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Native plant composition and soil microfauna in limestone post-mining land as potential for development of ruminant forage

HARWANTO[1,2], PANCA DEWI MANU HARA KARTI[3,♥], SUWARDI[4,5], LUKI ABDULLAH[3]

[1]Graduate School of Nutrition and Feed Technology, Faculty of Animal Science, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia

[2]Faculty of Animal Science, Universitas Jenderal Soedirman. Jl. Dr. Soeparno No. 60, Purwokerto Utara, Banyumas 53122, Central Java, Indonesia

[3]Department of Nutrition and Feed Technology, Faculty of Animal Science, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8622841, Fax.: +62-251-8622842, ♥email: pancadewi_fapetipb@yahoo.com

[4]Department of Soil Science and Land Resources, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia

[5]Center for Mine Reclamation Studies, Institut Pertanian Bogor. Jl Raya Pajajaran, Bogor 16143, West Java, Indonesia

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Abstract. *Harwanto, Karti PDMH, Suwardi, Abdullah L. 2023. Native plant composition and soil microfauna in limestone post-mining land as potential for development of ruminant forage. Biodiversitas 24: 6332-6342.* Limestone post-mining land is a potential marginal land, through revegetation and integration, as a forage provider. Therefore, utilizing an exploratory systematic random sampling, this research explored the native botanical composition that had the potential for forage and soil microfauna associated with plants in the limestone post-mining land of PT Sinar Tambang Arthalestari, Banyumas, Central Java, Indonesia from June to September 2023. The results showed that the limestone post-mining soil had a slightly alkaline pH and low fertility. The botanical composition comprised 16 grasses, four legumes, and 21 forbs. Native plants that have the highest Importance Value Index (IVI) were grasses; *Ischaemum rugosum, Imperata cylindrica, Paspalum scrobiculatum,* and legumes; *Mimosa pudica, Centrosema pubescens,* and *Calopogonium mucunoides*. Grasses contained 8.35-12.61% Crude Protein (CP) and 42.50-50.78% Total Digestible Nutrient (TDN), while legumes contained 17.06-17.74% CP, 62.12-64.75% TDN. *Mycorrhizal* colonization and populations of *Azotobacter*, *Azospirilum,* and *Rhizobium* were associated with native plants in the rhizosphere. Conclusively, the grasses *I. rugosum, I. cylindrica*, *P. scrobiculatum*, and the legumes *M. pudica, C. pubescens,* and *C. mucunoides* have the potential to forage on limestone post-mining land and are associated with soil microfauna. However, soil fertility requires further improvement by applying organic matter, nitrogen, and phosphorus fertilizers.

Keywords: Botanical composition, forage, limestone post-mining, microfauna, soil fertility

INTRODUCTION

Forage is an important component in the ruminant industry, requiring continuous study to provide livestock feed. The persistent problem for the industry is decreasing land area as a planting medium for forage plants. Land conversion for other purposes is prevalent (BPS 2021), and it affects the efforts to optimize the value of marginal soils used as the source of forage for ruminants, including limestone post-mining land.

The lime mining area in Indonesia has increased from 7,835,405 ha to 27,695,416 ha from 2013 to 2019 (BPS 2019). Mining activities have caused the loss of vegetation and topsoil, decreased soil organic matter, destroyed soil nutrients, and increased temperature and pH (Ortega et al. 2020). At high pH, the availability of phosphorus and micro minerals such as Fe, Mn, Zn, and Cu decreases, thus causing plant nutrient deficiencies (Roques et al. 2013). Efforts to repair post-mining land is called reclamation, which, according to previous studies (Pratiwi et al. 2021), can be done through recontouring, land processing, adding fertilizer, revegetation, and microbial utilization to improve its productivity. One potential use of the productive post-mining land is as a forage producer for ruminants.

Forage development on post-mining land needs to explore the presence of native plants. Pratiwi et al. (2021) state that native plants can grow naturally in mining areas, even in dry and infertile soil conditions. Khatiwada et al. (2020) stated that local plants such as grass, legumes, and forbs are equally feasible for ruminant feed. Grasses and legumes have more massive and deep roots in the soil than agricultural plants, making them more adaptive to marginal soil. Legumes can also increase forage production and soil nutrient quality and provide sufficient nutrients for ruminants (Voisin et al. 2013).

The adaptability of local plants is also influenced by the presence of soil microfauna such as arbuscular mycorrhizal fungi (Chen et al. 2018) and indigenous nitrogen-fixing bacteria like *Rhizobium*, *Azotobacter*, and *Azospirillum* (Fukami et al. 2018). Jaiswal et al. (2021) stated that *Rhizobium* is a symbiotic bacterium that forms nodules with the roots of legume plants, while *Azotobacter* and *Azospirilum* bacteria are non-symbiotic bacteria that can live freely in the soil and rhizosphere. Another soil microfauna is mycorrhiza, a fungus that can help absorb nutrients like phosphorus and water by infecting the roots and forming an intensive hypha, thereby increasing plant growth (Karti et al. 2018). Nitrogen-fixing bacteria convert free nitrogen into plant-available nitrogen through ammonification and nitrification (Grzyb et al. 2021). Microbes are essential in mineralizing macro and micro nutrients for plant growth, metabolism, and development (Meena et al. 2014). Jacoby et al. (2017) stated that soil microfauna is influenced by soil fertility, pH, macro and micronutrients, and host plants, requiring continuous study to know their association with plant species in a certain area.

This study explores the existence of botanical compositions that have the potential as forage for livestock and soil microfauna, likes Arbuscular Mycorrhizal Fungi (AMF) and indigenous nitrogen-fixing bacteria that are in association with native plants in limestone post-mining land in Banyumas District, Indonesia. We expect the results of this study can inform the possibility and feasibility of integrating forage plants to provide livestock feed into revegetation activity in limestone post-mining land.

MATERIALS AND METHODS

Study area

This research was carried out exploratively on a limestone post-mining land that belongs to PT. Sinar Tambang Arthalestari in Banyumas, Central Java, Indonesia, at the coordinates of 109°5'0-20"E to 7°25'50"-26'0" S, between June and September 2023. Lying 215 meters above sea level, Banyumas has an average temperature of 32.78°C, 17,750 lux sunlight intensity, and 86.78% relative humidity (primary data). The land area was two hectares and divided into 12 sampling locations (Figure 1).

