

RESEARCH ARTICLE

Application of bio and NPK fertilizer to improve yield soybean and acid sulfate soil properties in Indonesia

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ABSTRACT

In Indonesia, soybean (*Glycine max* (L.) Merr.) is the second most important staple food with low production compared to the demand. This problem can be overcome by using marginal land such as acid sulfate soil of tidal swampland, however, the optimal application of fertilizer in such soil is minimal. Therefore, this study aims to examine the application of fertilizer in acid sulfate soil in South Kalimantan, Indonesia. It was conducted using a split-plot design with three replicates and the main plot was biofertilizer, which consisted of no-biofertilizer (P1); biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria (P2), and biofertilizer consortium of N-fixing bacteria symbiotic and nonsymbiotic, P-solubilizing bacteria and phytohormone-producing bacteria (P3). Meanwhile, the subplot was NPK fertilizer dosage, consisting of no-fertilizer (A1), dose of 150% (A2), dose of 100% (A3), and dose of 50% (A4) of the recommendation. The results showed that the biofertilizer and NPK fertilization dose did not show an interaction with soil pH and Al³⁺. The highest N and K nutrient uptake was in P2 at 50% NPK fertilizer dosage, while P in P3 at 150% NPK fertilization dose. The highest soybean yields were obtained in the application of biofertilizer P3 with 150% NPK, i.e., 1.6 t ha⁻¹, followed by the 1.5 t ha⁻¹ in the application of P2 + 50% NPK. This showed that the use of P2 biofertilizer can reduce NPK fertilizer application to 50% in soybean plants in acid soil tidal lands. Furthermore, the application of biofertilizers can increase soybean productivity in acid sulfate soil in Indonesia.

Key words: Acid sulfate soil, biofertilizer, *Glycine max*, soybean.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is the staple food for Indonesians after rice and corn (Aminah et al., 2020), and the demands have exceeded the production capacity, leading to its importation to fulfill requirements (Aldillah, 2015). Meanwhile, one of the government's efforts to increase soybean production is to expand the planting area to marginal land (Aldillah, 2015). However, genetic and environmental factors affect soybean yield (Cubukcu et al., 2021).

Tidal swampland includes marginal land with great development potential as a soybean growing area. In Indonesia, the area of tidal swamps is around 20.12 million ha, which consists of 2.07 million ha potential land, 4.23 million hectares acid sulfate land, 10.89 million hectares peatland, and 0.44 million hectares saline land (Ritung et al., 2015). However, a careless reclamation of acid sulfate soils can lead to pyrite oxidation, which is oxidized to produce H⁺ ions and reduce pH of acid sulfate soils. High soil acidity increases the solubility of Fe, Al, and Mn, causing toxicity for plants, and P deficiency. This occurred because the soil acidity is tightly bound by Fe and Al, which lowers the soil base cations due to leaching (Shamshuddin et al., 2016).

Soybean commodities can be developed in the tidal swampland, specifically on acid sulfate soil that is not affected by tidal overflow but groundwater < 50 cm. However, the chemical properties of acid sulfate

soils such as high soil acidity, high Al^{3+} concentration, and low P and K nutrient availability (Fanning et al., 2017) inhibit the development. This makes land management important to improve soil acidity and nutrient availability. Nutrient management is optimized for plant growth and increasing soybean productivity in tidal swampland to approximately 2 t ha^{-1} (Anwar and Alwi, 2014). Meanwhile, improving the soil properties of acid tidal sulfate can be achieved through amelioration and fertilization such as the application of dolomite and manure, which increases the efficiency of NPK fertilization. According to Taufiq and Sundari (2012), soybean plants are sensitive to soil acidity, toxic elements, and salinity. The critical values of pH, Al, Mn, and salinity were pH 5.5, exc-Al $1.33 \text{ cmol}_{(+)} \text{ kg}^{-1}$, Mn $3.3 \mu\text{g g}^{-1}$, and EC 1.3 dS m^{-1} , respectively. The limit of Al and H saturation in acid soils for soybean plants is up to $2.26 \text{ cmol}_{(+)} \text{ kg}^{-1}$ soils (Fageria et al., 2014).

