

# Pioneer plants of calcareous land in its early succession and the existence of Arbuscular Mycorrhizal Fungi

SUHARNO<sup>1,\*</sup>, SUPENI SUFAATI<sup>1</sup>, DEWI WULANDARI<sup>2</sup>, MUHAMMAD BONDAN ALFARABBI<sup>2</sup>,  
SHERLITA MAULANI<sup>1</sup>, ANNISA ALFAADHILAH RUHANI<sup>1</sup>

<sup>1</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Cenderawasih. Jl. Kamp Wolker Perumnas 3, Jayapura 99351, Papua, Indonesia. Tel.: +62-967-583333, \*email: harn774@gmail.com

<sup>2</sup>Department of Environmental Engineering, Universitas Islam Indonesia. Jl. Kaliurang Km 14.5, Sleman 55584, Yogyakarta, Indonesia

Manuscript received: 16 October 2023. Revision accepted: 26 November 2023.

**Abstract.** Suharno, Sufaati S, Wulandari D, Alfarabbi MB, Maulani S, Ruhani AA. 2023. Pioneer plants of calcareous land in its early succession and the existence of Arbuscular Mycorrhizal Fungi. *Biodiversitas* 24: 6209-6217. Calcareous land is characterized with the high presence of calcium carbonate and classified as marginal land because it does not have the quality to support plant growth. The soil quality in calcareous land is poor physically, chemically, and biologically. Nonetheless, certain plants and Arbuscular Mycorrhizal Fungi (AMF) thrive in this area. This research aims to determine plant diversity and the existence of AMF on calcareous land in Jayapura City, Papua, Indonesia. Data were collected through field observations in former limestone mining areas for a year. The results show that 41 types of plants were found at the study site. Based on the important value index, the most important plants were *Bidens pilosa*, *Borreria* sp., *Piper aduncum*, *Pteris vittata*, *Polystichum* sp., *Axonopus compressus*, *Muntingia calabura*, *Pteris cretica*, *Cynodon* sp., and *Eleusine indica*. Family with the largest number of species were Poaceae followed by Fabaceae, Pteridaceae, Moraceae, and Asteraceae. Based on plant habitus, 14.64% were ferns, 34.15% grasses, 39.62% herbaceous plants, and 12.20% trees. Plants sampled for the presence of AMF included *B. pilosa*, *M. calabura*, and *P. aduncum*. These plant species had level of AMF colonization of 71.2, 71.4, and 63.3%, respectively. There were 13 morphospecies of AMF belonging to the genera *Glomus* (46.15%), *Claroideoglomus* (7.69%), *Acaulospora* (38.46%), and *Scutellospora* (7.69%). The mutually beneficial symbiotic relationship between plants and AMF can be capitalized for managing the revegetation of former mining land with calcareous soil.

**Keywords:** Calcareous soil, diversity, fungi, Papua, plant

## INTRODUCTION

Calcareous land is a land that contains high amounts of calcium carbonate. This land falls under the degraded land category because it lacks the quality to support plant growth, i.e., poor nutritional content (Singare et al. 2022). The poor quality of calcareous land, such as in former limestone mining areas, is not only in terms of chemical properties but also in physical and biological characteristics (Prayudyarningsih and Sari 2016; Singare et al. 2022). For example, calcareous land has low water availability in the soil, high soil compaction, and limited plant nutrient availability (Taalab et al. 2019; Singare et al. 2022). Soil formed from calcareous materials has a higher pH, ranging from 5.5-8. Although limestone dust soil has a pH of 7.5-8.7, but heavy rainfall can cause significant leaching of calcium from the soil, often reducing the pH to 6.5 (McPherson 2013).

In calcareous soil in former limestone mining land, various types of plants may grow naturally. The diversity of plant species in calcareous land is unique in each region (Akhalkatsi et al. 2018), with only a few pioneer plants growing at the beginning of vegetation succession (Akhalkatsi et al. 2018; Navarro-Ramos et al. 2022). Pioneer plants that are able to grow well at early succession stage might develop relationships with other organisms in the form of symbiotic mutualism, i.e., an association that can benefit

both symbionts (Hodge and Fitter 2013). Besides natural revegetation, degraded land can also be rehabilitated using plants that have been inoculated with various types of biofertilizer (Sheoran et al. 2010; Qureshi et al. 2019).

In terrestrial ecosystems, plants might change the conditions of biotic and abiotic components of soil through the effects caused by roots, microbes and litter. Both the biotic and abiotic components influence the growth, productivity and sustainability of plants, thereby influencing the dynamics of plant communities and the regulation of ecosystem functions (Zhu et al. 2022; Yan et al. 2023). In degraded land, the role of plants, microorganisms and litter (as source of nutrients) is very important for the land rehabilitation process (Suharno et al. 2020).

Fungi, including mycorrhiza, also play a role in revegetation of calcareous soil. They can associate naturally with plants. For example, the association between mycorrhiza and plants can be mutually beneficial (symbiotic mutualism). In particular, arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota) are naturally associated with plant root systems without human intervention (Souza 2015; Suharno et al. 2020). AMF association with plant roots occurs in its initial infection on the host plant roots. Moreover, indigenous AMF has greater potential to form a more adaptive association and is more tolerant of extreme environmental conditions (Husna et al. 2021; Ma et al. 2023; Samal et al. 2023).

The diversity and distribution of AMF have drawn the attention of botanists, agriculturalists, and environmental scientists, particularly the positive relationship between mycorrhiza and the plant root system that benefits the fungal partners by optimizing the absorption of nutrients and water (Püschel et al. 2020; Alrajhei et al. 2022). Moreover, AMF is capable to develop symbiosis with various types of plants with various benefits to their host plants, especially to increase disease resistance (Chanclud and Morel 2016; Wahab et al. 2023), and mobilize nutrients from organic substrates (Wang et al. 2022). These interactions can cause dramatic changes in the composition, structure, and function of plant communities. Comprehensive understanding of the interactions between AMF and plant communities is critical for successful conservation and ecosystem management (Wahab et al. 2023). An imbalance of beneficial microorganisms due to disruption of microbial populations has caused soil damage and decreased crop production. Changes in microbial community composition can also result in changes in soil nutrient cycles and reduced soil water retention, thereby causing further soil degradation (O'Callaghan et al. 2022).

