the metabolic activities of the bacterial communities (based on AWCD, functional diversity indices, and utilization of carbon sources), including the enzyme activities, soil nutrients, and root activity. As shown in Fig. 4, the selected variables accounted for 97.5% of the total variation in metabolic activities of the bacterial community. The greater distance between CK with T2 or T3 may be explained by the higher microbial metabolic activity and community diversity
in T2 and T3 than in CK. Among all the factors considered, soil nutrients explained most of the variation, i.e., 47.43%, whereas enzyme activities explained 41.66%, and root activity explained 10.91%. Among the nutrients, total N, available P, and available K significantly explained more of the variations in the bacterial community. Co-inoculation with KSB and K-feldspar enhanced soil total N, available P, and available K, thereby affecting the bacterial community diversity. Urease and acid phosphatase were the additional main factors associated with the variations in metabolic activities of the bacterial community. Urease and acid phosphatase participate in the cycling of N and P. Co-inoculation with KSB and K-feldspar affected the activities of these two enzymes, in turn influencing the utilization of carbon sources by microorganisms. Therefore, co-inoculation with KSB and K-feldspar could affect the variations in metabolic activities of the bacterial community mainly by enhancing the nutrient levels and enzyme activities in the soil.

5. Conclusion

Inoculation has been shown to enhance soil fertility and enzyme activities compared with the control, especially in co-inoculation treatment. Co-inoculation with K-feldspar and KSB enhanced the soil microbial metabolic activity and increased the diversity of the soil bacterial community, which was beneficial to the development of the soil microecology. The soil total N, available P, available K, urease, and acid phosphatase greatly affected the variations in metabolic activities of the bacterial community in kiwifruit planting soil. Because of the overall effects of co-inoculation with KSB and K-feldspar on soil nutrients, enzyme activities, metabolic activities of the bacterial community, co-inoculation with KSB and K-feldspar is feasible for use in kiwifruit orchards.
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**Figure legends:**

**Fig. 1** Changes in soil invertase (A), urease (B), acid phosphatase (C), and polyphenol oxidase (D) activities in the four treatments. Data points represent treatment means (n = 3), and different letters indicate significant differences (P < 0.05). CK: control (no addition); T1: K-feldspar, 1000 mg·kg⁻¹ soil; T2: KSB JK3, 50 mL·kg⁻¹ soil; T3: co-inoculation with K-feldspar (1000 mg·kg⁻¹ soil) and JK3 (50 mL·kg⁻¹ soil).

**Fig. 2** Root activities in the different treatments. Data points represent treatment means (n = 3), and different letters indicate significant differences (p < 0.05). CK: control (no addition); T1: K-feldspar, 1000 mg·kg⁻¹ soil; T2: KSB JK3, 50 mL·kg⁻¹ soil; T3: co-inoculation with K-feldspar (1000 mg·kg⁻¹ soil) and JK3 (50 mL·kg⁻¹ soil).

**Fig. 3** Loadings for the PCA of carbon sources utilization profiles of soil microbes in different treatments. Data points represent treatment means (n = 3), and different letters indicate significant differences (p < 0.05). CK: control (no addition); T1: K-feldspar, 1000 mg·kg⁻¹ soil; T2: KSB JK3, 50 mL·kg⁻¹ soil; T3: co-inoculation with K-feldspar (1000 mg·kg⁻¹ soil) and JK3 (50 mL·kg⁻¹ soil).

**Fig. 4** Redundancy analysis based on metabolic activities of the bacterial communities, soil enzyme activities, soil nutrients, and root activity. Metabolic activities of the bacterial communities (black arrows), including the AWCD values, functional diversity indices, and utilization ratios for six categories of
carbon sources. Soil enzyme activities (gray arrows) including invertase, urease, acid phosphatase, and polyphenol oxidase. Soil nutrients (gray arrows) including total N, available P, available K, organic matter, and pH.

Table 1 The soil nutrient contents from different treatments.