Microbes are used to support the requirement of plant nutrients naturally by applying microorganisms into the soil as inoculants to provide certain nutrients for plants. Bacteria such as *Rhizobium* in the nodules of legume plants fix N from the atmosphere (Manasikana et al., 2019) and are mainly needed on land that has not been cultivated for soybean. The symbiotic fixation of N_2 by a legume is affected by strains of *Rhizobium* (L'taief et al., 2020). Moreover, biofertilizer is a material used to increase soil fertility by using biological waste, to enrich the soil with microorganisms contents that produce organic nutrients for the soil and fights disease. The use of biofertilizers effectively improves fertilizer efficiency and increases productivity at a relatively low cost. Biotara biofertilizer is a type of biofertilizer, which consists of a microbial decomposer consortium (*Trichoderma* sp.), P-solubilizer (*Bacillus* sp.), and N-fixer (*Azospirillum* sp.) It is environmentally friendly and increases the efficiency of N and P fertilizers by more than 30% and rice yield to 20% (Hadi et al., 2017), however, this has not been proven in soybean. Agrimeth biological fertilizer is effective for soybean plants, where 200 g ha^{-1} have the potential to substitute 50% of the recommended inorganic fertilizer. At the use of 25% recommended fertilizer, the addition of 200 g ha^{-1} can substitute the application of 20 kg ha^{-1} Gliocompost to soybean cropping on dry land (Purba, 2016). Previous study showed that Iletrisoy, Agrimeth, Soybean Plus, Probio + compost, and Biopeat biofertilizers increase soybean yields compared to non-biofertilizer crops in non-acidic lands (Sucahyono and Harsono, 2015). The effectiveness of different types of biofertilizer for soybean plants in acid sulfate soils is unknown. Therefore, this study aims to examine the effect of biological fertilizer and NPK fertilization doses on soil properties, growth, and yield of soybean in acid sulfate soils.

MATERIALS AND METHODS

This study was conducted on acid sulfate soils in Kolam Kiri village, Wanaraya subdistrict, Barito Kuala Regency, South Kalimantan, Indonesia (Figure 1). The research was conducted from April to October 2019. The climatic conditions at the research site have an average temperature $26\text{-}27 \text{ }^\circ\text{C}$, the maximum temperature is $27.50 \text{ }^\circ\text{C}$ in October, while the minimum temperature is in July with temperatures reaching $26.50 \text{ }^\circ\text{C}$. The average rain every year is 2665 mm with 107 rainy days.

The characteristic soil of the study site was highly acidic and nutrient deficient (Table 1). The high soil acidity was indicated by the pH H_2O 3.48 and pH KCl 3.27. Moreover, the value of EC was 0.362 mS cm^{-1} , exchangeable K $0.247 \text{ cmol}_{(+)} \text{ kg}^{-1}$ was classified as low, exchangeable Na $0.762 \text{ cmol}_{(+)} \text{ kg}^{-1}$ was moderate, and Mg $0.972 \text{ cmol}_{(+)} \text{ kg}^{-1}$ was low, exchangeable H $80.90 \text{ cmol}_{(+)} \text{ kg}^{-1}$ was very high, and available P was very low. Sulfate and Al concentrations were classified as high, therefore, the application of chemical and biological fertilizers to increase the growth and yield of soybean (*Glycine max* (L.) Merr.) and soil fertility is required.

The research was designed using a split-plot with three replicates and the main plot treatment was biofertilizer: no biofertilizer (control) (P1), biofertilizer consortium of decomposer fungi (*Trichoderma* sp.), P-solubilizing bacteria (*Bacillus* sp.), N-fixing bacteria (*Azospirillum* sp.) (P2), and biofertilizer consortium of symbiotic N-fixing bacteria (*Rhizobium* sp., *Bradyrhizobium* sp.), nonsymbiotic (*Azotobacter* sp.), P-solubilizing bacteria (*Bacillus* sp.), and phytohormone-producing bacteria (*Methylobacterium* sp.) (P3). Moreover, subplot was NPK fertilizer doses: no NPK fertilizer (A1), NPK fertilizer 150% recommended dose (A2), NPK fertilizer 100% recommended dose (A3), and NPK fertilizer 50% recommended dose (A4). The doses of NPK fertilizer recommendation were urea 100 kg ha^{-1} , SP-36 75 kg ha^{-1} , and KCl 75 kg ha^{-1} .

Each treatment subplot was $10 \text{ m} \times 6 \text{ m}$, with 0.75 m between the plots and 1.5 m between the main plots. All plots received dolomite lime with a dose of 2 t ha^{-1} at 2 wk before application of ameliorant and biological fertilizer due to its effectiveness (Irwan and Nurmala, 2018). In this study, *Rhizobium* of the Legin brand with

a dose of 30 g inoculum 10 kg⁻¹ soybean seeds was used. The soybean variety used was ‘Anjasromo’, where 2-3 seeds were planted per hole with a spacing of 10 × 40 cm. Subsequently, the seeds that have been mixed with the inoculum according to each treatment were planted by immersing to a depth of 5-10 cm and covering them with soil. Thinning was carried out by leaving 1 plant per hole at 7-14 d after sowing and inorganic fertilizer was applied at planting by placing it 5 cm outside the planting hole at a depth of 7 cm. Urea fertilizer was applied twice with 2/3 dose at 7 d after sowing and 1/3 at maximum vegetation. Other cultivation such as land preparation, basic fertilizer application, control of weeds, pests, and diseases, as well as harvesting and processing were carried out based on recommendations.