Microbes are essential for sustainable soil fertility management in nutrient cycling, pest management, and soil structure, moreover in poor nutrients soil such as calcareous land. In various studies, attention has been paid to the important role of AMF symbiosis as a provider of ecosystem services to guarantee crop yields, and help develop sustainable agricultural systems (Diagne et al. 2020; Wahab et al. 2023). The AMF improves plant growth performance, including in the revegetation of degraded land and agro-industry development. AMF was found associated with several plants in the limestone quarry area (Prayudyarningsih and Sari 2016). Some areas in Papua are dominated by calcareous land. However, the study on diversity of microbes on the calcareous land in this area is

still limited. Therefore, this study aims to determine the diversity of pioneer plants and the presence of AMF in the early succession process on calcareous land. We expect that this preliminary data will contribute to the first step in efforts to revegetate degraded land, especially calcareous land.

## MATERIALS AND METHODS

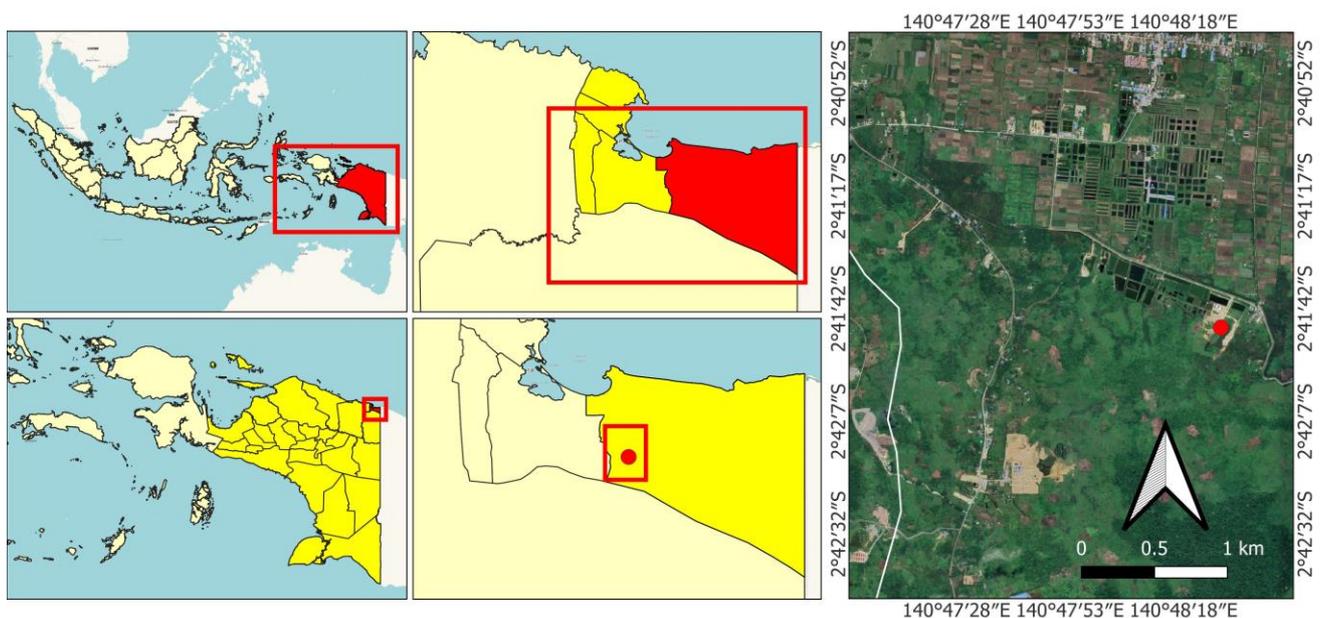
### Study area and period

This research was carried out from June to September 2023. Field observations were conducted in Muara Tami District, Jayapura, Papua, Indonesia (Figure 1). The geographical position is located at the coordinates S: 2°41'46.14" and E: 140°48'26.27". The number of rainy days in this area reaches 148-175 rainy days per year, temperatures range from 29-31.8°C, and humidity varies between 79-81%. Observations of plants on calcareous land were carried out between June and July 2023. During this time, the dry season had just started, although there was still a small amount of rain during the week. The soil and plant roots were sampled in June 2023 for soil fertility analysis and the presence of AMF.

### Data collection procedure

#### *Vegetation sampling*

Observations of the diversity of plant species growing on calcareous land were carried out using the transect method with sampling plot. The observation site chosen was one-year-old former limestone mining land. The transect was made in one line, with 18 square plots measuring 2x2 m, resulting in the total observation area was 72 m<sup>2</sup>. The plant samples were identified at the Biology Department Laboratory, the Faculty of Mathematics and Natural Sciences, Cenderawasih University, Jayapura.



**Figure 1.** Map of study area in Muara Tami Sub-district, Jayapura District, Papua, Indonesia

### Arbuscular Mycorrhizal Fungi (AMF) sampling

The presence of AMF in calcareous land was identified based on the presence or absence of spores in the plant rhizosphere. Meanwhile, the presence of association was determined by the presence of fungal colonization in the plant's root system. Spore observations were carried out by taking 1 kg of soil samples from the plant's rhizosphere. Of the various types of plants found in calcareous land areas, three dominant species were selected to be observed any association with AMF namely *Biden pilosa*, *Muntingia calabura*, and *Piper aduncum*.

The AMF spores were identified through spore extraction from the soil samples. A total of 10 g of soil samples was sieved using a multistage wet sieving technique. The spores were separated using a 60% sucrose solution by centrifugation at 1000 rpm for five minutes. The number of spores in the root's rhizosphere was counted using a dissecting microscope at 30–40x magnification.

Meanwhile, AMF colonization was identified using tryphane blue staining. The colonization of AMF in plant root systems can be seen from the structures built by AMF associations, i.e., the presence of intraradical hyphae, extraradical hyphae, vesicles, arbuscules, or spores formed in the roots. The roots to be observed were first fixed with FAA for one hour, washed and soaked in 10% KOH for 24 hours, and clarified with 2% HCl for 24 hours. The roots were then stained with 0.05% tryphane blue in lactoglycerol. The fungal colonization was determined using the slide method (Vierheilig et al. 2005; Sun and Tang 2012).

The taxonomical information of AMF was identified from the spore morphological characteristics, i.e., spore shape, color, attachment of hyphal stalk, wall, and the reaction of spore contents against Melzer's solution. Meanwhile, the identification from genus to species refers to the studies by Schenck and Perez (1990), Schüßler and Walker (2010), Suharno et al. (2020), and INVAM (2022).

