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Research Centre for the Management of Degraded and Mining Lands, and Soil Department, Faculty of Agriculture, Brawijaya University
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Journal of Degraded and Mining Lands Management (JDMLM): ISSN:2339-076X (p); 2502-2458 (e) is an international journal providing rapid publication of peer-reviewed articles concerned with aspects directed towards the management of degraded and mining lands covering landscape topography, soil and water quality, biogeochemistry, ecosystem structure and function, and environmental, economic, social and health impacts of degraded and mining lands. Papers dealing with the results of original research and critical reviews on the above aspects are welcome.

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Research Article

Soil properties change, and arbuscular mycorrhizal fungi associated with plants growing on the post-gold mining land of Bombana, Indonesia

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Abstract

This study aimed to investigate the effect of gold mining on soil properties. Soil samples were taken from the post-gold mining land, the property of PT Panca Logam Nusantara and PT Alam Buana Indonesia, and a nearby natural forest in Bombana, Southeast Sulawesi Province. The next step focused on specifying soil pH, total nitrogen (TN) and carbon (TC) concentration, C/N ratio, available phosphorus (P) concentration, cation exchange capacity (CEC), and exchangeable K, Na, Mg, Ca, Fe, Mn, Cd and Pb concentration, texture and spore amount, AMF resource and AMF colonization. The result shows that the pH in post-gold mining soil was higher than that in natural forest soil. Meanwhile, TN, TC, available P, and CEC of post-gold mining soil got lower compared with these of natural forest soil. The texture in the post-mining soil was clay loam, while that in natural forest soil was clay. Total of 10 AMF species belonging to five genera and three families were found in a post-gold mining area. Soil pH, CEC, soil texture, Mn, and total Fe had a negative relation with AMF colonization and spore count, while organic C, total N, C/N ratio, P_2O_5 and silt had a positive relation. Sand was proven to have a strong and positive correlation with the amount of AMF species. Adding organic matter and fertilization as well as applying mycorrhizal biofertilizers, were urgently required to support the effort in restoring post-gold mining soil.

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Introduction

Gold mining is a sector that has been cultivated for a long time in Indonesia. Furthermore, Indonesia is the sixth largest gold producer worldwide, with total production reaching 190 tons in 2018 (Retinitiv, 2019). Large enterprises and other small-scale groups (artisanal and small-scale gold mining, ASGM) have participated in gold mining management in Indonesia. Over two thousand gold mining locations can be found throughout Indonesia. Bombana, in Southeast Sulawesi Province, has the potential gold resource, while this area has been managed since mid-2008. This gold mining industry has made a considerable contribution to the country in social and economic terms (Martins et al., 2020). However, mining activities can negatively affect the environment, as proven by the landscape change, particularly damage and loss of natural vegetation (Tepanosyan et al., 2018), land degradation (Ahyani, 2011; Wawo et al., 2015; Mailendra and Buchori, 2019) and soil and water contamination (Basri et al., 2017; Sakakibara et al., 2017; Basri et al., 2020), human health (Arifin et al., 2015), and other social aspects (Upe et al., 2019). Besides, mining activities generally result in low fertility and degradation in post-mining land (Ma et al., 2019). It was reported that soil degradation occurred in almost all activities for mining nickel (Prematuri et al., 2020a), coal (Pandey et al., 2014, Ma et al., 2019), and bauxite (Prematuri et al., 2020b).

Reclamation to the post-gold mining land is significantly urgent, and it has been implemented for a long time in Indonesia. Some companies, namely PT ANTAM Tbk Pongkor, Bogor (Siregar et al., 2013) and PT Newmont Minahasa Raya, North Sulawesi (Pollo et al., 2012), have successfully carried out reclamation to the post-gold mining land. The success of a reclamation effort is determined by the commitment of the company to having the permit and knowledge of its technical aspects. Thalib et al. (2020) reported that almost all companies in the gold mining sector of Bombana have not yet performed reclamation for the post-mining land. On a small scale for research, a reclamation effort has been made by designing a conservation plot for the endangered legume species in accordance with mycorrhizal biofertilizers at PT Panca Logam Makmur (Arif et al., 2021; Husna et al., 2021a). Thus, the first step was the evaluation of soil properties and vegetation succession levels. Tree growth and the quality of the post-mining land should be considered in the evaluation process (Maiti, 2013).

The soil itself can be a limiting factor in the post-mining area and determine the success of revegetation in reclamation (Matichenkov and Bocharnikova, 2021; Prescot et al., 2021). Soil is believed to be a fundamental aspect in a restoration effort towards a landscape of degraded land, including post-mining land (Stanturf et al., 2021). Physical, chemical, and biological traits in post-mining land do not allow the plant to grow well in any restoration activity (Sheoran et al., 2010). In the long run, soil quality improvement in the post-mining area mainly aims to build a sustainable forest ecosystem through the improvement of soil nutrients and plant growth (Pietrzykowski et al., 2013). Therefore, soil properties on the gold post-mining land need to be identified to select an appropriate restoration method. Restoration of the post-mining land can be passively performed through natural regeneration and actively through human intervention. The active restoration can be performed by planting the tree. Thus, the data on soil properties can be made into the database for choosing tree species and appropriate soil manipulation methods. Beneficial soil microbes, arbuscular mycorrhizal fungi (Husna et al., 2021a), for example, can be applied as a method for soil manipulation. This study aimed to investigate the relationships between soil chemical and physical properties and mycorrhizal fungi in the rhizosphere of soils of plant species grown on the post-gold mining land of Bombana, Indonesia, and the soil restoration approach.

Materials and Methods

Sampling and preparation of soil material

Soil samples were collected around the root of an adaptive plant in a post-gold mining area owned by PT Panca Logam Nusantara (PLN) and PT Alam Buana Indonesia (ABI), Bombana Regency, Southeast Sulawesi Province (Figure 1). The samples were taken from the rhizosphere in every living plant at a weight of approximately 1 kg and a depth of 0-20 cm. The next step was to put a soil sample in a respective plastic bag and write the location code and the plant name for every sampling point or plot. The whole soil samples were air-dried in the laboratory, in which this stage was intended for the isolation and identification of AMF spores. Composite soil (± 1 kg) in every location was shipped to SEAMEO BIOTROP Soil and Plant Laboratory in Bogor City.

Laboratory analysis of soil

In SEAMEO BIOTROP's Soil and Plant Laboratory of Bogor, Indonesia, an analysis was conducted on physical and chemical properties in soil. Soil pH was determined using the method described in SNI 03-6787-2002 for the measurement process. Soil organic C was analyzed using a method of SNI 13-4720-1998 (Walkey-Black). Total N was measured using micro-Kjeldahl (SNI 13-4721-1998), while available phosphorus in the form of P_2O_5 was measured using Bray method I/II (SL-MU-TT-05). Furthermore, Ca, Mg, Na, K, and cation exchange capacity (CEC) were measured by SL-MU-TT-07c (buffer extract NH_4OAc 1.0 N pH 7.0). Texture analysis of three fractions of sand, silt, and clay was conducted using SL-MU-TT-10 (Hydrometer) method. Total metal (Mn, Fe_2O_3, Cd, and Pb) was measured by HNO_3-$HClO_4$-ASS.

Soil trap culture

The trapping technique was implemented with a method proposed by Brundrett et al. (1996) using open culture pots. The medium used for planting was a mixture of 50 g of soil sample and 150 g of zeolite rock. Weaning *Sorghum bicolor* sprouts were planted in the pot. Watering, nutrient administration (red hyponex 20 mL/pot), and manual pest control were carried out as maintenance efforts.

AMF colonization

A root sample was cleaned and put in an alcohol solution at 70%. Meanwhile, arbuscular mycorrhizal fungi (AMF) colonies were observed with the trypan blue stain (Phillips and Hayman, 1970). Mycorrhizal roots were analyzed to determine AMF percentage, using the formula as follows: [Σ mycorrhizal roots / Σ total observed field of view] x 100%.

