Exploration and isolation of indigenous arbuscular mycorrhizal fungi in post-landslide sandy and clayey silt for eco-friendly remediation

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Abstract. Critical lands are formed after landslides due to the erosion and stripping of the surface soil in the depletion zone and the formation of accumulation zones, requiring remediation efforts. Indigenous arbuscular mycorrhizal fungi help rehabilitate degraded lands, including post-landslide sites, by adapting to native soil conditions. This study investigates mycorrhiza's presence in post-landslide areas with sandy silt in Cililin and clayey silt in Citatah. Soil samples are collected at 30 cm depth using a corer near *Dysoxylum macrocarpum* in Citatah and *Gmelina arborea* in Cililin. Mycorrhiza isolation involves wet sieving and centrifugation. Local mycorrhiza genera identified are *Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora*. Sandy silt soil 2 exhibits the highest spore density of 178 per 100 grams, while clayey silt soil 1 has the lowest density of 148 per 100 grams. *Glomus* dominates in sandy silt with a 93 per 100 grams concentration and a 38-45 µm size. *Scutellospora* is scarce in clayey silt, with only 1 per 100 grams, 90-170 µm size. Isolated mycorrhiza can serve as compatible and adaptive inoculum. The discovery of mycorrhiza tolerant to post-landslide conditions is expected to play an important role in the remediation process of critical lands caused by landslides.

1 Introduction

Landslide disasters in Indonesia have been gradually increasing, leading to loss of life, material damage, and the emergence of critical land in landslide depletion zones. Additionally, these landslides contribute to soil deposition in landslide accumulation zones.

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Landslides are one of the factors causing land degradation, resulting in the loss or reduction of land potential and productivity. Landslides are also one of the leading causes of permafrost degradation in high-latitude areas [1]. The loss of vegetation, which serves as both water absorbers and surface protectors, contributes to the escalation of critical land and environmental degradation [2].

Post-landslide areas in disaster-prone regions require proper handling and technological input to create soil conditions that support the growth of landslide-resistant vegetation, stabilizing critical land and the surrounding landslide areas. Furthermore, the community can still maximally and safely utilize post-landslide land daily. Post-landslide land in Cililin is classified as sandy silt, whereas post-landslide land in Citatah is defined as clayey silt [3]. Both types of land have an acidic to neutral pH and low levels of C, N, the C/N ratio, and P_2O_5 content [3]. Efforts to improve the soil's biological, physical, and chemical properties can be carried out through remediation of the post-landslide land.

Arbuscular mycorrhizal fungi (AMF) are a group of soil microorganisms that associate with plant roots and benefit plant growth. The role of AMF in enhancing plant growth is through increased absorption of phosphorus (P) and other nutrients such as nitrogen (N), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn), as well as tolerance to water-deficiency conditions [4]. The use of indigenous AMF is one strategy that can be employed for the remediation of post-landslide land due to its adaptability. Mycorrhizae can provide nutrient and water access under suboptimal soil conditions. AMF infects and forms a symbiotic relationship with the roots of host plants, producing external hyphal networks that expand and penetrate deeper soil layers, thereby enhancing root capacity for nutrient and water uptake [5]. AMF improves soil aggregation, thereby increasing porosity [6], and enhances soil physical properties by improving soil structure. Based on the characteristics of AMF, plants associated with AMF can adapt well to critical lands with limited soil conditions, such as landslide-affected areas [7]. The seedling phase heavily depends on mycorrhizae. Therefore, providing suitable plant species associated with AMF for the specific conditions of critical land is vital to enhancing land remediation success. However, the AMF inoculum should preferably consist of indigenous strains adapted to the specific location. Using indigenous AMF strains is essential to producing AMF inoculum most suitable for the actual soil and climatic conditions [8].

Research is needed to assess AMF status and potential for indigenous AMF inoculum production in selected locations. Currently, there needs to be more information on AMF status in landslide-affected lands, specifically in the post-landslide areas of the Cililin sub-district (sandy silt) and the Citatah sub-district (clayey silt). This study aims to identify indigenous AMF status, including spore density and genus types, in both post-landslide sites. It also involves an inventory of pioneer and indigenous plants with potential landslide resistance in the surrounding areas. Methods

2 Methods

2.1 Soil Sampling

Soil sampling was carried out at two post-landslide sites, as follows:

- In the sandy silt soil of the post-landslide Cililin, where *Gmelina arborea* trees are present. Two specific sampling points are G1 (E 107°27'24" S 06°57'20.7") and G2 (E 107°27'24.4" S 06°57'20.3").
- In the clayey silt soil of the post-landslide Citatah, where *Dysoxilum macrocarpum* trees are present. Two specific sampling points are D1 (E 107°24'57.7" S 06°51'27.6" and D2 (E 107°24'56.3" S 5°128.1'57.5").