Exploration procedures

Soil chemical properties

Soil samples were taken in a representative manner at the post-mining reclamation land at a depth of 20 cm based on a method by Widawati and Sulasih (2019). The samples were analyzed for soil chemical properties that included pH, C-organic, Nitrogen (N), Potassium (K), Cation Exchange Capacity (CEC), Electrical Conductivity (EC), and soil texture based on Rowell (1994), Phosphorus (P) by Olsen principle (Olsen et al. 1954). The soil fertility criteria were classified based on Landon (1984).

Botanical composition

Exploration was undertaken, performing a systematic random sampling using a 1 m^2 quadrant in 12 location points to represent the reclamation area. The observations were aimed to identify the local plant species (Kumalasari et al. 2014; Xu and Zhou 2017). The plant diversity was calculated based on the Importance Value Index (IVI) (Mulya et al. 2021) and grouped into grass, legume, and forb (Guretzky 2005). The samples of each plant species were weighed as fresh weight, then dried at 60°C for 48 hours, and prepared for dry weight for Dry Matter (DM) basis analysis and nutrient analysis using near-infrared reflectance spectroscopy based on Despal et al. (2021). The nutrient profile analysis consisted of Crude Protein (CP), Crude Fiber (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Total Digestible Nutrients (TDN).

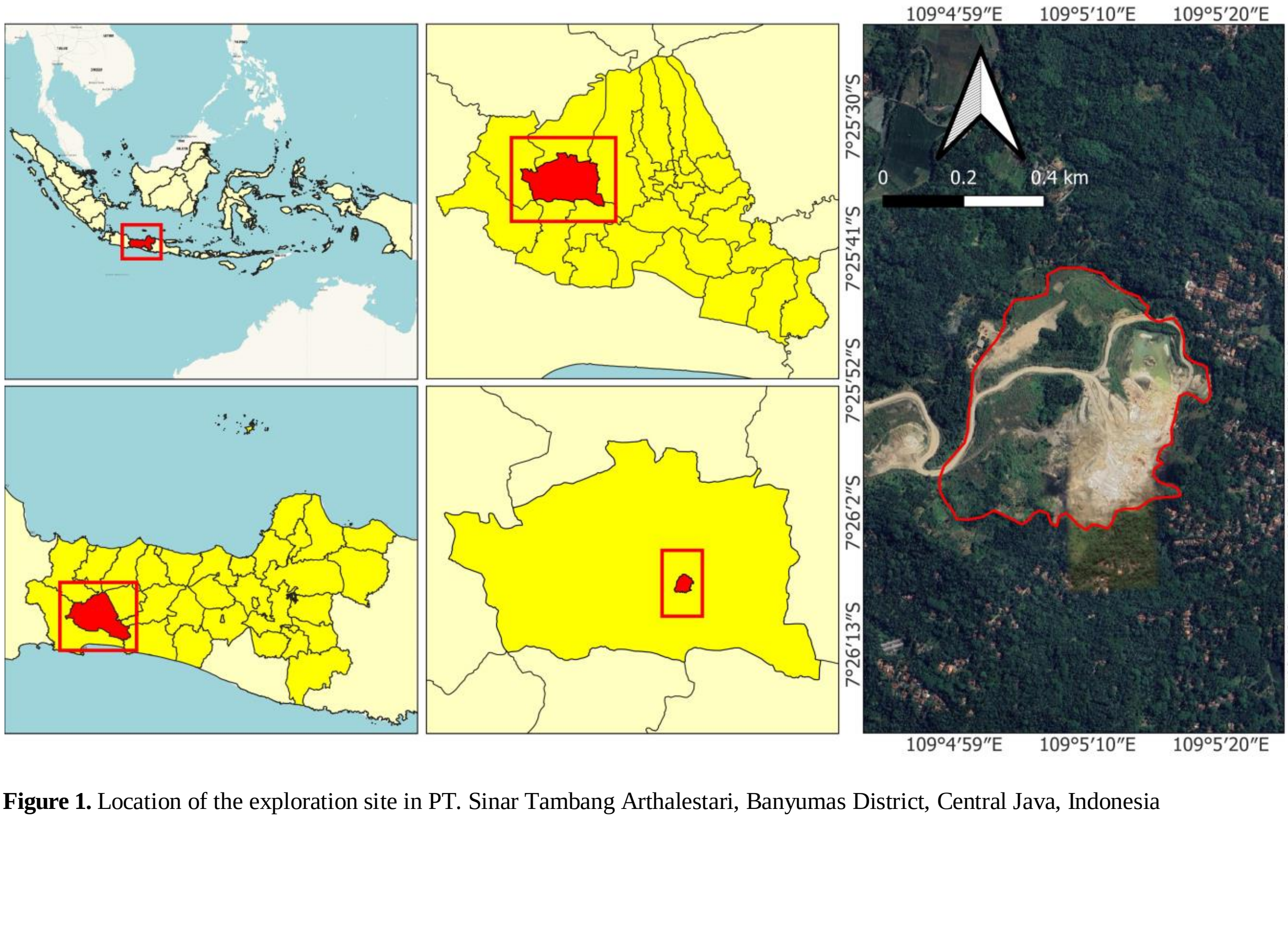

Figure 1. Location of the exploration site in PT. Sinar Tambang Arthalestari, Banyumas District, Central Java, Indonesia

Mycorrhiza identification

Mycorrhiza identification and colonization were carried out on native plants with the highest IVI in three grass and three legume species. The preparation for identifying and counting mycorrhizal spores applied the wet sieving techniques (Brundrett et al. 1996). Spores were counted under a microscope using a hand counter and expressed as a total of 100 g^{-1} spores of soil. Spore characteristics were identified by observing spore samples on glass slides under a microscope at 1000x magnification. Mycorrhizal spore types were grouped based on morphological characteristics at the morphospecies level.

