Diversity of Arbuscular Mycorrhizal Fungi as Affected by Time Consequences Revegetation Age in Post Coal Mine Area at PT Berau Coal Tbk, East Kalimantan Indonesia

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Abstract. Arbuscular Mycorrhizal Fungi (AMF) is one of the fungi that can be found in almost every ecosystem including post-coal mining land. This study aims to analyze the diversity of AMF in different age of revegetation of post-coal mining land area. Soil samples were collected from six different age of revegetation of post-coal mining area (unrevegetated area, 0, 2, 4, 6 and 8 years) and from natural forest as comparison. AMF spore isolation was carried out using wet-sieving method, while spore identification was carried out based on several morphological characters according to INVAM guideline. The results of the observation showed that with increasing of revegetation age will be able to increase the number of AMF populations ($R^2 = 84.96\%$). The highest AMF population was found at the age of 10 years after revegetation of land with the number of 492 spores per 20 grams soil, while the lowest AMF population was found in unrevegetated area with the number of 12 spores per 20 grams of soil. AMF genera found included Glomus, Acaulospora, Gigaspora, Scutellospora, and Dentiscutata. Glomus and Acaulospora were the dominant AMF in every land revegetation age (100% of frequency). AMF populations was correlated strongly with soil organic content ($r = 0.79$).

1. Introduction
Mycorrhiza is a symbiotic relationship between fungi with the roots of higher plants. The symbiosis that occurs is mutually beneficial for the host plant [1]. According to Smith and Read [2], generally mycorrhizae can be grouped into two groups, namely ectomycorrhiza and endomycorrhiza. Endomycorrhiza has a fairly wide range of hosts compared to ectomycorrhizae. Endomycorrhiza can also be called arbuscular mycorrhizal fungi (AMF). The AMF belongs to the Glomeromycota class which has four orders, 11 families and 17 genera [3]. AMF is an obligate fungus that requires a host to be transferred. Nearly more than 80% of plants are capable of symbiosis with AMF [4]. AMF is a potential natural resource that has an important role in the agricultural system [5].

AMF is one of the dominant types of endomycorrhiza and almost found in various ecosystems [6]. The presence of AMF in an ecosystem is strongly influenced by abiotic factors (climate and edaphic) and biotic factors (vegetation and microorganisms) [7, 8]. These factors can influence the distribution of AMF populations in the ecosystem.
AMF populations can be found in undisturbed ecosystems that are already disturbed. One of the disturbed ecosystems is post-mining lands. The results of several studies indicate that AMF can be found on coal mining areas [9, 10]. The population of AMF on coal mining areas varies according to the age of revegetation. Along with the increasing age of revegetation will increase the population of AMF [11] [12]. The presence of AMF in post-mining land is very helpful in the process of plant growth and helps the succession process that occurs [13]. The success of a reclamation and revegetation of the post-mining land is greatly influenced by soil conditions and host plants. According to Soka [15] AMF is very sensitive to changes soil and host plants. Generally, post-mining soils especially coal has low pH and Al, Fe and Mn are quite high. In addition, post-mining soils generally have a fairly high proportion of rocks, high bulk density and low infiltration [16, 17]. These conditions cause post-mining soils to have low fertility, so they are unable to support plant growth optimally.

There is very limited information regarding the diversity of AMFs in post-coal mining land in Indonesia, especially those focusing on various revegetation age classes. Differences in post-mining land age classes cause dynamics of AMF diversity [9]. Each type of plant has different characteristics, especially in forming symbiosis with AMF. In addition, each type of AMF has different abilities in forming symbiosis with plant roots [18]. According to Delvian [18] the relationship between AMF and plant roots can increase the success of revegetation activities in post-mining lands. Therefore, research is needed on the diversity of AMF in various age classes of post-coal mining revegetation. This study aims to examine the diversity of AMF in various age classes of post-coal mining revegetation, to examine the characteristics of soil properties in various age classes of post-coal mining revegetation and to analyze the relationship between soil properties with AMF diversity in various age classes of post-coal mining revegetation.

2. Method

2.1. Study site

The study was conducted in October 2018 at PT. Berau Coal, Berau Regency, East Kalimantan. PT Berau Coal is located between 01°52'26.67"LU-02° 2.5'09.78" LU and 117°07'44.52" BT-117°38'26.46 "BT. PT. Berau Coal has a concession area of 118,400 hectares consisting of three sites which are Lati, Sambarata, and Binungan. The study was conducted in the Binungan block and several revegetation age classes ranging from unrevegetated, 0, 2, 4, 6, 8 and 10 years. While natural forests were used as controls.

2.2. Procedure

2.2.1 Sample collection

Soil samples were taken from seven revegetation areas and from natural forests as a control. The plot used in soil sampling follows the method of Sahner et al. [20], the plot was established with an area of 50m x 50m and in it three sub-plots of 5m x 5m. Soil samples were taken from five points from each sub plot. The total sample of all revegetation areas is 120 samples (8 plots, 3 sub plots and 5 points).