AMF isolation and identification

AMF spores were isolated with a wet pour-strain technique (Pacioni. 1992), followed by the centrifugation technique of Brundrett et al. (1996). Healthy spores were selected, stored in a glass treated with PVLG and Melzer solution, and covered. AMF spores were identified by observing their morphology in terms of shape, size, color, carrier hyphae, spore ornaments, spore mother cells, as well as bulbous suspensors. The nomenclature of AMF spores was conducted following the guidelines of Schüßler and Walker (2010) and Redecker (2013). AMF spores were identified under a Nikon Eclipse 80i Microscope at the Cryptogam Laboratory, Centre for Biology Research, LIPI, Bogor City.

Parameters

This study observed physical and chemical properties in the soil, spore density, species richness (Tuheteru et al., 2020), and AMF colonization (Brundrett et al., 1996). The soil was restored following the guidelines developed by Stanturf et al. (2021) and Young et al. (2022).

Data analysis

The whole data were analyzed through Microsoft Excel. The relationships between soil properties, the amount of vegetation with AMF spore density, species resource, and root colonization were performed using Pearson's correlation.

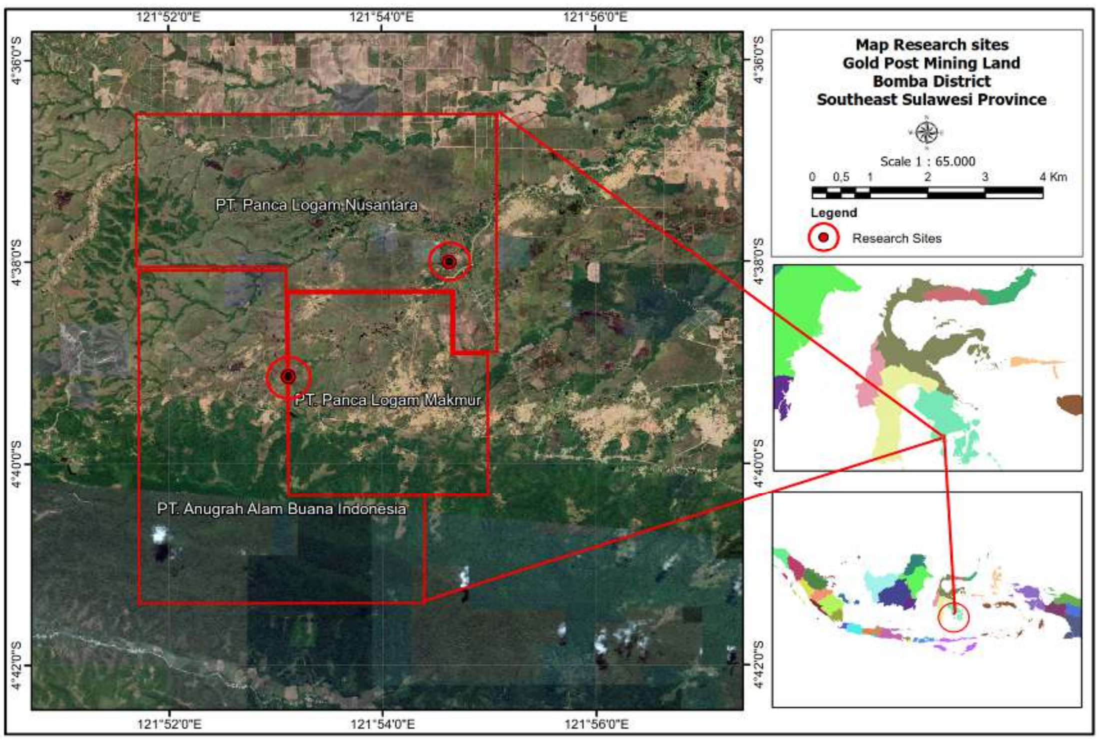

Figure 1. Location of the area studied.

Results and Discussion

Soil chemical and physical properties

The result of the analysis of physical and chemical properties in the soil is shown in Table 1. Soil pH was slightly alkaline in both post-gold mining locations. Total N content, C/N ratio, and P_2O_5 content were low, while soil organic C, Mn, and Fe contents were very low. However, soil K and Mg contents were very high. Soil pH, organic C, total N, C/N ratio, and P_2O_5 in post-mining soils were lower than in savannah soil, while Fe in the post-mining soil was higher than in savannah soil. CEC in the post-mining soil of PT Panca Logam Nusantara was lower than in savannah soil, while the total Mn of the post-mining soils was higher than that of savannah soil. Mining activities can lead to the damage of land as indicated by the pH, organic C, total N, P_2O_5 of the post-mining soils than savannah soil (Table 1). The damage to soil as a consequence of mining activities has been reported by several researchers, such as nickel mining (Prematuri et al., 2020a), coal mining (Pandey et al., 2014; Ma et al., 2019), and bauxite mining (Prematuri et al., 2020b). Soil and overburden of artisanal gold mining in

Sekotong provided very low-level plant nutrients (Siswanto et al., 2012). It was also proven that Ca<Mg, base saturation of less than 20%, CEC of less than 16 cmol/kg, and Ca<Mg in the area caused growth stagnation during reclamation. Soil texture in the savannah and both post-gold mining locations was clay loam. The proportion of sand in both post-mining soils was higher than in savannah soil, while silt and clay were lower (Table 1). Clay loam soil can support water flow and nutrients for most tree species (Osman, 2013). Clay loam soil was observed in artisanal gold mining land in West Lombok (Siswanto et al., 2012).

Table 1. Chemical and physical properties of soils.

No.	Parameter	Unit	Soil				
			Savannah (reference)	PT. Panca Logam Nusantara		PT. Alam Buana Indonesia	
				Value	Change (%)	Value	Change (%)
1	pH H_2O		8.07±0.15	7.8±0.39	- 0.96 (4)	7.7±0.12	-0.95 (5)
2	Organic C	%	0.76±0.16	0.6±0.11	- 0.79 (21)	0.5±0.11	- 0.66 (34)
3	Total N	%	0.11±0.0	0.1±0.00	- 0.91 (9)	0.1±0.01	- 0.91 (9)
4	C/N ratio		7±1.73	6.6±1.19	- 0.94 (6)	7.2±0.97	+1.03 (3)
5	P_2O_5	ppm	18.70±1.06	7.1±3.48	- 0.38 (62)	6.6±1.20	- 0.35 (65)
6	Ca	cmol/kg	nd	6.9±0.94	nd	3.6±0.20	nd
7	Mg	cmol/kg	nd	4.5±0.21	nd	4.7±0.17	nd
8	Na	cmol/kg	nd	0.4±0.13	nd	0.3±0.05	nd
9	K	cmol/kg	nd	0.2±0.00	nd	8.9±0.13	nd
10	CEC	cmol/kg	23.77±2.04	20.8±3.33	- 0.87 (13)	25.4±0.52	+1.07 (7)
11	Texture						
	Sand	%	35.07±3.50	44.6±4.61	+ 1.27 (27)	40.3±4.92	+1.15 (15)
	Silt	%	30.13±0.23	24.9±5.42	- 0.83 (17)	25.8±2.93	- 0.86 (14)
	Clay	%	34.80±3.41	30.5±1.91	- 0.87 (12)	33.9±2.04	- 0.97 (3)
12	Mn	ppm	853.0±89.11	1000±0.02	+ 1.17 (17)	1000±0.02	- 1.17 (17)
13	Fe	%	1.30±0.09	2.8±0.65	+ 2.15 (115)	3.3±0.11	+ 2.54 (154)
14	Cd	ppm	nd	1.2±0.10	nd	1.2±0.03	nd
15	Pb	ppm	nd	18.6±3.93	nd	24.8±0.57	nd

nd = no data.

AMF association with plant

AMF colonization and spore density

The microscopic observation of plant roots discovered AMF structures, including internal hyphae, vesicles, external hyphae, and coil hyphae (Figure 2). The average value of AMF colonization on plant roots ranged from 57% to 61% (Table 2). Table 3 shows the AMF colonization on plant roots. There were three AMF spores per 100 g of soil from the field. The highest amount of spores from the field was discovered in the savannah, which got lower after being cultured in the trapping culture media (Table 2). Johnson et al. (2013) and Wang (2017) reported that soil damage due to mining activities might reduce or lose AMF propagation and infectivity.