### Data analysis

#### Plant composition

The composition of plant community was analyzed using Importance Value Index (IVI), and was calculated as the sum of the relative frequency and relative density values. The equation by Mueller-Dombois and Ellenberg (1974) was used to calculate the Density (D) and Frequency (F) values as follow.

$$\text{Density of a species (D)} = \frac{\text{Number of individuals of a species}}{\text{Area of observation}}$$

$$\text{Relative Density (Dr)} = \frac{\text{Density of a species}}{\text{Density of all species}} \times 100\%$$

$$\text{Frequency of a species (F)} = \frac{\text{Number of plots occupied by a species}}{\text{Number of all observation plots}} \times 100\%$$

$$\text{Relative frequency (Fr)} = \frac{\text{Frequency of a species}}{\text{Frequency of all species}} \times 100\%$$

$$\text{Importance Value Index (IVI)} = \text{Relative Density (Dr)} + \text{Relative Frequency (Fr)}$$

### Soil analysis

Sample soil fertility analysis was conducted at the Testing Laboratory of the Application of Agricultural Instrument Standard, Yogyakarta (*Laboratorium Pengujian Balai Penerapan Standar Instrumen Pertanian Yogyakarta*). Soil pH parameters were measured by pH meter, C-organic content by Walkly & Black method, N-total by Kjeldahl method, phosphate (P<sub>2</sub>O<sub>5</sub>) by Olsen method, CEC by distillation, then for the availability of Ca, Mg, K, and Na analyzed by Atomic Absorption Spectrophotometry (AAS) method (Eviati and Sulaeman 2009).

## RESULTS AND DISCUSSION

### Diversity of plants on calcareous soil

The results showed that the calcareous land formerly used as a traditional mining site consisted 41 plant species. The mine has been non-operational for a year after being abandoned by the miners. The growth of pioneer plants has been sighted, such as ferns, grasses, herbs, shrubs, and even trees (Table 1). Plant species with the highest IVI (above 5.0) are *B. pilosa*, *Borreria* sp., *P. aduncum*, *Pteris vittata*, *Polystichum* sp., *Axonopus compressus*, *Muntingia calabura*, *Pteris cretica*, *Cynodon* sp., and *Eleusine indica*. Other plant species have IVI values lower than 5.0.

The majority of plant species recorded on the calcareous soil belong to the families Poaceae (12 species), Fabaceae (eight species), Pteridaceae, Moraceae, and Asteraceae (three species each), and Rubiaceae and Euphorbiaceae (two species each). Meanwhile, other families only have one species (Figure 2). Based on the plant habitus, the dominant plants are herbaceous plants and grasses. There were 16 species of herbaceous plants, 14 species of grasses, six species of ferns, and six species of trees. This shows that all groups of plants have grown on the calcareous land in the formerly mining area within a year.

Wibowo et al. (2020) found the dominant plant species with high IVI values at the limestone mining area of PT. Holcim Indonesia Tbk. in Narogong, Indonesia, were *Eleusine indica*, *Cyperus rotundus*, *Mimosa pudica*, *Bidens pilosa*, and *Ageratum conyzoides*. Suharno et al. (2014) also found that *Bidens pilosa* was predominant on gold mining land in Mimika Papua. Meanwhile, Piqueray et al. (2007) examined plant diversity in the calcareous grasslands in Southeast Belgium. They found plants that belong to the Festuco-Brometea class, such as *Festuca lemarii*, *Bromus erectus*, *Helianthemum nummularium*, and *Sanguisorba minor*.

At a degraded limestone mining area in Kavtiskhevi (Georgia), Akhalkatsi et al. (2018) found 114 plant species. Several species were only found in pioneer succession plots, namely *Chenopodium album* (Amaranthaceae), *Sisymbrium loeselii* (Brassicaceae), *Medicago lupulina* (Fabaceae), *Lactuca seriola* (Compositae), *Tussilago farfara* (Compositae), *Scorzonera biebersteinii* (Compositae), *Poa densa* (Poaceae), *Lotus corniculatus* (Fabaceae), *Melilotus officinalis* (Fabaceae) and *Taraxacum campyloides* (Compositae). This species lives mostly at mining sites where limestone material was extracted two to three years

previously. Akhalkatsi et al. (2018) stated that investigating the phytosociological process of natural revegetation will provide an important information for correctly managing and implementing rehabilitation processes. Information about the changes in species diversity in the revegetation succession can inform the selection of appropriate plant species for reforestation.

This research also shows that certain plant species can adapt to calcareous land in alkaline soil conditions with a pH ranging from 7.6 to 7.9 (Table 1). The organic C content was between 0.61-2.42% (low-medium), with an average of 1.71%, suggesting a medium level of organic C content. Meanwhile, the total N content ranged between 0.02 and 0.05%, with an average of 0.03%, meaning that the N content is low. By contrast, the C/N ratio was between 25.00 and 93.00, with an average of 54.33, implying that the C/N content is very high. According to

Sorensen (1993) and Warren et al. (2017), the level of C content is related to the value of carbon stock in an area.

The P content ( $P_2O_5$ ) ranged from 8.0 to 11.0 ppm, with an average of 9.33 ppm, which can be considered as low. Likewise, the K content ranged between 0.03 and 0.09  $cmol.kg^{-1}$ , with an average of 0.06  $cmol.kg^{-1}$ , which is also low. Mg was between 0.20 and 0.71  $cmol.kg^{-1}$  with an average of 0.46  $cmol.kg^{-1}$ , which also means low. The Cation Exchange Capacity (CEC) value of soil was between 15.59 and 16.50  $cmol.kg^{-1}$ , with an average of 15.95  $cmol.kg^{-1}$ , which also means low. The base saturation level was around 90.50-126.42% (very high). Meanwhile, the Ca content was between 13.91 and 19.94, with an average of 17.51  $cmol.kg^{-1}$ , which can be considered high. Notably, most of the soil texture in this calcareous land area is clay loam.

