

**Research Article**

## The potential use of rhizobacteria and cover crop residue for improving the growth of sorghum on post-nickel mining soil in Southeast Sulawesi

Sitti Leomo<sup>1\*</sup>, Syamsu Alam<sup>1</sup>, Muhidin<sup>2</sup>, Gusti R. Sadimantara<sup>2</sup>

<sup>1</sup> Department of Soil Science, Halu Oleo University, Kampus Baru Anduonohu, Kendari 93232, Indonesia

<sup>2</sup> Department of Agrotechnology, Halu Oleo University, Kampus Baru Anduonohu, Kendari 93232, Indonesia

\*corresponding author: sittleomouho@gmail.com

### Abstract

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The utilization of rhizobacteria and cover crop residue for improving plant productivity has been widely applied in agricultural land. However, the effectiveness of those treatments for increasing the growth performance of crops in the post-mining soil is still not evaluated. This study investigated the potential use of rhizobacteria and cover crop residue for improving the growth of *Sorghum* sp. developed in the post-nickel mining soil in Southeast Sulawesi. An experiment was established at the laboratory level using a completely randomized design with three replicates for every treatment. Four treatments were examined in this trial comprising of CE (control+*Eleusine indica*), CC (control+*Centrosema* sp.), BE (*Bacillus* sp.+*Eleusine indica*), and BC (*Bacillus* sp.+*Centrosema* sp.). Some parameters were observed to evaluate the growth of *Sorghum* sp., namely diameter, height, total leaves, leaf area, and biomass. The results demonstrated that the use of rhizobacteria and cover crop residue potentially improves the growth of *Sorghum* sp. in post-nickel mining soil, even though there was no significant statistical difference between treatments. The highest average biomass of *Sorghum* sp. was noted in BE (3.72±1.06 g), BC (3.20±0.50 g), CC (2.95±1.40 g), CE (2.81±0.33 g). These findings indicated that the treatment of BE was the best way to improve the growth performance of *Sorghum* sp. in the post-mining soil. According to the results, this study concluded that rhizobacteria and cover crops play a key role in soil fertility. The utilization of rhizobacteria and cover crop residue has the potential to support the growth of *Sorghum* sp. developed in the post-nickel mining soil.

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### Introduction

The management of post-mining areas to support agricultural development is currently one of the most strategic issues in Indonesia. Besides supporting environmental improvement, this effort also contributes to accelerating the implementation of a food security program at remote sites. However, this scenario is not easy to implement since the environmental quality in the post-nickel mining area is

dominated by soil with high acidity and low fertility (Paramasivam and Anbazhagan, 2019; Fernandez-Caliani et al., 2021; Pratiwi et al., 2021; Nugroho et al., 2022). Consequently, the land capability in the post-nickel mining site is naturally not suitable for supporting the growth performance of crops. To anticipate these constraints, the utilization of rhizobacteria and cover crop residue has the potential to become an alternative method to facilitating the effort of crop development in the post-nickel mining

soil. Several studies report the use of rhizobacteria and cover crop residue for crop cultivation provides many benefits for supporting plant growth and development (Backer et al., 2018; Mustikaningrum et al., 2018; Scavo et al., 2022; Andrade et al., 2023).

The application of rhizobacteria on crops can stimulate the production of phytohormones that play essential roles as growth regulators, including auxins, cytokinins, and gibberellins (Vejan et al., 2016; Santos et al., 2020). It also increases the crop's ability for nutrient absorption since the existence of rhizobacteria can accelerate the mineralization process of macronutrients such as nitrogen, phosphate, and potassium (Gouda et al., 2018; Saeed et al., 2021; Igiehon et al., 2024). Meanwhile, the utilization of cover crop residue as additional organic matter can improve soil structure, maintain soil moisture, and increase nutrient availability (Adetunji et al., 2020; Kocira et al., 2020; Koudahe et al., 2022). Interestingly, the addition of organic matter into the soil, like cover crop residue, can also generate a complex compound that binds heavy metals, particularly in the contaminated soil (Muddarisna and Siahaan, 2014; Leomo et al., 2021; Rashid et al., 2023). Therefore, both treatments have been widely applied in commercial agriculture to improve crop productivity (Purwanto and Alam, 2020; Kumar et al., 2021; Quintarelli et al., 2022; Hasan et al., 2024). Unfortunately, the effectiveness of those treatments for supporting crop cultivation in the post-mining soil is rarely evaluated despite the fact that it looks like the high potential to facilitate this effort.