Total AMF types in field and trap culture

A total of ten AMF species, including five genera viz, were identified at two sites. *Acaulospora, Glomus, Sclerocystis, Gigaspora*, and *Scutellospora* (Table 4 and Figure 3). Data presented in Table 4 show that 70% of the total 10 AMFs belong to Glomeraceae, followed by Gigasporaceae and Acaulosporaceae. The amount of AMF species in the savannah soil was lower than that in gold post-mining soil. Results of this study showed that the dominant AMF family was Glomeraceae at a percentage of 70%. Several studies also showed the same results, in which Glomeraceae was dominant in the post-mining area of nickel (Prayudyaningsih et al., 2019), coal (Ezeokoli et al., 2020), asphalt (Tuheteru et al., 2022), gold (Tuheteru et al., 2020), and limestone (Suting and Devi, 2021).

Table 2. AMF colonization and spore density.

Site	Plant richness	AMF Colonization (%)	Spore density	
			Field (100 g soil)	Trap culture (50 g media)
Savannah	14	72	12	17
PT. Panca Logam Nusantara	24	57	3	541
PT. Alam Buana Indonesia	36	61	3	320
Mean		59		

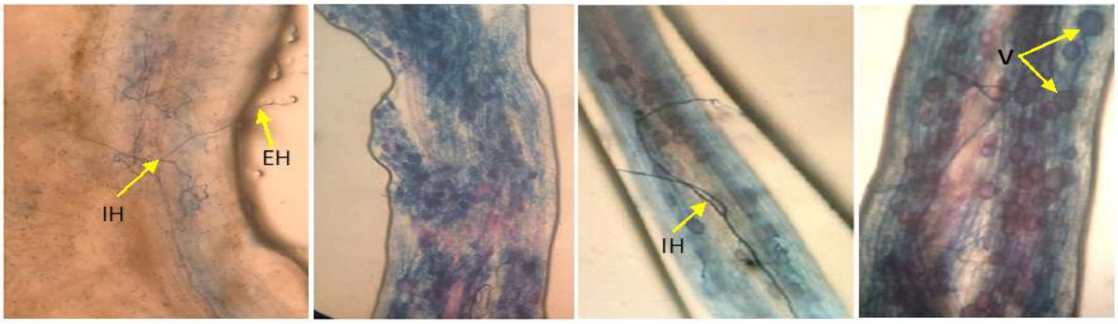

Figure 2. AMF colonization in adaptive plant roots *Codariocalyx* sp. *Acacia mangium*, *Calopogonium mucunoides* and *Imperata cylindrica* (IH Internal hyphae, EH external hyphae, V Vesicle).

Table 3. Percentage of AMF colonization on plant roots.

No	Species	Family	AMF colonization (%)	
			PT. Panca Logam Nusantara	PT. Alam Buana Indonesia
1	*Chromolaena odorata*	Asteraceae	-	53
2	*Fimbristylis dichotoma*	Cyperaceae	53	-
3	*Rhynchospora* sp.		93	-
4	Cyperaceae (sp. 1)		-	88
5	*Merremia hederacea*	Convolvulaceae	93	-
6	*Euphorbia* sp.	Euphorbiaceae	50	53
7	*Euphorbia hyssopifolia*		73	-
8	*Euphorbia heterophylla*		-	98
9	*Acacia mangium*	Fabaceae	68	-
10	*Aeschynomene americana*		-	43
11	*Alysicarpus vaginalis*		100	17
12	*Crotalaria* sp.		5	43
13	*Codariocalyx* sp.		63	-
14	*Indigofera* sp.		-	93
15	*Mimosa pudica*		95	30
16	*Uraria* sp.		30	68
17	*Melochia odorata*	Malvaceae	68	-
18	*Phyllanthus* sp.	Phyllanthaceae	100	68
19	*Calopogonium mucunoides*	Poaceae	30	73
20	*Chrysopogon* sp.		53	-
21	*Digitaria* sp.		-	100
22	*Imperata cylindrica*		50	93
23	*Saccharum* sp.		-	63
24	*Sorghum* sp.		-	50
25	Poaceae (sp. 1)		-	95
26	Poaceae (sp. 2)		-	100
27	*Oldenlandia diffusa*	Rubiaceae	43	-

Glomeracecae was reported to be dominant in the rhizosphere of several tropical tree species (Husna et al., 2021b). In Indonesia, Glomeraceae was reported to be dominant, having 36 species or 53% of 72 AMF species (Husna et al., 2021b). Glomeracecae was dominant for having AMF species that were tolerant and adaptive to the diverse condition of soil and environment, as well as the capability to survive in acid and alkaline soil to produce small spores in a shorter time, compared with *Gigaspora* and *Scutellospora*. *Glomus* had the most species in the phylum Glomeromycota (Schüßler and Walker, 2010). The Glomeraceae family was widely distributed and discovered in four climatic zones, seven continents, seventeen biomes, and many countries (Strumer et al., 2018). This study shows that the amount of AMF species in post-mining soil was higher compared with savanna soil. *G. coronatum, Glomus* sp. 1, and *S. sinousa* were discovered in savannah and post-mining soil.