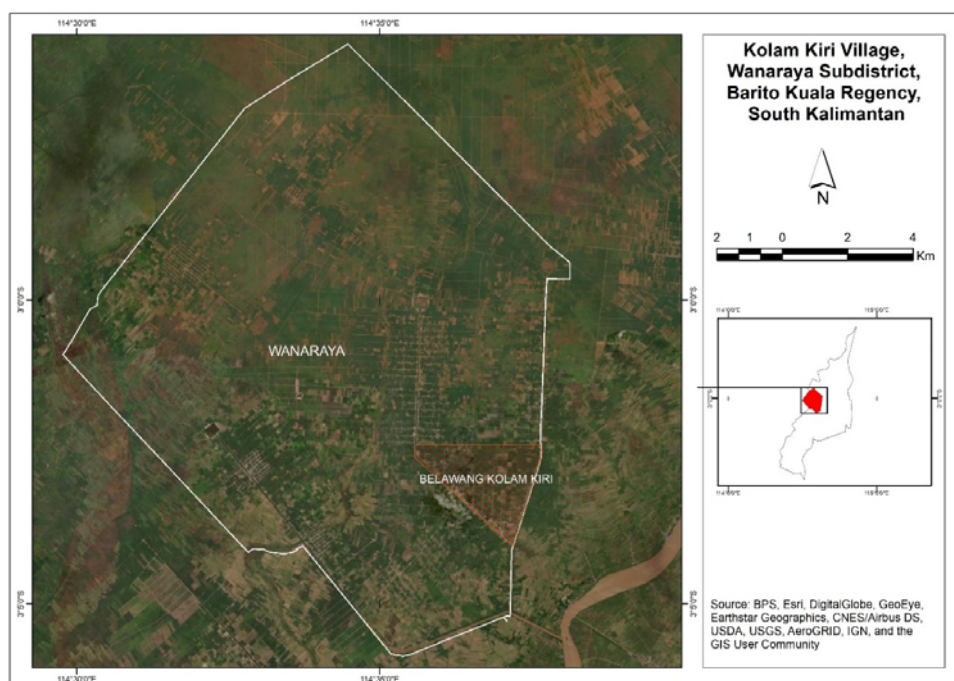


Figure 1. Research location map.

Table 1. Initial soil analysis of the study site.

| Soil properties | Analysis results |
|---------------------------------------------------------|------------------|
| pH H ₂ O | 3.480 |
| pH KCl | 3.270 |
| Electrical conductivity, mS cm ⁻¹ | 0.362 |
| Exchangeable K, cmol ₍₊₎ kg ⁻¹ | 0.247 |
| Exchangeable Na, cmol ₍₊₎ kg ⁻¹ | 0.762 |
| Exchangeable Ca, cmol ₍₊₎ kg ⁻¹ | 0.680 |
| Exchangeable Mg, cmol ₍₊₎ kg ⁻¹ | 0.972 |
| Exchangeable H, cmol ₍₊₎ kg ⁻¹ | 80.980 |
| Available P, μ g ⁻¹ | 6.184 |
| Fe, μ g ⁻¹ | 243.411 |
| SO ₄ , μ g ⁻¹ | 456.579 |
| FeS ₂ , % | 0.182 |
| Al ³⁺ , cmol ₍₊₎ kg ⁻¹ | 26.704 |
| Water content, % | 105.010 |

The measurement of the plant growth was carried out at 4 and 6 wk after planting (WAP) to determine the height. At the maximum vegetative (8 WAP), five plant samples were taken destructively at each plot to measure plant dry weight, levels of N, P, K and uptake, and the number of effective nodules. During harvest, the yield component measured included the percentage of filled pods, number of pods per clump, soybean yield in form of dry seed weight per tile 2 m × 2.5 m. The total N in plant tissue was measured using the Kjeldahl method with wet distillation (H₂SO₄), while the total P and K used the 25% HCl extraction method and measured with a spectrophotometer. Nutrient uptake per plant was determined by converting nutrient content and plant dry weight, while the total microbial population was analyzed at the beginning and finishing (at harvest).