**Table 1.** Diversity and the importance value index (IVI) of plant species on calcareous land in Muara Tami District, Jayapura

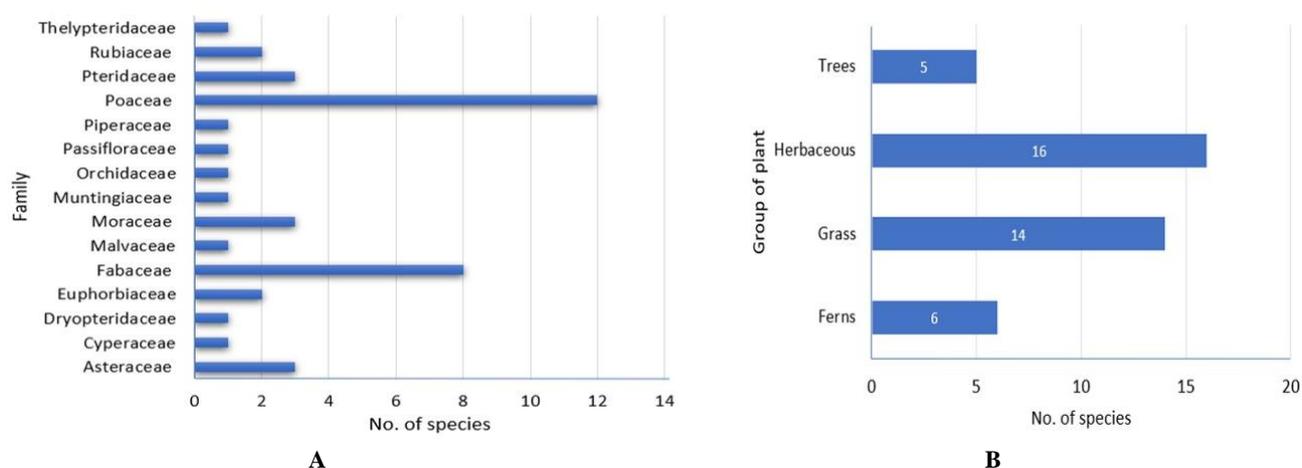
Plant species	$\Sigma$ individual	D	F	Dr	Fr	IVI
<i>Bidens pilosa</i> L.	569	7.90	0.89	32.85	12.40	45.25
<i>Borreria</i> sp.	476	6.61	0.72	27.48	10.08	37.55
<i>Piper aduncum</i> L.	159	2.21	0.61	9.18	8.53	17.71
<i>Pteris vittata</i> L.	111	1.54	0.33	6.41	4.65	11.06
<i>Polystichum</i> sp.	122	1.69	0.28	7.04	3.88	10.92
<i>Axonopus compressus</i> (Sw.) P.Beauv.	69	0.96	0.44	3.98	6.20	10.18
<i>Muntingia calabura</i> L.	40	0.56	0.33	2.31	4.65	6.96
<i>Pteris cretica</i> L.	52	0.72	0.22	3.00	3.10	6.10
<i>Cynodon</i> sp.	22	0.31	0.28	1.27	3.88	5.15
<i>Eleusine indica</i> (L.) Gaertn.	21	0.29	0.28	1.21	3.88	5.09
<i>Pueraria</i> sp.	7	0.10	0.28	0.40	3.88	4.28
<i>Ficus</i> sp.	11	0.15	0.17	0.63	2.33	2.96
<i>Desmodium repandum</i> (Vahl.) DC.	6	0.08	0.17	0.35	2.33	2.67
<i>Digitaria sanguinalis</i> (L.) Scop.	5	0.07	0.17	0.29	2.33	2.61
<i>Phalaris canariensis</i> L.	6	0.08	0.11	0.35	1.55	1.90
<i>Crotalaria</i> sp.	5	0.07	0.11	0.29	1.55	1.84
<i>Chromolaena odorata</i> L.	4	0.06	0.11	0.23	1.55	1.78
<i>Mallotus penangensis</i> Müll.Arg.	3	0.04	0.11	0.17	1.55	1.72
<i>Pteris</i> sp.	3	0.04	0.11	0.17	1.55	1.72
<i>Cyperus rotundus</i> L.	3	0.04	0.11	0.17	1.55	1.72
<i>Mimosa invisa</i> L.	2	0.03	0.11	0.12	1.55	1.67
<i>Ficus septica</i> Burm.	2	0.03	0.11	0.12	1.55	1.67
<i>Cenchrus purpureus</i> (Schumach.) Morrone.	2	0.03	0.11	0.12	1.55	1.67
<i>Panicum</i> sp.	6	0.08	0.06	0.35	0.78	1.12
<i>Imperata cylindrica</i> L.	6	0.08	0.06	0.35	0.78	1.12
<i>Calopogonium mucunoides</i> Desv.	4	0.06	0.06	0.23	0.78	1.01
<i>Mimosa pudica</i> L.	2	0.03	0.06	0.12	0.78	0.89
<i>Ficus nodosa</i> Teijsm. & Binn.	1	0.01	0.06	0.06	0.78	0.83
<i>Erigeron canadensis</i> L.	1	0.01	0.06	0.06	0.78	0.83
<i>Passiflora foetida</i> L.	1	0.01	0.06	0.06	0.78	0.83
<i>Alisycarpus vaginalis</i> (L.) DC	1	0.01	0.06	0.06	0.78	0.83
<i>Brachypodium</i> sp.	1	0.01	0.06	0.06	0.78	0.83
<i>Cynodon dactylon</i> Pers.	1	0.01	0.06	0.06	0.78	0.83
<i>Thelypteris</i> sp.	1	0.01	0.06	0.06	0.78	0.83
<i>Paspalum</i> sp.	1	0.01	0.06	0.06	0.78	0.83
<i>Spathoglottis</i> sp.	1	0.01	0.06	0.06	0.78	0.83
<i>Sida rhombifolia</i> L.	1	0.01	0.06	0.06	0.78	0.83
<i>Leucaena leucocephala</i> (Lam.) de Wit	1	0.01	0.06	0.06	0.78	0.83
<i>Digitaria</i> sp.	1	0.01	0.06	0.06	0.78	0.83
<i>Spermacoce remota</i> Lam.	1	0.01	0.06	0.06	0.78	0.83
<i>Euphorbia hirta</i> L.	1	0.01	0.06	0.06	0.78	0.83
Total		24.06	7.17	100.00	100.00	200.00

**AMF colonization in plants on calcareous land**

The association between AMF and plants can be recognized from fungal infection on plant roots. The AMF colonization on plant roots also shows in plant growth. This research shows that the levels of AMF colonization varied in different plants. In *B. pilosa*, the percentage of AMF colonization reached an average of 71.2% (high). Meanwhile, the colonization in *M. calabura* and *P. aduncum* plants was up to 71.5% (high) and 63.3% (high) (Figure 4). Generally, the levels of AMF colonization were high, ranging between 50 and 80%.