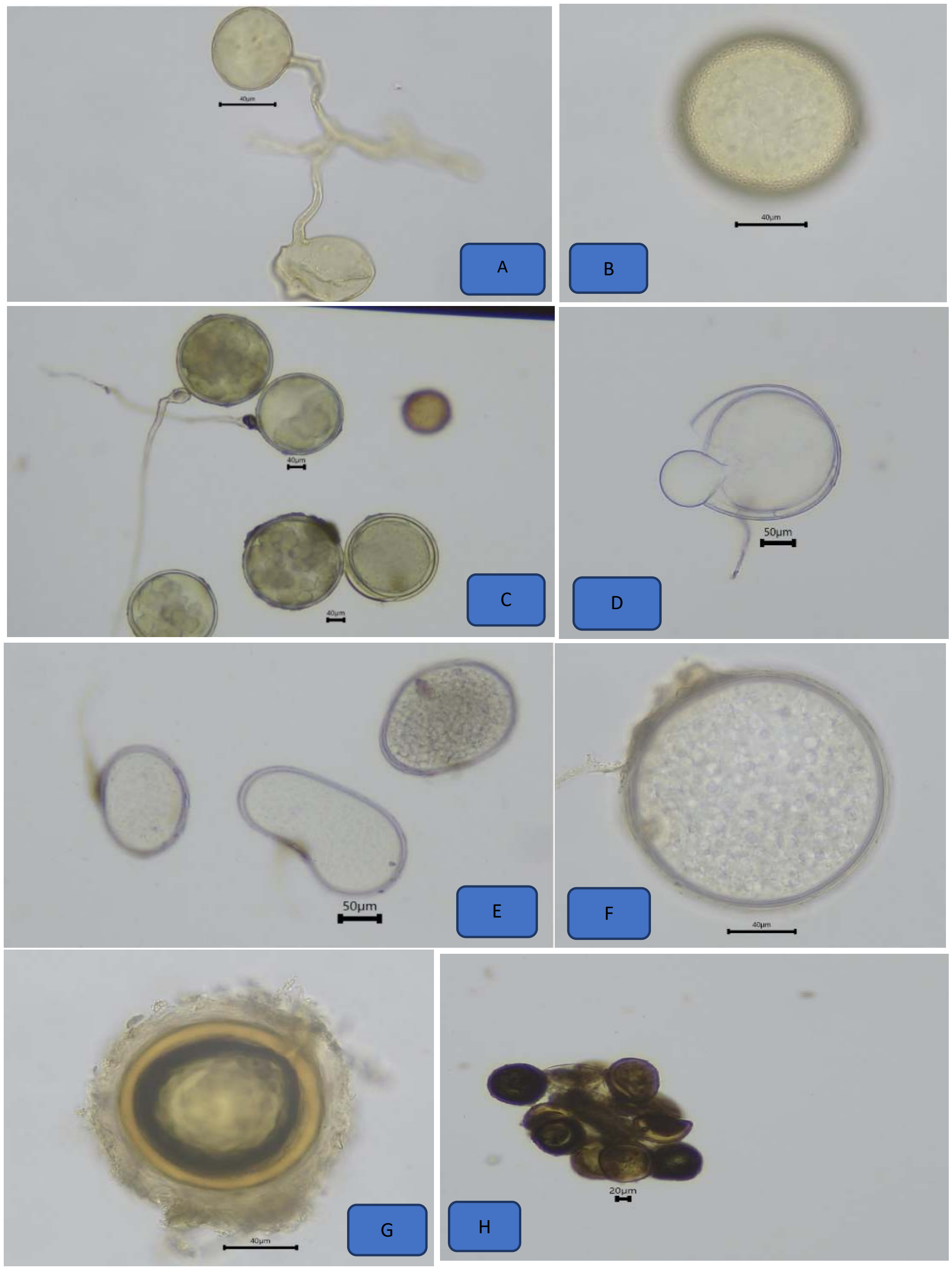

Figure 3. Types of AMF found A = *Glomus aggregatum*, ABI., B = *A. scrobiculata*, ABI., C = *Gigaspora* sp. ABI, D = *Gigaspora* sp., ABI, E = *Gigaspora* sp, PLN, F = *Gigaspora* sp. ABI, G = *Glomus coronatum*, H = *Glomus rubiforme*).

A. scrobiculata was discovered in post-gold mining owned by PT Panca Logam Makmur and a mining community in Bombana (Tuheteru et al., 2020). AMF discovered in three locations was *Glomus* sp. 1. Wide distribution of *Glomus* sp. 1 was attributed to a small size relatable to a high sporulation capacity, adaptability to the soil, climate, and diverse plant (Morales et al., 2019).

Relation between soil properties and arbuscular mycorhizal fungi parameters.

The whole soil properties were negatively related to an amount of AMF species, excluding its total Mn, total Fe, and amount of plants. Soil pH, organic C, total N, P_2O_5, silt, and clay positively related with AMF colonization and spore count (Table 5), while total Mn and Fe, sand, and vegetation were negatively related. The difference in the AMF types from every location was arguably caused by diverse ecological preferences in every AMF type. One of the factors affecting AMF abundance and diversity was soil property. This study shows that soil pH, CEC, soil texture, total Mn and Fe, as well as vegetation had a negative relation with AMF colonization and spore count. Meanwhile, organic C, total N, C/N ratio, P_2O_5, and silt had positive and strong correlations. Sand had a strong and positive correlation with the amount of AMF species. Soil pH determines spore density and AMF distribution (Bainard et al., 2015). High pH in the soil is capable of reducing the abundance of AMF species (Bainard et al., 2015). The result of this research is in line with another study by Wei et al. (2014) and Zu et al. (2018), which showed that soil Mn concentration is negatively related to AMF diversity. This research showed that P was positively correlated to spore amount and AMF colonization. The result of this research, however, is in contrast to previous research conducted by Wei et al. (2014), Soka and Ritchie (2018), and Chiomento et al. (2019), that low P was capable of improving AMF diversity. Johnson et al. (2013) reported that intense use of land was capable of changing soil properties and resulted in a decrease in species and AMF diversity.

Table 4. Glomeromycota species recovered from field soil and trap culture.

Family	AMF species	Savannah*		PT. Alam Buana Indonesia		PT. Panca Logam Nusantara	
		Fs	Tc	Fs	Tc	Fs	Tc
Acauloporaceae	*Acaulospora scrobiculata*			√	√		
Glomeraceae	*Glomus coronatum*	√		√	√	√	√
	Glomus agrregatum			√	√		
	Glomus sp.1	√		√	√	√	√
	Glomus sp.2			√		√	
	Sclerocystis rubiformis			√			
	Sclerocystis sinousa	√		√		√	
	Sclerocytis clavispora			√			
Gigasporaceae	*Gigaspora* sp.			√		√	
	Scutellospora sp.					√	
	Total	3		9		6	
				10			

*Tuheteru et al. (2020), Fs (Field soils), Tc (Trap cultures).

Restoration of soil/land

The restoration is required to improve soil conditions in post-gold mining land and Bombana. Restoration of the damaged and degraded land can be carried out in a passive way through natural regeneration or in an active one by involving human intervention (Bandyopadhyay and Maiti, 2019). Martins et al. (2020) proposed six ways to recover degraded land due to mining activities, in which two of the six ways are planting tree seedlings and natural regeneration. Restoration methods can be selected by determining ecosystem resilience, restoration purpose, landscape context, and expenditure for the restoration project (Festin et al., 2019). Referring to this option, most of the corporates having a gold-mining permit are not willing to carry out the mine reclamation (Thalib et al., 2020). Natural regeneration in the post-gold mining field in Bombana has been reported. Post-gold mining area was dominated by grass and covered with various trees, including *Acacia mangium, Alstonia scolaris, Neolamarckia cadamba, N. macrophyllus*, and *Nauclea orientalis* (Tuheteru et al., 2021; Albasri et al., 2021a,b). This study indicated six species of trees that grew naturally, namely *Anacardium ocidentale*, *A. mangium, Hibiscus* sp. *Leucaena leucocephala, Neolamarckia cadamba*, and *Tamarindus indicus*. However, all companies were asked to reclaim the post-mining land by implementing an active restoration effort as stipulated in Law No. 3 of 2020 concerning Mineral and Coal Mining. A form of reclamation in post-mining land is revegetation which is mostly determined by the option of tree species and the condition of the mining soil (Ahirwal and Maiti, 2021). The right choice of tree species was capable of improving the success rate of reclamation activities on post-nickel mining land. Reclamation for the post-mining land required 1) the use of locally adaptive species, 2) the use of relatively fast-growing tree species, 3) sufficient light and low nutrients provided, 4) the use of tree species having a lot of decomposable litter, 5) the use of tree species in catalytic type, 6) the use of tree species easy to propagate, 7) the use of tree species having a low cost for planting and maintenance, and 8) the use of tree species easy to manage (Maiti, 2013; Pancel, 2015). Common tree species selected for revegetation include Fabaceae (Legume) family (Maiti, 2013). Legume species capable of growing well on post-gold mining land in Bombana were *Pterocarpus indicus, Pericopsis mooniana,* and *Kalappia celebica* (Arif et al., 2021; Husna et al., 2021a). As described above, local tree species had the potency to grow on post-gold mining land.

Table 5. The relationship among soil properties, colonization, number of species, and amount of AMF spores.

	pH H_2O	Organic C (%)	Total N (%)	C/N ratio	P_2O_5 (ppm)	CEC (cmol/kg)	Texture			Total Mn (ppm)	Total Fe (%)	Plant
							Sand (%)	Silt (%)	Clay (%)			
AMF colonization	0.864	0.794	0.707	0.437	0.956	0.416	-0.979	0.995	0.839	-0.966	-0.875	-0.669
Species richness	-0.705	-0.610	-0.866	-0.655	-0.847	-0.637	0.998	-0.935	-0.951	0.866	0.721	0.454
Spore number	0.965	0.924	0.500	0.189	0.999	0.166	-0.893	0.987	0.669	-1.000	-0.971	-0.839

To ensure the success of revegetation in the post-mining land, soil manipulation is required. This kind of manipulation can be performed by adding soil amendment in the form of organic matter input, mulch, fertilization, and mycorrhizal inoculum (Stanturf et al., 2021). Soil amendment, namely biochar, and compost, can improve soil conditions in plant growth (Ghosh and Maiti, 2021; Worlanyo and Jiangfeng, 2021). Arbuscular mycorrhizal fungi are beneficial soil microbes belonging to an Endomycorrhizae group. AMF served as a tool for restoring the degraded land and the disturbed ecosystem (Asmelash et al., 2016), and for the post-mining land (de Moura et al., 2022) in particular. Several studies showed that AMF was capable of accelerating the succession of natural vegetation in several post-mining land conditions worldwide, including coal (Husin et al., 2017), gold (Tuheteru et al., 2020), and asphalt (Tuheteru et al., 2022). According to previous reports, AMF could improve the growth of tropical tree species in soil media from a post-gold mining land at a nursery and field scale in Bombana (Arif et al., 2021; Husna et al., 2021a). On a small scale, the reclamation of a post-gold mining land owned by PT Panca Logam Makmur in Bombana has used mycorrhizal biofertilizers on four species from local and endangered legumes, while the result was proven to be excellent. The reclamation effort can be implemented in another area of Bombana, Southeast Sulawesi.