This study investigated the potential use of rhizobacteria and cover crop residue to support the cultivation of *Sorghum* sp., which was developed at the post-nickel mining soil in Southeast Sulawesi. As one of the crops, *Sorghum* sp. is a species commonly developed by local communities in this area. However, the development of *Sorghum* sp. in Southeast Sulawesi has not been intensively conducted since it is highly difficult to find land with good fertility (Karimuna et al., 2020). The majority area in Southeast Sulawesi is dominated by nickel mining (Leomo et al., 2019; Pratiwi et al., 2021; Syaf et al., 2022); wherein during

the past five years, the activity of mine closure has been conducted on a large scale. Consequently, there is a large area of degraded land in this region. On the other hand, the effort of agriculture development is required to guarantee food security in this province. Therefore, this study is exceptionally required to integrate the activity of mining reclamation and agriculture development in Southeast Sulawesi.

## Materials and Methods

### Study area

This study was carried out at the laboratory level to support an easier monitoring process on a regular basis. The trial was established in the field laboratory managed by the Department of Soil Science, Faculty of Agriculture, Halu Oleo University. The land configuration was quite flat, with a slope level of 0-8%. The elevation reached 55 m above sea level. The average daily temperature was 27.6 °C with a mean air humidity of 80.9%. The annual rainfall ranged from 1,600 to 2,500 mm year<sup>-1</sup> during the past five years, from 2016 to 2020. The highest rainfall occurred in January. Dry periods are relatively longer than 5 months, from May to September.

### Experimental design

A factorial experiment comprising two levels of rhizobacteria and cover crop residue was set up in a completely randomized design with four replicates for every treatment. In this context, rhizobacteria treatment consisted of two factors, i.e., control plots that did not receive rhizobacteria application (C) and inoculation plots that received rhizobacteria application (B). The species of rhizobacteria used in this study was *Bacillus* sp., which has the natural ability to fix nitrogen. Meanwhile, two types of cover crop residues were applied in this study, i.e., *Eleusine indica* (E) and *Centrosema* sp. (S). Both species were generally used as cover crops in the commercial plantations around the study site. The details of the combination of every treatment are presented in Table 1.

Table 1. Treatment application in the experiment.

Treatment	Component of treatment		Replications
	Rhizobacteria	Cover crops	
CE	Control	<i>Eleusine indica</i>	4
CS	Control	<i>Centrosema</i> sp.	4
BE	<i>Bacillus</i> sp.	<i>Eleusine indica</i>	4
BS	<i>Bacillus</i> sp.	<i>Centrosema</i> sp.	4

The soil materials utilized in this study were collected from the post-nickel mining area located in Konawe District. Before starting the experiment, the soil characteristics were analyzed to evaluate its quality. This stage was important to obtain the initial information about soil quality from the post-mining

site as basic data to measure the effectiveness of treatment application for supporting the soil improvement process. In natural, better soil quality would provide more suitable environmental conditions for facilitating crop growth and development. Several parameters were used to quantify soil quality, namely

soil acidity, soil organic carbon, total nitrogen, exchangeable magnesium, exchangeable potassium, iron, and manganese (Table 2). The measurement of soil acidity was conducted using a digital pH meter, and soil organic carbon was quantified using the Walkley and Black method. Total nitrogen was calculated using the Kjeldahl method, while exchangeable potassium was analyzed using a flame photometer. The exchangeable magnesium and heavy metals contents (iron, manganese, and zinc) were measured using atomic absorption spectrophotometry. The soil analysis protocol was processed following the guidance of soil and water analysis (Estefan et al., 2013).

Table 2. Soil characteristics from post-nickel mining area used in this experiment.