According to Parihar et al. (2019) and Campo et al. (2020), mycorrhizal colonization in plant’s roots affects nutrient uptake, the plant’s tolerance to pests and diseases, drought, exposure to heavy metals, and the plant’s ability

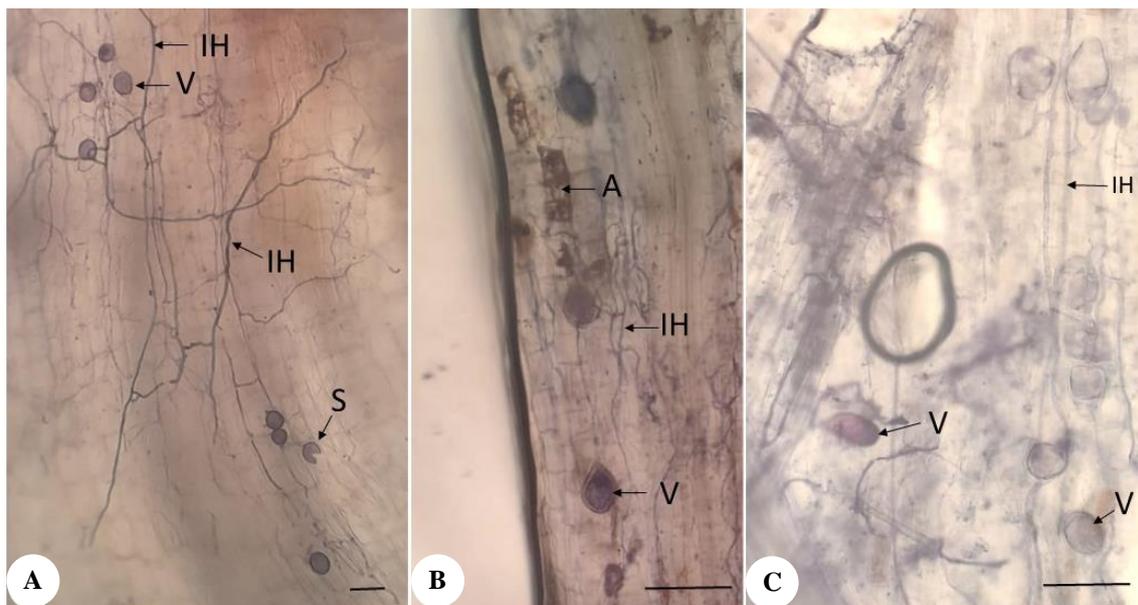
to adapt to abiotic environmental conditions. However, Suharno et al. (2021) revealed that, at the early stages of growth, AMF colonization might slow the plant’s growth and only positively affects the growth at the later stages. In the case of the Barley plant, Beslemes et al. (2023) revealed that the presence of AMF increases growth parameters, including the absorption and efficient use of phosphorus and nitrogen. The AMF species *Rhizophagus clarus* and *Claroideoglossum etunicatum* inoculated on *P. aduncum* can form symbiont associations. The microscopic analysis of the roots of the inoculated plants showed the presence of hyphae, arbuscules, and vesicles. The inoculation also affects plant height growth and root length at 30 and 90 days after inoculation (de Oliveira et al. 2019).



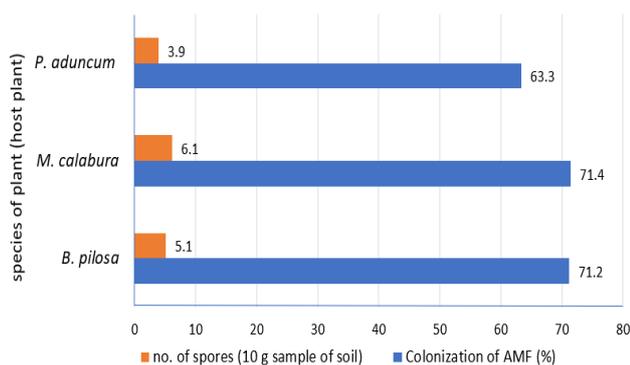
**Figure 2.** Number of plant species found on the calcareous soil area based on: A. Family, and B. Plant habitus

**Table 2.** Physico-chemical characteristics of calcareous soil in Muara Tami District, Jayapura, Papua

Physico-chemical characteristics of soil	Sample code			Average
	KG-01	KG-02	KG-03	
pH (H <sub>2</sub> O)	7.62	7.79	7.85	7.75
pH (KCl)	7.37	7.53	7.53	7.48
Organic C (%)	2.42	0.61	2.10	1.71
N total (%)	0.05	0.02	0.02	0.03
Ratio C/N	45.0	25.0	93.0	54.33
P (available) (ppm)	9.00	11.00	8.00	9.33
K-dd (cmol.kg <sup>-1</sup> )	0.09	0.05	0.03	0.06
Ca-dd (cmol.kg <sup>-1</sup> )	19.94	18.69	13.91	17.51
Na-dd (cmol.kg <sup>-1</sup> )	0.54	0.25	0.12	0.30
Mg-dd (cmol.kg <sup>-1</sup> )	0.47	0.71	0.20	0.46
KTK (cmol.kg <sup>-1</sup> )	16.50	15.59	15.76	15.95
Base Saturation (%)	> 100	126,42	90.50	108.97
<i>Al-HadKCl IN:</i>				
Al-dd (me.100g <sup>-1</sup> )	0.18	0.00	0.00	0.06
H-dd (me.100g <sup>-1</sup> )	0.18	0.12	0.40	0.23
Soil texture				
Sand (%)	26	37	34	32.33
Dust (%)	56	27	30	37.67
Clay (%)	18	36	36	30.00
	Silt loam	Clay loam	Clay loam	Clay loam



**Figure 3.** Colonization of AMF on plant roots in calcareous land. A. Colonization in *B. pilosa*, B. Colonization in *M. calabura*, C. Colonization in *P. aduncum*. V: vesicle, A: arbuscule, IH: intraradical hyphae, S: intraradical spore. Scale bar: 100 µm



**Figure 4.** Number of AMF spores in rhizosphere and percentage of AMF colonization in plants

The plants found on calcareous land are those often used as traditional medicinal plants, such as *P. aduncum* (Taher et al. 2020), *B. pilosa* (Bartolome et al. 2013), and *M. calabura* (Upadhye et al. 2021). The role of AMF in plant growth and disease resistance influences the production of secondary metabolite compounds (Suharno et al. 2022). Therefore, the finding of this study might indicate that calcareous land is more suitable for the development of medicinal plants than agricultural crops.

