

# IMPROVING SOIL FERTILITY AND SOYBEAN PRODUCTION BY GROWTH-PROMOTING BACTERIA IN DRYLANDS

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(Received 30<sup>th</sup> Dec 2023; accepted 25<sup>th</sup> Mar 2024)

**Abstract.** Declining arable land for crop production is a major threat to Indonesia's food security. Utilizing marginal land such as dryland for crop production is crucial. However, dryland has many limitations, such as low productivity and high variability of soil fertility. This study aimed to explore the beneficial effect of plant growth-promoting bacteria isolated from different organic materials such as compost in improving dryland fertility and soybean production. This study was started with compost preparation from maize and soybean biomass and plant growth-promoting (PGP) bacterial isolation and identification: indole acetic acid production, non-symbiotic N-fixing, and P-solubilizing. PGP assay was done qualitatively and quantitatively. Thus, a pot experiment was conducted in a completely randomized design with six treatments and four replications, including unsterilized and sterilized soil. This study showed that PGP bacteria inoculation, *Bacillus aerophilus* and *B. subtilis*, improved soybean production, specifically on seed dry weight (9.30%), N uptake (9.51%), and the number of soybean root nodules (17.24%). Compost application and the bacterial inoculation improved soil fertility, precisely the number of bacteria in unsterilized (29.03%) and sterilized soil (39.34%). However, a long-term application of organic materials and beneficial microorganisms (as biofertilizers) is crucial for supplying organic C and essential nutrients in drylands.

**Keywords:** *biofertilizers, food security, marginal lands, non-pathogenic bacteria, sustainable agriculture*

## Introduction

The increasing world population increases food demand for staple food commodities such as rice, corn, and soybean, specifically in Indonesia. The Agricultural Data Center and Information System (2020) reported that imports from other countries fulfill the demand for soybeans up to 2.7 to 7 million tonnes within five years (2014-2019) (Direktorat Jenderal Tanaman Pangan Pertanian, 2020). Due to the high demand and import concerns, Indonesia government's national strategic plans have been focused on increasing domestic soybean production. The program is "Pajale," with the main goal to increase rice, corn, and soybean production in Indonesia. One approach prepared to achieve the program goals is expanding the area for production (extensification). Thus,

it can be referred that the increasing food demand is in line with the increasing demand for arable lands. However, arable land for crop production has declined because of land use changes for non-agricultural use, such as settlements that threatens Indonesia's food security and sustainability (FAO, 2017; Putri et al., 2019).

There is an urgent need to utilize marginal land, such as dryland, in exchange for arable land for crop production. Dryland in Indonesia has the potential to support Indonesia as a world food barn in 2045 (Mulyani and Agus, 2017). The dryland area in Indonesia covers 144.5 million ha or 75.61% of the total land area of Indonesia (Mulyani et al., 2016). However, dryland has many limitations, such as water scarcity, limited adaptive plant species, low productivity, and high variability of soil fertility (Maestre et al., 2016; McLeod et al., 2016). Moreover, dryland has low organic matter and limitations of microbial processing for releasing nutrients for plants (Oswald and Harris, 2016).

Considering the high potential of drylands for food production in Indonesia, the demand for applying many inventions from previous studies in managing dryland fertility is crucial. Previous studies reported that applying organic matter increases organic C content in drylands (Yustika and Muchtar, 2016; Sufardi et al., 2020; Suminarti et al., 2021). Moreover, organic matter plays an important and beneficial role in ameliorating soil, so the soil is conducive as a growth medium due to organic soil amendment provides plant-available macronutrients and creates the optimum ecological conditions for crops over the years (Eagleton, 2017). Also, applying plant growth-promoting microorganisms supports and provides essential nutrients for plants. Microorganisms with plant growth-promoting (PGP) traits, specifically bacteria, improve plant growth even in harsh conditions such as the presence of heavy metals (Ustiatik et al., 2022a). The bacteria have the potential to be biofertilizer that are suitable for any soil conditions, especially in drylands (Dal'rio et al., 2022).

As biofertilizers, bacteria with PGP traits need a carrier before the bacteria can be applied to the soil (Bashan et al., 2014). Usually, the carrier is organic materials such as compost (Faesal et al., 2020). Many studies on compost quality reported that compost provides essential nutrients for carried bacteria, thus the bacteria provide available nutrients for plants (Masters-Clark et al., 2020). However, only a few studies reported on the use of PGP bacteria isolated from compost using biomass harvested from the drylands. Based on its quality, organic material consists of low-quality organic matter and high-quality organic matter (Rahmadaniarti and Mofu, 2020). This study aimed to explore the beneficial effect of plant growth-promoting bacteria (non-symbiotic N-fixing, P-solubilizing, and IAA-producing bacteria) isolated from different organic material qualities such as compost (low and high quality) in improving dryland fertility and soybean production.

## **Materials and methods**

### ***Study site description and soil sample collection and analysis***

This study was conducted in a Screen House of the Faculty of Agriculture, Universitas Brawijaya, Malang City, Indonesia (7°54'43.3 S and 112°37'44.0 E). Soil samples were collected from Jatikerto Agro Techno Park of Universitas Brawijaya, Malang Regency, Indonesia (8°07'43.5" S 112°31'42.3" E). Compost was prepared in the composting unit of Universitas Brawijaya. Analysis of soil, manure, compost, and plant biomass were conducted in the laboratory of Soil Science Department, Faculty of Agriculture, Universitas Brawijaya. Bacterial isolation and plant growth-promoting

assay were conducted at the Microbiology Research laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya.