Soil parameters	Symbol	Units	Value
Soil acidity	pH		5.64
Soil organic carbon	C-org	%	0.07
Total nitrogen	N-tot	%	0.03
Exchangeable magnesium	Mg-dd	cmol(+) kg <sup>-1</sup>	0.04
Exchangeable potassium	K-dd	cmol(+) kg <sup>-1</sup>	0.22
Iron	Fe	cmol(+) kg <sup>-1</sup>	7.44
Manganese	Mn	cmol(+) kg <sup>-1</sup>	7.44
Zinc	Zn	cmol(+) kg <sup>-1</sup>	2.68

After obtaining the preliminary soil information, the soil was placed into buckets and given a name tag to differentiate the treatments. Then, site preparation was done to arrange a homogeneous condition in the experiment. It was required to minimize the bias observation due to the effect of environmental conditions outside the treatment. Afterward, 10 mL suspension of *Bacillus* sp. was inoculated into the bucket following the layout of the experimental design. The cover crop residue was also mixed in the bucket with a dose of 200 g for every treatment (Figure 1). A month later, the seeds of *Sorghum* sp. were planted in the bucket. This plant was selected as an alternative crop in this study because it was commonly cultivated by local communities in Southeast Sulawesi as the main food ingredient besides corn. The application of fertilizer was not conducted to observe the natural effect of rhizobacteria and cover crop residue on the growth performance of *Sorghum* sp., which was planted in the post-nickel mining soil. However, maintenance activities like manual weed control and watering processes were implemented to support the growth of *Sorghum* sp. in every treatment plot.

#### Data collection

The process of data collection was divided into two stages, i.e., soil improvement evaluation and crop productivity measurement. The evaluation of soil improvement aimed to assess the effectiveness of

treatment application for supporting soil improvement transition. It was conducted by re-analyzing soil quality a month after providing treatment application. To facilitate this stage, the soil sample from every treatment plot was collected before the planting process. Then, the soil sample was composited into four groups following the type of treatment application before re-analyzing its quality.

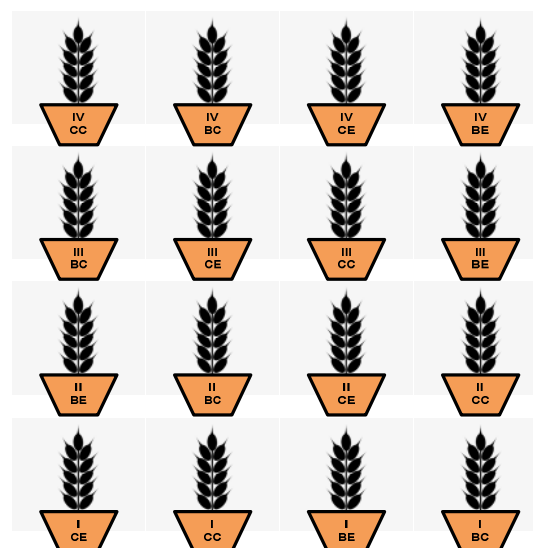


Figure 1. Layout of experimental design.

Afterward, the measurement of crop productivity was done periodically every two weeks. The time period for monitoring the growth performance of *Sorghum* sp. was ten weeks. Several parameters were measured to evaluate the growth performance of *Sorghum* sp. in each treatment, including height, diameter, total leaves, and leaf area. Moreover, the harvesting process was also undertaken at the end of observation to measure biomass accumulation in every crop. This process was done step by step in a chronological manner. After crops were harvested, their components were separated into three parts, namely roots, shoots, and flowers. The fresh weight of every component was determined using a hanging balance. Then, the crop components were brought to the laboratory for the drying process using an air oven at 70 °C for 48 h before being weighed for biomass determination (Wirabuana et al., 2021).

#### Data analysis

Statistical analysis was processed using a significant level of 5%. A descriptive test was conducted to identify data attributes, including minimum, maximum, mean, and standard deviation (Mishra et al., 2019). The normality of data was evaluated by the Shapiro-Wilk test (Ghasemi and Zahediasl, 2012). The homogeneity of variance among treatments was examined using the Fligner-Killeen test (Beyene, 2016). A comparison of soil improvement among treatments was presented descriptively using the actual

value and percentage unit for every soil parameter. Meanwhile, the comparison analysis of *Sorghum* sp. productivity among treatments was tested by the Kruskal-Wallis test and followed by the Kruskal-Nemenyi test (Sadono et al., 2021).

