BIODIVERSITAS
Volume 23, Number 12, December 2022
Pages: 6327-6334

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231229

Diversity of arbuscular mycorrhizal fungi in asphalt post-mining land in Buton Island, Indonesia

FAISAL DANU TUHETERU[1,♥], HUSNA[1], ALBASRI[1], HENI MANGUN EFFENDY[1], ASRIANTI ARIF[1], BASRUDIN[1], EDY JAMAL TUHETERU[2], SRI MULYONO[3], RAGIL SB IRIANTO[4]

[1]Department of Forestry, Faculty of Forestry and Environmental Science, Universitas Halu Oleo. Jl. H.E.A. Mokodongan, Anduonohu, Kendari 93232, Southeast Sulawesi, Indonesia. Tel./Fax.: +62-401-3190105, ♥email: faisal.danu.tuheteru_fhut@uho.ac.id

[2]Department of Mining Engineering, Faculty of Earth and Energy Technology, Universitas Trisakti. Jl. Letjen S. Parman No. 1, Jakarta Barat 11440, Jakarta, Indonesia

[3]PT. WIKA Bitumen. Banabungi, Pasar Wajo, Buton 93752, Southeast Sulawesi, Indonesia

[4]Research Center for Applied Microbiology, National Research and Innovation Council of Indonesia. Jl. Raya Jakarta Bogor Km. 46, Cibinong, Bogor 16911, West Java, Indonesia

Manuscript received: 29 October 2022. Revision accepted: 8 December 2022.

Abstract. *Tuheteru FD, Husna, Albasri, Effendy HM, Arif A, Basrudin, Tuheteru EJ, Mulyono S, Irianto RSB. 2022. Diversity of arbuscular mycorrhizal fungi in asphalt post-mining land in Buton Island, Indonesia. Biodiversitas 23: 6327-6334.* Studies on the presence and diversity of Arbuscular Mycorrhizal Fungi (AMF) on overburden (OB) and asphalt post-mining areas in Buton Island are still limited. The purpose of this study was to examine the presence and diversity of AMF on OB and asphalt post-mining stockpiles in Buton Island, Southeast Sulawesi Province. The sampling of soil and roots of successional plants was carried out at eight post-mining sites at the IUP Kabungka (Pasarwajo) (6 sites) PT. WIKA Aspal and IUP Lawele (2 sites), PT. WIKA Bitumen. The AMF spores were isolated using the wet filter pour method, followed by the identification of AMF which was done by observing the morphology of AMF spores. The results showed that all successional plants were in symbiosis with AMF, which was indicated by the existence of AMF structure in plant roots with the percentage of AMF colonization ranging from 5-95%. The number of spores per 50 g ranged from 1 to 9 spores. A total of 9 AMF species were found to belong to five genera, i.e., *Claroideoglomus, Haloatospora, Glomus, Gigaspora,* and *Cetraspora* from three families. *Claroideoglomus claroideum* was found to be the most dominant species isolated with the high occurrence and the highest Importance and Shannon-Wiener Diversity Indices followed by *Haloatospora* cf. *pansihalos*, *Glomus* sp. 5, and *Glomus* sp. 4. This study also showed that the AMF diversity index differed among locations. The presence of AMF can accelerate the emergence and development of adaptive plants on post-asphalt mining land in Buton Island.

Keywords: Asphalt, Buton, Glomeraceae, passive restoration

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) (phylum Glomeromycota) is one of the important soil microbes that establish mutual symbiotic associations with many plant species (Brundrett and Tedersoo 2018) and can accelerate the formation and development of plants for restoring degraded land after mining practices (de Moura et al. 2022). AMF are reported to have the ability to accelerate the restoration of various post-mining lands in the world. Acceleration mechanisms by AMF include increasing nutrient and water acquisition and plant tolerance, improving soil structure and quality, sequestration of heavy metals, and maintaining ecosystem stability and function (Lone et al. 2016; Wang 2017; Husna et al. 2021a, b; Tuheteru et al. 2020a; Manjula et al. 2022). In particular, publications related to AMF as a restoration tool have been reported by several other researchers (Asmelash et al. 2016; Wang 2017; Neuenkamp et al. 2019; Aavik et al. 2021). A study conducted by Martins et al. (2020) showed that the trend of post-mining land restoration research and mycorrhizal research in supporting the restoration effort is also increasing.

The use of local AMF isolated from damaged lands, such as post-mining land, is more tolerant toward stress conditions, especially pollutants, and has good adaptation to local habitats due to long-term natural selection (Kodre et al. 2017). Several studies also reported that the use of AMF isolated from local species is more effective than that from exotic species (Husna et al. 2016, 2019, 2021a; Tuheteru et al. 2017, 2021) and is more efficient and cost-effective. The local AMF has the potential to be developed for revegetation, reforestation, and restoration programs of damaged land and forests. To support these efforts, it is necessary to explore AMF existence and abundance on degraded lands to determine AMF composition and diversity and to implement the use of AMF on a wider scale.

Understanding the composition and diversity of Arbuscular Mycorrhizal Fungi (AMF) is imperative for potentially enhancing their ecological roles in different terrestrial ecosystems (Tuheteru et al. 2021). AMF composition and species richness in a habitat or ecosystem are highly dependent on the types of vegetation, soil properties, climate, and damage regimes (Tuheteru et al. 2020b). Therefore, it is crucial to identify AMF species abundance in disturbed environments (Jean-Philippe et al. 2011).

Studies related to AMF symbiosis with successional plants on post-mining land have been widely reported. Wang (2017) reported that from 98 studies, more than 80% of plants observed on post-mining land were colonized by AMF. A study conducted by Tuheteru et al. (2019) showed that 100% of vegetation on gold tailings fields was colonized by AMF. In Indonesia, studies on AMF diversity and colonization in adaptive plants have been reported in gold (Suharno et al. 2016, 2017; Tuheteru et al. 2019 2020b), nickel (Husna et al. 2015), and coal post-mining areas (Tomo and Prasetyo 2021). Twenty species of 75 AMF species in Indonesia were found on post-mining land (Husna et al. 2021). In Southeast Sulawesi, exploration and identification of AMF in adaptive plants are still limited to nickel (Husna et al. 2015) and gold post-mining areas (Tuheteru et al. 2019, 2020b).