### **Compost preparation**

Compost was made from maize biomass and soybean biomass (at a ratio 1:1 (w/w), then chopped to 2–3 cm (total 500 kg biomass). The copped biomass was mixed with cow manure at a ratio of cow manure and plant biomass was 1:20 (w/w), then tap water was added for 100% (w/v) moisture content. The compost was incubated in a box with plastic covered for 30 days. Every two days, the compost was stirred and mixed thoroughly. Compost temperature (Thermometer; TPI 312C), pH (pH meter; OHAUS AB33PH-B), organic C (Walkley and Black method), total N (Kjeldahl method), C/N ratio, and available P (Bray method) were measured every seven days. The characteristics and nutrient composition of the plant biomass (maize and soybean) and manure used for composting are shown in *Table 1*.

**Table 1.** The characteristics and nutrients composition of compost materials

| Parameter             | Maize | Soybean | Manure |
|-----------------------|-------|---------|--------|
| pH (H <sub>2</sub> O) | 7.20  | 8.00    | 7.20   |
| Organic C (%)         | 34.28 | 22.83   | 33.67  |
| Total N (%)           | 2.10  | 1.83    | 1.98   |
| C/N                   | 16.00 | 12.00   | 17.00  |
| Total P (%)           | 0.21  | 0.81    | 0.35   |
| Total K (%)           | 1.06  | 1.73    | 0.41   |
| Na (%)                | 0.28  | 0.42    | 0.34   |
| Ca (%)                | 0.82  | 3.13    | 1.77   |
| Mg (%)                | 0.34  | 0.51    | 0.68   |

### **Plant growth-promoting assay**

Bacterial isolation and plant growth-promoting assay were conducted inside Laminar Air Flow chamber (*Fig. 1A*). Fifty grams of the prepared compost was suspended into 450 mL of sodium chloride solution (NaCl 0.85% w/v; Merck), which was known as dilution 10<sup>-1</sup>, and then the suspension was transferred into a test tube for serial dilution at a ratio 1:9 (1 mL of sample suspension and 9 mL of NaCl solution). The serial dilution was made up to 10<sup>-7</sup>. Serial dilution of 10<sup>-3</sup>, 10<sup>-5</sup>, and 10<sup>-7</sup> were used for plant growth-promoting bacteria isolation (Ustiatik et al., 2021).

### **Non-symbiotic N-fixing assay**

For qualitative analysis, non-symbiotic N-fixing bacteria were isolated using nitrogen-free bromothymol blue (NfB) semisolid medium that was freshly prepared with a composition of K<sub>2</sub>HPO<sub>4</sub> 0.5 g/L, FeCl<sub>3</sub>·6H<sub>2</sub>O 0.015 g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.2 g/L, NaCl 0.1 g/L, Malic Acid 5 g/L, KOH 4.8 g/L, Yeast Extract 0.05 g/L, Bromothymol Blue 0.1% (w/v) (Merck). Bacteriological agar, 0.3% (w/v) (Merck), was added as a solidifying agent. The pH of the medium was adjusted to a neutral condition (pH 6.8) by adding HCl 1 N or NaOH 2 N. The serial dilution of 10<sup>-3</sup>, 10<sup>-5</sup>, and 10<sup>-7</sup> (0.1 mL) were

inoculated into test tubes containing NfB semisolid medium. The inoculated media were incubated in a dark place at 30 °C for seven days. A positive result was indicated with the changing color of the medium from green to dark blue.

For quantitative analysis, the N-fixing activity of selected bacteria (*Fig. 1B*) was analyzed using the NfB liquid medium that was prepared without agar and bromothymol blue. An aliquot of propagated isolate (24 h old) on Luria Bertani (LB) medium (5 mL;  $10^7$  cells/mL) was inoculated into a 45 mL liquid NfB medium. The inoculated media were incubated on an incubator shaker (120 rpm) at 30 °C. After seven days, the suspension (5 mL) was removed, then centrifuged at 10,000 rpm, 28 °C for 15 min. The supernatant (1 mL) was mixed with 1 mL of Nessler Reagent (10 g HgCl<sub>2</sub>, 7 g KI, 16 g NaOH (Merck), and 100 mL ammonia-free water). The supernatant and Nessler Reagent were made to 10 mL using distilled water, then incubated at room temperature for 30 min. The absorbance of the suspension was measured using a UV-Vis spectrophotometer (425 nm), and the ammonium concentration was calculated based on an ammonium standard curve (Kanimozhi and Panneerselvam, 2010; Setia et al., 2018; Ustiatik et al., 2022).

### ***P-solubilizing assay***

For qualitative analysis, P-solubilizing bacteria were isolated using Pikovskaya medium (*Fig. 1C*) that was prepared with a composition of Yeast Extract 0.5 g/L, Dextrose 10 g/L, Calcium Phosphate 5 g/L, Ammonium Sulphate 0.5 g/L, Potassium Chloride 0.2 g/L, Magnesium Sulphate 0.1 g/L, Manganese Sulphate 0.0001 g/L, Ferrous Sulphate 0.0001 g/L, and Agar 15 g/L (Sigma-Aldrich). The serial dilution of  $10^{-3}$ ,  $10^{-5}$ , and  $10^{-7}$  (0.1 mL) were inoculated onto Petri dishes containing Pikovskaya medium (9 mL). The inoculated media were incubated at 30 °C for nine days. The positive result was indicated with a clear zone around the colony (*Fig. 1D*) (Atekan et al., 2014).