2.2.2 Analysis of soil chemical properties

Each parameter of soil properties was analyzed using different methods. The soil parameters measured and the method of soil analysis are presented in Table 1.

Table 1. Parameter and soil analysis method
SOC: soil organic carbon; CEC: cation exchange capacity.

2.2.3 Spore isolation
Spores were isolated using the wet-sieving method and continued with centrifugation [21]. Soil samples were weighed as much as 20 grams, then put into a measuring cup and added with water as much as 2/3. The soil was stirred for a few minutes and allowed to stand for a few seconds then spilled into a spore filter (500 μm, 125 μm and 45 μm). Repeat the process until the suspension of the soil in the measuring cup is clear enough. Soil deposits retained in two sieve (125 μm) and one (45 μm) were then inserted into a centrifuge tube. The glucose solution was then added to the tube approximately two-thirds of the volume of the tube. The sample was then centrifuged at a speed of 3,000 rpm (rotation per minute) for ± 3 minutes. Supernatant solution was poured into a petri dish to calculate the number of spores and observe the identification of spores under a microscope.

2.2.4 Spore identification
AMF spores were identified by the method of Schenck and Perez [22] based on morphological characters ranging from shape, size, color, carrier hyphae, wall layers, ornamentation of spores (spore jewelry) and their reaction with melzer solution. In addition, the AMF spores are matched with the spores on the website https://invam.wvu.edu/ and from the results of several recent studies.

2.2.5 Data analysis
The diversity of AMF was calculated starting from the number of spores and the frequency of the spores. In addition, regression and correlation analyzes were performed to see the relationship between revegetation age and the number of spores and soil properties and the number of spores.

3. Result and Discussion

3.1 Characteristics of Soil Chemical Properties in Post Coal Mining Revegetation area
The chemical properties of soil from post-mining revegetation areas are presented in Table 2. The nature of soil clams at various revegetation ages varies greatly (Table 2). The varied chemical properties of the soil cause inconsistent development of soil fertility in each revegetation age class [9]. In addition, this condition has hampered the recovery of post-mining area fertility. Restoring soil fertility on post-coal mining land requires very high inputs with considerable time [12]. Low soil fertility in revegetation land is due to low input of nutrients supplied to the soil.

| Revegetation age (year) | pH  | SOC (%) | N-Total (%) | P-Available (ppm) | P-Total (ppm) | K (cmol(+) /kg) | CEC (cmol(+) /kg) |
|-------------------------|-----|---------|-------------|-------------------|---------------|----------------|------------------|
| Natural forest          | 3.68| 1.02    | 0.13        | 1.48              | 37.22         | 0.16           | 33.14            |
| Unrevegetated 0         | 4.08| 0.34    | 0.19        | 1.41              | 54.32         | 0.12           | 38.77            |
|                         | 5.04| 2.50    | 0.11        | 1.22              | 111.70        | 0.26           | 31.23            |
Revegetation activities can increase the productivity of degraded soils such as post-mining lands through planting various types of plants (development of extensive root systems), the use of soil microbes which can later provide nutrients into the soil [23]. Soil analysis results in revegetation activities can increase soil pH and soil organic carbon. Even the pH and c-organic value of soil in revegetation age class is higher than in forest land. However, the pH value at each revegetation age class is still very acid to acid [24]. This is because open mining has caused an atmospheric oxidation process that results in contamination of ground water. This condition causes the concentration of pyrite in the soil to increase which causes the soil to become acidic [25]. Provision of organic fertilizer in improving soil fertility is able to increase soil pH and soil organic carbon. In addition, the increase in soil organic carbon content is also influenced by the presence of vegetation that grows on every land of revegetation age class. Even the provision of organic materials accelerate the process of succession and formation of AMF [12]. Total N, available P, total P, total K and CEC showed different variations in each revegetation age class. Revegetation activities have been able to increase total N, available P, total P, total K and CEC. However, the increase is not consistent in every increase in revegetation age class.

3.2 AMF population in various age classes of revegetation post-coal mining area

AMF spore populations describe the number of spores present in an ecosystem. Revegetation activities are able to increase the number of AMF spores in the post-coal mining area. The number of AMF spores in revegetation land increased from 0 to 6 years, decreased in the 8 year age class and then increased again in the 10 year age class (R² = 84.96%) (Figure 1).

![Graph showing AMF spore population](image)

**Figure 1.** Number of AMF spores in various age classes of revegetation of post-coal mining lands

The development of AMF is not optimal due to heterogeneous soil fertility conditions in various revegetation age classes. According to Sieverding [26], the AMF population in an ecosystem is strongly influenced by climate and soil fertility. In soils where the level of soil fertility is high enough the role of AMF is not active. Conversely, in lands with low soil fertility, such as post-mining land, the role of the AMF is very active [27].
The results of the correlation between the number of spores with the chemical properties of the soil refers only to C-organic soil which has a significant effect on the number of spores (Table 3). This condition is due to the variation in some of the chemical properties of the soil in each revegetation age class. The AMF population is strongly correlated with soil organic carbon \((r = 0.794)\). These results are in accordance with research by Bath et al. [28] which shows that soil organic carbon has a significant positive correlation with the number of spores. The AMF population increases with increasing soil organic carbon content [29]. The other hand, soil organic carbon content will increase with increasing AMF population in the soil. This is due to cells from AMF being able to release exudates in the form of organic carbon [30]. Soil organic carbon is able to stimulate the presence of AMF in the soil [31].