Conclusion

The pH level in post-mining soil was lower than that of savannah soil. Total N, total Carbon, available P, and CEC in post-gold mining soil were lower than those of savannah soil. Texture of post-mining soil and savannah was clay loam. Ten AMF species from five genera and three families were discovered in the post-gold mining area. Soil pH, CEC, soil texture, total Mn, and Fe had a negative relation with AMF colonization and spore count. Soil restoration can be performed by applying soil conditioners (organic matter), mycorrhizal biofertilizers, and appropriate tree species selection.

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Soil properties change, and arbuscular mycorrhizal fungi associated with plants growing on the post-gold mining land of Bombana, Indonesia

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Research Article

Soil properties change, and arbuscular mycorrhizal fungi associated with plants growing on the post-gold mining land of Bombana, Indonesia

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Abstract

This study aimed to investigate the effect of gold mining on soil properties. Soil samples were taken from the post-gold mining land, the property of PT Panca Logam Nusantara and PT Alam Buana Indonesia, and a nearby natural forest in Bombana, Southeast Sulawesi Province. The next step focused on specifying soil pH, total nitrogen (TN) and carbon (TC) concentration, C/N ratio, available phosphorus (P) concentration, cation exchange capacity (CEC), and exchangeable K, Na, Mg, Ca, Fe, Mn, Cd and Pb concentration, texture and spore amount, AMF resource and AMF colonization. The result shows that the pH in post-gold mining soil was higher than that in natural forest soil. Meanwhile, TN, TC, available P, and CEC of post-gold mining soil got lower compared with these of natural forest soil. The texture in the post-mining soil was clay loam, while that in natural forest soil was clay. Total of 10 AMF species belonging to five genera and three families were found in a post-gold mining area. Soil pH, CEC, soil texture, Mn, and total Fe had a negative relation with AMF colonization and spore count, while organic C, total N, C/N ratio, P_2O_5 and silt had a positive relation. Sand was proven to have a strong and positive correlation with the amount of AMF species. Adding organic matter and fertilization as well as applying mycorrhizal biofertilizers, were urgently required to support the effort in restoring post-gold mining soil.

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Introduction

Gold mining is a sector that has been cultivated for a long time in Indonesia. Furthermore, Indonesia is the sixth largest gold producer worldwide, with total production reaching 190 tons in 2018 (Retinitiv, 2019). Large enterprises and other small-scale groups (artisanal and small-scale gold mining, ASGM) have participated in gold mining management in Indonesia. Over two thousand gold mining locations can be found throughout Indonesia. Bombana, in Southeast Sulawesi Province, has the potential gold resource, while this area has been managed since mid-2008. This gold mining industry has made a considerable contribution to the country in social and economic terms (Martins et al., 2020). However, mining activities can negatively affect the environment, as proven by the landscape change, particularly damage and loss of natural vegetation (Tepanosyan et al., 2018), land degradation (Ahyani, 2011; Wawo et al., 2015; Mailendra and Buchori, 2019) and soil and water contamination (Basri et al., 2017; Sakakibara et al., 2017; Basri et al., 2020), human health (Arifin et al., 2015), and other social aspects (Upe et al., 2019). Besides, mining activities generally result in low fertility and degradation in post-mining land (Ma et al., 2019). It was reported that soil degradation occurred in almost all activities for mining nickel (Prematuri et al., 2020a), coal (Pandey et al., 2014, Ma et al., 2019), and bauxite (Prematuri et al., 2020b).

Reclamation to the post-gold mining land is significantly urgent, and it has been implemented for a long time in Indonesia. Some companies, namely PT ANTAM Tbk Pongkor, Bogor (Siregar et al., 2013) and PT Newmont Minahasa Raya, North Sulawesi (Pollo et al., 2012), have successfully carried out reclamation to the post-gold mining land. The success of a reclamation effort is determined by the commitment of the company to having the permit and knowledge of its technical aspects. Thalib et al. (2020) reported that almost all companies in the gold mining sector of Bombana have not yet performed reclamation for the post-mining land. On a small scale for research, a reclamation effort has been made by designing a conservation plot for the endangered legume species in accordance with mycorrhizal biofertilizers at PT Panca Logam Makmur (Arif et al., 2021; Husna et al., 2021a). Thus, the first step was the evaluation of soil properties and vegetation succession levels. Tree growth and the quality of the post-mining land should be considered in the evaluation process (Maiti, 2013).

The soil itself can be a limiting factor in the post-mining area and determine the success of revegetation in reclamation (Matichenkov and Bocharnikova, 2021; Prescot et al., 2021). Soil is believed to be a fundamental aspect in a restoration effort towards a landscape of degraded land, including post-mining land (Stanturf et al., 2021). Physical, chemical, and biological traits in post-mining land do not allow the plant to grow well in any restoration activity (Sheoran et al., 2010). In the long run, soil quality improvement in the post-mining area mainly aims to build a sustainable forest ecosystem through the improvement of soil nutrients and plant growth (Pietrzykowski et al., 2013). Therefore, soil properties on the gold post-mining land need to be identified to select an appropriate restoration method. Restoration of the post-mining land can be passively performed through natural regeneration and actively through human intervention. The active restoration can be performed by planting the tree. Thus, the data on soil properties can be made into the database for choosing tree species and appropriate soil manipulation methods. Beneficial soil microbes, arbuscular mycorrhizal fungi (Husna et al., 2021a), for example, can be applied as a method for soil manipulation. This study aimed to investigate the relationships between soil chemical and physical properties and mycorrhizal fungi in the rhizosphere of soils of plant species grown on the post-gold mining land of Bombana, Indonesia, and the soil restoration approach.

Materials and Methods

Sampling and preparation of soil material

Soil samples were collected around the root of an adaptive plant in a post-gold mining area owned by PT Panca Logam Nusantara (PLN) and PT Alam Buana Indonesia (ABI), Bombana Regency, Southeast Sulawesi Province (Figure 1). The samples were taken from the rhizosphere in every living plant at a weight of approximately 1 kg and a depth of 0-20 cm. The next step was to put a soil sample in a respective plastic bag and write the location code and the plant name for every sampling point or plot. The whole soil samples were air-dried in the laboratory, in which this stage was intended for the isolation and identification of AMF spores. Composite soil (± 1 kg) in every location was shipped to SEAMEO BIOTROP Soil and Plant Laboratory in Bogor City.

Laboratory analysis of soil

In SEAMEO BIOTROP's Soil and Plant Laboratory of Bogor, Indonesia, an analysis was conducted on physical and chemical properties in soil. Soil pH was determined using the method described in SNI 03-6787-2002 for the measurement process. Soil organic C was analyzed using a method of SNI 13-4720-1998 (Walkey-Black). Total N was measured using micro-Kjeldahl (SNI 13-4721-1998), while available phosphorus in the form of P_2O_5 was measured using Bray method I/II (SL-MU-TT-05). Furthermore, Ca, Mg, Na, K, and cation exchange capacity (CEC) were measured by SL-MU-TT-07c (buffer extract NH_4OAc 1.0 N pH 7.0). Texture analysis of three fractions of sand, silt, and clay was conducted using SL-MU-TT-10 (Hydrometer) method. Total metal (Mn, Fe_2O_3, Cd, and Pb) was measured by HNO_3-$HClO_4$-ASS.

Soil trap culture

The trapping technique was implemented with a method proposed by Brundrett et al. (1996) using open culture pots. The medium used for planting was a mixture of 50 g of soil sample and 150 g of zeolite rock. Weaning *Sorghum bicolor* sprouts were planted in the pot. Watering, nutrient administration (red hyponex 20 mL/pot), and manual pest control were carried out as maintenance efforts.