For quantitative analysis, the P-solubilizing activity of selected bacteria was measured using a modified method by Setia et al. (2018). Five milliliters of selected isolates, propagated on LB medium for 24 h ( $10^7$  cells/mL), were inoculated into 45 mL Pikovskaya broth (pH 7.0) without agar, then supplemented with 0.5% Ca<sub>3</sub>PO<sub>4</sub> (w/v) (Merck) and incubated on an incubator shaker (120 rpm, 28 °C). The uninoculated medium was used as a control. After nine days, 10 mL of bacterial suspension were harvested and then centrifuged at 10,000 rpm for 10 min. One milliliter of the supernatant was mixed with 10 mL of molybdic acid ((NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> 15 g dissolved in 400 mL of warm deionized water) and 0.1 mL of Chlorostannous acid (SnCl<sub>2</sub>·2H<sub>2</sub>O (Merck) 2.5 g dissolved in 10 mL of concentrated HCl (Sigma-Aldrich), then added deionized water to 100 mL). The suspension was diluted and homogenized using distilled water to 25 mL, then incubated for 10 min. The absorbance of samples was measured using a UV-Vis spectrophotometer at 690 nm. The phosphate solubilization was calculated according to a phosphate standard curve (Setia et al., 2018; Senthilkumar et al., 2021).

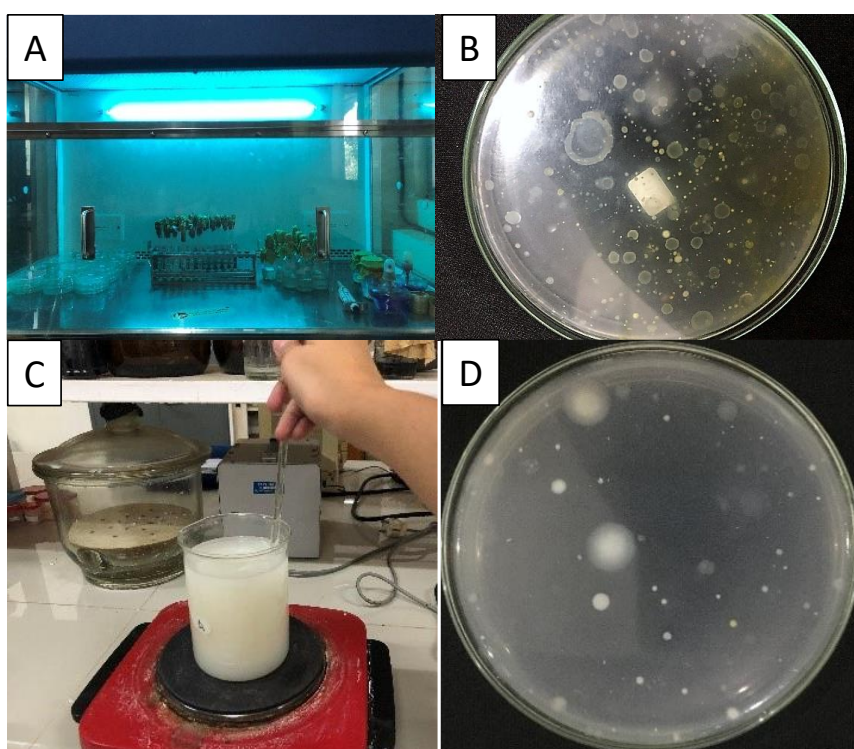
### ***Indole acetic acid (IAA) assay***

Isolates with positive results of non-symbiotic N-fixing and P-solubilizing bacteria were further propagated on the LB medium. An aliquot (5 mL) of broth culture that was incubated for 24 h (overnight) with a cell density of  $10^7$  Cells/mL was inoculated into 50 mL Tryptic Soy Broth (Merck) for IAA assay. The inoculated media were incubated at 30 °C for 5 h on a shaker incubator (120 rpm), then centrifuged for 10 min (4 °C) at 10,000 rpm. Salkowski reagent (2 mL), with the composition of 1.351 g FeCl<sub>3</sub>·6H<sub>2</sub>O 0.5 M, 100

mL H<sub>2</sub>SO<sub>4</sub> (Merck), and sterile distilled water up to a total volume of 250 mL, was added into the supernatant (1 mL) and incubated in the dark place for 30 min before the absorbance measurement using a UV-Vis Spectrophotometer at 535 nm (Uv mini-1240 Shimadzu, Japan). The IAA concentration was calculated according to the IAA standard curve (Setia et al., 2018; Ustiatik et al., 2022).

### ***Antagonistic test for bacterial consortium***

An antagonistic test was carried out to screen bacterial isolates with synergistic characteristics when applied as a consortium. The test was done using dual culture on Nutrient Agar (NA) medium (Oxoid) in a Petri dish. The plate was divided into two sides by drawing a line on the bottom of the petri dish using a marker. Each bacterial isolate (log phase) was inoculated in one-half of the petri dish by streaking the isolate using an inoculation loop with a minimum distance of 2 cm between each streak of isolate. The inoculated media were incubated at 30 °C for 24 to 48 h. The positive result was indicated by the growth of each isolated colony that is close to another colony, which means the isolates have synergistic characteristics and potential for a consortium (Muniaraj et al., 2008; Hadi et al., 2021).