However, until now, studies related to the existence and diversity of AMF in asphalt post-mining land in the world, especially in Southeast Sulawesi Province, Indonesia have not been carried out. Indonesia is among countries with the largest natural asphalt deposits in the world (Widhiyatna et al. 2007). Natural asphalt rock in Indonesia is only found in Buton Island, Southeast Sulawesi Province with an average asphalt content of 20% (Suaryana 2016), while the Buton asphalt reserves (Asbuton) is around 667 million tonnes. Exploration and exploitation of asphalt mines by private companies and state-owned enterprises have long been carried out on Buton Island. Asphalt mining activities are generally carried out by land clearing, stripping, and stockpiling of Overburden (OB) and asphalt mining. At the OB stockpile location, successional vegetation usually be overgrown. The presence and growth of successional vegetation on OB stockpiles are thought to be accelerated by the presence of arbuscular mycorrhizal fungi. Therefore, it is necessary to conduct research to obtain information on AMF colonization, presence and diversity, and adaptive plants in asphalt post-mining land on Buton Island. The long-term goal is to obtain a collection of local AMF species for supporting active restoration of asphalt post-mining land in the tropics. In this study, we hypothesized that: 1) AMF diversity indices are low; 2) AMF colonization, diversity indices, and species richness vary at different sites.

MATERIALS AND METHODS

Soil and roots samples in the field

Sampling of soil and roots of adaptive plants was carried out at 8 (eight) sites of OB stockpiles and ex-mining sites in the Kabungka IUP (six observation sites created on overburden soils with age of 1 year-K1, 2 years-K2, 5 years-K5, 7 years-K7, 10 years-K10 and 20 years-K20) and IUP Lawele (post mining-land with no overburden soils-LM 10 and overburden with age of 10 years-L10) belong to PT. WIKA Bitumen Buton, Southeast Sulawesi in July-September 2022 (Table 1, Figure 1). Root samples were taken from the rhizosphere of each plant species and soil of each plot per site to a depth of 20 cm (Husna et al. 2015). Soil samples for each plot were put into labeled sample plastic bags. Roots samples were preserved with 70% alcohol and then put into a film tube. The soil and roots samples were then taken to the laboratory. Soil (± 1 kg) for each location was sent to the SEAMEO BIOTROP's Soil and Plant Laboratory in Bogor.

AMF isolation and identification from soil sample

AMF spores were extracted from 50 g of the soil sample by using the wet filter pour method (Gerdemann and Nicolson 1963). The supernatant was then centrifuged with the addition of 50% sugar solution (Brundrett et al. 1996). The AMF spores obtained from the extraction were then identified and counted under a dissecting microscope with 35x magnification. AMF spores were identified based on morphological characteristics such as shape, size, colour, wall ornamentation,etc. using identification keys of International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://www.invam.caf.wvu.edu) and AMF phylogeny (www.amf-phylogeny.com). The AMF extraction, counting, and identification were carried out at the Laboratory of Mycorrhiza Interface of the Center for Standardization of Sustainable Forest Management Instruments of the Ministry of Environment and Forestry of the Republic of Indonesia.

Table 1. Description of the research site

Site	Size (ha)	GPS	Altitude (m asl.)	Dominant vegetation	Annual rainfall (mm)
IUP Kabungka					
K1	0,5	122^0 53' 38,76"-5^0 23' 8,28"	153,99	*Blechnum finlaysonianum, Muntingia calabura, Pteris wallichiana*	2.340,19
K2	1	122^0 53' 40,44"-5^0 23' 10,61"	159,21	*M.calabura* L. *Imperata cylindrica, Neolamarckia macrophylla*	
K5	3	122^0 53' 33,96"-5^0 23' 8,13"	163,77	*M. calabura* L. *N.macrophylla*	
K7	1	122^0 52' 23,48"-5^0 23' 42,14"	205,46	*M.calabura* L. *P. wallichiana*	
K10	3	122^0 53' 11,12"-5^0 24' 19,98"	173,78	*N. macrophylla*	
K20	3	122^0 53' 1,17"-5^0 23' 46,97"	166,89	*Microcos* sp. *Alstonia spectabilis*	
IUP Lawele					
L10	1,5	122^0 53' 38,76"-5^0 23' 8,28"	35,24	*Mallotus paniculatus, N. macrophylla, Nauclea orientalis*	2.076,48
LM10	2	122^0 53' 40,44"-^{0} 23' 10,61"	41,32	*Chromolaena odorata, N.orientalis*	