Preparing for mycorrhizal colonization testing on roots used the staining technique based on Phillips and Hayman (1970). The staining solution to observe the colonization comprised 400 mL of glycerol, 400 mL of lactic acid, 200 mL of distilled water, and 0.05 g of trypan blue. Microscopic observations were carried out at 400x magnification with approximately ten fields for each root section. The percentage of infected roots was calculated as follows:

$$\% \text{ Mycorrhizal colonization} = \frac{\text{Infected mycorrhiza area}}{\text{Sum of view observed}} \times 100\%$$

Indigenous nitrogen-fixing bacteria

The nitrogen-fixing bacteria analysis was applied to *Azotobacter*, *Azospirilum*, and *Rhizobium*. Samples were taken in the rhizosphere area of the soil at a depth of 0-20 cm using a method by Widawati and Sulasih (2019). Testing for *Azotobacter* and *Azospirilum* was carried out on the rhizosphere of local plants, which had the highest IVI in three grass species and three legume species. Meanwhile, testing for *Rhizobium* was only carried out on the rhizosphere of three legume species. The native plant species with the highest IVI are the dominant ones in the respective area.

Bacterial isolation was carried out on selected media according to Husen et al. (2022) *Azotobacter* on Ashby mannitol, *Rhizobium* on yeast mannitol agar, and *Azospirilum* on Caceres medium. About 10 g of rhizosphere soil from each plant was put into an Erlermeyer flask containing 90 mL of distilled water and mixed in a rotary shaker at 120 rpm for 30 minutes. The solution was serially diluted from 10^{-2} to 10^{-7}. Next, 1 mL of each serial dilution was taken, inoculated into the bacterial growth selection medium in a sterile petri dish, and incubated at room temperature for 3-5 days until colonies formed. Growing colonies were counted using the total plate count. The observed morphological characteristics of bacteria were size, pigmentation, optical characteristics, shape, elevation, surface, and margins (Holt et al. 1994). Cell shape and gram staining were carried out based on Claus (1992), and the accuracy of bacterial isolates was determined using Bergey's Systematic Bacteriology based on Krieg and Dobereiner (1984).

Data analysis

Data on botanical compositions, mycorrhiza spore types, and indigenous nitrogen-fixing bacteria were presented in figures and tables, subjected to descriptive analysis, and compared with findings from previous studies. Data *Mycorrhiza* colonization was analyzed statistically using analysis of variance and significantly different results were analyzed using Duncan multiple range test.

RESULTS AND DISCUSSION

Soil chemical properties

The physical and macronutrient soil properties illustrated in Table 1 showed that the limestone post-mining soil had slightly alkaline and low fertility characteristics with 0.52% organic carbon content (very low), 0.24% total N (low), 16.13 ppm of P (low), and 42.20 $cmol^{(+)}$ kg^{-1} of cation exchange capacity (moderate). According to Shafer et al. (2001), organic matter is important for soil quality because it influences the soil's ability to bind and provide plant nutrients and water, encourages and maintains root growth, creates biotically suitable habitats, and resists degradation. Alkaline soil causes low absorbability of phosphorus elements because it is bound by calcium. High calcium levels will cause phosphate deposition because it is more reactive with Ca^{2+} ions to form $Ca_3(PO_4)_2$, which is difficult to dissolve (Whitehead 2000).

Cation Exchange Capacity (CEC) is a chemical property of soil closely related to soil fertility because only with low CEC can soil absorb and provide nutrients for plant growth (Landon 1984). The soil texture of limestone post-mining soil in this study was sandy clay, meaning a greater ability to bind water than sand-textured soil. It was related to its adsorptive surface area, in which the finer the texture, the greater its water-holding capacity. The chemical and physical characteristics of limestone post-mining soil will likely influence the diversity of local plants and indigenous nitrogen-fixing bacteria. Similarly, Mulya et al. (2021) report that plant and bacterial diversity is influenced by nutrient availability and soil texture.

Botanical composition

The results showed that 41 species with 12 families of 16 grasses, four legumes, and 21 forbs were found in limestone post-mining land (Table 2). The dominance of growing plants can be observed from the parameter of the plant's role in the community or the Important Value Index (IVI). In this study, the IVI was divided into 96.41 grass, 41.15 legumes, and 62.44 forbs. The highest IVI were observed in three grass species (*Ischaemum rugosum*, *Imperata cylindrica*, *Paspalum scrobiculatum)* three legume species (*Mimosa pudica*, *Centrosema pubescens*, *Calopogonium mucunoides*), and three forb species (*Chromolaena odorata*, *Euphorbia hirta*, *Spermacoce laevis*). Each plant species shows particular adaptation ability and tolerance to environmental conditions (Pratiwi et al. 2021), and the species with the highest IVI are the dominant ones in the respective area (Razali et al. 2014); an important value index above 10% indicates a substantial role in the community. Overall, *I. rugosum* has the highest IVI in limestone post-mining soil, while the local plant with the lowest IVI is *Ageratum conyzoides*. Therefore, this study showed grasses' adaptability to grow and develop in low-fertility soil (Table 1).

In this study (Table 2), fresh biomass weight and dry weight were higher in grasses (552.17 g m^{-2} and 177.25 g m^{-2}) than in legumes (198.93 g m^{-2} and 62.26 g m^{-2}) and forbs (342.26 g m^{-2} and 75.41 g m^{-2}). The highest dry weight was identified in *I. cylindrica* grass, *C. pubescens* legume, and *C. odorata* forb. The results showed that these plants are adaptive in low-fertility soil and can compete with other local plants, absorb soil nutrients, and convert them into higher biomass. According to Gulwa et al. (2018), grass-legume can mutually increase productivity; as a carbon source, grasses and legume plants can form a symbiotic relationship with nitrogen-fixing bacteria, which results in fixing free nitrogen into available nitrogen and increasing soil porosity and fertility.

According to Crowder and Chheda (1982), the pasture quality was classified as ideal if the proportion of grass dry biomass to legumes is 3:2. While the comparison of grass and legume in this study was 177.25 g m^{-2} vs 62.27 g m^{-2} or equivalent to 3:1. These results indicate that the proportion of native plant for livestock was still low; grass species and forbs dominate more than legumes. The grass is an energy source, while legumes are a protein source. A solution that can be taken to improve the quality is by improving soil fertilizers and introducing non-native plants into limestone post-mining land so that the proportion of grass and legumes provides sufficient forage for livestock.

