The density and diversity of Arbuscular mycorrhizal spores on land covers with different tree canopy densities at the UNS educational forests

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Abstract. Mycorrhiza is a 'super-organism,' potential to support plants facing increment temperatures due to more open tree canopy cover. This research aimed to study how three different land covers affect the abundance and diversity of mycorrhiza spores in the UNS educational forests. The research was descriptive-explorative with a survey approach on three land covers with different canopy densities, i.e.: (1) mahogany, (2) pine, and (3) pine replanting, with a canopy cover of 95.56%, 80.93%, and 16.50%, respectively. Soil samples were taken by purposive random sampling at several points at a soil depth of 0-30 cm in a 200 m² transect, then composited. Repeated each land cover was four times. The results showed that land cover with a more open canopy had a higher density of mycorrhiza spores than a dense canopy. The spores' density on the land cover of Pine replanting > Pine > Mahogany was 865, 761, and 608 spores/100 g of soil, with a diversity index (H’) 1.00; 0.63; and 0.88, respectively. The types of spores found were Gigaspora sp., Glomus sp., and Archaeospora sp. Mycorrhizal spore density was related to soil temperature (r = 0.56*) and C-organic (r = -0.68**). Mycorrhizae provide new hope to support plant tolerance facing higher temperatures due to climate changes at the UNS educational forests.

1. Introduction
The UNS educational forests often referred to as KHDTK Gunung Bromo, is a forest area whose management was handed over from the Ministry of Environment and Forestry (KLHK) to UNS in 2018, based on the Decree (SK) of the Minister of Environment and Forestry No.177/MENLHK/SETJEN/PLA.0/4/2018 [1],[2]. The forest area is 122.78 ha. These forests were originally managed by Perhutani as limited production forests that produces pine sap so that Pinus merkusii was the dominant tree [3]. The types of land covers in the UNS educational forest can be grouped into six, including mixed land for various types of trees, pine, mahogany, mahogany-pine, pine replanting (open land), and an annual crop. Each of them has a different tree canopy density so that it gives a different dynamic effect of temperature and soil moisture [4].

Tropical forests, like the UNS educational forests, have important potential in mitigating climate change because they act as carbon sinks with a large amount of stable soil organic matter [5]. Forests also function to control water systems, reduce land degradation and desertification, and provide livelihoods and various products for people living around them. However, the unsustainable use of...
forests and the increase of drought frequency can reduce forest covers, soil biota and ecosystem services provided [6].

Mycorrhiza fungal tissue plays a vital role in forest ecosystems since almost all vegetation in the forest network through these below-ground fungi. The changes in composition and diversity of plant communities above the soil surface can affect the processes and functions of soils through changes in the tree canopy, the soil temperature and moisture, the litter production, the root exudates, the provision of habitat for soil biota, and the diversity and activity of soil microbes [7]. Forests that were less disturbed with high densities of trees and shrubs had high densities of arbuscular mycorrhiza (AMF) spores and root colonization [6]. In contrast, disturbed forests with low densities of vegetation had low spores abundance and AMF colonization. The presence of AMF is significant for plant communities on dry land or in ecosystems that experience dry season changes [8].

The ecosystem function is directly and indirectly influenced by the processes carried out by soil biota [7]. AMF affect various ecosystem processes and functions, both in natural and managed ecosystems [9]. AMF are beneficial fungi that form a symbiosis with more than 80-90% of terrestrial plant roots [10],[11]. AMF facilitates and increase nutrient and water uptake by plants, while host plants provide carbohydrates and lipids to AMF [12]. These fungi are a member of Phylum Glomeromycota [13], have the ability to promote plant growth and maintain plant health through increasing mineral nutrition and plant tolerance towards abiotic and biotic stresses [14].

AMF have arbuscular structures, vesicles, hyphae, and intraradical and extraradical spores [6]. Spores are a form of mycorrhiza propagules that are resistant and able to survive in unfavorable conditions [15]. The higher the stress level, the more spores are formed. Factors that can influence the existence of mycorrhizal associations are (a) root characteristics, (b) edaphic or ecological factors, (c) soil bio-dynamics, and (d) compatibility of fungi with the host [16]. The formation of AMF spores is highly influenced by the season, while the physicochemical characteristics of the soils give a specific location effect [8]. The results of research by [9] showed that soil characteristics, especially pH, nitrogen and micronutrients of Zn and Cu as well as root habitat, significantly affected the AMF community, while the host plant and climate were only slightly described.

Land use has a substantial effect on mycorrhiza, both species composition and diversity, but some of these influences have not been widely studied in the tropics [17]. Research on the density and diversity of AMF spores has never been conducted in the UNS educational forests. How is the effect of several land covers with different tree canopy density levels on the density and diversity of AMF spores? What factors influence the density of AMF spores? These questions will be answered through this paper.

2. Methods
Using a descriptive exploratory research with a survey approach, this study was conducted in the UNS educational forests "KHDTK Gunung Bromo" in Karanganyar Regency. The geographical position was located at 7°35'21.93" - 7°35'38.09" S and 110°59'40.39 - 111°04'39.36" E, with altitudes ranging from 200-337.5 m above the sea level and with an average air temperature of 26.5 °C and humidity ranging from 70-83%. The type of soils in the research location was Alfisols. The composition of clay, sand and dust fractions were 59.74%, 23.44%, and 14.82%, respectively. In-depth surveys were carried out on three types of land covers including: (1) Mahogany, (2) Pine, and (3) Pine replanting. The characteristics of canopy and soil covers are presented in Table 1 and Table 2.