The soil sampling method for indigenous AMF isolation is done randomly, which involves taking samples from diagonal points within an area, with a total of 2 sampling points. Soil samples are collected using a soil corer, approximately 100 grams, around the roots at a depth of 30 cm. The soil samples were then placed in labeled plastic bags as an indication for transportation to the laboratory.

2.2 Indigenous AMF isolation

Mycorrhizal spores were obtained using the wet sieving technique and centrifugation. Soil samples (100g) were mixed with 500 ml water and settled for 10 minutes. The mixture was sieved through 250, 90, and 38 μm sieves. The filtrate was combined with a glucose solution in a centrifuge tube. After rinsing the supernatant on the 38 μm sieve to remove sucrose, the spores were transferred to a petri dish for counting and identification under a stereomicroscope (100x magnification). Further analysis was conducted using a microscope at magnifications of 400-1000x[9].

2.3 Indigenous AMF identification

Mycorrhizal spore slides are prepared to aid in the identification process, focusing on their morphological characteristics and subcellular structure to determine the genus of vesicular arbuscular mycorrhizae. The isolated spores are examined under a microscope at 400x magnification for genus-level identification. References such as the guidebook "Working with Mycorrhizas in Forestry and Agriculture" and INVAM are used [10]. Spores are carefully collected using a microscope and tweezers, considering morphological features like bulbous suspensor, spore shape, size, color, and wall composition. These observations guide the identification of indigenous vesicular-arbuscular mycorrhizae.

2.4 Data Analysis

The community structure of indigenous AMF was analyzed using several diversity parameters such as isolate frequency, genus/type abundance, spore density, and species richness (Table 1). Isolate frequency reflects the distribution status of indigenous AMF based on the percentage of their presence in the samples [11]. Relative abundance can reveal the strength or weakness of sporulation ability among different AMF species in the ecosystem.

3 Results and Discussion

3.1 Critical land in post-landslide sandy silt and clayey silt

Landslides in Citatah occur on sedimentary rock (sandstone, siltstone, claystone, and sedimentary breccia), while Cililin experiences landslides on Andesitic intrusion rock (Figure 1). Weathering of these rocks formed the soil, with the sliding plane between completely weathered and moderately to slightly weathered rocks. The depletion zone lost the humus-containing soil layer, while the accumulation zone accumulated new soil (Figure 1). Post-landslide land management is necessary, using biotechnical methods or vegetation to stabilize slopes and benefit the local community. Some vegetation may not be suitable for improving slope stability. Local-specific information and support for appropriate biotechnical methods are required. Utilizing indigenous mycorrhizae can aid in the recovery of critical landslide areas and accelerate plant growth.

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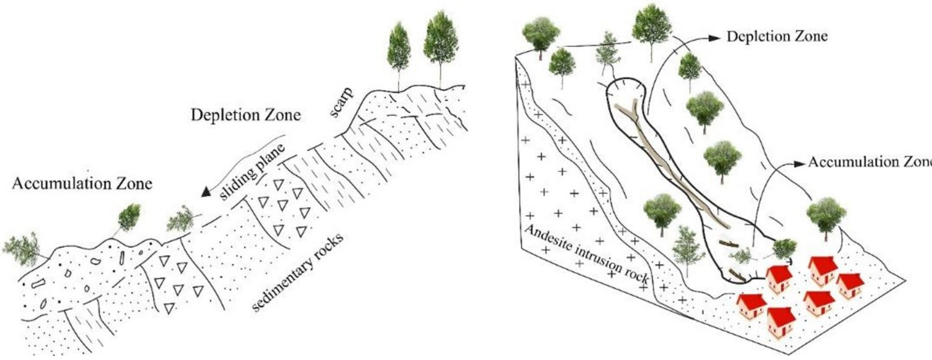


Figure 1. The schematic diagram of landslides in Citatah (left) and Cililin (right) shows the depletion and accumulation zones.