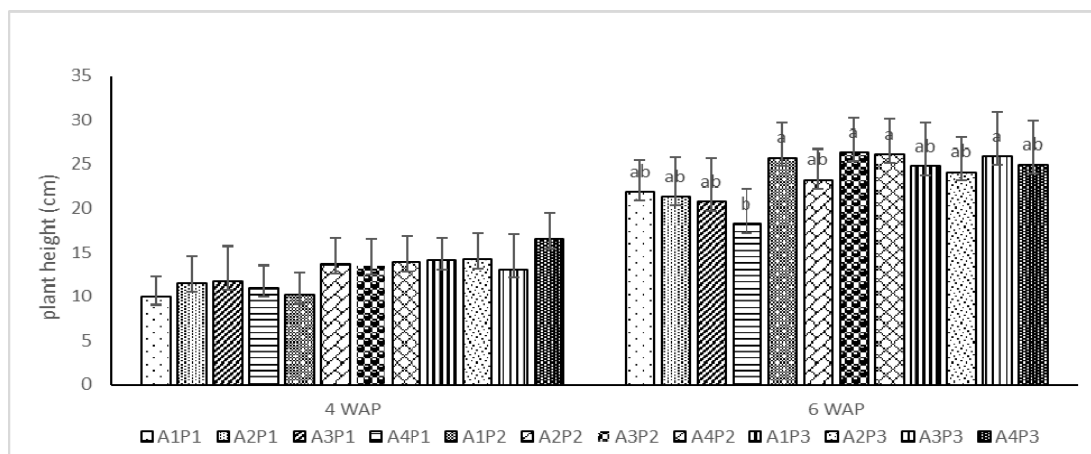


Figure 2. Effects of biofertilizer and NPK fertilizer dosage on soybean plant height. A1: Without NPK fertilizer; A2: 150% NPK; A3: 100% NPK; A4: 50% NPK; P1: without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria, P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohormone-producing bacteria WAP: weeks after planting. Different letters indicate significantly different mean Duncan's multiple range test $p < 0.05$.

Table 2. Effects of biofertilizer and NPK fertilizer dosages on the number of effective root nodules. P1: Without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria; P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohilizing bacteria. Values followed by the same letter are not significantly different based on the Duncan's multiple range test ($p < 0.05$).

| NPK Fertilizer dose | P1 | P2 | P3 | Average |
|-----------------------------------|-------|-------|-------|---------|
| A1 = Control (without fertilizer) | 2.3b | 3.2ab | 2.5ab | 2.5a |
| A2 = 150% NPK | 2.1b | 2.7ab | 3.2ab | 2.7a |
| A3 = 100% NPK | 2.0b | 2.8ab | 2.7ab | 2.7a |
| A4 = 50% NPK | 2.6ab | 4.3a | 3.9ab | 3.6a |
| Average | 3.0a | 2.8a | 2.8a | |

The number of nodules per plant was evaluated by counting the number of root nodules per plant, while the number of effective root nodules by counting the number of effective roots per plant. The effective root nodules were marked with a blood-red color in the center of the nodules. Meanwhile, the observed growth and yield parameters included root dry weight per plant, stover dry weight per plant, pod dry weight per plant, seed dry weight per plot, and weight of 1000 seeds.

The periodic soil properties, namely pH and Al³⁺ at 4, 8, and 12 wk after planting were analyzed. The pH was analyzed using a pH meter and Al using NH₄OAc extraction. In addition, during harvest, the population of

microorganisms such as decomposer fungi and N-fixing bacteria in the soil was observed. The observation data were analyzed using ANOVA at the 5% alpha level, while Duncan's multiple range test was carried out at the 5% alpha level to determine the difference between treatments.

RESULTS AND DISCUSSION

Soybean growth and productivity

The results of the analysis of plant height at 4 and 6 WAP are shown in Figure 2. At 4 WAP, there was nonsignificant effect of biological and NPK fertilizers on the height of soybean plants, while at 6 WAP, a significant effect occurred compared to non-treatment. Apart from N fixation, fertilizer is also needed for optimum production. Therefore, N fertilization is needed as a trigger before nodules form to when they are formed and achieve development that fulfills their N requirements. They are also used as supplement fertilizer to fulfill the high N requirements during pods filling height.

The effect of biofertilizers and NPK fertilizer dose on root nodules are shown in Table 2. All treatments showed nonsignificant effect on the number of nodules, except A1P1, A2P1, and A3P1 that had a lower number of nodules than A4P2. Moreover, P2 biofertilizer contains microbes such as phosphate-solubilizing bacteria that affect plant growth directly or indirectly. Tagore et al. (2013) stated that there is a synergic relationship between Rhizobium and P-solubilizing bacteria that can affect nodulation. This showed that inoculation of phosphate-solubilizing bacteria with P fertilizer enhanced the supply of P for nodulation. Furthermore, the number of nodules on soybean root is reduced at flooded conditions (Koesrini et al., 2015), while high soil acidity and Al toxicity influence root weight and nodule formation (Bakar et al., 2020).