A study in Iran shows that not all medicinal plants can be colonized by AMF. According to Sinegani and Yeganeh (2017), colonization and spore density of perennial plants are slightly higher than annual plants and vary between plant families. Soil texture and the availability of phosphorus are the most important soil properties influencing fungal colonization and spore numbers.

#### Diversity of AMF on calcareous land

This research shows that, based on the morphospecies identification, there were 13 species of AMF found on calcareous land at the studied area (Table 3; Figure 5). Genera with the largest number of species were *Glomus* (six species) and *Acaulospora* (five species), while other genera, *Claroideoglomus* and *Scutellospora*, only had one species each. An average of six to seven species were found in the rhizosphere of *B. pilosa*, *M. calabura*, and *P. aduncum* plants. *Scutellospora* sp., and *Glomus* sp6 were only found in the rhizosphere of *B. pilosa*. Meanwhile, *Acaulospora* sp1. was found in all three plants observed. The level of AMF diversity in this study is similar to the results of observations on the kava plant (*Piper methysticum*) in the lowland area of Merauke, Papua, where 13 species of AMF based on the morphospecies were found (Suharno et al. 2018). More morphospecies (18) were found in the kebar grass (Suharno et al. 2018; Suharno et al. 2022). This condition shows that at the beginning of the succession period, the calcareous land can facilitate the growth and development of AMF species.

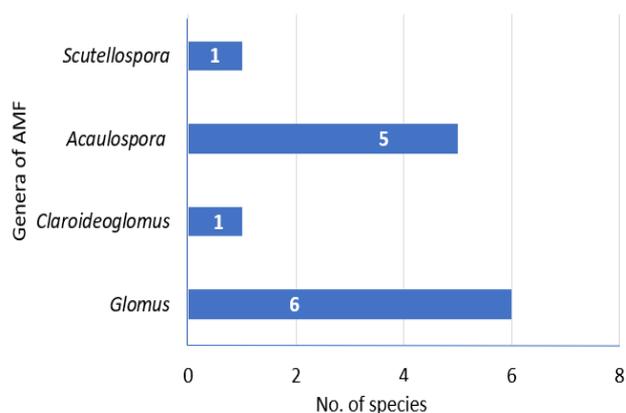
According to Ma et al. (2023) and Kumalawati et al. (2021), the available P in soil is an important predictor of the AMF diversity and abundance in an area. A study by Parihar et al. (2019) in the hot sub-humid ecoregion of the Central Gangetic Plain of India found six genera and eight species of AMF in soils with neutral to alkaline pH. The AMF were *Acaulospora*, *Cetraspora*, *Entrophospora*, *Funneliformis* (*F. geosporum* and *F. mosseae*), *Glomus*, *Rhizoglomus* (*R. intraradices* and *R. fasciculatum*), found in the rhizosphere of salt-tolerant native vegetation. Meanwhile, Liu et al. (2016) revealed that using phosphorus fertilizer did not significantly affect AMF richness except in deeper soils. High P use reduces root colonization, whereas optimal P increases colonization and fungal richness.

**Table 3.** Diversity of AMF found in several dominant plants on calcareous land in Muara Tami District, Jayapura, Papua

Family	Genus	Species	Host plant species		
			<i>B. pilosa</i>	<i>M. calabura</i>	<i>P. aduncum</i>
Glomaceae	<i>Glomus</i>	<i>Glomus aggregatum</i>	+	+	-
		<i>Glomus</i> sp2.	-	+	-
		<i>Glomus</i> sp3.	+	-	+
		<i>Glomus</i> sp4.	-	+	+
		<i>Glomus</i> sp5.	-	+	-
		<i>Glomus</i> sp6.	+	-	-
Acaulosporaceae	<i>Claroideoglomus</i>	<i>Claroideoglomus etunicatum</i>	-	+	-
	<i>Acaulospora</i>	<i>Acaulospora spinosa</i>	-	+	+
		<i>Acaulospora</i> sp1.	+	+	+
		<i>Acaulospora</i> sp2.	-	-	+
		<i>Acaulospora</i> sp3.	+	-	+
Gigasporaceae	<i>Scutellospora</i>	<i>Acaulopsosa</i> sp4.	-	-	+
		<i>Scutellospora</i> sp.	+	-	-
Total			6	7	7



**Figure 5.** AMF morphospecies found on calcareous land in Muara Tami District, Jayapura, Papua. A. *Acaulospora* sp1., B. *Scutellospora* sp., C. *Claroideoglomus* sp., D. *Acaulospora spinosa*, E. *Glomus* sp1., F. *Glomus aggregatum*, G. *Glomus* sp3., and H. *Acaulospora* sp2. Scale bar: 50 µm



**Figure 6.** The genus and number AMF species found on calcareous soil in Muara Tami District, Jayapura, Papua

Furthermore, Parihar et al. (2019) revealed that *R. fasciculatum* (*Glomus fasciculatum*) is the most widely distributed species in strong alkaline conditions (52.96%). Meanwhile, *Funneliformis mosseae* lower (22.99%) to moderate (35.78%) distribution in alkaline conditions. Soil alkalinity is known to be detrimental to AMF, but the members of Glomeraceae might present more frequently (~65%) with higher spore densities. This indicates a strong adaptation, so it can be explored to aid the recovery of degraded areas due to moderate to strong alkaline soil conditions.

In general, it can be concluded that natural revegetation can take place on calcareous land, as shown by the ability of plants to develop in alkaline soil conditions. In degraded soil, such as calcareous land, plants can cooperate with AMF. This adaptation ability can be leveraged to accelerate revegetation and rehabilitation of degraded land.

## ACKNOWLEDGEMENTS

We thank the Head of Research Institutions and Community Service, Cenderawasih University and the Head of the Indonesian Islamic University (Universitas Islam Indonesia/UII) Research Institute for supporting this research. The research was funded by Domestic Cooperation Research (*Penelitian Kerjasama Dalam Negeri/PKDN*), DGHE, Ministry of Higher Education the Republic of Indonesia, 2023, and Laboratory of Mycology, Cenderawasih University, Jayapura, and UII Environmental Engineering Laboratory, Yogyakarta.