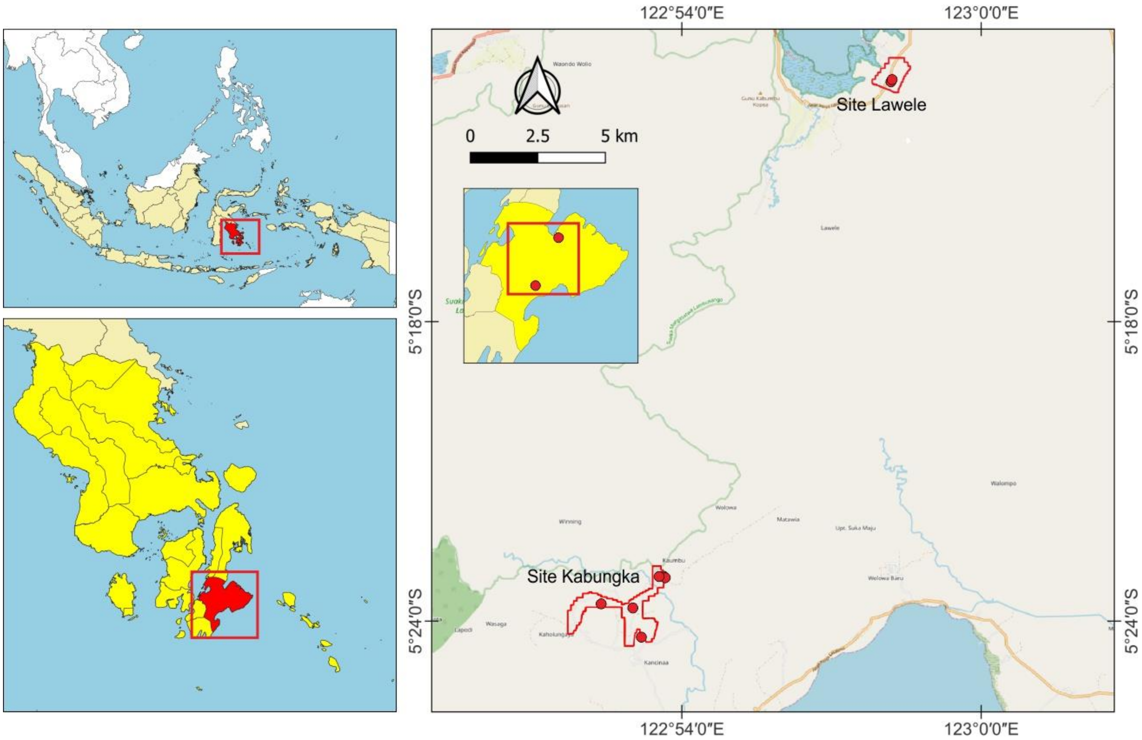

Figure 1. Map of study area showing observation sites in two locations (i.e., IUP Kabungka and IUP Lawele) in Buton Island, Southeast Sulawesi Province, Indonesia

Table 2. Parameters of AMF diversity and calculation methods

Parameter	Formula	References
Frequency of isolation	[the amount of soil samples where AMF presence/total sample] x 100%	Gaonkar and Rodrigues (2020)
Relative abundance	Percentage of spores amount from a species or a genus	
Importance Value (IV)	(Isolation frequency + Relative abundance)/2. Importance Index $\geq$ 20 means dominant species or genus	Husna et al. (2015)
Spore densities	Spores amount per 100 g soil	
Species richness	Species in each soil sample	
Shannon-Wiener Index	$H' = -\Sigma pi \ln pi$	Gaonkar and Rodrigues (2020)
Evenness Index	$E = H'/H'max$	
Simpson's Index	$D = \Sigma[n_i(n_i-1)/N(N-1)]$	
AMF colonization	[Σ mycorrhizal field of view/total field of view observed] x 100%	Brundrett et al. 1996

Note: Pi = ni / N, ni = the number of AMF spores per species; N = the total number of spores that are identified; H 'max = ln S, S = the total number of species identified

Observation of roots colonization

To study the roots colonization, was done using the root staining technique of Brundrett et al. (1996). A total of 30 pieces of fresh roots (± 1 cm long) were randomly taken from plant roots. The roots were cleaned in 10% KOH for 2 days, then immersed in H_2O_2 solution for 10-20 minutes and rinsed thoroughly. The roots were then soaked in 0.2% HCl solution for 20 minutes and were put into 0.05% Trypan Blue solution. Ten roots samples were placed on a glass slide and covered with a cover glass and then observed under a microscope.

Soil laboratory analyses

The analyses of soil physical and chemical properties were conducted at the SEAMEO BIOTROP's Soil and Plant Laboratory in Bogor, Indonesia.

AMF diversity indices

Diversity data observed in this study were isolation frequency, relative abundance, importance value, spore density, species richness, Shannon-Wiener index, evenness and Simpson's index as presented in Table 2.

Data analysis

All data were statistically analyzed using MS Excel. The correlation analyses between soil properties, number of vegetation and age OB with AMF spore number, roots colonization and diversity indices were carried out using Pearson's correlation.

RESULTS AND DISCUSSION

AMF colonization and spore density

Adaptive plant roots in all study sites were confirmed to be colonized by AMF (Figure 2). The range of colonization percentage was 5-95% with a mean of 45%. AMF colonization with percentage of more than 40% was found at the Kabungka site age 1 year (K1), 2 years (K2) and Lawele site age 10 years (L10) (Figure 2). The AMF structures found were internal hyphae, external hyphae, vesicles and arbuscule. The highest number of spores per 50 g of soil sample was shown in the ex mining-land site with no overburden soils (LM10) (9 spores).

AMF species

A total of 9 AMF species belonging to five genera, i.e. *Claroideoglomus, Halonatospora, Glomus, Gigaspora* and *Cetraspora,* from three Family were identified during present study (Table 3, Figure 3). Six species belongs to Glomeraceae family, two species belong to Gigasporaceae family and one species belongs to Claroideoglomeraceae family. *Claroideoglomus claroideum* and *Glomus* sp. 5 were each found in the 5 locations. *Cetraspora pellucida, Glomus* sp. *1, Glomus* sp. 2, *and Glomus* sp. 3 were found at a different location.

Claroideoglomus claroideum was found to be the most dominant species isolated with high occurrence and the highest Importance and Shannon-Wiener Diversity Indices followed by *Halonatospora* cf. *pansihalos, Glomus* sp. 5 and *Glomus* sp. 4 (Table 4). The richness and diversity of AMF species differed among locations (Figure 3). Site Kabungka age 10 years (K10) had the highest species richness followed by Site Kabungka age 5 years (K5), 20 years (K20) and Lawele age 10 years (L10). The species richness found was between 1-3 species per 50 g soil sample.

Diversity indices

The Simpson’s index of diversity (D) ranged from 0.24 to 1, Shannon-Wiener diversity index (H) ranged from 0.12 to 1.24, and Evenness index (E) ranged from 0.13 to 0.98 (Figure 4) was observed in present study. Site Kabungka age 10 years (K10) had the highest species diversity (H'-Shannon-Wiener index) followed by Site Kabungka age 20 years (K20) and Lawele age 10 years (L10). The uniformity and the Simpson's dominance indices (D) were higher at the Lawele site age 10 years (L10), while the uniformity and Simpson’s dominance indices (D) were the lowest at the Kabungka site age 7 years (K7).

Soil properties

The chemical and physical properties of the soil samples presented in Table 5 showed that the soil pH was slightly alkaline (7.7-8.1). The C-org, N Total, P_2O_5 and Ca tended to be high at the Kabungka site age 10 (K10) and 20 years (K20), at the Lawele site age 10 years (L10) and at the ex-mining land site with no overburden soils (LM10). The P_2O_5 levels were categorized as very high at both sites in the Lawele IUP (L10 and LM10).