Plant dry weight indicated the accumulation of assimilates from plant growth activity, while shoot dry weight showed the ability of plants to produce carbohydrates through photosynthesis, and root dry weight represented the ability of roots to absorb, provide nutrients, and water that function in plant metabolism. The results showed that there was an interaction between biofertilizer and NPK dose to the root dry weight and shoot of soybean plants, with the highest root dry weight and shoot height shown by A4P2.

The effect of biofertilizers and NPK fertilizer doses on NPK uptake is shown in Figure 3. The results showed that the amount of N, P, and K content of soybean plants is related to the level of yield achieved. Based on N nutrient analysis, all treatments are generally deficient in N. Gaspar et al. (2017) stated that at a certain yield level, the optimal nutrient requirements of N, P, and K for soybean plants are linear to yield. With the assistance of bacteria, soybeans can take N_2 from the air but need to be supported by environmental conditions that support plant health such as pH, N nutrient levels, water content, and temperature, where the highest N uptake was shown in A4P2 treatment (Figure 3). Meanwhile, the application of P2 biofertilizer can reduce the use of inorganic N fertilizers by 50%, as indicated by the highest N uptake by soybeans in A2P4 treatment. The application of N fertilization is required for N fixation starter; however, the administration of excessive doses has suppressed its fixation and the activity of N-fixing bacteria. Previous study showed that the application of N as a starter fertilizer increased soybean yield (Gai et al., 2017). The level of effectiveness of N fertilization in soybean plants is higher when N-fixing bacteria cannot grow and develop properly (Ayuni et al., 2015). Although N fertilization at various doses does not affect soybean yields, it led to higher N levels and reduced N_2 fixation. Furthermore, N fixation in soybean plants ranged from 0% to 98% of the total N uptake, 0-337 kg $N ha^{-1}$, which depends on rhizobia activity (Ciampitti and Salvaggiott, 2018).

The P nutrient in acid soils is a major limiting element in crop productivity, including soybean (Sabilu et al., 2015). This is because high acidity ($pH < 4.0$) causes the solubility of Al, Fe, and Mn to increase, together with the hydrogen sulfide and saltwater (sodium). Furthermore, Al toxicity occurs under oxidation conditions and is accompanied by P deficiency because P is bound to insoluble aluminum phosphate (Nazemi et al., 2012). In addition, P affects soybean plant root development, flowering, and fruiting (Lingaraju et al., 2016).

The effect of biofertilizer on K nutrient uptake by soybean was not significantly different and the impact of dosage of NPK fertilization was not in line with K uptake by soybean. The response of soybean plants due to the application of K fertilizer is not continuously consistent because of the nature of K elements that are mobile (Adisarwanto, 2014). Moreover, K nutrients are needed by soybean for various plant metabolic activities such as assimilation, transformation, protein synthesis, and stress tolerance (Hasanuzzaman et al., 2018). It is a very mobile element in plant tissue, through phloem vessels, it is transported to the top of the plant and the bottom. Furthermore, it affects several physiological processes in plant meristem tissue growth, water content in plant tissue, photosynthesis, and transportation processes in plant tissue, which influence the quality of crop yields

(Wang et al., 2015). Potassium does not only affect plant physiological processes, but also metabolism in plant tissues (Hasanuzzaman et al., 2018).

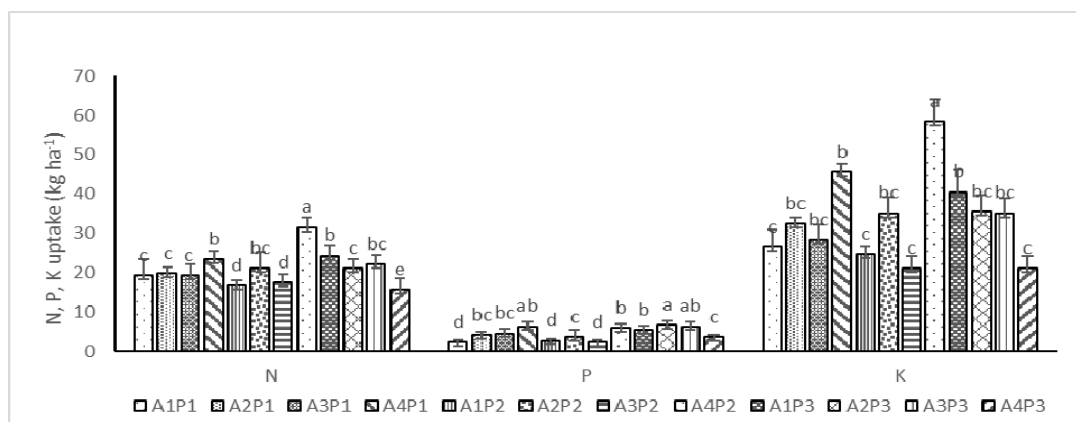


Figure 3. Effect of biofertilizer and NPK fertilizer dosage on NPK uptake at harvest time. A1: Without NPK fertilizer; A2: 150% NPK; A3: 100% NPK; A4: 50% NPK; P1: without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria; P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohormone-producing bacteria. Different letters indicate significantly different mean Duncan's multiple range test $p < 0.05$.