## REFERENCES

- Akhalkatsi M, Arabuli G, Togonidze N. 2018. Plant species composition and diversity on the calcareous soils in around of the limestone quarry in Georgia, Caucasus region. *MOJ Food Process Technol* 6 (3): 251-256. DOI: 10.15406/mojfpt.2018.06.00172.
- Alrajhei K, Saleh I, Abu-Dieyeh MH. 2022. Biodiversity of arbuscular mycorrhizal fungi in plant roots and rhizosphere soil from different arid land environment of Qatar. *Plant Direct* 6 (1): e369. DOI: 10.1002/pld3.369.
- Bartolome AP, Villaseñor IM, Yang W-C. 2013. *Bidens pilosa* L. (Asteraceae): Botanical properties, traditional uses, phytochemistry, and pharmacology. *Evid Based Complement Alternat Med* 2013: 340215. DOI: 10.1155/2013/340215.
- Beslemes D, Tigka E, Roussis I, Kakabouki I, Mavroeidis A, Vlachostergios D. 2023. Effect of arbuscular mycorrhizal fungi on nitrogen and phosphorus uptake efficiency and crop productivity of two-rowed barley under different crop production systems. *Plants* 12 (9): 1908. DOI: 10.3390/plants12091908.
- Campo S, Martín-Cardoso H, Olivé M, Pla E, Catala-Forner M, Martínez-Eixarch M, Segundo BS. 2020. Effect of root colonization by arbuscular mycorrhizal fungi on growth, productivity and blast resistance in rice. *Rice* 13 (1): 42. DOI: 10.1186/s12284-020-00402-7.
- Chanclud E, Morel J-B. 2016. Plant hormones: A fungal point of view. *Mol Plant Pathol* 17 (8): 1289-1297. DOI: 10.1111/mpp.12393.
- de Oliveira JSF, Xavier LP, Lins A, Andrade EHA, Maia JGS, de Mello AH, Setzer WN, Ramos AR, da Silva JKR. 2019. Effects of inoculation by arbuscular mycorrhizal fungi on the composition of the essential oil, plant growth, and lipoxigenase activity of *Piper aduncum* L. *AMB Express* 9 (1): 29. DOI: 10.1186/s13568-019-0756-y.
- Diagne N, Ngom M, Djighaly PI, Fall D, Hoche V, Svistoonoff S. 2020. Roles of arbuscular mycorrhizal fungi on plant growth and performance: Importance in biotic and abiotic stressed regulation. *Diversity* 12 (10): 370. DOI: 10.3390/d12100370.
- Eviati, Sulaeman. 2009. Analisis kimia tanah, tanaman, air, dan pupuk. Balai Penelitian Tanah, Bogor. [Indonesian]
- Hodge A, Fitter AH. 2013. Mechanisms of plant competition: Microbial mediation of plant competition and community structure. *Funct Ecol* 27 (4): 865-875. DOI: 10.1111/1365-2435.12002.
- Husna, Tuheteru FD, Arif A. 2021. 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.
- INVAM. 2022. International culture collection of (vesicular) arbuscular mycorrhizal Fungi. <https://invam.ku.edu/>.
- Kumalawati Z, Muliani S, Asmawati A, Kafrawi K, Musa Y. 2021. Exploration of arbuscular mycorrhizal fungi from sugarcane rhizosphere in marginal land. *Plant Tropika: J Agro Sci* 9 (2): 126-135. DOI: 10.18196/pt.v9i2.4026.
- Liu W, Zhang Y, Jiang S, Deng Y, Christie P, Murray PJ, Li X, Zhang J. 2016. Arbuscular mycorrhizal fungi in soil and roots respond differently to phosphorus inputs in an intensively managed calcareous agricultural soil. *Sci Rep* 6: 24902. DOI: 10.1038/srep24902.
- Ma X, Xu X, Geng Q, Luo Y, Ju C, Li Q, Zhou Y. 2023. Global arbuscular mycorrhizal fungal diversity and abundance decreases with soil available phosphorus. *Glob Ecol Biogeogr* 32 (8): 1423-1434. DOI: 10.1111/geb.13704.
- McPherson JJ. 2013. Conservation assessment of calcareous ecosystems in Pennsylvania. Pennsylvania Natural Heritage Program at Western Pennsylvania Conservancy. Pittsburgh, PA. Report to Wild Resources Conservation Program, Harrisburg, PA.
- Mueller-Dombois D, Ellenberg H. 1974. *Aims and Methods of Vegetation Ecology*. John Wiley & Sons, Inc, New York.
- Navarro-Ramos SE, Sparacino J, Rodríguez JM, Filippini E, Marsal-Castillo BE, García-Cannata L, Renison D, Torres RC. 2022. Active revegetation after mining: What is the contribution of peer-reviewed studies? *Heliyon* 8 (3): e09179. DOI: 10.1016/j.heliyon.2022.e09179.
- O'Callaghan M, Ballard RA, Wright D. 2022. Soil microbial inoculants for sustainable agriculture: Limitations and opportunities. *Soil Use Manag* 38 (3): 1340-1369. DOI: 10.1111/sum.12811.
- Parihar M, Rakshit A, Singh HB, Rana K. 2019. Diversity of arbuscular mycorrhizal fungi in alkaline soils of hot sub humid eco-region of Middle Gangetic Plains of India. *Acta Agric Scand B - Soil Plant Sci* 69 (5): 386-397. DOI: 10.1080/09064710.2019.1582692.
- Piqueray J, Bisteau E, Bottin G, Mahy G. 2007. Plant communities and species richness of the calcareous grasslands in Southeast Belgium. *Belg J Bot* 140 (2): 157-173. DOI: 10.2307/20794637.
- Prayudyaningsih R, Sari R. 2016. The application of arbuscular mycorrhizal fungi (AMF) and compost to improve the growth of teak seedlings (*Tectona grandis* Linn.F.) on limestone post-mining soil. *Jurnal Penelitian Kehutanan Wallacea* 5 (1): 37-46. DOI: 10.18330/jwallacea.2016.vol5iss1pp37-46. [Indonesian]
- Püschel D, Bitterlich M, Rydlová J, Jansa J. 2020. Facilitation of plant water uptake by an arbuscular mycorrhizal fungus: A Gordian knot of roots and hyphae. *Mycorrhiza* 30 (2-3): 299-313. DOI: 10.1007/s00572-020-00949-9.
- Quoreshi AM, Suleiman MK, Manuvel AJ, Sivadasan MT, Jacob S, Thomas R. 2019. Biofertilizers for agriculture and reclamation of disturbed lands: An eco-friendly resource for plant nutrition. *J Mech Cont Math Sci* 4: 231-243. DOI: 10.26782/jmcs.spl.4/2019.11.00023.
- Samal SI, Mansur I, Junaedi A. 2023. Exploration of indigenous arbuscular mycorrhizal fungi on *Arenga pinnata* Merr in post-mining land. *Indones Min J* 26: 39-47. DOI: 10.30556/imj.Vol26.No1.2023.1285.
- Schenck NC, Perez Y. 1990. *Manual for the identification of VA mycorrhizal fungi*. Synergis Publication, Gainesville, USA.
- Schüßler A, Walker C. 2010. *The Glomeromycota. A species list with new families and new genera*. With correction on July 2011. [online]. The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University.
- Sheoran V, Sheoran AS, Poonia P. 2010. Soil reclamation of abandoned mine land by revegetation: A review. *Intl J Soil Sediment Water* 3 (2): 13.
- Sinegani AAS, Yeganeh ME. 2017. The occurrence of arbuscular mycorrhizal fungi in soil and root of medicinal plants in Bu-Ali Sina garden in Hamadan, Iran. *Biol J Microorganism* 5 (20): 43-59.
- Singare PB, Meshram NA, Kausadikar HK. 2022. Calcareous soil and their management-A review. *Agriculture* 3 (4): 1-6.
- Souza T. 2015. *Handbook of arbuscular mycorrhizal fungi*. Switzerland: Springer International Publishing.
- Sorensen KW. 1993. Indonesian peat swamp forests and their role as a carbon sink. *Chemosphere* 27 (6): 1065-1082. DOI: 10.1016/0045-6535(93)90068-G.
- Warren M, Hergoualc'h K, Kauffman JB, Murdiyoso D, Kolka R. 2017. An appraisal of Indonesia's immense peat carbon stock using national peatland maps: Uncertainties and potential losses from conversion. *Carbon Balance Manag* 12: 12. DOI: 10.1186/s13021-017-0080-2.
- Suharno S, Rahayu I, Tanjung RHR, Sufaati S. 2022. New record of Arbuscular Mycorrhizal Fungi (AMF) association with kebar grass (*Biophytum petersianum* Klotzsch.) in the grassland area of Kebar, Tambrau Regency, West Papua, Indonesia. *J Trop Biodivers Biotechnol* 7 (2): jtbb70021. DOI: 10.22146/jtbb.70021.
- Suharno S, Sancayaningsih RP, Kasiamdari RS, Soetarto ES. 2021. The growth response of pokem (*Setaria italica* L.) inoculated with Arbuscular Mycorrhizal Fungi (AMF) from tailings area. *J Degrad Min Land Manag* 8 (4): 2873-2880. DOI: 10.15243/jdmlm.2021.084.2873.
- Suharno S, Sancayaningsih RP, Soetarto ES, Kasiamdari RS. 2014. The presence of arbuscular mycorrhizal fungi in the tailings of mining gold Timika as an attempt of environmentally friendly. *Jurnal Manusia dan Lingkungan* 21 (3): 295-303. DOI: 10.22146/jml.18556. [Indonesian]