**Figure 1.** PGP bacterial bioassay activities; (A) Laminar air flow chamber for aseptic condition; (B) N-fixing bacterial isolation; (C) Pikovskaya medium preparation; (D) P-solubilizing bacterial isolation

### ***Identification of potential plant growth-promoting bacterial based on 16S rRNA sequence***

The potential bacterial isolates were chosen for DNA (Deoxyribonucleic acid) extraction. Twenty-four hours old pure culture in NA medium (3 loops) was extracted

using Quick-DNATM Fungal/Bacterial Miniprep Kit (USA) according to the kit procedure. Polymerase chain reaction (PCR) (Thermal Cycler) for amplification and sequencing of 16S rRNA of the isolated were performed using primer 1387R primers (5'-GGGCGGWGTGTACAAGGC-3') and 63F primers (5'-CAGGCCTAACACATGCAAGTC-3'). 16S rRNA amplification was performed for 35 cycles, and the PCR cycle was set as pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, elongation at 72 °C for 90 s, post-elongation at 72 °C for 10 min, and cooling at 4 °C for 5 min (Santosa et al., 2018). Amplicons of 16S rRNA were purified and sequenced in First BASE, Malaysia. The 16S rRNA sequences were compared with sequences in GenBank using the BLAST program. The 16S rRNA sequences were aligned with reference sequences using the MEGA V.6 program. Phylogeny trees were constructed and inferred with the Neighbor-joining algorithm based on the Tamura-Nei model using 1000 replicates bootstraps (Setia et al., 2018; Ustiatik et al., 2022).

### **Pot experiments**

Soil for pot trial was collected at a depth of 0-20 cm using purposive random sampling with soil order of Alfisol. Soil chemical and biological properties were analyzed: soil pH, total N, available P, organic C, C/N ratio, total bacteria (standard plate count on NA medium). The soil was air-dried and sieved to 2 mm. Each polybag was filled with 10 kg of soil. For sterile treatment, the soil was sterilized using an autoclave (Tomy) for 20 min at 121 °C (Berns et al., 2008). The characteristics of the soil used for the pot trial are shown in *Table 2*.

**Table 2.** Chemical and biological properties of the soil for pot trial

| Parameter             | Result                      | Classification* |
|-----------------------|-----------------------------|-----------------|
| pH (H <sub>2</sub> O) | 6.2                         | Slightly acidic |
| Organic C             | 0.31%                       | Very low        |
| Total N               | 0.05%                       | Low             |
| C/N                   | 6                           | Low             |
| Available P           | 17.12 mg/kg                 | Low             |
| Total bacteria        | 1.1 x 10 <sup>6</sup> CFU/g | -               |

\*Classification according to Indonesia Soil Research Institute (2006)

The pot trial was arranged in a completely randomized design in the screen house, there were six treatments with four replications. The pot trial was conducted in a non-climate-controlled screen house with an average temperature 25 °C, relative humidity 80.8%, and light intensity 4.4%. The treatments consist of unsterilized soil (K0) and sterilized soil (K0S) as control, compost + bacterial consortium applied on unsterilized soil (KK) and sterilized soil (KKS), cow manure applied on unsterilized soil (KC) and sterilized soil (KCS). The prepared compost was inoculated with the potential bacterial isolates as a consortium (10<sup>7</sup> cells/mL). The compost and cow manure were added at a dose of 84 g/polybag (20 tons/ha). After compost and cow manure application, the soil was incubated for seven days before planting soybean seeds *Wilis* variety (2 seeds in each polybag). Base fertilizer was applied with a dose

of 0.21 g/polybag (50 kg Urea/ha) and 0.65 g/polybag (SP36 150 kg/ha). The pots were watered every day using sterilized deionized water at 50% soil water content up to the seed germination and then reduced to 20% soil water content, mimicking dryland soil moisture.

Soil chemical and biological properties were analyzed after the experiments that consisted of soil pH, total N, available P, total bacteria, and organic C. Plant fresh biomass were oven dried at 50 °C for > 24 h until the constant weight was achieved to measure plant dry biomass (gravimetric method). Seed dry weight was weighted after harvest when the soybean seed emerged from the pod. Root nodules were destructively counted after harvest by pulling the plant out of the polybag, separating the plant from the growth medium, then soybean root was washed with tap water to clean the root of remaining debris and counted the number of root nodules. Soybean N and P uptake were analyzed according to the total N and P on soybean biomass (seed and root-shoot biomass) and multiplied by the weight of the dry biomass.