Nutrient profile of native plants

The nutrient profile of native plants growing on limestone land is presented in Table 3. The crude protein content of native grasses, legumes, and forbs ranged between 8.35 and 22.01% (Table 3), higher than that of *Brachiaria brizantha* (6% to 8%) for grazing ruminants reported by Guerra et al. (2019). This result indicates that native plants growing on limestone post-mining have the potential to provide CP for livestock grazing. The average of the CP values was higher in legumes (17.37%) than in grasses (11.11%) and forbs (14.99%). As per native plant species, the highest IVI, *I. rugosum* grass, contained CP at 10.99%, *C. pubescens* legume contained at 17.54%, and *C. odorata* forb contained at 18.14%. Meanwhile, the lowest CP was found in *I. cylindrica* (8.35%). The crude protein of native plants in this study was higher than local plants in the coal post-mining (6.05%) with acidic soil, as Ansori et al. (2013) reported. According to Onjai-uea et al. (2022), protein is the most influential aspect in ruminal fermentation, nutrient digestibility, and ruminant productivity: species, age at pruning, leaf proportion, and soil quality influenced forage protein content.

Information on the nutrient profile content of native plants is crucial to evaluate their potential as ruminant feed. Native plants are a potential source of forage, especially protein for ruminants. Native legumes such as *C. pubescens* (17.54% CP) and *C. mucunoides* (17.74% CP) are prospective protein supplements to fulfill nitrogen deficiency in ruminants. This means that the CP of local plants in limestone post-mining areas can be used as a forage provider for ruminants. In contrast to Forb, *C. odorata* has a high CP (18.14%) and 10.07 IVI, but based on Daru et al. (2023), *C. odorata* is not palatable by livestock, so it is not recommended as ruminant forage. Other forb plants, including *Asystasia gangetica* and *Biden pilosa*, that grow on post-limestone mining land have higher CP (16.43% and 16.38%) and are palatable for ruminants (Yang et al. 2021; Daru et al. 2023) but have low IVI which correlates with low adaptation. Melo et al. (2022) stated that the contributing factors to local plant nutrient content are the climatic conditions during growth and harvest, especially temperature, light intensity, rainfall, and soil conditions.

In this study, grasses had higher crude fiber, NDF, and ADF (31.49%; 64.51%; 35.98%) than legumes (20.83%; 47.02%; 33.25%) and forbs (25.37%; 52.01%; 33.43%). Fiber will be degraded into volatile fatty acids and absorbed by ruminants through ruminal fermentation, producing energy for ruminants. Shi et al. (2023) stated that NDF indicates CF quality in ruminant diets. It plays a critical role in the digestive process of ruminants, as it affects feed intake, rumen fermentation, and nutrient metabolism. Increasing NDF content affects a decrease in feed intake. The NDF content of native plants in this study is similar to that of corn silage and local oat hay (47.39% and 64.38%) reported by Shi et al. (2023), but lower than in natural pasture (69.58%) reported by Yalew et al. (2020). The differences in NDF might be attributed to plant species, soil, forage processing, and climatic conditions. This result indicates the need to combine NDF of native plants (grasses-legume-forbs) so that they could be the nutrient provider for livestock. This result is similar to Gulwa et al. (2018), that grasses and legumes are needed to complement each other's nutritional elements to serve as livestock feed.

Table 1. Chemical characteristics of post-mining limestone soil

Soil property	Value	Landon's category (1984)
pH H_2O	8.08±0.14	Slightly Alkaline
pH KCl	7.32±0.03	Slightly Alkaline
C-Organic (%)	0.52±0.11	Very low
N-Total (%)	0.24±0.11	Low
NO_3^+ (%)	0.07±0.02	Very low
NH_4^+ (%)	0.03±0.02	Very low
P_2O_5 (Olsen) ppm	16.13±6.45	Low
Ca (cmol$^{(+)}$ kg^{-1})	58.90±5.24	High
Mg (cmol$^{(+)}$ kg^{-1})	2.12±0.79	High
K (cmol$^{(+)}$ kg^{-1})	0.52±0.20	Moderate
Na (cmol$^{(+)}$ kg^{-1})	0.19±0.08	Low
Cation Exchange Capacity (cmol$^{(+)}$ kg^{-1})	42.20±3.88	Moderate
Electrical conductivity (μS/cm)	220±42.19	Moderate
Texture		
Sand (%)	45.21±4.97	Sandy clay
Dust (%)	24.19±4.97	
Clay (%)	30.51±6.71	

Note: C: Carbon, NO_3^+: Nitrate, NH_4^+: Ammonium, P_2O_5: Phosphorus pentoxide, Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium

The average of the TDN values was higher in legumes (63.20%) than in grasses (46.54%) and forbs (57.13%). According to Kearl (1982), ruminants require 50% TDN of feed for basic life. Local grasses in this study contained the highest biomass but low TDN, so they must be combined with other plants to provide TDN ruminants. These results show that native plants in limestone post-mining areas in Banyumas have met the criteria as ruminant feed (Table 3), although lower than those of weeds in paddy areas reported by Kumalasari et al. (2014). The nutrient profile of native plants in this study might be influenced by the post-limestone mining soil chemical properties (Table 1). Khalil et al. (2014) stated that plant growth largely depends on the soil quality where it grows, and different species express and respond to their tolerance differently. These results indicate that levels of organic carbon, available nitrogen (NO_3^+ and NH_4^+), phosphorus, magnesium, potassium, and sodium in soil (Table 1) influence CP, CF, NDF, ADF, and TDN of native plants (Table 3). The soil characteristics will produce differences in the productivity and quality of plants.