- Suharno S, Sufaati S, Agustini V, Tanjung RHR. 2018. Arbuscular mycorrhizal fungi associated with wati (*Piper methysticum*), a medicinal plant from Merauke Lowland, Papua, Indonesia. *Biosaintifika J Biol Biol Educ* 10 (2): 260-266. DOI: 10.15294/biosaintifika.v10i2.14303.
- Suharno, Tanjung RHR, Sufaati S. 2020. Arbuscular Mycorrhizal Fungi Accelerate the Rehabilitation of Mining Areas. UGM Press, Yogyakarta.
- Sun X-G, Tang M. 2012. Comparison of four routinely used methods for assessing root colonization by arbuscular mycorrhizal fungi. *Botany* 90 (11): 1073-1083. DOI: 10.1139/b2012-084.
- Taalab AS, Ageeb GW, Siam HS, Mahmoud SA. 2019. Some characteristics of calcareous soils. A review. *Middle East J Agric Res* 8 (1): 96-105.
- Taher M, Amri MS, Susanti D, Kudus MBA, Nor NFAM, Syukri Y. 2020. Medicinal uses, phytochemistry, and pharmacological properties of *Piper aduncum* L. *Sains Malays* 49 (8): 1829-1851. DOI: 10.17576/jsm-2020-4908-07.
- Upadhye M, Kuchekar M, Pujari R, Kadam S, Gunjal P. 2021. *Muntingia calabura*: A comprehensive review. *J Pharm Biol Sci* 9 (2): 81-87. DOI: 10.18231/j.jpbs.2021.011.
- Vierheilig H, Schweiger P, Brundrett M. 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiol Plant* 125 (4): 393-404. DOI: 10.1111/j.1399-3054.2005.00564.x.
- Wahab A, Muhammad M, Munir A, Abdi G, Zaman W, Ayaz A, Khizar C, Reddy SPP. 2023. Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. *Plants* 12 (17): 3102. DOI: 10.3390/plants12173102.
- Wang F, Zhang L, Zhou J, Rengel Z, George TS, Feng G. 2022. Exploring the secrets of hyphosphere of arbuscular mycorrhizal fungi: Processes and ecological functions. *Plant Soil* 481: 1-22. DOI: 10.1007/s11104-022-05621-z.
- Wibowo C, Mulyana D, Wildasari W. 2020. Species composition of understorey vegetation and soil properties in ex mining land silica and lime closure PT Holcim Indonesia Tbk. *Jurnal Silvikultur Tropika* 11 (2): 82-88.
- Yan P, Hou H, Lv Y, Zhang H, Li J, Shao L, Xie Q, Liang Y, Li J, Ni X. 2023. Diversity characteristics of arbuscular mycorrhizal fungi communities in the soil along successional altitudes of Helan Mountain, arid, and semi-arid regions of China. *Front Microbiol* 14: 1099131. DOI: 10.3389/fmicb.2023.1099131.
- Zhu S-C, Zheng H-X, Liu W-S, Liu C, Guo M-N, Huot H, Morel JL, Qiu R-L, Chao Y, Tang Y-T. 2022. Plant-soil feedbacks for the restoration of degraded mine lands: A review. *Front Microbiol* 12: 751794. DOI: 10.3389/fmicb.2021.751794.