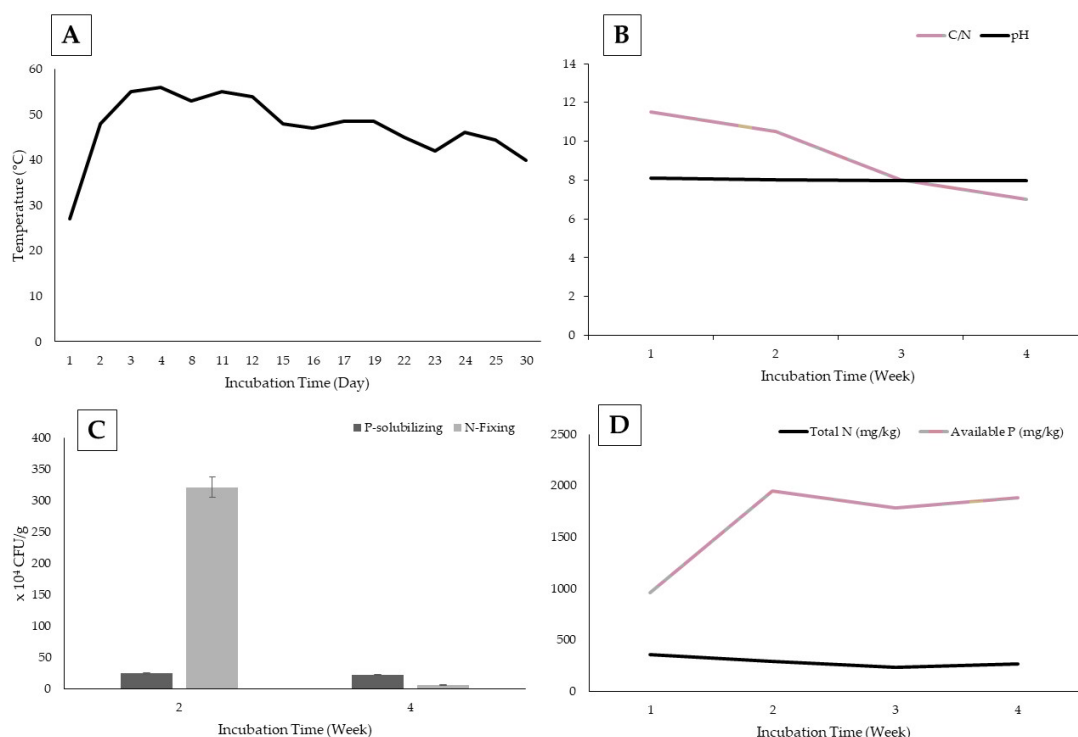
### ***Data analysis***

Statistical analysis was conducted using SPSS 13. The obtained data were subjected to a data normality test using Shapiro-Wilk's test. The data that was not in normal distributions were subsequently transformed using square root (Sqrt) or logarithm (Log10) (Ustiatik et al., 2022a). The effect of the treatments on the measured variables or parameters was statistically analyzed using a one-way ANOVA. The differences between treatment means were tested using the Duncan Multiple Range Test (DMRT) at 5% significance level.

## **Results**

### ***Compost characteristics***

Compost temperature increased after two days of incubation, then peaked at 4 to 12 days after incubation. The highest compost temperature was 56 °C (*Fig. 2A*). The temperature started to decrease and stable. The lowest compost temperature was at the end of incubation time (Day 30), which was 40 °C. The C/N ratio of the compost on the initial day of incubation was 11.5, then decreased over the incubation time, with the lowest C/N ratio was 7 (*Fig. 2B*). Compost pH was stable at 8.1 from the initial day of incubation up to the last day of incubation (7.9). The bacterial population of P-solubilizing bacteria was lower than the N-fixing bacteria population in two weeks of compost incubation,  $24.15 \times 10^4$  CFU/g and  $321.15 \times 10^4$  CFU/g, respectively (*Fig. 2C*). Non-symbiotic N-fixing bacteria population decreased at four weeks after incubation up to  $5.65 \times 10^4$  CFU/g. However, the P-solubilizing bacteria population was stable ( $22 \times 10^4$  CFU/g). Total N was decreased over incubation time (1 to 3 weeks after incubation). The lowest N total content was 238.50 mg/kg (at week 3). On the other hand, the available P of the compost increased (*Fig. 2D*) and peaked at two weeks after incubation (1952.07 mg/kg). According to the Indonesia Ministry of Agriculture Standard of Organic Fertilizer (Decree No 261 Year 2019), the compost met the standard, the pH was in the range of 4-9 and C/N was lower than 25.



**Figure 2.** Compost characteristics: (A) Compost temperature; (B) Compost pH and C/N ratio; (C) Compost functional bacterial population (non-symbiotic N-fixing and P-solubilizing bacteria); (D) Compost total N and available P

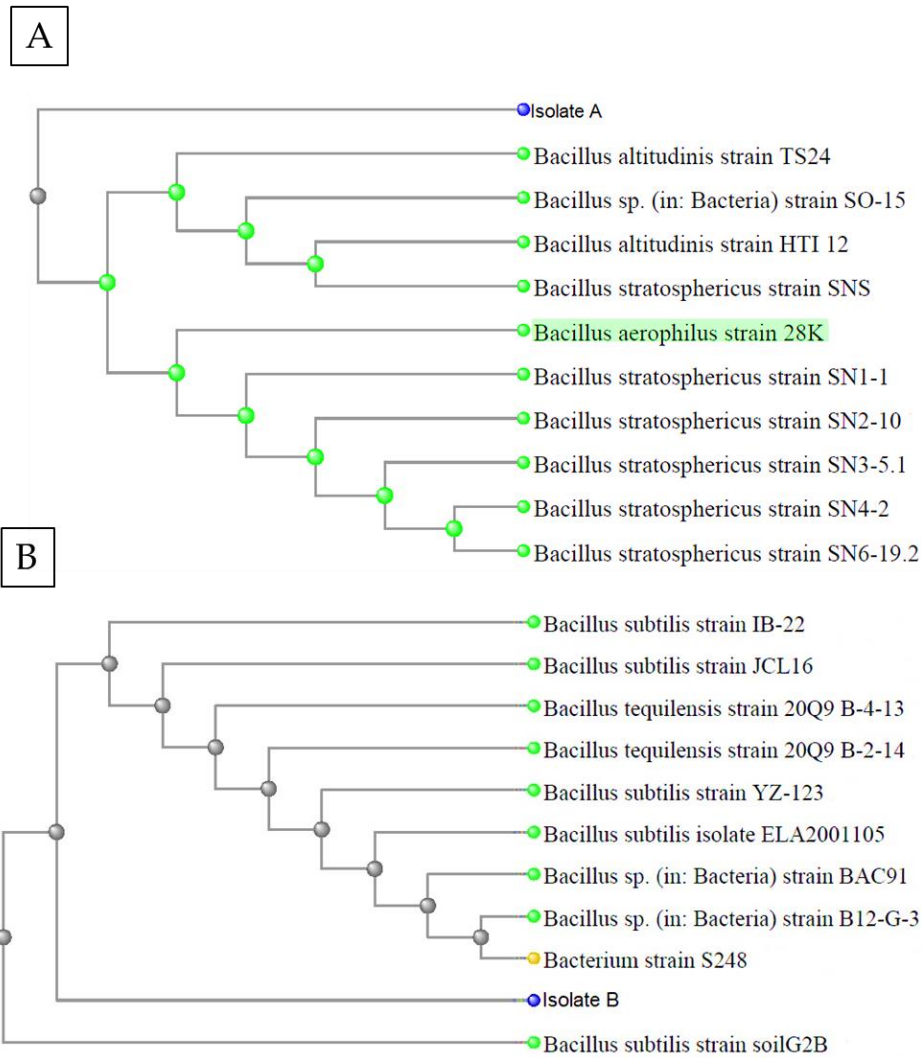
### Plant growth-promoting traits of the selected isolates