Table 2. Botanical Composition in limestone post-mining land

Species	Family	IVI	Fresh weight (g m^{-2})	Dry weight (g m^{-2})	Dry matter basis (%)
Grass					
Ischaemum rugosum	Poaceae	22.12	75.16	24.29	32.32
Imperata cylindrica	Poaceae	19.71	175.00	52.62	30.07
Paspalum scrobiculatum	Poaceae	7.52	19.70	7.19	30.48
Saccharum spontaneum	Poaceae	6.74	135.73	49.05	36.14
Paspalum dilalatum	Poaceae	5.77	17.15	6.32	36.84
Digitaria longiflora	Poaceae	5.47	44.03	13.26	30.12
Eleusine indica	Poaceae	4.88	23.05	6.83	29.65
Cyperus michelianus	Cyperaceae	4.45	11.29	2.96	26.19
Melinis repens	Poaceae	4.02	16.31	4.80	29.46
Paspalum conjugatum	Poaceae	3.47	7.47	2.70	36.10
Digitaria ternata	Poaceae	3.34	13.32	2.77	20.77
Themeda arguens	Poaceae	2.26	5.48	1.84	33.57
Echinochloa colona	Poaceae	1.94	3.03	0.76	25.10
Paspalum notatum	Poaceae	1.63	1.84	0.49	26.68
Lolium perenne	Poaceae	1.60	0.93	0.24	25.71
Polytrias indica	Poaceae	1.48	2.68	1.13	42.19
	Sub-total	96.41	552.17	177.25	32.10±5.57
Legume					
Mimosa pudica	Fabaceae	16.10	26.92	9.32	34.62
Centrosema pubescens	Fabaceae	14.77	118.46	37.80	31.91
Calopogonium mucunoides	Fabaceae	8.09	36.20	11.21	30.98
Vigna trilobata	Fabaceae	2.19	17.35	3.93	22.67
	Sub-total	41.15	198.93	62.26	31.30±5.15
Forb					
Chromolaena odorata	Asteraceae	10.07	170.60	38.06	22.31
Euphorbia hirta	Euphorbiaceae	5.17	23.82	5.82	24.44
Spermacoce laevis	Rubiaceae	4.68	3.01	0.54	17.8
Tridax procumbens	Asteraceae	4.30	15.22	2.86	18.82
Conyza sumatrensis	Asteraceae	4.15	6.47	1.22	18.94
Emilia sonchifolia	Asteraceae	3.61	13.09	2.13	16.29
Acanthispermum hispidum	Asteraceae	3.47	20.38	4.11	20.15
Fimbristylis autumnalis	Cyperaceae	2.86	26.24	6.57	25.05
Asystasia gangetica	Acanthaceae	2.73	3.03	0.59	19.55
Cuphea sp.	Lythraceae	2.69	5.71	1.27	22.15
Cyathula prostrata	Amaranthaceae	2.59	1.51	0.25	16.68
Bidens pilosa	Asteraceae	2.52	1.42	0.28	19.85
Eclipta prostrata	Asteraceae	2.23	5.39	0.97	18.05
Acmella uliginosa	Asteraceae	1.95	13.91	3.05	21.96
Mikania micrantha	Asteraceae	1.67	1.13	0.18	15.66
Porophyllum ruderale	Asteraceae	1.63	3.23	0.67	20.87
Alternanthera philoxeroides	Amaranthaceae	1.56	2.97	0.63	21.22
Spigelia anthelmia	Loganiaceae	1.56	0.43	0.07	15.65
Melastoma sp.	Melastomataceae	1.24	13.64	3.97	29.09
Ipomoea triloba	Convolvulaceae	0.96	10.18	2.02	19.82
Ageratum conyzoides	Asteraceae	0.82	0.88	0.15	17.35
	Sub-total	62.44	342.26	75.41	22.03±3.35

Note: IVI: Importance Value Index

Table 3. Nutrient profile of native plants that grow on limestone post-mining

Species	**CP (%)**	**CF (%)**	**NDF (%)**	**ADF (%)**	**TDN (%)**
Grass					
I. rugosum	10.99	33.43	66.13	35.03	45.72
I. cylindrica	8.35	33.08	70.48	42.51	45.93
P. scrobiculatum	11.44	32.60	64.75	37.84	45.22
S. spontaneum	9.70	30.56	64.29	39.44	46.59
P. dilalatum	10.94	31.13	61.99	32.44	43.62
D. longiflora	11.58	31.86	63.01	33.57	46.35
E. indica	10.01	32.09	67.58	35.47	42.50
F. autumnalis	10.51	33.81	66.06	36.39	45.39
M. repens	12.61	30.45	64.83	35.25	47.00
P. conjugatum	10.81	30.99	65.14	37.79	47.72
D. ternata	11.81	31.75	64.21	36.90	45.74
T. arguens	12.15	30.05	62.13	34.12	48.26
E. colona	12.60	30.65	62.46	33.01	47.17
P. notatum	11.00	29.82	63.48	34.76	47.75
L. perenne	11.91	30.64	62.52	33.72	48.83
P. indica	11.42	30.95	63.17	37.43	50.78
Average	11.11±1.11	31.49±1.22	64.51±2.25	35.98±2.63	46.54±1.99
Legume					
M. pudica	17.06	21.18	44.76	26.54	63.29
C. pubescens	17.54	18.25	47.97	38.08	64.75
C. mucunoides	17.74	21.59	46.91	33.80	62.12
V. trilobata	17.15	22.29	48.45	34.56	62.65
Average	17.37±0.32	20.83±1.78	47.02±1.64	33.25±4.84	63.20±1.14
Forb					
C. odorata	18.14	23.49	40.46	35.99	63.04
E. hirta	14.89	28.01	54.63	26.03	51.99
S. laevis	15.63	26.30	45.28	31.08	59.18
C. michelianus	10.97	29.43	64.43	34.25	53.93
T. procumbens	15.14	26.00	44.83	40.00	55.52
C. sumatrensis	14.86	21.72	45.20	33.95	59.63
E. sonchifolia	10.32	22.59	70.95	26.34	54.62
A. hispidun	11.78	25.33	49.76	32.13	50.86
A. gangetica	16.43	23.9	49.61	34.07	60.25
Cuphea sp.	15.83	22.79	46.68	37.14	62.91
C. prostrata	14.75	23.06	43.34	38.40	61.03
B. pilosa	16.38	23.51	43.69	35.75	60.13
E. prostrata	22.01	24.03	39.96	29.02	62.31
A. uliginosa	20.31	22.42	39.25	31.66	70.31
M. micrantha	11.25	31.80	66.32	39.86	48.20
P. ruderale	14.93	23.53	56.19	30.73	56.48
A. philoxeroides	18.38	24.02	59.12	33.12	59.65
S. anthelmia	11.42	32.39	65.84	33.00	46.19
Melastoma sp.	14.10	27.19	53.67	37.86	59.76
I. triloba	14.67	26.85	51.82	31.37	56.87
A. conyzoides	12.54	24.51	61.21	30.28	46.85
Average	14.99±3.05	25.37±3.00	52.01±9.59	33.43±3.95	57.13±5.99

Note: CP: Crude Protein, CF: Crude Fiber, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, TDN: Total Digestible Nutrients

Mycorrhizal diversity

The presence and density of mycorrhizal spores in the diversity of native plants were calculated on limestone post-mining land in Table 4. The spore density of *I. rugosum, I. cylindrica*, *P. scrobiculatum, M. pudica, C. pubescens*, and *C. mucunoides* plants, respectively was 10-415, 40-625, 30-325, 60, 45-275, and 15-60 spores/100 g of soil. We found three mycorrhizal genera associated with native forage species, namely *Acaulospora* sp., *Gigaspora* sp., and *Glomus* sp., comprising 17 spore morphospecies. It shows that the genus and morphospecies of mycorrhiza are adaptive in post-limestone mining soil where low nutrients and high pH are expected. According to Suharno and Sancayaningsih (2013), *Glomus* sp. is a mycorrhiza that contributes to the resilience and increase of plant growth in rehabilitating mining land. Our effort to discover indigenous mycorrhiza in limestone post-mining areas is environmentally friendly, and we have successfully identified adaptive, symbiotic plants. This finding is expected to improve marginal land and promote plant growth.