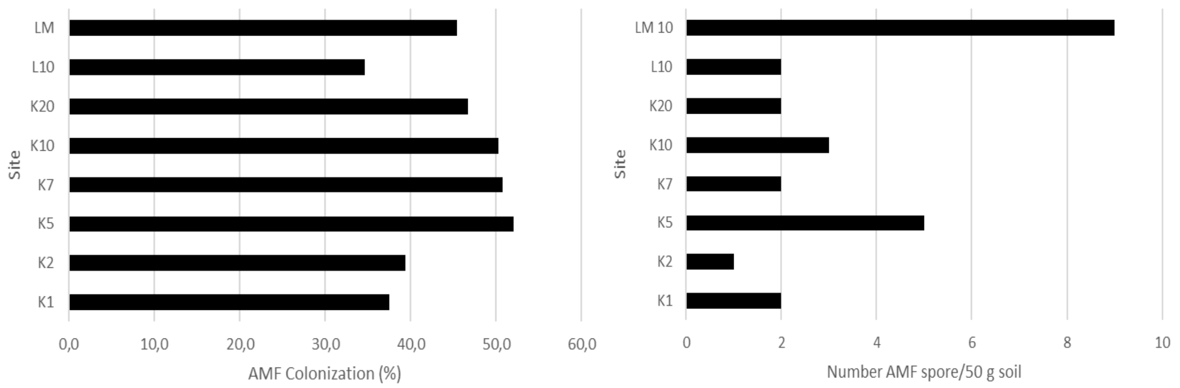

Figure 2. AMF colonization and spore density

Table 3. Glomeromycota species recovered from field soils

Family	AMF Species	Color	Shape	Size (µm)	Site
Claroideoglomeraceae	*Claroideoglomus claroideum*	Brownish	Globose	85-118 (285) x 85.915-104 (276)	K5, K10, K20, L10, LM10
Glomeraceae	*Halonatospora* cf. *pansihalos*	Brownish	Globose	83-121 x 87-118	K5, K7
	Glomus sp. 1	Yellowish	Globose	55-80 x 56-71	K10
	Glomus sp. 2	Yellowish	Globose	108-128 x 101-123	K20
	Glomus sp. 3	Yellowish	Oval	76.126-103.929	K2
	Glomus sp. 4	Brownish	Globose	97-113 x 43-145	K20, L10
	Glomus sp. 5	Brownish	Globose	127-147 x 109-143	K2, K5, K7, K10, LM10
Gigasporaceae	*Gigaspora* sp.	Pale yellow	Globose	198 x 190	K10, L10
	Cetraspora pellucida	Pale yellow	Globose	245-286 x 240-275	K1

Table 4. frequency of isolation, relative density, importance and diversity indices of AMF species

AMF species	Frequency of isolation (FI)	Relative abundance (RA)	Importance value (IV)	Shannon-Wiener Index (H)
Claroideoglomus claroideum	35.29	49.25	42.27	0.35
Halonatospora cf. *pansihalos*	11.76	13.43	12.60	0.27
Glomus sp. 1	5.88	4.48	5.18	0.14
Glomus sp. 2	5.88	2.99	4.43	0.10
Glomus sp. 3	2.94	2.99	2.96	0.10
Glomus sp. 4	11.76	7.46	9.61	0.19
Glomus sp. 5	14.71	10.45	12.58	0.24
Gigaspora sp.	5.88	2.99	4.43	0.10
Cetraspora pellucida	5.88	5.97	5.93	0.17

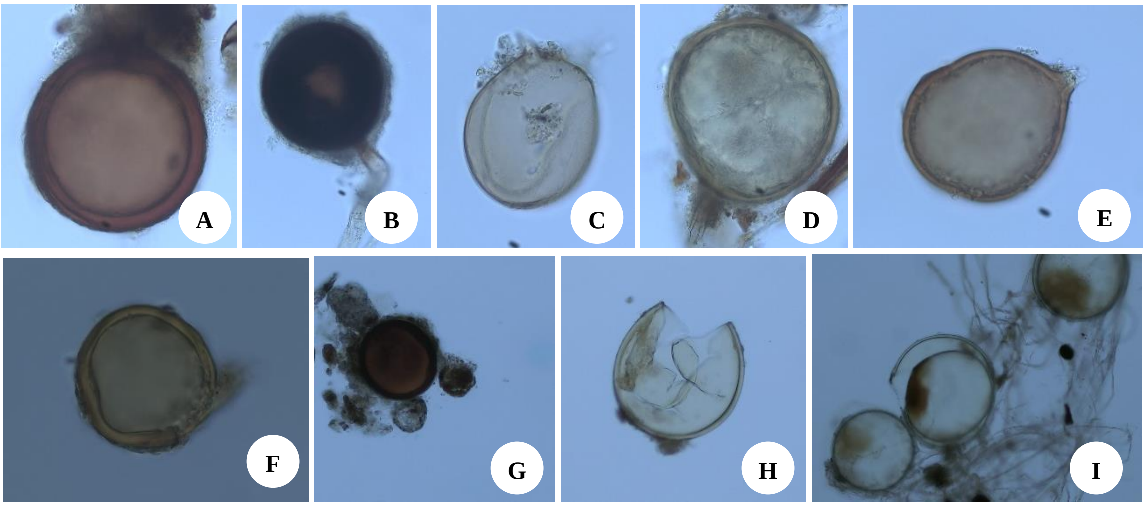

Figure 3. The types of AMF found in the rhizosphere of pioneer plants that grow naturally in asphalt post mining land site at PT. Wika Bitumen, Buton, Southeast Sulawesi, Indonesia. A. *Claroideoglomus claroideum,* B. *Halonatospora* cf. *pansihalos,* C. *Glomus* sp. 1, D. *Glomus* sp. 2, E. *Glomus* sp. 3, F. *Glomus* sp. 4, G. *Glomus* sp. 5, H. *Gigaspora* sp., I. *Cetraspora pellucida*