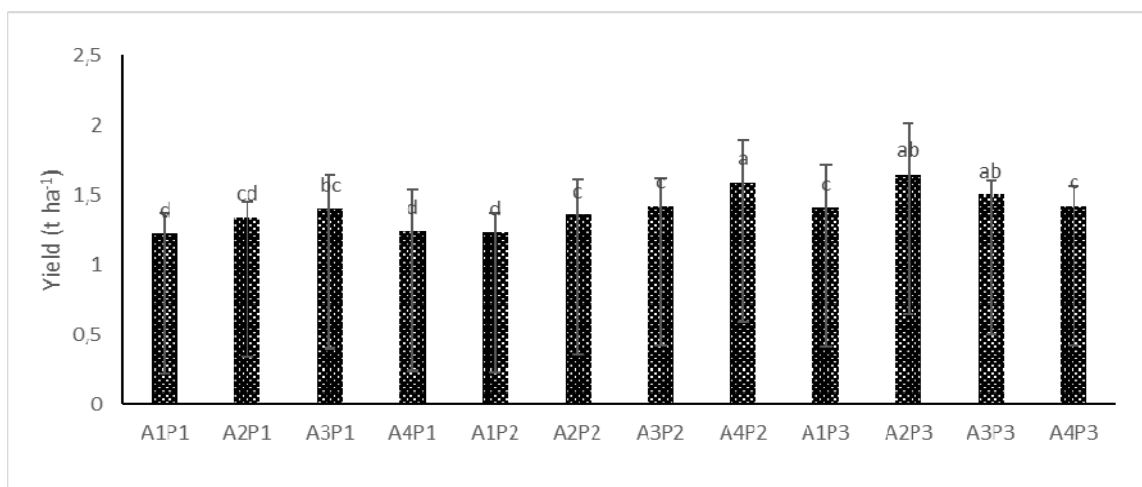


Figure 4. Effect of biofertilizer and NPK fertilizer doses on soybean yields. A1: Without NPK fertilizer; A2: 150% NPK; A3: 100% NPK; A4: 50% NPK; P1: without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria; P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohormone-producing bacteria. Different letters indicate significantly different mean Duncan's multiple range test $p < 0.05$.

The effect of biofertilizer and NPK fertilizer doses on soybean yields is shown in Figure 4. The highest soybean yield was shown by P3 biofertilizer and NPK 150% of recommendation (A2P3) treatment reaching 1.6 t ha^{-1} . This was not significantly different from P2 biofertilizer and 50% NPK (A4P2) treatment reaching 1.5 t ha^{-1} , as well as the potential yield of 'Anjasmoro', which was $2.03\text{-}2.25 \text{ t ha}^{-1}$. Vegetative organ growth affects crop yields, therefore, greater vegetative growth, which function as a producer of assimilation (source)

increases the growth of user organs (sinks) and ultimately produced greater output. The use of P1 biofertilizer substituted the application of NPK fertilizer by 50% and increased yield by 30% compared to control (without biofertilizer). According to study by Lubis et al. (2021) the application of biofertilizer and chicken manure increased organic C, total N, availability of P in acid sulfate soils and increased N and P uptake by soybeans.

Change of soil properties

Soil properties changes that were measured included pH H₂O (Table 3) and Al³⁺ (Table 4) in the period of 4, 8, and 12 wk after planting (WAP). Biofertilizer treatment and NPK fertilization dose did not show significant interaction with soil pH, however, there was an increase in pH during the observation period (Table 3). P2 biofertilizers also have a better effect on soil pH than P3 biofertilizers. This is because the microbes contained in P2 fertilizers were isolated from swamplands, which increased their compatibility. Soybean is a plant sensitive to low pH, therefore, the acidic pH of acid sulfate soil caused a reduction in soybean yields. The main problems in acid sulfate tidal swampland for soybean cultivation include high soil acidity (pH 3-4), nutrient deficiency of Ca, P, K, and Mg, as well as the presence of toxic elements Al³⁺. High soil acidity also inhibits the growth and propagation of Rhizobium, leading to a decrease in the formation of root nodules.