Grass plants and legumes with the highest spore density were *I. cylindrica* and *C. pubescens*, respectively. The size of the spores varies from each plant, ranging from 70 to 270 µm. Mycorrhizae is globose to oval shaped and has a wall and bulbous suspensor spores with yellowish to brownish color (Figure 2). The diversity of mycorrhizal morphospecies in limestone post-mining land was higher than in asphalt post-mining land (9 morphospecies) reported by Tuheteru et al. (2022) and gold post-mining land (6 morphospecies) reported by Suharno et al. (2014). The presence of mycorrhizal spores in the soil is influenced by two factors: soil chemical conditions and host plants where the latter is reported by Trouvelot et al. (2015) to affect the spores through their ability to form roots in the soil.

Imperata cylindrica plants had the highest mycorrhizal colonization on the roots, namely 38.11 ($P<0.05$) compared to other plants (Table 5). However, the colonization of all plants in this study was under 50% (low category), requiring an optimized symbiosis or introduction of non-indigenous mycorrhizae. Mycorrhizal colonization in this study was lower than that of *Glirisidia cepium* plants (100%) in coal post-mining areas (Salim et al. 2020) but higher than *Setaria* sp. plants (23.33%) in gold post-mining land in Timika (Suharno et al. 2014).

Table 4. Mycorrhizal morphospecies and density

Native plant	Morphospecies	Density (100 g soil)	Spore size (µm)	Form	Color
I. rugosum	*Gigaspora* sp.3	10	160-180	Boulbus suspensor	Yellowish
	Glomus sp.4	415	130-180	Boulbus suspensor	Yellowish
I. cylindrica	*Acaulospora* sp.1	625	120-130	Globose	Transparent
	Acaulospora sp.2	80	100-150	Oval	Transparent
	Gigaspora sp.1	40	70-130	Globose	Transparent
	Glomus sp.1	115	90-130	Globose	Transparent
P. scrobiculatum	*Gigaspora* sp.2	30	70-100	Globose	Yellowish
	Glomus sp.2	130	200-270	Globose	Yellowish
	Glomus sp.3	325	150-200	Spore-wall	Yellowish
M. pudica	*Gigaspora* sp.4	60	90-100	Spore-wall	Yellow
	Glomus sp.5	60	90- 150	Spore-wall	Yellowish
C. pubescens	*Acaulospora* sp.3	275	70-70	Spore-wall	Brownish
	Acaulospora sp.4	45	70-100	Globose	Yellowish
	Glomus sp.6	45	120-140	Globose	Transparent
	Glomus sp.7	55	80-140	Globose	Yellowish
C. mucunoides	*Acaulospora* sp.5	60	150-180	Spora-wall	Brownish
	Glomus sp.8	15	90-150	Globose	Brownish-yellowish