AMF colonization

A root sample was cleaned and put in an alcohol solution at 70%. Meanwhile, arbuscular mycorrhizal fungi (AMF) colonies were observed with the trypan blue stain (Phillips and Hayman, 1970). Mycorrhizal roots were analyzed to determine AMF percentage, using the formula as follows: [Σ mycorrhizal roots / Σ total observed field of view] x 100%.

AMF isolation and identification

AMF spores were isolated with a wet pour-strain technique (Pacioni. 1992), followed by the centrifugation technique of Brundrett et al. (1996). Healthy spores were selected, stored in a glass treated with PVLG and Melzer solution, and covered. AMF spores were identified by observing their morphology in terms of shape, size, color, carrier hyphae, spore ornaments, spore mother cells, as well as bulbous suspensors. The nomenclature of AMF spores was conducted following the guidelines of Schüßler and Walker (2010) and Redecker (2013). AMF spores were identified under a Nikon Eclipse 80i Microscope at the Cryptogam Laboratory, Centre for Biology Research, LIPI, Bogor City.

Parameters

This study observed physical and chemical properties in the soil, spore density, species richness (Tuheteru et al., 2020), and AMF colonization (Brundrett et al., 1996). The soil was restored following the guidelines developed by Stanturf et al. (2021) and Young et al. (2022).

Data analysis

The whole data were analyzed through Microsoft Excel. The relationships between soil properties, the amount of vegetation with AMF spore density, species resource, and root colonization were performed using Pearson's correlation.

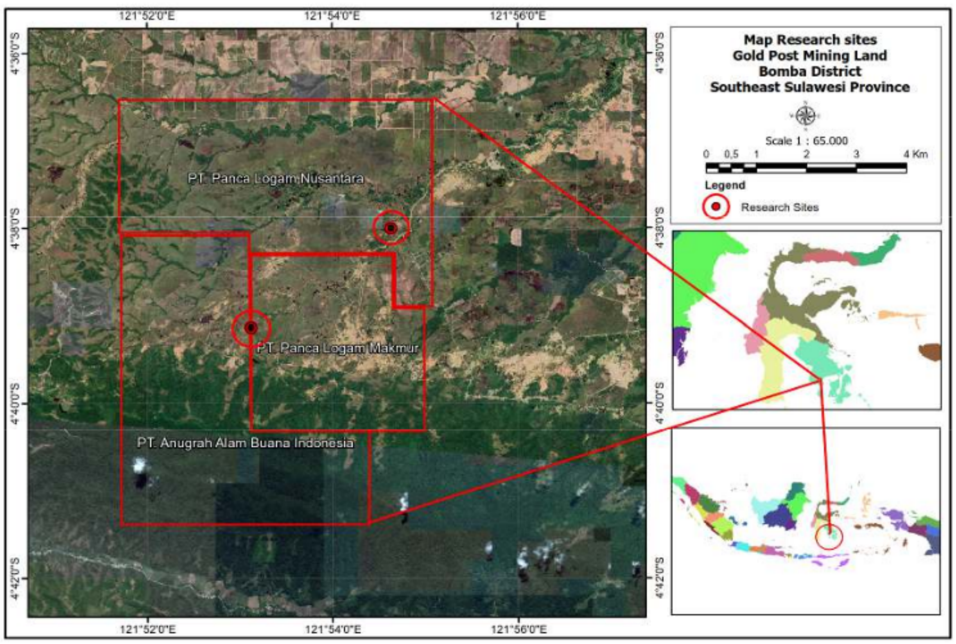

Figure 1. Location of the area studied.

Results and Discussion

Soil chemical and physical properties

The result of the analysis of physical and chemical properties in the soil is shown in Table 1. Soil pH was slightly alkaline in both post-gold mining locations. Total N content, C/N ratio, and P_2O_5 content were low, while soil organic C, Mn, and Fe contents were very low. However, soil K and Mg contents were very high. Soil pH, organic C, total N, C/N ratio, and P_2O_5 in post-mining soils were lower than in savannah soil, while Fe in the post-mining soil was higher than in savannah soil. CEC in the post-mining soil of PT Panca Logam Nusantara was lower than in savannah soil, while the total Mn of the post-mining soils was higher than that of savannah soil. Mining activities can lead to the damage of land as indicated by the pH, organic C, total N, P_2O_5 of the post-mining soils than savannah soil (Table 1). The damage to soil as a consequence of mining activities has been reported by several researchers, such as nickel mining (Prematuri et al., 2020a), coal mining (Pandey et al., 2014; Ma et al., 2019), and bauxite mining (Prematuri et al., 2020b). Soil and overburden of artisanal gold mining in

Sekotong provided very low-level plant nutrients (Siswanto et al., 2012). It was also proven that Ca<Mg, base saturation of less than 20%, CEC of less than 16 cmol/kg, and Ca<Mg in the area caused growth stagnation during reclamation. Soil texture in the savannah and both post-gold mining locations was clay loam. The proportion of sand in both post-mining soils was higher than in savannah soil, while silt and clay were lower (Table 1). Clay loam soil can support water flow and nutrients for most tree species (Osman, 2013). Clay loam soil was observed in artisanal gold mining land in West Lombok (Siswanto et al., 2012).

Table 1. Chemical and physical properties of soils.

No.	Parameter	Unit	Soil				
			Savannah (reference)	PT. Panca Logam Nusantara		PT. Alam Buana Indonesia	
				Value	Change (%)	Value	Change (%)
1	pH H_2O		8.07±0.15	7.8±0.39	- 0.96 (4)	7.7±0.12	-0.95 (5)
2	Organic C	%	0.76±0.16	0.6±0.11	- 0.79 (21)	0.5±0.11	- 0.66 (34)
3	Total N	%	0.11±0.0	0.1±0.00	- 0.91 (9)	0.1±0.01	- 0.91 (9)
4	C/N ratio		7±1.73	6.6±1.19	- 0.94 (6)	7.2±0.97	+1.03 (3)
5	P_2O_5	ppm	18.70±1.06	7.1±3.48	- 0.38 (62)	6.6±1.20	- 0.35 (65)
6	Ca	cmol/kg	nd	6.9±0.94	nd	3.6±0.20	nd
7	Mg	cmol/kg	nd	4.5±0.21	nd	4.7±0.17	nd
8	Na	cmol/kg	nd	0.4±0.13	nd	0.3±0.05	nd
9	K	cmol/kg	nd	0.2±0.00	nd	8.9±0.13	nd
10	CEC	cmol/kg	23.77±2.04	20.8±3.33	- 0.87 (13)	25.4±0.52	+1.07 (7)
11	Texture						
	Sand	%	35.07±3.50	44.6±4.61	+ 1.27 (27)	40.3±4.92	+1.15 (15)
	Silt	%	30.13±0.23	24.9±5.42	- 0.83 (17)	25.8±2.93	- 0.86 (14)
	Clay	%	34.80±3.41	30.5±1.91	- 0.87 (12)	33.9±2.04	- 0.97 (3)
12	Mn	ppm	853.0±89.11	1000±0.02	+ 1.17 (17)	1000±0.02	- 1.17 (17)
13	Fe	%	1.30±0.09	2.8±0.65	+ 2.15 (115)	3.3±0.11	+ 2.54 (154)
14	Cd	ppm	nd	1.2±0.10	nd	1.2±0.03	nd
15	Pb	ppm	nd	18.6±3.93	nd	24.8±0.57	nd

nd = no data.

AMF association with plant

AMF colonization and spore density

The microscopic observation of plant roots discovered AMF structures, including internal hyphae, vesicles, external hyphae, and coil hyphae (Figure 2). The average value of AMF colonization on plant roots ranged from 57% to 61% (Table 2). Table 3 shows the AMF colonization on plant roots. There were three AMF spores per 100 g of soil from the field. The highest amount of spores from the field was discovered in the savannah, which got lower after being cultured in the trapping culture media (Table 2). Johnson et al. (2013) and Wang (2017) reported that soil damage due to mining activities might reduce or lose AMF propagation and infectivity.