The NPK fertilizer dose treatment and type of biological fertilizer did not show interactions with Al³⁺ in soil solution. The lowest Al³⁺ concentration at week 4 was shown by the use of P3 biofertilizer. However, at the 8 and 12 WAP, it was indicated by the P2 biofertilizer treatment, while the NPK fertilizer dose did not show any difference to the Al³⁺ concentration (Table 4). Treatment P2 contains microbial consortia such as decomposers (*Trichoderma* sp.), P-solvent (*Bacillus* sp.), and N (*Azospirillum* sp.) inhibitors which can bind N, increase the availability of soil P nutrients, decompose organic residues, and spur growth. *Azospirillum* bacteria live in plant roots and breed mainly in the area of root and length extension. They prefer energy from organic acids such as malate, succinate, lactate, and pyruvate (Kantikowati et al., 2019). Microbes contained in biofertilizers provide nutrients for plants, facilitate the absorption of nutrients, support the decomposition of organic matter, provide a better rhizosphere environment that ultimately supports growth, and increase crop production (Saraswati, 2012).

Table 3. Changes in pH of H₂O due to biofertilizer treatment and NPK fertilizer application. P1: Without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria; P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohormone-producing bacteria; WAP: weeks after planting. Values followed by the same letter are not significantly different based on the Duncan's multiple range test (p < 0.05).

| NPK Fertilizer dose | P1 | P2 | P3 | Average |
|-----------------------------------|-------|--------|-------|---------|
| Period of observation 4 WAP | | | | |
| A1 = Control (without NPK) | 3.51 | 4.02 | 3.67 | 3.73a |
| A2 = 150% NPK | 3.57 | 3.54 | 3.60 | 3.57b |
| A3 = 100% NPK | 3.58 | 3.89 | 3.52 | 3.67ab |
| A4 = 50% NPK | 3.64 | 3.95 | 3.70 | 3.76a |
| Average | 3.57b | 3.85a | 3.62b | |
| Period of observation 8 WAP | | | | |
| A1 = Control (without fertilizer) | 3.60 | 3.85 | 3.80 | 3.75a |
| A2 = 150% NPK | 3.53 | 3.64 | 3.75 | 3.64b |
| A3 = 100% NPK | 3.64 | 3.63 | 3.79 | 3.69ab |
| A4 = 50% NPK | 3.65 | 3.79 | 3.88 | 3.77a |
| Average | 3.61b | 3.73ab | 3.80a | |
| Period of observation 12 WAP | | | | |
| A1 = Control (without fertilizer) | 3.68 | 3.72 | 3.73 | 3.71b |
| A2 = 150% NPK | 3.93 | 3.79 | 3.68 | 3.80a |
| A3 = 100% NPK | 3.71 | 4.07 | 3.78 | 3.85a |
| A4 = 50% NPK | 3.64 | 3.81 | 3.69 | 3.71b |
| Average | 3.74b | 3.85a | 3.72b | |

Table 4. Changes in Al³⁺ concentration due to biofertilizer treatment and NPK fertilization dose. P1 Without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria, P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohormone-producing bacteria; WAP: weeks after planting. Values followed by the same letter are not significantly different based on the Duncan's multiple range test ($p < 0.05$).

| NPK Fertilizer dose | Al ³⁺ concentration | | | Average |
|------------------------------|--------------------------------|-------|-------|---------|
| | P1 | P2 | P3 | |
| | cmol(+) kg ⁻¹ | | | |
| Period of observation 4 WAP | | | | |
| A1 = Control (without NPK) | 0.34 | 0.28 | 0.41 | 0.34a |
| A2 = 150% NPK | 0.37 | 0.43 | 0.37 | 0.39a |
| A3 = 100% NPK | 0.59 | 0.52 | 0.35 | 0.49a |
| A4 = 50% NPK | 0.32 | 0.42 | 0.28 | 0.34a |
| Average | 0.40a | 0.41a | 0.35b | |
| Period of observation 8 WAP | | | | |
| A1 = Control (without NPK) | 0.41 | 0.51 | 0.62 | 0.51a |
| A2 = 150% NPK | 0.69 | 0.35 | 0.61 | 0.55a |
| A3 = 100% NPK | 0.32 | 0.34 | 0.73 | 0.46a |
| A4 = 50% NPK | 0.50 | 0.57 | 0.54 | 0.54a |
| Average | 0.48b | 0.44b | 0.63a | |
| Period of observation 12 WAP | | | | |
| A1 = Control (without NPK) | 0.763 | 0.491 | 0.376 | 0.51a |
| A2 = 150% NPK | 0.379 | 0.709 | 0.647 | 0.55a |
| A3 = 100% NPK | 0.634 | 0.396 | 0.668 | 0.46a |
| A4 = 50% NPK | 0.736 | 0.555 | 0.804 | 0.54a |
| Average | 0.48b | 0.44b | 0.63a | |