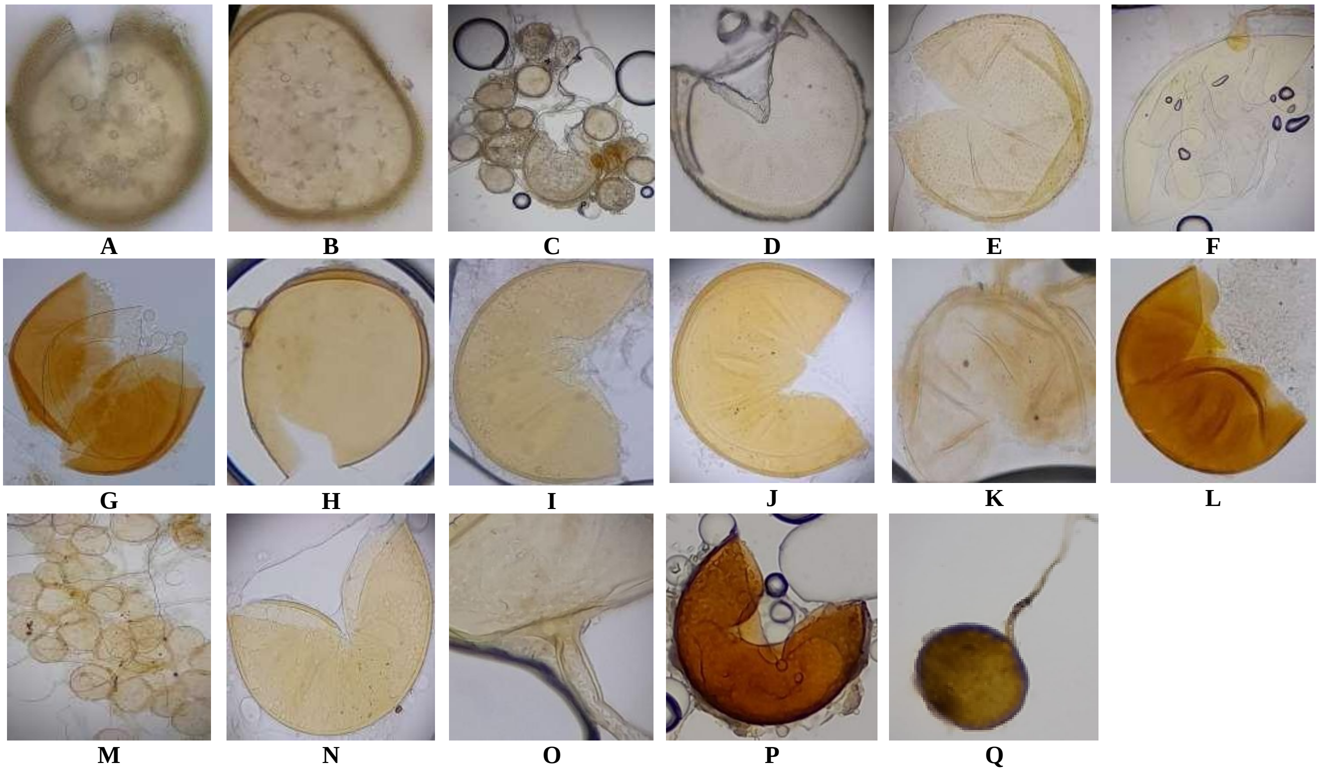

Figure 2. Mycorrhizal spore types in limestone post-mining with 1000x magnification A. *Acaulospora* sp.1, B. *Acaulospora* sp.2, C. *Acaulospora* sp.3, D. *Acaulospora* sp.4, E. *Acaulospora* sp.5, F. *Gigaspora* sp.1, G. *Gigaspora* sp.2, H. *Gigaspora* sp.3, I. *Gigaspora* sp.4, J. *Glomus* sp.1, K. *Glomus* sp.2, L. *Glomus* sp.3, M. *Glomus* sp.4, N. *Glomus* sp.5, O. *Glomus* sp.6, P. *Glomus* sp.7, Q. *Glomus* sp.8

The root shape of vegetation influences the level of mycorrhizal colonization in plants. According to Choi et al. (2018), plants with broad and deep root systems can likely form colonies with mycorrhiza. Mycorrhizal colonization begins with hyphae infection in the roots, forming vesicles and arbuscular in the root tissue. Vesicles are a place to store food, while arbuscular is where nutrients between the mycorrhiza and the host plant are exchanged (Chen et al. 2018). Trouvelot et al. (2015) stated that soil environmental factors, including abiotic and biotic factors, influence mycorrhiza's effectiveness. The former includes nutrient concentration, pH, water content, temperature, soil processing, and fertilizer use, while the latter are interactions between microbes, fungus species, host plants, and host plant root types.

Indigenous *Azotobacter* sp.

The Azotobacter population in the local plant rhizosphere with the highest IVI can be seen in Table 6. The results showed that *the Azotobacter* sp. population of the legume rhizosphere is higher than the grass. *Azotobacter* populations were found in the rhizosphere of *M. pudica (*4.65 x 10^5 cfu g^{-1} soil), *C. pubescens (*4.75 x 10^5 cfu g^{-1} soil), and *C. mucunoides* (3.95 x 10^5 cfu g^{-1} soil), or averagely 4.45 x 10^5 cfu g^{-1} soil. Meanwhile, *Azotobacter* populations were found in the grass of *I. cylindrica (*2.95 x 10^4 cfu g^{-1} soil), *P. scrobiculatum* (4.55 x 10^4 cfu g^{-1}), and *I. rugosum* (2.60 x 10^4 cfu g^{-1}), or averagely 3.37 x 10^4 cfu g^{-1} soil. Therefore, *C. pubescens* is associated with the highest *Azotobacter* sp. compared to other plants. This result showed that *Azotobacter* can live in limestone post-mining soil with an alkaline pH (8.08) and low fertility (Table 1). The *Azotobacter* population in this study was lower than in neutral soil (1.0 x 10^6 cfu g^{-1} soil) reported by Widawati and Sulasih (2019) but higher than in acidic soil (1.0 x 10^3 cfu g^{-1} soil) reported by Sulaiman et al. (2022).

Sumbul et al. (2020) stated that pH greatly influences the growth and activity of bacteria in the soil; most bacteria live and have high activity at neutral pH. *Azotobacter* sp. is widely distributed in soil, although in relatively limited amounts in certain soil conditions. According to Holt et al. (1994), the *Azotobacter* genus can grow at a pH ranging from 4.8 to 8.5, while the optimum pH for growth and nitrogen fixation is 7.0-7.5. In addition to soil pH, other contributing factors to *Azotobacter* populations are organic matter, chemical properties, plant associations, and other microorganisms (Aasfar et al. 2021).

The observation showed that *Azotobacter* sp. is a Gram-negative bacterium with a coccus cell shape. *Azotobacter* sp. has a small-moderate size, white pigmentation, optically translucent characteristics, circular-irregular colony shape, convex elevation, smooth-slimy surface, and entire-undulate margins. This research is similar to Zhengtao et al. (2019), the genus *Azotobacter* is a Gram-negative bacterium with small colonies, moist-transparent, and irregular form. According to Ward and Jensen (2014), *Azotobacter* sp. is a non-symbiotic bacterium associated with various plants and can fix N as an element available to plants. This result shows that the presence of *Azotobacter* is positively correlated with adaptation and biomass produced by plants in Table 2. Widawati and Sulasih (2019) added that the physical properties of soil influence the bacterial population's level, the availability of organic material, including macro and microelements and plant species, and the increase of plant growth.

Indigenous *Azospirilum* sp.

Table 7 showed that grass-legume growing on limestone post-mining land (pH 8.08) was associated with the *Azospirilum* sp. that was found in the rhizosphere of *I. cylindrica* (3.20 x 10^4 cfu g^{-1}), *P. scrobiculatum* (2.60 x 10^4 cfu g^{-1}), and *I. rugosum* 3.20 x 10^4 cfu g^{-1}, so averagely 3.00 x 10^4 cfu g^{-1} soil. Meanwhile, *M. pudica* was found at (3.20 x 10^4 cfu g^{-1}), *C. pubescens* (3.90 x 10^4 cfu g^{-1}*)*, and *C. mucunoides* (3.30 x 10^4 cfu g^{-1}), or averagely 3.47 x 10^4 cfu g^{-1} soil. However, the *Azospirilum* population in this research was lower than in neutral pH (3.6 x 10^5 cfu g^{-1} soil) reported by Nur'ainy et al. (2020) but higher than in acidic soil pH (1.0 x 10^3 cfu g^{-1} soil) reported by Widawati and Suliasih (2019).