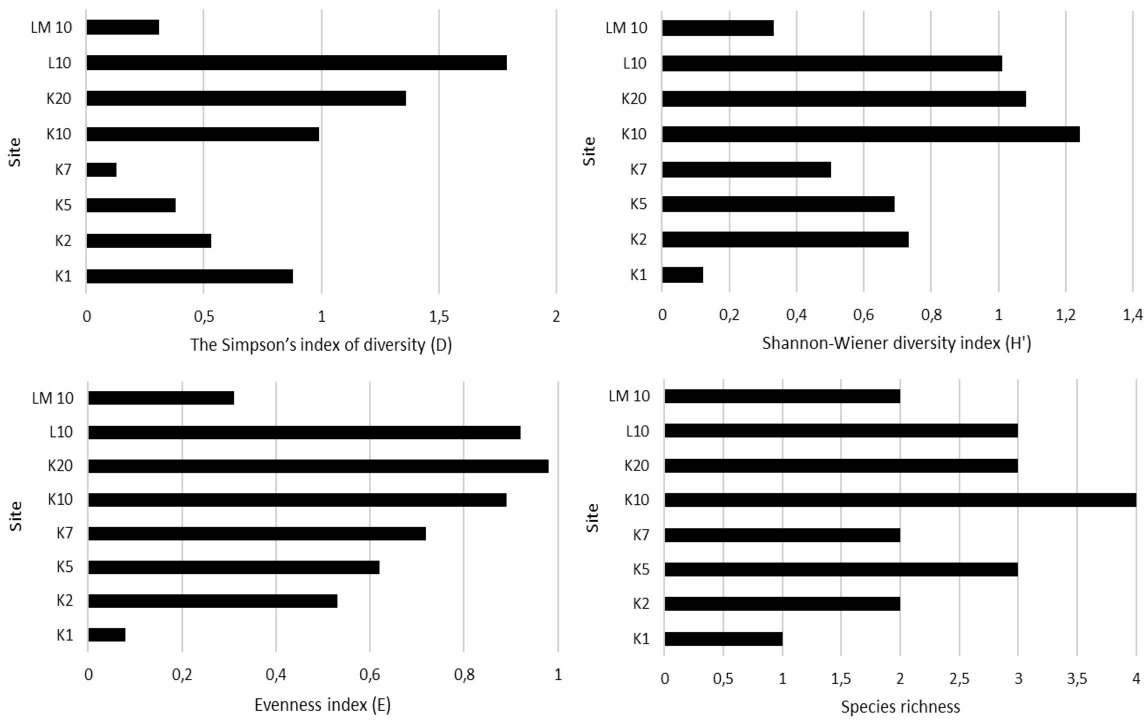

Figure 4. Diversity indices of AMF species in the natural habitat of *Kalappia celebica*, Southeast Sulawesi Province, Indonesia

Table 5. Soil chemical and physical properties

Site	pH	C org (%)	N Total (%)	P_2O_5 (ppm)	Ca (Cmol/kg)	Mg (Cmol/kg)	Texture		
							Sand (%)	Silt (%)	Clay (%)
	SNI 03-6787-2002	SNI 13-47211-1998 (Walkey & Black)	SNI 13-4721-1998 (Kjeldahl)	SL-MU-TT-05 (Bray I/II)	SL-MU-TT-07		SL-MU-TT-10 (Hidrometer)		
K1	8.1	1.11	0.08	1.92	34.64	0.60	41	20.4	38.6
K2	8.1	1.1	0.11	1.37	34.52	0.16	37.5	23.9	38.6
K5	8	1.38	0.11	1.63	27.25	0.16	44	20.7	35.3
K7	7.9	2.07	0.13	3.3	33.78	0.59	42.8	31.1	26.1
K10	7.7	2.83	0.25	9.46	36.8	0.59	40.8	29.6	29.6
K20	7.8	3.93	0.33	10.3	41.83	0.59	42.3	34.6	23.1
L10	7.8	4.2	0.35	16.6	47.59	0.65	24.2	26.7	49.1
LM10	7.8	3.19	0.23	19	49.9	1.12	42.9	34.3	22.8

Note: IUP Kabungka, six observation sites created on overburden soils with age of 1 year (K1), 2 years (K2), 5 years (K5), 7 years (K7), 10 years (K10), and 20 years (K20), and IUP Lawele (ex mined-land with no overburden soils-LM 10 and overburden with age of 10 years-L10)

Table 6. Pearson-correlation matrix for edaphic variables associated with AMF abundance

Parameter	Soil									Vegetation number	Age OB
	pH	C org	N Total	P_2O_5	Ca	Mg	Sand	Silt	Clay		
H	-0.61	0.55	0.68	0.24	0.10	-0.23	-0.34	0.29	0.06	-0.20	0.59
E	-0.74	0.68	0.72	0.34	0.20	-0.04	-0.19	0.58	0.22	-0.23	0.46
D	0.44	-0.44	-0.75	-0.13	-0.02	0.40	0.35	-0.21	-0.12	0.07	-0.50
SN	-0.29	0.15	0.05	0.52	0.36	0.57	0.35	0.30	-0.43	-0.26	0.55
SR	-0.71	0.52	0.61	0.30	0.05	-0.13	-0.16	0.28	-0.06	-0.18	0.11
Col	-0.31	-0.09	-0.41	-0.19	-0.42	-0.08	0.74	0.31	-0.71	-0.02	0.25

Note: Correlations above 0.75 are strong, 0.35-0.75 moderate, and less than 0.35 weak

The Ca level was categorized as very high for all study sites. The Mg level was in the moderate category at the LM10 site. Soil texture was divided into loam (at the K1, K2 and K5 sites) and clay loam (at other sites).

The correlation between soil properties and AMF diversity index, number of spores, and AMF species richness is presented in Table 6. The soil pH was negatively correlated with all parameters except D. The C organic and total N were positively correlated with H, E and species richness, while negatively correlated with D. The P_2O_5 and Mg were positively correlated with spore number. The total N, Ca and clay were negatively correlated with AMF colonization. The sand texture was positively correlated with AMF colonization. The correlation between the number of plants and the diversity parameter was weak (< 0.35). The stockpile age OB was positively correlated with H, E and number of species, while negatively correlated with D (Table 6).