## Results and Discussion

### Soil improvement

Summarized results of the experiment indicated the use of rhizobacteria and cover crop residue potentially accelerated the improvement process of soil quality from the post-mining site (Table 3). The application of treatments demonstrated a high potential to increase nutrients. This study recorded the highest trend of soil improvement from all treatments, which was found in total nitrogen (700-833%), followed by soil organic carbon (414-500%) and exchangeable magnesium (250-425%). In contrast, the lowest improvement process was noted in soil acidity level with a ranging from 20 to 28%. These findings indicated that the combination of rhizobacteria and cover crop residue was effective in helping the soil improvement effort at the post-mining site. This study also observed that the

use of BS treatment resulted in higher soil improvement than others, particularly related to the soil organic carbon, total nitrogen, exchangeable magnesium, and manganese content (Table 3).

The application of this treatment increased soil organic carbon by 500%, total nitrogen by 833%, and exchangeable magnesium by 425%. On the other hand, this treatment reduced the manganese content in the post-mining soil by around 88.43%. The results could have occurred since the BS treatment was composed of a combination of *Bacillus* sp. and *Centrosema* sp. Several studies reported that *Bacillus* sp. was a rhizobacteria species that had high ability for nitrogen fixation and phosphorus dissolution (Kashyap et al., 2019; Widawati and Suliasih, 2019; Miljaković et al., 2020; Saxena et al., 2020). The addition of *Bacillus* sp. in the soil could accelerate nitrogen mineralization and phosphorus dissolution (Widdig et al., 2019; Stepien et al., 2022; Iqbal et al., 2024). It explained why the application of *Bacillus* sp. in the post-mining soil could contribute to accelerating the soil improvement process. Meanwhile, *Centrosema* sp. is a cover crop species that had good ability for nitrogen fixation (John et al., 2011; Indriani et al., 2019; Solis et al., 2019; Pipai et al., 2023).

Table 3. Soil quality improvement of every treatment a month after application.

Symbol	Unit	CE		CS		BE		BS	
		Actual	IMP (%)	Actual	IMP (%)	Actual	IMP (%)	Actual	IMP (%)
pH		6.82	20.92	6.97	23.58	7.25	28.55	7.05	25
C-org	%	0.36	414.29	0.36	414.29	0.36	414.29	0.42	500
N-tot	%	0.24	700	0.27	800	0.27	800	0.28	833
Mg-dd	cmol(+) kg <sup>-1</sup>	0.14	250	0.16	300	0.18	350	0.21	425
K-dd	cmol(+) kg <sup>-1</sup>	0.86	290.91	0.74	236.36	0.71	222.73	0.74	236.36
Fe	cmol(+) kg <sup>-1</sup>	3.07	(58.71)	3.6	(51.58)	4.12	(44.59)	4.67	(37.19)
Mn	cmol(+) kg <sup>-1</sup>	1.26	(83.05)	1.12	(84.94)	0.99	(86.68)	0.86	(88.43)
Zn	cmol(+) kg <sup>-1</sup>	0.9	(66.36)	1.05	(60.75)	1.21	(54.77)	1.37	(48.78)

Note: pH (soil acidity); C-org (soil organic carbon); N-tot (total nitrogen); Mg-dd (exchangeable magnesium); K-dd (exchangeable potassium); Fe (iron); Mn (manganese); Zn (Zinc); IMP (soil improvement level); CE (control+*Eleusine indica*); CS (control+*Centrosema* sp.); BE (*Bacillus* sp.+*Eleusine indica*); BS (*Bacillus* sp.+*Centrosema* sp.). The number between the brackets indicates the reduction percentage.