3.2 Results of exploration and isolation of indigenous AMF spores

The morphological characteristics used to identify types of spores are shape, colour, spore wall, ornamentation, and spore size. In this study, the taxonomy of arbuscular mycorrhizal fungi is limited to the genus level. Identifying spore types based on morphological characteristics and the response to Melzer's reaction yielded four genera: *Glomus, Gigaspora, Scutellospora,* and *Acaulospora.* The results of arbuscular mycorrhizal isolation from two soil samples, namely sandy silt and clayey silt, post-landslide, are presented in Figure 2 and Table 1.

No	Spore Shape	Characteristics
1	Glomus sp.   20 µm	The spores of Glomus are globose, sub-globose, ovoid, or obovoid, with spore walls consisting of more than one layer. They can be hyaline t yellow, reddish-brown, brown, or black in color D Q G U D Q J H L Q V L] H [12]. The spore surface is relatively smooth, with thin spore walls, and the diameter of the Glomus spores found in this study ranged from 38 t P [13].
2	Gigaspora sp.   80 µm	This genus possesses distinctive characteristi including the production of single spores in th soil, the absence of an inner spore wall laye the presence of a bulbous suspensor, and globose or sub-globose shape. The spores ar typically cream to yellow in color and range in size from 125 to 600m [12]. This study found the Gigaspora genus to have a diameter rangin from 90 to 210 µm.

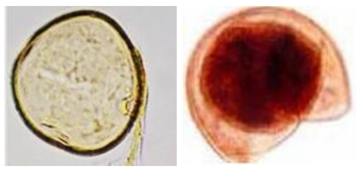
3	Scutellospora sp.  80 µm	This genus exhibits several distincti characteristics, including 2-3 spore walls. Spore are formed on the lateral side of the sporifer saccule neck and are globose to ellipsoidal. spores can be hyaline, yellow, or reddish yellow, with a size ranging from 100 to 400 m [14]. The rupture of the hyphal tip leaves a small h called a cicatrix This study found the Acaulospora genus with a diameter ranging fro 90 to 120 µm.
4	Acaulospora sp.  80 µm	Single spores inside the sporocarp; spores laterally attached to hyphae with swollen ti with sizes almost equal to the spores; sp shapes can be rounded, subglobular, ellipsoi broadly fusi form. The distinguishing featur between Gigaspora and Scutellospora is the presence of a germination shield in Scutellospora [15]. This study found Scutellospora genus with a diameter ranging from 90 to 170 µm.

Figure 2. Mycorrhizal spores at 400x magnification

Table 1. The abundance of mychorrhizae from post-landslide sandy silt and clayey silt

Genus	Post-landslide sandy-silt			Post-landslide clayey silt		
	Sample	Density (spores per 100g of soil)	Diameter	Sample	Density (spores per 100g of soil)	Diameter
Glomus, sp	G1	79	38-45	D1	93	38-45
Gigaspora, sp		64	90-210		42	90-190
Scutellospora, sp		11	90-190		1	90-170
Acaulospora sp.		17	90-120		12	90-100
Total		171	Total		148	
Glomus, sp	G2	83	38-45	D2	99	38-45
Gigaspora, sp		63	90-210		44	90-190
Scutellospora, sp		13	90-190		3	90-170
Acaulospora sp.		19	90-120		11	90-118
Total		178	Total		157	

3.3 Indigenous AMF in post-landslide sandy and clayey silt

Indigenous AMF isolations were conducted in sandy silt (Cililin) and clayey silt (Citatah) post-landslide locations, yielding the genera *Glomus, Gigaspora, Scutellospora*, and *Acaulospora* (Table 1, Figure 1). Spore population dynamics and frequency of spore-type changes varied between locations. *Glomus* was the dominant genus in both areas, indicating its wider distribution than other genera. *Glomus* predominantly dominated the post-landslide site in the Citatah district. The *Glomus* domination is because the texture of the soil sample is classified as clayey silt [3]. Soil dominated by the clay fraction provides suitable conditions for developing *Glomus* spores [16]. The Cililin post-landslide location falls under the sandy silt category [3]. From soil samples taken at this location, it was found that the genus *Gigaspora* is more abundant compared to Citatah.

Mycorrhizae can sustain their life in this spore form, and spores can germinate when conditions are favourable, initiating the root infection process. Mycorrhizal development begins when mycorrhizae are in the soil as spores and eventually infect plant roots. Mycorrhiza thrives in well-drained soils and flooded lands such as rice paddies [17]. It has been stated that mycorrhiza can demonstrate existence even in nutrient-poor environments or environments contaminated with hazardous waste. The highest diversity of arbuscular mycorrhizal types is found in grass-covered lands, followed by lands with low to intermediate inputs, and the lowest in lands continuously and intensively planted with corn [18].