Table 2 Functional diversity indices of soil bacterial communities in different treatment
Fig. 1
Fig. 2

![Graph showing root activity (µg·g·h⁻¹) for different treatments.](image_url)

- CK
- T1
- T2
- T3

Root activity (µg·g·h⁻¹)
Fig. 4
| Treatments | Sampling time (d) | Total N (%) | Available P (mg·kg⁻¹) | Available K (mg·kg⁻¹) | Organic matter (mg·kg⁻¹) | pH  |
|------------|------------------|-------------|------------------------|----------------------|-------------------------|-----|
| CK         | 0                | 0.21±0.01a  | 41.01±2.46a            | 125.00±5.57a         | 1.77±0.08a              | 6.44±0.06a |
| T1         | 0                | 0.22±0.03a  | 44.49±5.06a            | 125.33±14.29a        | 1.80±0.16a              | 6.48±0.03a |
| T2         | 0                | 0.21±0.02a  | 42.30±6.59a            | 124.33±11.06a        | 1.80±0.05a              | 6.44±0.05a |
| T3         | 0                | 0.22±0.03a  | 42.93±3.18a            | 127.00±15.10a        | 1.80±0.16a              | 6.48±0.02a |
| CK         | 30               | 0.25±0.01b  | 92.48±3.82a            | 124.33±16.26c        | 1.86±0.02d              | 6.42±0.09a |
| T1         | 30               | 0.27±0.03ab | 97.46±25.05a           | 132.00±10.54bc       | 2.33±0.01c              | 6.47±0.09a |
| T2         | 30               | 0.31±0.01a  | 103.27±4.89a           | 157.67±11.93ab       | 3.77±0.15a              | 6.23±0.09b |
| T3         | 30               | 0.31±0.02a  | 117.83±39.55a          | 163.67±19.01a        | 2.73±0.09b              | 6.29±0.09b |
| CK         | 60               | 0.22±0.02b  | 158.79±4.70c           | 200.34±23.76a        | 1.93±0.02c              | 6.32±0.01a |
| T1         | 60               | 0.23±0.05b  | 174.35±29.99b          | 206.00±2.65a         | 2.54±0.19b              | 6.25±0.09ab |
| T2         | 60               | 0.30±0.00a  | 195.49±20.49ab         | 211.33±22.90a        | 3.45±0.61a              | 6.12±0.07c |
| T3         | 60               | 0.28±0.01a  | 202.78±21.75a          | 217.53±25.11a        | 2.62±0.01c              | 6.14±0.05bc |
| CK         | 90               | 0.22±0.02b  | 45.64±0.71b            | 128.33±1.53c         | 1.88±0.46c              | 6.30±0.06a |
| T1         | 90               | 0.23±0.05ab | 59.52±9.17a            | 134.67±5.69bc        | 2.59±0.17b              | 6.29±0.06a |
| T2         | 90               | 0.25±0.05a  | 62.59±4.92a            | 143.67±1.15ab        | 2.86±0.13a              | 6.17±0.02b |
| T3         | 90               | 0.26±0.05a  | 66.28±5.07a            | 147.68±10.12a        | 2.77±0.08ab             | 6.18±0.02b |

Note: the values are the means of three replicates (means ± SD), and different letters indicate significant differences among different treatments (P < 0.05). CK: control (no K-feldspar or bacteria applied); T1: K-feldspar powder, 1000 mg·kg⁻¹ soil; T2: KSB JK3, 50 mL·kg⁻¹ soil; T3: mixed application of K-feldspar powder (1000 mg kg⁻¹ soil) and JK3 (50 mL·kg⁻¹ soil).
| Treatment | Shannon index (H) | Evenness index (E) | Simpson index (D) | McIntosh index (U) |
|-----------|------------------|--------------------|-------------------|-------------------|
| CK        | 3.23±0.06b       | 0.89±0.01b         | 0.95±0.06a        | 7.04±0.42a        |
| T1        | 3.30±0.08b       | 0.90±0.04b         | 0.95±0.01a        | 7.43±0.96a        |
| T2        | 3.98±0.95b       | 0.94±0.02a         | 0.95±0.00a        | 7.49±0.82a        |
| T3        | 5.06±0.38a       | 0.96±0.01a         | 0.96±0.04a        | 8.00±1.08a        |

Note: The values are the means of three replicates (means ± SD), and different letters indicate significant differences ($p < 0.05$)