The principal utilization of *Centrosema* sp. as the organic matter would increase the availability of nutrients in the soil. A study explained that every 100 g biomass of *Centrosema* sp. residue contained 3.18 g N, 0.12 g P, 0.3 g K, and 0.38 g Mg (Teitzel and Burt, 1976). This fact confirmed why the application of BE treatment generated a higher improvement in soil nitrogen and exchangeable magnesium than other treatments. Moreover, the use of *Centrosema* sp. as the additional organic matter could also reduce the manganese content in soil because when it was decomposed, it formed complex compounds and brought the heavy metals immobile (Egli et al., 2010). In the context of mining reclamation, the decreasing heavy metals content become the primary challenge since they became limiting factors that inhibit the revegetation process (Yang et al., 2010; Sanchez-

Castro et al., 2023; Zhakypbek et al., 2024). Therefore, the utilization of cover crop residue like *Centrosema* sp. could become a good solution for accelerating soil improvement at the post-mining site.

### Growth performance of sorghum

Results of this study showed that there was no significant difference in the growth performance of *Sorghum* sp. among treatments applied both in single or combination treatments (Table 4). Nevertheless, the use of rhizobacteria and cover crop residue to support the growth performance of *Sorghum* sp. in post-mining soil still demonstrated prospective results. For example, without inoculating rhizobacteria in the post-mining soil, the biomass accumulation of *Sorghum* sp. was relatively lower, around 16.76%, than in the treatment of rhizobacteria application.

Table 4. Growth performance of *Sorghum* sp. among different treatment applications.

Treatment	Height (cm)	Diameter (cm)	Total Leaves	Leaf Area (cm <sup>2</sup> )	Biomass component (g)			Total biomass (g)
					Root	Shoot	Flower	
<b>R response</b>								
Control	76.28±3.59 a	2.35±0.21 a	6.16±0.40 a	687.28±75.73 a	0.77±0.14 a	1.98±0.82 a	0.12±0.03 a	2.88±0.91 a
<i>Bacillus</i> sp.	77.66±7.57 a	2.45±0.20 a	6.33±0.51 a	667.29±190.69 a	0.82±0.33 a	2.45±0.85 a	0.18±0.08 a	3.46±0.79 a
p-value	0.748 ns	0.320 ns	0.523 ns	0.872 ns	0.999 ns	0.336 ns	0.332 ns	0.262 ns
<b>C response</b>								
<i>Eleusine indica</i>	76.43±5.99 a	2.41±0.21 a	6.16±0.40 a	705.12±89.88 a	0.80±0.17 a	2.33±0.91 a	0.13±0.02 a	3.27±0.86 a
<i>Centrosema</i> sp.	77.51±5.89 a	2.38±0.22 a	6.33±0.51 a	649.44±180.0 a	0.79±0.32 a	2.10±0.81 a	0.17±0.08 a	3.07±0.95 a
p-value	0.748 ns	0.561 ns	0.523 ns	0.521 ns	0.872 ns	0.872 ns	0.258 ns	0.872 ns
<b>R * C response</b>								
Control * <i>Eleusine indica</i>	74.30±3.74 a	2.33±0.25 a	6.00±0.00 a	699.06±84.55 a	0.80±0.04 a	1.89±0.27 a	0.11±0.02 a	2.81±0.33 a
Control * <i>Centrosema</i> sp.	78.26±2.54 a	2.36±0.23 a	6.33±0.57 a	675.49±82.29 a	0.73±0.21 a	2.07±1.26 a	0.14±0.03 a	2.95±1.40 a
<i>Bacillus</i> sp. * <i>Eleusine indica</i>	78.56±7.87 a	2.50±0.17 a	6.33±0.57 a	711.19±113.74 a	0.80±0.26 a	2.77±1.19 a	0.15±0.00 a	3.72±1.06 a
<i>Bacillus</i> sp. * <i>Centrosema</i> sp.	76.76±8.87 a	2.40±0.26 a	6.33±0.57 a	623.40±268.68 a	0.84±0.45 a	2.14±0.27 a	0.21±0.11 a	3.20±0.50 a
p-value	0.691 ns	0.682 ns	0.747 ns	0.838 ns	0.988 ns	0.715 ns	0.451 ns	0.588 ns

Note: R (rhizobacteria); C (cover crop residue); \*(interaction); ns (non-significant difference based on the ANOVA test); a similar letter in column indicates a non-significant difference based on post hoc test.