The bacteria used in the study were chosen according to the highest potential production of PGP substances and synergistic traits for the bacterial consortium. The identified bacterial isolates according to the 16s rRNA sequence were *Bacillus aerophilus* (isolate A; Fig. 3A) and *Bacillus subtilis* (isolate B; Fig. 3B). According to this study, *B. subtilis* was better at producing ammonium and indole acetic acid (IAA), 94.4 mg/L and 3.78 mg/kg, respectively, compared to *B. aerophilus*. The clear zone index on qualitative P-solubilizing activity (Pikovskaya agar medium) of *B. aerophilus* was higher than *B. subtilis*. However, quantitative P-solubilizing activity showed the opposite result. P-solubilizing activity on Pikovskaya liquid medium of *B. subtilis* was higher than *B. aerophilus*, 5.26 mg/L and 0.37 mg/L, respectively (Fig. 4).

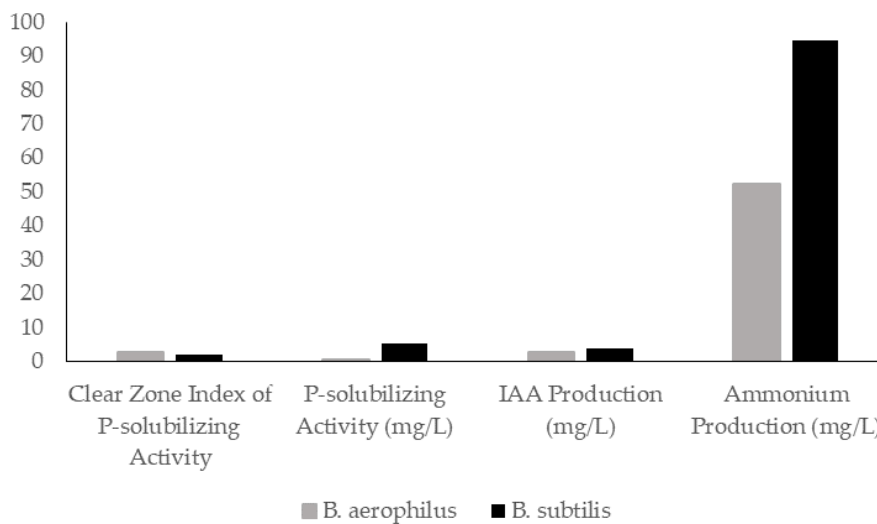
### Soybean growth and production

There were no significant differences between the treatments ( $p > 0.05$ ), therefore the application of isolates and compost did not affect the soybean biomass (Fig. 5A). Application of bacterial isolates, compost, and manure increased seed dry weight both on unsterilized and sterilized soils ( $p < 0.05$ ), but there were no significant difference on the increase of seed dry weight on sterilized soil (Fig. 5B). The application of compost inoculated with bacterial isolates increased the number of root nodules ( $p < 0.05$ ). However, no significant differences ( $p > 0.05$ ) were found in the number of root nodules on sterilized soil (Fig. 5C).

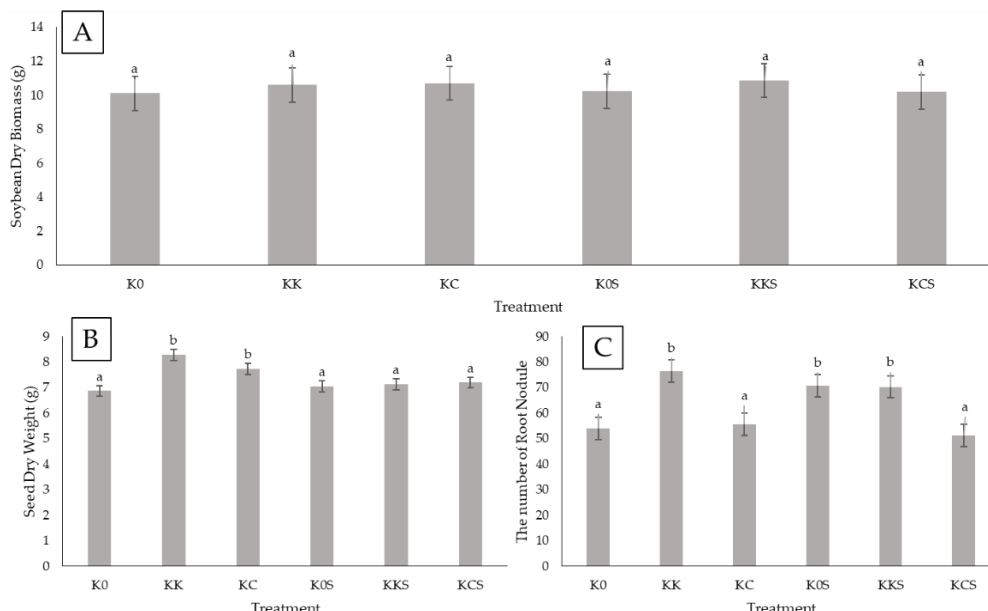




**Figure 3.** Phylogeny tree of (A) *Bacillus aerophilus*; (B) *Bacillus subtilis* and reference strains based on 16S rRNA sequence similarity