Discussion

A total of 9 species of AMF from 5 genera and 3 families were isolated and identified in the present study. The Glomeraceae family was found to be dominant with 6 species. The Glomeraceae family is reported to have a very wide distribution globally. The Glomeraceae family is found in 4 climatic zones, 7 continents, 17 biomes (Strümer et al. 2018). *Glomus* is a genus that is tolerant and adaptive to various soil and environmental conditions. *Glomus* can survive in acidic to alkaline soils, produces small spores in a short time compared to Gigaspora and Scutellospora and *Glomus* has the highest number of species in the phylum Glomeromycota (www.amf-phylogeny.com). *Glomus* is also dominantly found in gold post-mining areas (Tuheteru et al. 2019, 2020b) and limestone mining spoils (Suting and Devi 2021). *C. claroideum* was the most dominant species isolated with high occurrence and the highest Importance and Shannon-Wiener Diversity Indices. This species is thought to be dominant because of: 1) having a small spore size 2) having the ability to sporulate (spore production) in various environmental conditions; 3) having the ability to adapt to various soil and climatic conditions as well as the ability to produce inoculum (propagules) (Shukla et al. 2013; Husna et al. 2015; Tuheteru et al. 2019, 2020a). *Gigaspora* is also found on post-mining land which is polluted with pollutants (Husna et al. 2016; Tuheteru et al. 2020b). *Cetraspora pellucida* (syn. *Scutellopora pellucida*) has been reported in post-mining fields (Buck et al. 2019; Tuheteru et al. 2019; Rodríguez-Rodríguez et al. 2021).

Adaptive plant roots collected on all overburden stockpiles and asphalt ex-mining sites in Buton Island were colonized by AMF. The forms of colonization found were internal and external hyphae, vesicles and arbuscules. Each AMF structure found has different roles in supporting plant growth and development on degraded land. External hyphae play a role in absorbing water and nutrients (especially P) needed by plants. Roots of the *Ageratum conizoides* (L.) L., *Calopogonium mucunoides* Desv, *Hyptis capitata* Jacq, *Neolamarckia macrophylla* (Roxb.)

Bosser, *Neolamarckia cadamba* (Roxb.) Miq., *Solanum torvum* Sw., *Chormolaena odorata* (L.) R.M. King & H. Rob., *Imperata cylindrica* L., *Leucaena leucocephala* (Lam.) de Wit, and *Muntingia calabura* L. plants were also reported in gold post-mining areas (Tuheteru et al. 2019). The average spore density was observed in the range of 1-9 spores per 50 g of soil. The highest number of spores per 50 g of soil sample was shown at ex mining-land site with no overburdened soils (LM10) (9 spores). The number of spores was higher at LM10, presumably because it had high P_2O_5 and Mg levels. Most terrestrial plant species associate with AM fungi, which have a fundamental role in ecosystem functioning through enhancing plant nutrition and tolerance to abiotic and biotic stress (Aavik et al. 2021).

AMF diversity index, the number of spores, and species richness are influenced by soil properties. Some contributing soil properties include pH, organic C, N, P_2O_5, Mg, Ca, and soil texture. Soil pH in all study sites was categorized as slightly alkaline (7.7-8.1). Soil pH was negatively correlated with all parameters except D (Table 6). However, soil pH and available P did not correlate with AMF colonization and spore density. The organic C and total N were positively correlated with H, E and species richness and negatively correlated with D. The P_2O_5 and Mg were positively correlated with spore number. High concentration of available P decreases the diversity of AMF (Husna et al. 2015; Abdedaiem et al. 2020). On the other hand, low P can increase AMF diversity (Wei et al. 2014; Bainard et al. 2015; Soka and Ritchie 2018; Chiomento et al. 2019). The total N, Ca and clay were negatively correlated with AMF colonization. The sand texture was positively correlated with AMF colonization (Table 6). Several factors have been identified that may influence AMF distributions, including abiotic factors (e.g., soil physicochemical properties, latitude, and climate) (Chaudhary et al. 2018; Melo et al. 2019).

The Simpson's dominance index (D) ranged from 0.24 to 1, Shannon-Wiener diversity index (H) ranged from 0.12 to 1.24 (low-moderate), and the Evenness index (E) ranged from 0.13 to 1.79 was observed here in this study (Figure 3). Site Kabungka age 10 years (K10) had the highest species diversity (Shannon-Wiener index) followed by Site Kabungka age 20 years (K20) and Lawele age 10 years (L10). The Lawele site age 10 years (L10) had higher uniformity and the Simpson's dominance indices (D), while the lowest uniformity and Simpson's dominance indices were found at the Kabungka site age 7 years (K7). The D, E, and H in the OB embankment area tended to increase with the age of the OB embankment (Table 6). Species richness and AMF diversity may be related to host type, life cycle, and site-specific conditions (Öpik et al. 2006). The number of plant species is very weakly correlated with all parameters. This research is different from the research of Moraes et al. (2019) that the diversity of AMF species increases along with the increase in plant species diversity. Although plant species richness is not always correlated with AMF species richness (Cuenca et al. 1998). Host plant genetic diversity and arbuscular mycorrhizal fungal (AMF) diversity and composition can be affected by environmental and landscape context (Aavik et al. 2021).

In conclusion, adaptive plants that grow on overburden stockpiles and asphalt ex-mining sites in Buton Island are in symbiosis with Arbsula Mycorrhizal Fungi (AMF). AMF colonizes plant roots as an indication that the presence of AMF can accelerate passive restoration on asphalt post-mining land. Therefore, the identified AMF types can be developed as environmentally friendly biological fertilizers to support post-mining reclamation activities in Indonesia.

ACKNOWLEDGEMENTS

The authors wish to thank the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research and Technology of the Republic of Indonesia (No. 28/UN29.20/PG/2022). The authors would also like to thank the Director, Head of Mining Engineering and the staff of PT. WIKA Bitumen, Buton, Southeast Sulawesi Province, and the Head of the Center for Standardization of Sustainable Forest Management Instruments, Ministry of Environment and Forestry of the Republic of Indonesia for the research permission.

REFERENCES

Abdedaiem R, Rejili M, Mahdhi M, Lajudie dP, Mars M. 2020. Phylogeny and distribution of arbuscular mycorrhizal fungi associated with *Vachellia tortilis* ssp. *raddiana* in relation to soil properties under arid ecosystems of Tunisia. Mycol Prog 19: 495-504. DOI: 10.1007/s11557-020-01572-w.