The presence of AMF populations in the soil is strongly influenced by soil organic carbon [32]. Generally the development of microorganisms in the soil is stimulated by the presence of organic matter. The organic matter is able to change soil conditions which later can be useful for the development of AMF [33]. Based on the results of the study of Hammer et al [34] organic matter is able to support the growth of external mycelium from AMF.

| Chemical properties of soil | pH | SOC (%) | N-Total (%) | P-available (ppm) | P-Total (ppm) | K (cmol(+)kg) | CEC (cmol(+)kg) |
|-----------------------------|----|---------|-------------|------------------|---------------|---------------|----------------|
| Number of Spores            | 0.07 ns | 0.79*   | 0.28 ns     | 0.44 ns          | 0.06 ns       | -0.27 ns      | 0.7 ns         |

*: There was significant at the 95% level; ns: not significant.

### 3.3 Diversity of AMF in various age classes of revegetation of post-coal mining

The diversity of AMF in various age classes of revegetation of post-coal mines identified up to the genera level. The identification results showed that there were five AMF genera found in the post-coal mining area (Table 4).

**Table 4.** Diversity of the AMF genera in various age classes of revegetation post-coal mining revegetation.

| Revegetation age | Genere                        |
|------------------|-------------------------------|
| Natural forest   | Acaulospora, Dentiscutata, Gigaspora, Glomus, Secutellospora |
| Unrevegetated    | Acaulospora, Glomus           |
| 0 year           | Acaulospora, Glomus, Dentiscutata, Gigaspora |
| 2 year           | Acaulospora, Glomus, Dentiscutata |
| 4 year           | Acaulospora, Dentiscutata, Gigaspora, Glomus, Secutellospora |
| 6 year           | Acaulospora, Dentiscutata, Glomus, Secutellospora |
| 8 year           | Acaulospora, Glomus, Secutellospora |
| 10 year          | Acaulospora, Glomus, Secutellospora |

Based on Table 4, the diversity of the AMF genera in revegetation land aged 4 years is equivalent to the diversity of the AMF genera in natural forest. This shows that the presence of post-mining revegetation activities has been able to increase the diversity of the AMF genera. Improvement of soil fertility and planting of various types of plants is one factor that is able to restore the diversity of AMF in the post-coal mine. According to Wubet et al. [35] host plants, AMF species and seasons greatly influence the diversity of AMF in the soil. According to Erikson [36], plant species can influence AMF diversity. Several species of plants that exist above the ground surface can be a host for the
survival of AMF [37]. Generally the presence of disturbance such as coal mining activities may reduce the diversity of AMF [38, 39]. Generally high AMF diversity is on undisturbed lands [40].

The genera AMF Acaulospora and Glomus have a relative frequency of 100%. This shows that the two genera were found in each age class of revegetation after coal mining (Figure 2). The genera Acaulospora and Glomus are able to adapt to extreme environmental conditions, such as in post-mining lands. High adapted genera are able to survive in disturbed soils compared to vulnerable genera [41]. According to Santri et al. [42], the genera Acaulospora and Glomus are the genera that is almost found in every ecosystem. The genera Acaulospora is able to grow and develop well on acid soils [25]. In addition, the genera Acaulospora dominates in degraded lands [15]. While the genera Glomus is able to dominate in post-mining lands and has the ability to adjust sporulation patterns to environmental conditions, so that it is able to dominate around the rhizosphere [43]. Other genera such as Dentiscutata, Gigaspora and Secutellspora have a narrower spread compared to the genera Acaulospora and Glomus. Therefore, these genera do not dominate much in revegetation areas after coal mining. Examples of genera morphology found in coal mining lands can be seen in Figure 3.

![Figure 2](image-url)

**Figure 2.** The relative frequency of each genera AMF

![Figure 3](image-url)

**Figure 3.** Morphology of the Genera AMF found, (A) Glomus, (B) Dentiscutata, (C) Gigaspora, (D) Acaulospora and (E) Secutellspora.

4. Conclusion
The population of AMF spores increased with revegetation activities. The diversity of AMF in the 4 year revegetation age class has equaled the diversity of the AMF in natural forests. There are 4 genera of AMF found on revegetation land and natural forest namely Acaulospora, Dentiscutata, Gigaspora, Glomus and Secutellspora. Revegetation land has different soil fertility in each age class. Revegetation activities increase soil pH and c-organic. The AMF spore population has a close relationship with the soil organic carbon.

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