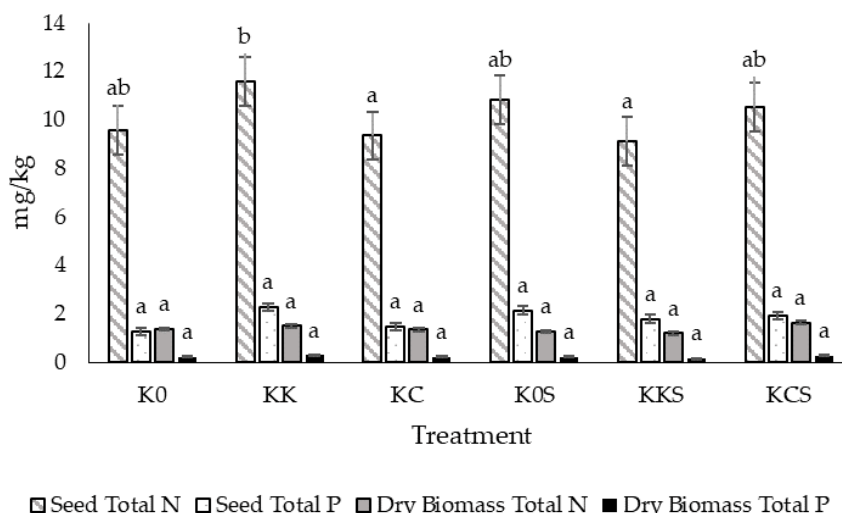


**Figure 4.** Plant growth promoting (PGP) activity of *B. aerophilus* and *B. subtilis*



**Figure 5.** The effects of compost application and bacterial inoculation on soybeans: (A) dry biomass; (B) seed dry weight; (C) the number of root nodules. K0 = control of unsterilized soil; K0S = control of sterilized soil; KK = compost + bacterial consortium application on unsterilized soil; KKS = compost + bacterial consortium application on sterilized soil; KC = cow manure application on unsterilized soil; KCS = cow manure application on sterilized soil

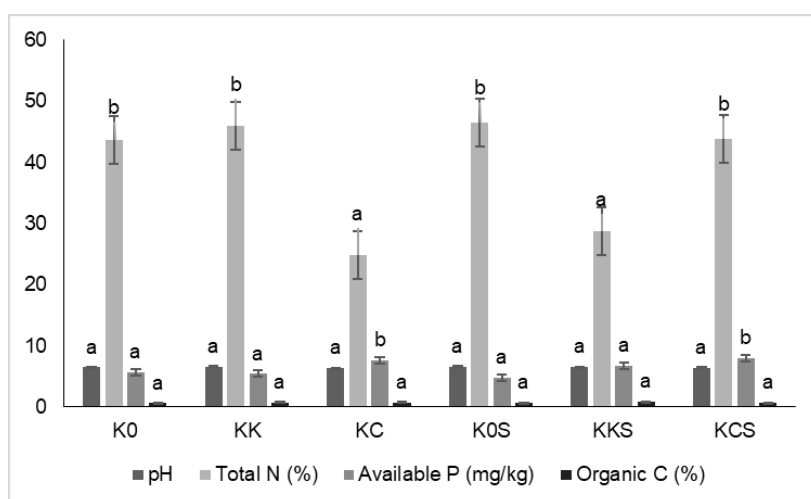
This study revealed that the application of compost inoculated with bacterial isolates affected to nutrients uptake (N and P) both in seed and plant biomass ( $p < 0.05$ ). The highest nutrient uptake was found in the seed, specifically N uptake (Fig. 6). However, there were no significant different on the N uptake on both sterilized and unsterilized soils compared to control ( $p > 0.05$ ).



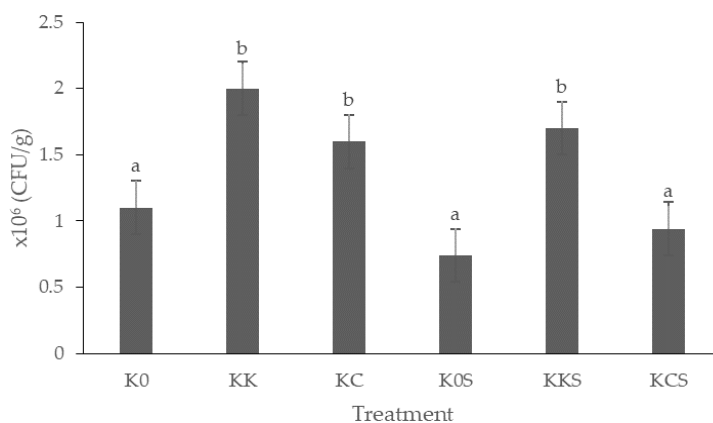
**Figure 6.** Nutrients (N and P) uptake of soybean (biomass and seed). K0 = control of unsterilized soil; K0S = control of sterilized soil; KK = compost + bacterial consortium application on unsterilized soil; KKS = compost + bacterial consortium application on sterilized soil; KC = cow manure application on unsterilized soil; KCS = cow manure application on sterilized soil