Table 5. Effect of types of biofertilizers and NPK fertilizer doses on fungi population. P1: Without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria; P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohormone-producing bacteria. Values followed by the same letter are not significantly different based on the Duncan's multiple range test ($p < 0.05$).

| NPK Fertilizer dose | Fungi population | | | Average |
|-----------------------------------|-------------------------------------|----------|----------|---------|
| | P1 | P2 | P3 | |
| | 10 ⁶ cfu g ⁻¹ | | | |
| A1 = Control (without fertilizer) | 12.00abc | 11.60abc | 23.00a | 15.50a |
| A2 = 150% NPK | 4.00c | 9.00bc | 11.30abc | 13.60ab |
| A3 = 100% NPK | 11.60abc | 10.60abc | 10.60abc | 11.00ab |
| A4 = 50% NPK | 8.60bc | 19.60ab | 12.60abc | 8.10b |
| Average | 9.00a | 12.70a | 14.40a | |

Table 6. Effects of biofertilizers and NPK fertilizer doses on populations of N-fixing bacteria. P1: Without biofertilizer; P2: biofertilizer consortium of decomposer fungi, P-solubilizing bacteria, N-fixing bacteria; P3: biofertilizer consortium of symbiotic N-fixing bacteria, nonsymbiotic, P-solubilizing bacteria, and phytohormone-producing bacteria. Values followed by the same letter are not significantly different based on the Duncan's multiple range test ($p < 0.05$).

| NPK Fertilizer dose | N-fixing bacteria | | | Average |
|-----------------------------------|----------------------------|-------|-------|---------|
| | P1 | P2 | P3 | |
| | 10^6 cfu g ⁻¹ | | | |
| A1 = Control (without fertilizer) | 2.0b | 2.6ab | 2.6ab | 2.4a |
| A2 = 150% NPK | 2.0b | 2.6ab | 1.9b | 2.1a |
| A3 = 100% NPK | 3.0ab | 3.0ab | 1.6c | 5.1a |
| A4 = 50% NPK | 1.6c | 5.0a | 2.3ab | 3.0a |
| Average | 2.1a | 3.3a | 4.0a | |

Microorganism population

The effect of biological and NPK fertilizer doses on the population of fungi and N-fixing bacteria are shown in Tables 5 and 6, respectively. The highest fungal population was obtained in P3 biofertilizer without NPK fertilization, and the lowest was in A2P1 (without biofertilizer at dosage 150% NPK from recommendation). Furthermore, the highest population of N-fixing bacteria was in the A4P2 treatment and the lowest at A2P1 were not significantly different from A4P3. Treatment P2 contains decomposers, P-solubilizing bacteria, and N₂-fixing bacteria (*Azospirillum* sp.) which are inoculated from swampland (Lubis et al., 2021). This causes that the N₂-fixing microbes in P2 biofertilizers be more adaptable compared to P3 biofertilizers. Meanwhile, microorganisms in P3 biofertilizer include *Azotobacter vinelandii* (non-symbiotic N₂ inhibitors and anti-pathogenic solvents), *Azospirillum* sp. (non-symbiotic N₂ inhibitors and phytohormone-producing), *Bacillus cereus* (soil P-solvent and anti-pathogenic solvent), and *Methylobacterium* sp. (phytohormone-producing).

CONCLUSIONS

The highest N and K uptake in biofertilizer from microbial consortium of fungi, P-solubilizing bacteria, N-fixing bacteria (P2) was combined with 50% recommended NPK fertilization dose, while P uptake in biofertilizer from microbial consortium of N-fixing bacteria symbiotic and nonsymbiotic, P-solubilizing bacteria and phytohormone-producing bacteria (P3) was at 150% NPK fertilizer dose. The highest soybean yield (1.6 t ha⁻¹) was obtained with P3 at 150% NPK fertilizer dosage. This was not significantly different from 1.5 t ha⁻¹ with P2 and 50% NPK fertilizer dosage. Therefore, the use of P2 biofertilizers can reduce the concentration of Al³⁺, and the application of NPK fertilizer to 50% in soybean plants in acid sulfate soils. The biofertilizers can reduce the use of inorganic fertilizers and increase soybean productivity of acid sulfate soils in Indonesia.

Author contributions

Methodology: A.S., M.M. Software: V.K. Validation: M.M., Y.S. Formal analysis: M.M., Y.S. Resources: A.S., Y.L. Data curation: A.S., Y.L. Writing-original draft: E.M. Writing-review & editing: E.M. Supervision: Y.S. Project administration: A.S. Funding acquisition: Y.S. All co-authors reviewed the final version and approved the manuscript before submission.

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