Total AMF types in field and trap culture

A total of ten AMF species, including five genera viz, were identified at two sites. *Acaulospora, Glomus, Sclerocystis, Gigaspora*, and *Scutellospora* (Table 4 and Figure 3). Data presented in Table 4 show that 70% of the total 10 AMFs belong to Glomeraceae, followed by Gigasporaceae and Acaulosporaceae. The amount of AMF species in the savannah soil was lower than that in gold post-mining soil. Results of this study showed that the dominant AMF family was Glomeraceae at a percentage of 70%. Several studies also showed the same results, in which Glomeraceae was dominant in the post-mining area of nickel (Prayudyaningsih et al., 2019), coal (Ezeokoli et al., 2020), asphalt (Tuheteru et al., 2022), gold (Tuheteru et al., 2020), and limestone (Suting and Devi, 2021).

Table 2. AMF colonization and spore density.

Site	Plant richness	AMF Colonization (%)	Spore density	
			Field (100 g soil)	Trap culture (50 g media)
Savannah	14	72	12	17
PT. Panca Logam Nusantara	24	57	3	541
PT. Alam Buana Indonesia	36	61	3	320
Mean		59		

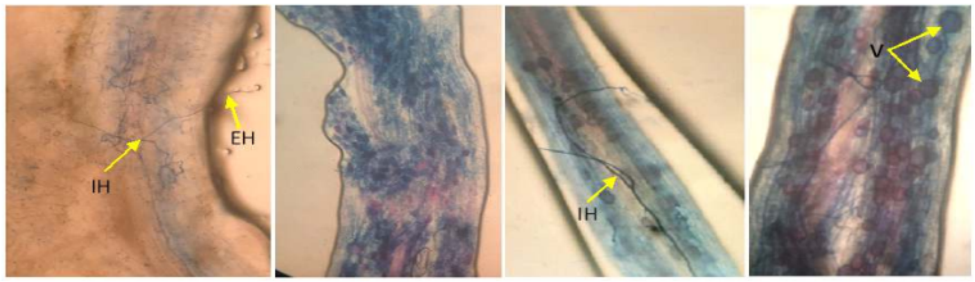

Figure 2. AMF colonization in adaptive plant roots *Codariocalyx* sp. *Acacia mangium*, *Calopogonium mucunoides* and *Imperata cylindrica* (IH Internal hyphae, EH external hyphae, V Vesicle).

Table 3. Percentage of AMF colonization on plant roots.

No	Species	Family	AMF colonization (%)	
			PT. Panca Logam Nusantara	PT. Alam Buana Indonesia
1	*Chromolaena odorata*	Asteraceae	-	53
2	*Fimbristylis dichotoma*		53	-
3	*Rhynchospora* sp.	Cyperaceae	93	-
4	Cyperaceae (sp. 1)		-	88
5	*Merremia hederacea*	Convolvulaceae	93	-
6	*Euphorbia* sp.		50	53
7	*Euphorbia hyssopifolia*	Euphorbiaceae	73	-
8	*Euphorbia heterophylla*		-	98
9	*Acacia mangium*		68	-
10	*Aeschynomene americana*		-	43
11	*Alysicarpus vaginalis*		100	17
12	*Crotalaria* sp.	Fabaceae	5	43
13	*Codariocalyx* sp.		63	-
14	*Indigofera* sp.		-	93
15	*Mimosa pudica*		95	30
16	*Uraria* sp.		30	68
17	*Melochia odorata*	Malvaceae	68	-
18	*Phyllanthus* sp.	Phyllanthaceae	100	68
19	*Calopogonium mucunoides*		30	73
20	*Chrysopogon* sp.		53	-
21	*Digitaria* sp.		-	100
22	*Imperata cylindrica*	Poaceae	50	93
23	*Saccharum* sp.		-	63
24	*Sorghum* sp.		-	50
25	Poaceae (sp. 1)		-	95
26	Poaceae (sp. 2)		-	100
27	*Oldenlandia diffusa*	Rubiaceae	43	-

Glomeracecae was reported to be dominant in the rhizosphere of several tropical tree species (Husna et al., 2021b). In Indonesia, Glomeraceae was reported to be dominant, having 36 species or 53% of 72 AMF species (Husna et al., 2021b). Glomeracecae was dominant for having AMF species that were tolerant and adaptive to the diverse condition of soil and environment, as well as the capability to survive in acid and alkaline soil to produce small spores in a shorter time, compared with *Gigaspora* and *Scutellospora. Glomus* had the most species in the phylum Glomeromycota (Schüßler and Walker, 2010). The Glomeraceae family was widely distributed and discovered in four climatic zones, seven continents, seventeen biomes, and many countries (Strumer et al., 2018). This study shows that the amount of AMF species in post-mining soil was higher compared with savanna soil. *G. coronatum, Glomus* sp. 1, and *S. sinousa* were discovered in savannah and post-mining soil.

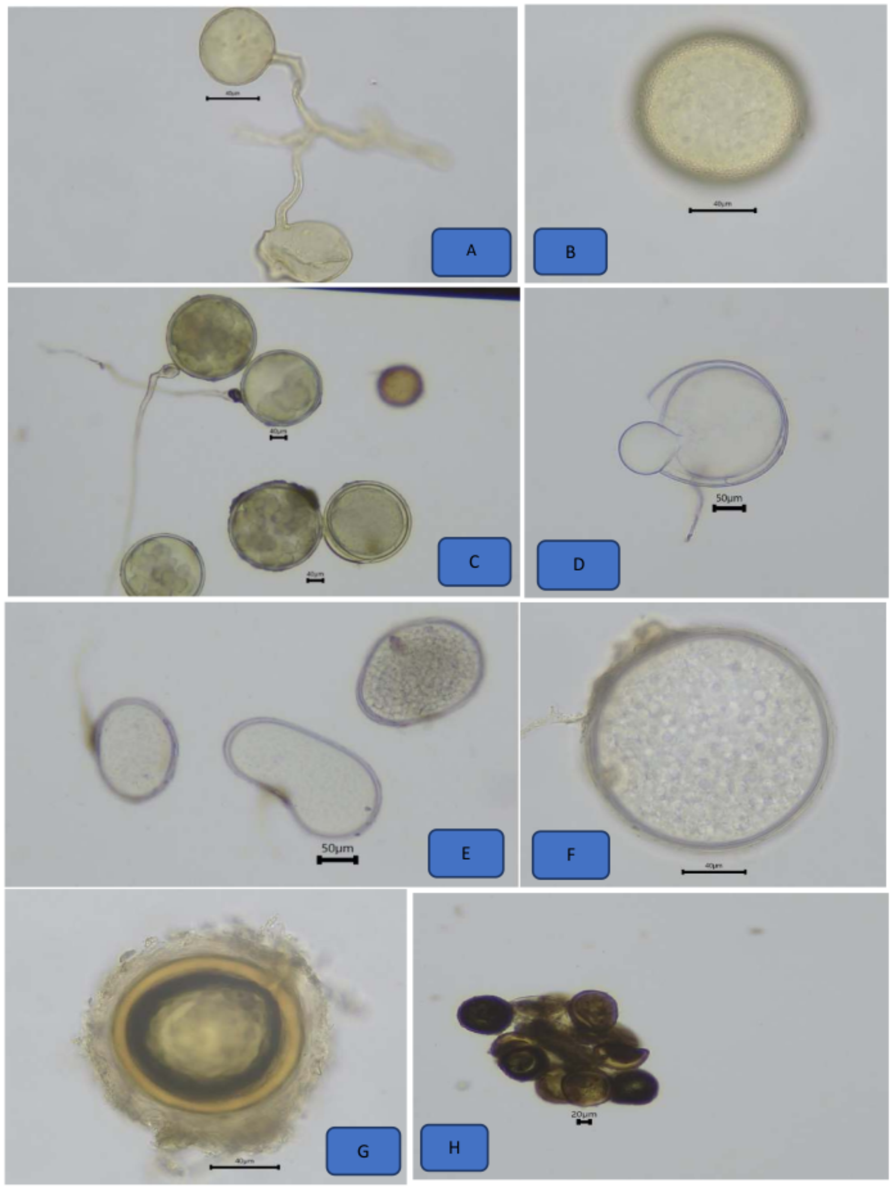

Figure 3. Types of AMF found A = *Glomus aggregatum*, ABI., B = *A. scrobiculata*, ABI., C = *Gigaspora* sp. ABI, D = *Gigaspora* sp., ABI, E = *Gigaspora* sp, PLN, F = *Gigaspora* sp. ABI, G = *Glomus coronatum*, H = *Glomus rubiforme*).