### Soil fertility status after compost and PGP bacteria application

The study found that the treatments affected soil fertility status, namely soil pH, total N, available P, organic C, and bacterial population ( $p < 0.05$ ) (Fig. 7). There were significant differences in soil total N after the treatments ( $p < 0.05$ ), the highest N total was 49.97 mg/kg (KK treatment). Moreover, the highest available P was on KC and KCS treatment (~7.82 mg/kg). Compared to control (K0 and KOS), organic C was higher on the given treatments, application of compost + bacterial inoculation as well as cow manure. The result is in line with the bacterial population that was higher in the treatments than in the control (Fig. 8). There were significant differences in the bacterial population in unsterilized and sterilized soil treatment ( $p < 0.05$ ). The highest bacterial population was KK (unsterilized soil:  $2 \times 10^6$  CFU/g) and KCS (sterilized soil:  $1.7 \times 10^6$  CFU/g) treatment.



**Figure 7.** Soil fertility status after given treatments. K0 = control of unsterilized soil; KOS = control of sterilized soil; KK = compost + bacterial consortium application on unsterilized soil; KKS = compost + bacterial consortium application on sterilized soil; KC = cow manure application on unsterilized soil; KCS = cow manure application on sterilized soil



**Figure 8.** Bacterial population after given treatments. K0 = control of unsterilized soil; KOS = control of sterilized soil; KK = compost + bacterial consortium application on unsterilized soil; KKS = compost + bacterial consortium application on sterilized soil; KC = cow manure application on unsterilized soil; KCS = cow manure application on sterilized soil

## Discussion

This study proved that compost application and selected PGP bacteria inoculation, *B. aerophilus* and *B. subtilis*, improved soybean seed dry weight and N uptake on soybean seed up to 9.30% and 9.51%, respectively. These results highlight the positive influence of bacterial isolates, compost, and cow manure on overall plant growth and production. The study demonstrated that the effect occurred in both sterilized and unsterilized soils, indicating the treatments' robustness. The study found that using compost inoculated with bacterial isolates significantly increased nutrient uptake, especially for nitrogen (N) and phosphorus (P). The seeds showed the highest nutrient uptake, with a specific emphasis on N. These treatments not only promote plant growth but also improve the nutritional quality of the harvested seeds.

The inoculated bacterium, *B. aerophilus* produces IAA, fixing N from the air non-symbiotically and solubilizing P, which is essential for plant growth. A previous study also reported that PGP traits of *B. aerophilus* are not only the substance mentioned before but also produce siderophore for acquiring iron and 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) that is crucial in supporting plant growth and survival under harsh conditions (Kumar et al., 2021). For *B. subtilis*, the bacterium known as PGP rhizobacterium, besides the beneficial role in producing PGP substances, the bacterium also alters biotic stress due to plant pathogens by producing enzymes and metabolites that support plants against plant pathogens (Hashem et al., 2019). Moreover, this study proved that compost application and the selected PGP bacterial inoculation had a positive impact on the number of root nodules formed by soybean plants. The number of soybean root nodules improved by up to 17.24%. Root nodules are essential for nitrogen fixation in leguminous plants, and the observed increase suggests improved nitrogen-fixing capabilities in the presence of these treatments. Root nodules in soybeans are vital for symbiotic N-fixing bacteria and supporting the plant in acquiring N from the air, which is essential for plant growth and production (Lu et al., 2022).

Compost application and selected PGP bacteria inoculation to improve soil fertility. However, the improvement is still far from a great improvement. A clear improvement can be seen in the number of total bacteria after compost treatment and PGP bacteria inoculation, both in unsterilized and sterilized soil, which improved up to 29.03% and 39.34%, respectively. This study is in line with a previous report by PGP bacteria application (known as biofertilizers), which is a key player in enhancing soil fertility and plant productivity (Itelima et al., 2018). In this study, removing soil microorganisms by sterilization has harmful effects on soil fertility. On the contrary, a previous study revealed that soil sterilization leads to a healthier rhizosphere microbiome re-colonization due to the absence of pathogenic microorganisms (Li et al., 2019). This study highlighted that compost and the application of PGP bacteria to sterilized soil are insufficient to soil fertility after soil sterilization. Thus, long-term application of organic materials, such as compost and biofertilizer, might be needed to supply organic C to the soil in drylands. Also, adding more beneficial microorganisms will support plant growth by acquiring essential nutrients and reducing water scarcity for plants in drylands. Further studies are needed to elucidate the mineralization and time-released nutrients from the compost that are available when needed by the plants so optimal plant growth can be achieved. Also, further diverse PGP bacteria exploration might be beneficial in solving dryland problems in the future.

## Conclusion

This study proved that PGP bacteria inoculation, *B. aerophilus* and *B. subtilis*, improved soybean production (seed dry weight and N uptake, 9.30% and 9.51%, respectively) and the number of soybean root nodules (17.24%). Compost application enriched the selected PGP bacteria inoculation potentially improved soil fertility and health, due to the increase in the number of bacteria in both unsterilized and sterilized soil (29.03% and 39.34%, respectively), as the bacteria may provide ecosystem services such as providing soil nutrients to plants by mineralizing essential nutrients from soil organic carbon. Thus, long-term application of organic materials (compost) and beneficial microorganisms (biofertilizers), is needed to provide organic C and essential nutrients to drylands soil.

**Acknowledgements.** The authors thank staff and assistants of the laboratory of Soil Science Department, Faculty of Agriculture, and Biology Department, Faculty of Mathematics and Natural Science, Universitas Brawijaya. Atiqah Aulia Hanuf and Maydella Vista Putri Rinaldy, thank you for helping with data tabulation.

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