Isolated indigenous AMFs can be multiplied, and they have the potential to help with post-landslide land remediation by acting as a biofertilizer to promote plant growth. It is expected to adapt quickly, accelerating land recovery and the growth of slope-strengthening plants. The carbon within glomalin, produced by mycorrhiza through its external root structures, significantly contributes to the increase of soil organic carbon and positively impacts soil improvement [19]. Mycorrhiza could enhance the soil's cation exchange capacity (CEC) [20]. Increased CEC indicates that the soil can retain nutrients, improving nutrient availability for plant growth. Additionally, plants in symbiosis with mycorrhiza can enhance nutrient and water uptake, increase drought tolerance, and prevent infection from pathogenic organisms [21].

The activity of mycorrhiza is influenced by factors such as organic matter, aeration, pH, and nutrient availability. Mycorrhiza thrives well in well-aerated soils, such as sandy soils. Under unfavourable environmental conditions, mycorrhiza exhibits higher performance. With limited nutrient availability, mycorrhiza will exert more effort in infecting plants. Previous research findings indicate that post-landslide sandy silt in Cililin has a pH range from acidic to neutral (5.3-6.8). In contrast, post-landslide clayey silt in Citatah has an acidic pH of 5.9. Both locations have low levels of organic carbon, nitrogen, the C/N ratio, and P_2O_5. Post-landslide sandy silt contains C-organic = 1.17-1.72%, N = 0.11-0.19%, C/N ratio = 9, and P_2O_5 = 6.6–11 ppm, whereas clayey silt contains organic carbon = 2.98%, N = 0.28%, C/N ratio = 11, and P_2O_5 = 7–7.2 ppm [3]. Due to the low nutrient content of sandy and clayey silt, mycorrhiza is expected to work harder to infect plants.

The presence of native mycorrhizal fungi and their potential for remediation in post-landslide sandy and clayey silt areas is expected to assist in restoring slope stability following sliding events that require immediate attention. The discovery of mycorrhizae through exploration, isolation, and identification around *Gmelina arborea* plants in sandy silt and around *Dysoxylum macrocarpum* plants in clayey silt post-landslide indicates that these plants have positive mycorrhizal symbiosis.

A replanting plan is an important step in slope stabilisation. The selection of plant species is critical in reestablishing slope stability after a landslide. The plant selection can involve employing local species found in the respective areas as a land improvement effort. Local plants in sandy silt in Cililin encompass *Gmelina arborea, Swietenia macrophylla* (mahogany), *Acacia mangium* (acasia), *Bambusa,* sp. (bamboo), and *Paraserianthes falcataria* (albizia). Meanwhile, local plants in clayey silt in Citatah include *Gmelina arborea*, *Anthocephalus cadamba* (jabon), *Paraserianthes falcataria* (albizia), *Bambusa*, sp. (bamboo), *Acacia mangium* (acasia), *Parkia speciosa* (stink bean). These local plant species are deemed more effective due to their adaptability to their respective locations. Based on analyses of root growth rate and biomass, *Gmelina arborea* can be recommended as the best woody plant for slope stabilisation in both the post-landslide sandy silt area in Cililin and the post-landslide clayey silt area in Citatah [3]. As a result, the *Gmelina arborea* should be preserved and used to stabilise slopes in post-landslide areas.

The symbiosis between local plants and indigenous AMF can accelerate the remediation process in post-landslide areas. Furthermore, using indigenous AMF serves the purpose of eco-friendly ecosystem remediation, returning the ecosystem to its original state. This approach contributes to the long-term sustainability of the natural ecosystem.

4 Conclusion

Four mycorrhizal spore genera, *Glomus, Gigaspora, Acaulospora*, and *Scutellospora*, were successfully isolated and identified during the investigation of indigenous mycorrhizal spores in post-landslde sandy silt in Cililin with *Gmelina arborea* as host trees and post-landslide clayey silt in Citatah with *Dysoxylum macrocarpum* as host trees.

The sandy silt soil samples collected from the post-landslide location in Cililin, characterized by sandy soil with humus content, exhibited a higher abundance of the *Gigaspora* genus than the post-landslide location in Citatah. In contrast, the clayey silt samples from the post-landslide in Citatah were predominantly dominated by the *Glomus* genus.

The application of indigenous mycorrhiza for post-landslide land remediation is expected to have more adaptability, resulting in faster mycorrhiza performance. Similarly, selecting fast-growing local plants will make it possible for easier adaptation and accelerate the slope stabilization process.

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