Moreover, the addition of residue of *E. indica* as organic matter potentially increased the total biomass of *Sorghum* sp. by approximately 8.79% compared to the utilization of cover crop residue from *Centrosema* sp. Interestingly, the relative contribution of crop components to total biomass accumulation was

relatively similar among treatment applications (Figure 2). However, the results indicated that the combination of *Bacillus* sp. and *Eleusine indica* generated the highest productivity of *Sorghum* sp. than others. This condition could have happened since both factors have a complementary interaction.

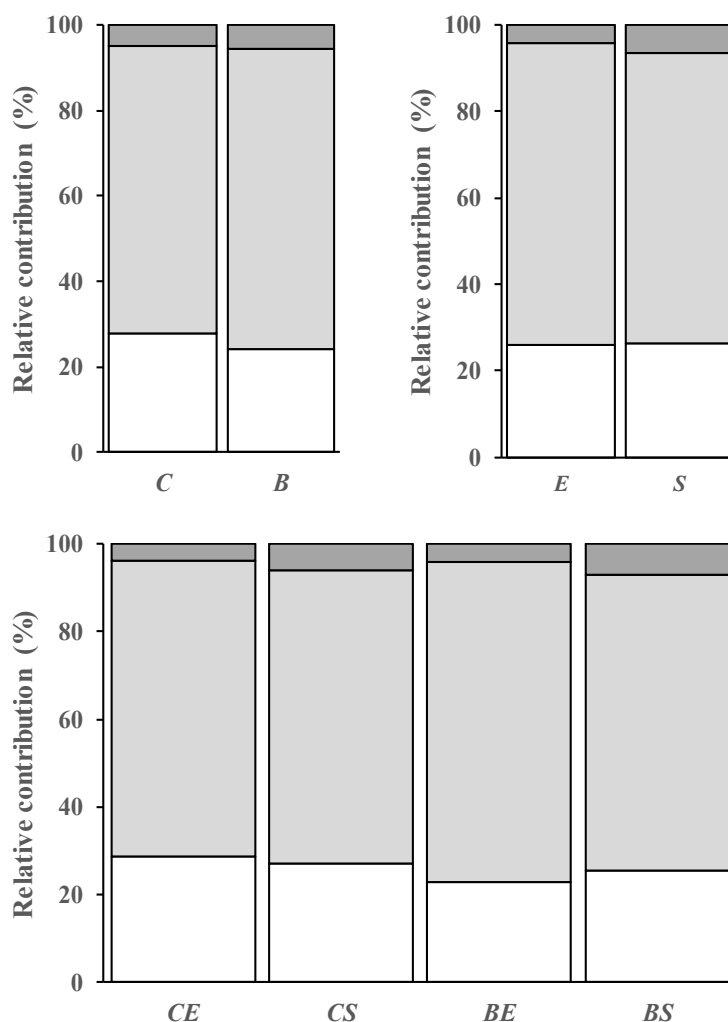


Figure 2. Relative contribution of every crop component into total biomass accumulation among different treatment applications.

As one of the rhizobacteria species, the addition of *Bacillus* sp. in the post-mining soil would accelerate the mineralization process (Grzyb et al., 2020); thus, the plant could absorb nutrients efficiently, primarily for nitrogen. In addition, the symbiotic relationship between *Bacillus* sp. and plant roots could stimulate the production of auxins that are classified as essential phytohormones for plants (Asari et al., 2017). On the other hand, the use of *Eleusine indica* residue as organic matter could accelerate the phytoremediation process for heavy metals (Hamzah et al., 2017). Therefore, the toxicity level of soil would decline, and it also increased the opportunity for plants to survive in marginal conditions. Finally, this study realized that the utilization of rhizobacteria and cover crop residue

offered prospective results for improving crop productivity in post-nickel mining soil.

## Conclusion

This study concluded that the utilization of rhizobacteria and cover crop residue demonstrated a high potential to support the growth of *Sorghum* sp., which developed in the post-mining soil in Southeast Sulawesi. The application of both treatments potentially improved soil quality by increasing nutrient availability and reducing heavy metals content. Based on the results, the combination treatment of *Bacillus* sp. and *Eleusine indica* generated relatively higher productivity of *Sorghum* sp. than other treatments.

Thereby, this treatment could be recommended to support the effort of agriculture development at the post-mining soil in Southeast Sulawesi.

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