Asmelash F, Bekele T, Birhane E. 2016. The potential role of arbuscular mycorrhizal fungi in the restoration of degraded lands. Front Microbiol 7: 1095. DOI: 10.3389/fmicb.2016.01095.

Aavik T, Träger S, Zobel M, Honnay O, Van Geel M, Bueno CG, Koorem K. 2021. The joint effect of host plant genetic diversity and arbuscular mycorrhizal fungal communities on restoration success. Funct Ecol 35: 2621-2634. DOI: 10.1111/1365-2435.13914.

Bainard LD, Dai M, Furrazola-Gómez E, Torres-Arias Y, Bainard JD, Sheng M, Eilers W, Hamel C. 2015. Arbuscular mycorrhizal fungal communities are influenced by agricultural land use and not soil type among the chernozem great groups of the Canadian Prairies. Plant Soil 387: 351-362. DOI: 10.1007/s11104-014-2288-1.

Brundrett M, N Bougher, B Dell, T Grove, N Malajczuk. 1996. Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agriculture Research, Canberra.

Brundrett MC, Tedersoo L. 2018. Evolutionary history of mycorhhizal symbiosis and global host plant diversity. New Phytol 220: 1108-1111. DOI: 10.1111/nph.14976.

Buck MT, Straker CJ, Mavri-Damelin D, Weiersbye IM. 2019. Diversity of arbuscular mycorrhizal (AM) fungi colonising roots of indigenous Vachellia and Senegalia trees on gold and uranium mine tailings in South Africa. S Afr J Bot 121: 34-44. DOI: 10.1016/j.sajb.2018.10.014.

Chaudhary VB, Cuenca G, Johnson NC. 2018. Tropical-temperate comparison of landscape-scale arbuscular mycorrhizal fungal species distributions. Divers Distrib 24: 116-128. DOI: 10.1111/ddi.12664.

Chiomento JLT, Stürmer SL, Carrenho R, da Costa RC, Scheffer-Basso SM, Antunes LEC, Nienow AA, Calvete EO. 2019. Composition of arbuscular mycorrhizal fungi communities signals generalist species in soils cultivated with strawberry. Sci Hortic 253: 286-294. DOI: 10.1016/j.scienta.2019.04.029.

Cuenca G, De Andrade Z, Escalante G. 1998. Diversity of Glomalean spores from natural, disturbed and revegetated communities growing on nutrient-poor tropical soils. Soil Biol Biochem 30 (6): 711-719. DOI: 10.1016/S0038-0717(97)00191-0.

De Moura MA, Oki Y, Arantes-Garcia L, Cornelissen T, Nunes YRF, Fernandes GW. 2022. Mycorrhiza fungi application as a successful tool for worldwide mine land restoration: Current state of knowledge and the way forward. Ecol Eng 178: e106580. DOI: 10.1016/j.ecoleng.2022.106580.

Gaonkar S, Rodrigues BF. 2020. Diversity of arbuscular mycorrhizal (AM) fungi in mangroves of Chorao Island, Goa, India. Wetl Ecol Manag 28: 765-778. DOI: 10.1007/s11273-020-09747-8.

Gerdemann JW, Nicolson TH. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Brit Mycol Soc 46: 235-244. DOI: 10.1016/S0007-1536(63)80079-0.

Husna, Budi SWR, Mansur I, Kusmana C. 2015. Diversity of arbuscular mycorrhizal fungi in the growth habitat of kayu kuku (*Pericopsis mooniana* Thw.) in Southeast Sulawesi. Pak J Bio Sci 18 (1): 1-10. DOI: 10.3923/pjbs.2015.1.10.

Husna, Budi SWR, Mansur I, Kusmana C. 2016. Growth and nutrient status of Kayu Kuku (*Pericopsis mooniana* Thw.) with mycorrhiza in soil media of nickel post mining. Pak J Biol Sci 19: 158-170. DOI: 10.3923/pjbs.2016.158.170.

Husna FD Tuheteru, Arif A. 2021b. Arbuscular mycorrhizal fungi to enhance the growth of tropical endangered species *Pterocarpus indicus* and *Pericopsis mooniana* in post gold mine field in Southeast Sulawesi, Indonesia. Biodiversitas 22 (9): 3844-3853. DOI: 10.13057/biodiv/d220930.

Husna, Mansur I, Budi SWR, Tuheteru FD, Arif A, Tuheteru EJ, Albasri. 2019. Effects of arbuscular mycorrhizal fungi and organic material on growth and nutrient uptake by *Pericopsis mooniana* in coal mine. Asian J Plant Sci 18: 101-109. DOI: 10.3923/ajps.2019.101.109.

Husna, Tuheteru FD, Arif A, Basrudin, Albasri. 2021c. Biodiversity of arbuscular mycorrhizal fungi in tropical Indonesia. In: Wu et al. (editors): An Introduction to mycroorganisms. Nova Science Publishers, Inc.

Husna, Tuheteru FD, Arif A. 2021a. The potential of arbuscular mycorrhizal fungi to conserve *Kalappia celebica*, an endangered endemic legume on gold mine tailings in Sulawesi, Indonesia. J For Res 32: 675-682. DOI: 10.1007/s11676-020-01097-8.

Jean-Philippe SR, Franklin JA, Buckley DS, Hughes K. 2011. The effect of mercury on trees and their mycorrhizal fungi. Environ Pollut 159: 2733-2739. DOI: 10.1016/j.envpol.2011.05.017.

Kodre A, Arcon I, Debeljak M, Potisek M, Likar M, Vogel-Mikuš K. 2017. Arbuscular mycorrhizal fungi alter Hg root uptake and ligand environment as studied by X-ray absorption fine structure. Environ Exp Bot 133: 12-23. DOI: 10.1016/j.envexpbot.2016.09.006.

Lone R, Shuab R, Malla NA, Gautam AK, Koul KK. 2016. Beneficial effects of arbuscular mycorrhizal fungi on underground modified stem propagule plants. J New Biol Rep 5 (1): 41-51.