A. scrobiculata was discovered in post-gold mining owned by PT Panca Logam Makmur and a mining community in Bombana (Tuheteru et al., 2020). AMF discovered in three locations was *Glomus* sp. 1. Wide distribution of *Glomus* sp. 1 was attributed to a small size relatable to a high sporulation capacity, adaptability to the soil, climate, and diverse plant (Morales et al., 2019).

Relation between soil properties and arbuscular mycorhizal fungi parameters.

The whole soil properties were negatively related to an amount of AMF species, excluding its total Mn, total Fe, and amount of plants. Soil pH, organic C, total N, P_2O_5, silt, and clay positively related with AMF colonization and spore count (Table 5), while total Mn and Fe, sand, and vegetation were negatively related. The difference in the AMF types from every location was arguably caused by diverse ecological preferences in every AMF type. One of the factors affecting AMF abundance and diversity was soil property. This study shows that soil pH, CEC, soil texture, total Mn and Fe, as well as vegetation had a negative relation with AMF colonization and spore count. Meanwhile, organic C, total N, C/N ratio, P_2O_5, and silt had positive and strong correlations. Sand had a strong and positive correlation with the amount of AMF species. Soil pH determines spore density and AMF distribution (Bainard et al., 2015). High pH in the soil is capable of reducing the abundance of AMF species (Bainard et al., 2015). The result of this research is in line with another study by Wei et al. (2014) and Zu et al. (2018), which showed that soil Mn concentration is negatively related to AMF diversity. This research showed that P was positively correlated to spore amount and AMF colonization. The result of this research, however, is in contrast to previous research conducted by Wei et al. (2014), Soka and Ritchie (2018), and Chiomento et al. (2019), that low P was capable of improving AMF diversity. Johnson et al. (2013) reported that intense use of land was capable of changing soil properties and resulted in a decrease in species and AMF diversity.

Table 4. Glomeromycota species recovered from field soil and trap culture.

Family	**AMF species**	**Savannah***		**PT. Alam Buana Indonesia**		**PT. Panca Logam Nusantara**	
		Fs	**Tc**	**Fs**	**Tc**	**Fs**	**Tc**
Acauloporaceae	*Acaulospora scrobiculata*			√	√		
Glomeraceae	*Glomus coronatum*	√		√	√	√	√
	Glomus agrregatum			√	√		
	Glomus sp.1	√		√	√	√	√
	Glomus sp.2			√		√	
	Sclerocystis rubiformis			√			
	Sclerocystis sinousa	√		√		√	
	Sclerocytis clavispora			√			
Gigasporaceae	*Gigaspora* sp.			√		√	
	Scutellospora sp.					√	
	Total	**3**		**9**		**6**	
				10			

*Tuheteru et al. (2020), Fs (Field soils), Tc (Trap cultures).

Restoration of soil/land

The restoration is required to improve soil conditions in post-gold mining land and Bombana. Restoration of the damaged and degraded land can be carried out in a passive way through natural regeneration or in an active one by involving human intervention (Bandyopadhyay and Maiti, 2019). Martins et al. (2020) proposed six ways to recover degraded land due to mining activities, in which two of the six ways are planting tree seedlings and natural regeneration. Restoration methods can be selected by determining ecosystem resilience, restoration purpose, landscape context, and expenditure for the restoration project (Festin et al., 2019). Referring to this option, most of the corporates having a gold-mining permit are not willing to carry out the mine reclamation (Thalib et al., 2020). Natural regeneration in the post-gold mining field in Bombana has been reported. Post-gold mining area was dominated by grass and covered with various trees, including *Acacia mangium, Alstonia scolaris, Neolamarckia cadamba, N. macrophyllus*, and *Nauclea orientalis* (Tuheteru et al., 2021; Albasri et al., 2021a,b). This study indicated six species of trees that grew naturally, namely *Anacardium ocidentale*, *A. mangium, Hibiscus* sp. *Leucaena leucocephala, Neolamarckia cadamba*, and *Tamarindus indicus*. However, all companies were asked to reclaim the post-mining land by implementing an active restoration effort as stipulated in Law No. 3 of 2020 concerning Mineral and Coal Mining. A form of reclamation in post-mining land is revegetation which is mostly determined by the option of tree species and the condition of the mining soil (Ahirwal and Maiti, 2021). The right choice of tree species was capable of improving the success rate of reclamation activities on post-nickel mining land. Reclamation for the post-mining land required 1) the use of locally adaptive species, 2) the use of relatively fast-growing tree species, 3) sufficient light and low nutrients provided, 4) the use of tree species having a lot of decomposable litter, 5) the use of tree species in catalytic type, 6) the use of tree species easy to propagate, 7) the use of tree species having a low cost for planting and maintenance, and 8) the use of tree species easy to manage (Maiti, 2013; Pancel, 2015). Common tree species selected for revegetation include Fabaceae (Legume) family (Maiti, 2013). Legume species capable of growing well on post-gold mining land in Bombana were *Pterocarpus indicus, Pericopsis mooniana,* and *Kalappia celebica* (Arif et al., 2021; Husna et al., 2021a). As described above, local tree species had the potency to grow on post-gold mining land.

Table 5. The relationship among soil properties, colonization, number of species, and amount of AMF spores.

	pH H_2O	Organic C (%)	Total N (%)	C/N ratio	P_2O_5 (ppm)	CEC (cmol/kg)	Texture			Total Mn (ppm)	Total Fe (%)	Plant
							Sand (%)	Silt (%)	Clay (%)			
AMF colonization	0.864	0.794	0.707	0.437	0.956	0.416	-0.979	0.995	0.839	-0.966	-0.875	-0.669
Species richness	-0.705	-0.610	-0.866	-0.655	-0.847	-0.637	0.998	-0.935	-0.951	0.866	0.721	0.454
Spore number	0.965	0.924	0.500	0.189	0.999	0.166	-0.893	0.987	0.669	-1.000	-0.971	-0.839

To ensure the success of revegetation in the post-mining land, soil manipulation is required. This kind of manipulation can be performed by adding soil amendment in the form of organic matter input, mulch, fertilization, and mycorrhizal inoculum (Stanturf et al., 2021). Soil amendment, namely biochar, and compost, can improve soil conditions in plant growth (Ghosh and Maiti, 2021; Worlanyo and Jiangfeng, 2021). Arbuscular mycorrhizal fungi are beneficial soil microbes belonging to an Endomycorrhizae group. AMF served as a tool for restoring the degraded land and the disturbed ecosystem (Asmelash et al., 2016), and for the post-mining land (de Moura et al., 2022) in particular. Several studies showed that AMF was capable of accelerating the succession of natural vegetation in several post-mining land conditions worldwide, including coal (Husin et al., 2017), gold (Tuheteru et al., 2020), and asphalt (Tuheteru et al., 2022). According to previous reports, AMF could improve the growth of tropical tree species in soil media from a post-gold mining land at a nursery and field scale in Bombana (Arif et al., 2021; Husna et al., 2021a). On a small scale, the reclamation of a post-gold mining land owned by PT Panca Logam Makmur in Bombana has used mycorrhizal biofertilizers on four species from local and endangered legumes, while the result was proven to be excellent. The reclamation effort can be implemented in another area of Bombana, Southeast Sulawesi.

Conclusion

The pH level in post-mining soil was lower than that of savannah soil. Total N, total Carbon, available P, and CEC in post-gold mining soil were lower than those of savannah soil. Texture of post-mining soil and savannah was clay loam. Ten AMF species from five genera and three families were discovered in the post-gold mining area. Soil pH, CEC, soil texture, total Mn, and Fe had a negative relation with AMF colonization and spore count. Soil restoration can be performed by applying soil conditioners (organic matter), mycorrhizal biofertilizers, and appropriate tree species selection.

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