Table 5. Mycorrhizal colonization on roots

Native plant	Mycorrhizal colonization (%)
I. rugosum	$31.96^{ab} \pm 3.98$
I. cylindrica	$38.11^{b} \pm 5.88$
P. scrobiculatum	$32.62^{ab} \pm 4.33$
M. pudica	$29.63^{a} \pm 1.35$
C. pubescens	$28.49^{a} \pm 3.50$
C. mucunoides	$34.17^{ab} \pm 4.31$

Note: ^{ab}Different superscripts in the same column indicate significant differences (P<0.05)

Table 6. Characteristics of indigenous *Azotobacter* on native plant rhizosphere

Variable	Grass			Legume		
	I. cylindrica	***P. scrobiculatum***	***I. rugosum***	***M. pudica***	***C. pubescens***	***C. mucunoides***
Population (cfu g^{-1} soil)	2.95 x 10^4	4.55 x 10^4	2.60 x 10^4	4.65 x 10^5	4.75 x 10^5	3.95 x 10^5
Gram	Negative	Negative	Negative	Negative	Negative	Negative
Cell shape	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus
Size	Small	Moderate	Small	Moderate	Moderate	Moderate
Pigmentation	Turbid white	Turbid white	White	White	Turbid	Turbid
Optical	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
Form	Ireguler	Ireguler	Circular	Circular	Ireguler	Ireguler
Elevation	Convex	Convex	Convex	Convex	Convex	Convex
Surface	Smooth glossy-slimy	Smooth glossy-slimy	Smooth glossy-slimy	Smooth glossy-slimy	Smooth glossy-slimy	Smooth glossy-slimy
Margin	Undulate	Undulate	Entire	Entire	Undulate	Undulate

Table 7. Characteristics of indigenous *Azospirilum* on local plant rhizosphere

Variable	Grass			Legume		
	I. cylindrica	***P. scrobiculatum***	***I. rugosum***	***M. pudica***	***C. pubescens***	***C. mucunoides***
Population (cfu g^{-1} soil)	3.20 x 10^4	2.60 x 10^4	3.20 x 10^4	3.20 x 10^4	3.90 x 10^4	3.30 x 10^4
Gram	Negative	Negative	Negative	Negative	Negative	Negative
Cell shape	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus
Size	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Pigmentation	White	White	White	White	White	White
Optical	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
Form	Circular	Circular	Circular	Circular	Circular	Circular
Elevation	Convex	Convex	Convex	Convex	Convex	Convex
Surface	Smooth-shiny	Smooth-shiny	Smooth-shiny	Smooth-shiny	Smooth-shiny	Smooth-shiny
Margin	Entire	Entire	Entire	Entire	Entire	Entire

Table 8. Characteristics of indigenous *Rhizobium* sp. on native plant rhizosphere

Variable	***M. pudica***	***C. pubescens***	***C. mucunoides***
Population (cfu g^{-1} soil)	2.40 x 10^5	2.05 x 10^5	1.35 x 10^5
Gram	Negative	Negative	Negative
Cell shape	Coccus	Coccus	Coccus
Size	Small	Moderate	Moderate
Pigmentation	Milky white	Milky white	Milky white
Optical	Translucent	Translucent	Translucent
Form	Circular	Circular	Circular
Elevation	Convex	Convex	Convex
Surface	Smooth shiny	Smooth shiny	Smooth shiny
Margin	Entire	Entire	Entire

The observation showed that *Azospirillum* sp. is a gram-negative bacterium with a coccus shape, moderate size, white pigmentation, translucence, circular colony shape, convex elevation, smooth surface, and entire margins. This result is similar to Nur'ainy et al. (2020) that genus *Azospirillum* is small-to-moderate, white-creamy, and has lobate-entire surfaces. Fukami et al. (2018) state that *Azospirillum* sp. is a non-symbiotic *Rhizobacter* that lives in the soil around the roots but can help provide nitrogen through fixation. This result indicates that the presence of *Azospirillum* is positively correlated with local plant adaptation and biomass (Table 2). The *Azospirillum* helps provide nitrogen for native plants in the limestone post-mining to grow and produce biomass. According to Pedraza et al. (2020), *Azospirillum* sp. plays a substantial role in plant growth, such as forming lateral roots, adventitious roots, elongating primary roots, and determining the number of root hairs. This result indicates that *Azospirilum* sp. can be associated with local forage for integration with post-mining land reclamation.

Indigenous *Rhizobium* sp.

The results showed that legumes growing on limestone post-mining land are associated with *Rhizobium* sp. (Table 8). Their populations were found in the rhizosphere of *M. pudica (*2.40 x 10^5 cfu g^{-1}), *C. pubescens* (1.35 x 10^5 cfu/g*)*, and *C. mucunoides* (2.05 x 10^5 cfu g^{-1}), so averagely 1.93 x 10^5 cfu g^{-1}soil. The results of this study were comparable to Muller and Denison (2018) on legumes at neutral pH (10^5-10^6 cfu g^{-1} soil) and higher than Dhiman et al. (2019) on acidic soil (7.76 x 10^3 cfu g^{-1}soil). Zhou et al. (2017) reported that differences influence variations in bacterial populations in soil physical-chemical quality, environmental conditions, and host plants.

Rhizobium bacteria have a coccus cell morphology, small-moderate size, milky white pigmentation, translucent optical characteristics, circular shape, smooth surface convex elevation, and entire margins. These results (Table 2) follow the morphology of Paudyal et al. (2021), which showed that *Rhizobium* positively impacts native plant biomass. According to Concha and Doerner (2020), *Rhizobium* sp. can fix free nitrogen into ammonia, which will be converted into nitrogen compounds used by plants to grow and develop, improve nutrient quality, and increase soil fertility. In contrast, bacterial growth is influenced by soil chemistry, host plants, soil acidity, and environmental factors, such as temperature and water availability (Fahde et al 2023).

This study concludes that limestone post-mining soil in Banyumas has low fertility and slightly alkaline pH, so treatment is required to improve organic matter, nitrogen, and phosphorus fertilizers. Natural plants that have potential as forage for ruminants are grasses (*Ischaemum rugosum*, *Imperata cylindrica*, *Paspalum scrobiculatum*) and legumes (*Mimosa pudica*, *Centrosema pubescens*, *Calopogonium mucunoides*). Microfaunae such as mycorrhiza, *Azotobacter* sp., *Azospirilum* sp., and *Rhizobium* sp. have prospective development to support plant growth in post-limestone mining land. Mycorrhizal colonization on roots below 50% indicates a low category; therefore, an optimized symbiosis or introduction of non-indigenous mycorrhiza needs to be implemented in the development of forage plants in post-mining land. The population of indigenous nitrogen-fixing bacteria is positively correlated with adaptation and biomass produced by native plants.

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