Martins WB, Lima MD, Junior UD, Amorim LS, de Assis Oliveira F, Schwartz G. 2020. Ecological methods and indicators for recovering and monitoring ecosystems after mining: A global literature review. Ecol. Eng 145: 105707. DOI: 10.1016/j.ecoleng.2019.105707.

Manjula, Aishwarya, Payal, Avasthi S, Verma RK, Gautam AK. 2022. Effects of arbuscular mycorrhizal fungi on growth parameters of *Pisum sativum*. Asian J Mycol 5 (2): 1-10. DOI: 10.5943/ajom/5/2/1.

Melo CD, Walker C, Krüger C, Borges PA, Luna S, Mendonça D, Fonseca HM, Machado AC. 2019. Environmental factors driving arbuscular mycorrhizal fungal communities associated with endemic woody plant *Picconia azorica* on native forest of Azores. Ann Microbiol 69: 1309-1327. DOI: 10.1007/s13213-019-01535-x.

Moraes JMA de Souza, Zanchi CS, Pires GC, Moretti CF, Barbosa MV, Silva AO, Pacheco LP, Carneiro MAC, Oliveira RL, Kemmelmeier K, Souza ED. 2019. Arbuscular mycorrhizal fungi in integrated crop livestock systems with intercropping in the pasture phase in the Cerrado. Rhizosphere 11: 100165. DOI: 10.1016/j.rhisph.2019.100165.

Neuenkamp L, Prober SM, Price JN, Zobel M, Standish RJ. 2019. Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. Fungal Ecol 40: 140-149. DOI: 10.1016/j.funeco.2018.05.004.

Öpik M, Moora M, Liira J, Zobel M. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. J Ecol 778: 778-790. DOI: 10.1111/j.1365-2745.2006.01136.x.

Rodríguez-Rodríguez RM, Kemmelmeier K, de Fátima Pedroso D, et al. 2021. Native arbuscular mycorrhizal fungi respond to rehabilitation in iron ore mining areas from the Eastern Brazilian Amazon. Pedobiol-J Soil Ecol 89: 150768. DOI: 10.1016/j.pedobi.2021.150768.

Shukla A, Vyas D, Anuradha J. 2013. Soil depth: an overriding factor for distribution of arbuscular mycorrhizal fungi. J Soil Sci Plant Nutr 13 (1): 23-33. DOI: 10.4067/S0718-95162013005000003.

Soka GE, Ritchie ME. 2018. Arbuscular mycorrhizal spore composition and diversity associated with different land uses in a tropical savanna landscape, Tanzania. Appl Soil Ecol 125: 222-232. DOI: 10.1016/j.apsoil.2018.01.013.

Stürmer SL, Bever JD, Morton JB. 2018. Biogeography of arbuscular mycorrhizal fungi (Glomeromycota): A phylogenetic perspective on species distribution patterns. Mycorrhiza 28: 587-603. DOI: 10.1007/s00572-018-0864-6.

Suaryana. 2016. Performance evaluation of stone matrix asphalt using indonesian natural rock asphalt as stabilizer. Intl J Pavement Res Technol 9 (5): 387-392. DOI: 10.1016/j.ijprt.2016.09.007.

Suharno, Kasiamdari RS, Soetarto ES, Sancayaningsih RP. 2016. Presence of arbuscular mycorrhizal fungi on fern from tailing deposition area of gold mine in Timika, Indonesia. Intl J Environ Bioremediat Biodegrad 4 (1): 1-7. DOI: 10.12691/ijebb-4-1-1.

Suharno, Soetarto ES, Sancayaningsih RP, Kasiamdari RS. 2017. Association of arbuscular mycorrhizal fungi (AMF) with *Brachiaria precumbens* (Poaceae) in tailing and its potential to increase the growth of maize (*Zea mays*). Biodiversitas 18: 433-441. DOI: 10.13057/biodiv/d180156.

Suting EG, Devi O. 2021. Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from limestone mining sites and un-mined forest soil of Mawsmai, Meghalaya. Trop Ecol 62: 525-537. DOI: 10.1007/s42965-021-00144-7.

Tomo Y, Prasetya B. 2021. Exploration of arbuscular mycorrhiza on various soil depths in bull grass rooting zone on post-mining land. JTSL 8 (2): 341-347. DOI: 10.21776/ub.jtsl.2021.008.2.5.

Tuheteru FD, Arif A, Husna, Mansur I, Tuheteru EJ, Jusniar, Basrudin, Albasri, Hadijah MH, Karepesina S. 2020a. Arbuscular mycorrhizal fungal inoculation improves *Nauclea orientalis* L. growth and phosphorus uptake in gold mine tailing soil media. J Degraded Mining Land Manag 7 (3): 2193-2200. DOI: 10.15243/jdmlm.2020.073.2193.

Tuheteru FD, Arif A, Wulan SA, Kramadibrata K. 2019. Arbuscular mycorrhizal fungi associated with adaptive plants in gold mine tailing. Biodiversitas 20 (11): 3398-3404. DOI: 10.13057/biodiv/d201137.

Tuheteru FD, Husna, Albasri, Arif A, Kramadibrata K, Soka G. 2020b. Composition and diversity of arbuscular mycorrhizal fungi spore associated with different land-use types in tropical gold mine. J Degrade Min Land Manag 8 (1): 2503-2512. DOI: 10.15243/jdmlm.2020.081.2503.

Wang F. 2017. Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: Mechanisms and applications. Crit Rev Environ Sci Technol 47: 20: 1901-1957. DOI: 10.1080/10643389.2017.1400853.

Wei Y, Hou H, Li J, Shangguan Y, Xu Y, Zhang J, Zhao L, Wang W. 2014. Molecular diversity of arbuscular mycorrhizal fungi associated with an Mn hyperaccumulator *Phytolacca americana*, in Mn mining area. Appl Soil Ecol 82: 11-17. DOI: 10.1016/j.apsoil.2014.05.005.

Widhiyatna D, Hutamadi R, Sutrisno. 2007. Tinjauan konservasi sumberdaya aspal buton. Buletin Sumberdaya Geologi 2 (3): 42-49. DOI: 10.47599/bsdg.v2i3.218. [Indonesian]