2.1. Sampling of canopy cover characteristics
Canopy density was set up on the transect of 200 m². The canopy cover area of all trees on the transect was described using the forest simulator (Sexi-FS) application. The percentage of canopy density was determined based on the proportion of the total area of tree canopy covers compared to the total area [18]. Light intensity under the tree canopy was measured using a luxmeter, between 9 AM and 1 PM. Litter thickness at soil surface was measured on the transect with a size of 50 cm x 50 cm, by measuring the height of litter 10 times at different points. In each 200 m² transect, the thickness of the
litter is measured 5 times. The soil temperature is measured 3 times at a depth between 0-30 cm on the same transect with litter thickness measurements. The canopy characters of various types of land covers are presented in Table 1.

2.2. Sampling of soil characteristics
Some of the soil characteristics including C-organic, P availability, pH H₂O and soil moisture were measured using the method established by the Soil Research Institute [19]. Soil samples were composite soil samples taken by purposive random sampling at 5 different points, at a depth of 0-30 cm. Soil characteristics of various types of land cover are presented in Table 2.

2.3. AMF spore isolation and identification
Fungal spores were extracted from 100 g of composite soil samples taken at 0-30 cm depth at the same location as the canopy density measurements. In each repetition, 2 sub repetitions were carried out. For each type of land covers, it was repeated 4 times. The isolation of AMF spores was carried out using the wetsieving-decanting and sucrose centrifugation methods as practiced by Gerdemann and Nicolson [8]. The identification of spores uses the method of Brundrett et al. [20].

2.4. Some mycorrhizal index
The density and diversity of AMF spores was determined based on: (1) Shannon-Weiner Diversity Index (H'), using the formula: 
\[ H' = -\sum p_i \ln p_i \], where \( H' \) is the Shannon-Weiner diversity index, \( p_i \) is the number of individuals of type \( i \) (\( n_i \)) compared to the total number of individuals (\( N \)); (2) Simpson’s Dominance Index (D), using the formula: 
\[ D = \frac{\sum [n_i (n_i-1)]}{N (N-1)} \], where \( D = \) Simpson Dominance Index, \( n_i = \) the number of individuals 1 to \( i \) and \( N = \) total number of individuals of all types; and (3) Evenness (E), using the formula \( E = H'/H'_{max} \), where \( H' = \) the Shannon-Wiener diversity index, \( H'_{max} = \ln S \), where \( S = \) the number of species found [21],[17].

2.5. Statistical analysis
The data were analyzed using the variance test (ANOVA) with a confidence level of 95%. If the land cover had a significant effect (\( p <0.05 \)) on the parameter then it was followed by Duncan's Multiple Range Test (DMRT). The closeness of the relationship between parameters was tested using Pearson correlation analysis, while the patterns of the relationship were tested using regression analysis.

3. Results and discussion
The characteristics of the three types of land covers are presented in Table 1 and Table 2. The pine replanting of land covers has the most open canopy density (16.50%), followed by pine land (80.94%) and mahogany land with the densest canopy cover (95.56%). This causes the light intensity received in the pine replanting area (48910.5 lux) to be higher than two other land covers which are denser. The amount of light intensity received by the pine replanting land has an impact on the high soil temperature, which is 32.96 °C. It is higher than the other two land covers with an average of around 25.50 °C (Table 1). The results of measuring the characteristics of this canopy are in accordance with the results of research by Ariyanto et al. [4] which show that the pine replanting land is the land with the most open canopy cover in the UNS educational forest area. This has an effect on the high intensity of light it receives, and successively affects the high air temperature and soil temperature. The highest air and ground temperatures ever achieved are 35 °C and 33 °C. The land cover type of mahogany has a litter thickness of 0.73 cm. It is higher than the other two land covers with an average of about 0.30 cm (Table 1). This is understandable because mahogany is a tropical plant that sheds its leaves during the dry season to reduce transpiration, resulting in a high thickness of litter on the forest floor.
Table 1. Land cover characteristics.

| Land cover          | Canopy density (% | Litter thickness (cm) | Light intensity (lux) | Soil Temperature (°C) |
|---------------------|-------------------|-----------------------|------------------------|------------------------|
| Mahogany            | 95.56             | 0.73                  | 10993.75               | 25.08                  |
| Pine                | 80.94             | 0.41                  | 18955.75               | 25.98                  |
| Pine replanting     | 16.50             | 0.20                  | 48910.50               | 32.96                  |

Regarding the characteristics of soils in the three types of land covers in the UNS educational forests, it is shown that: the organic C content is low to high; the availability of P and the content of soil moisture are very low; and the pH is slightly acidic (Table 2). The land cover of pine replanting has the lowest soil properties and is significantly different (p <0.05) when compared to pine and mahogany land covers. This is thought to be the reason why in general the production of pine sap in the UNS educational forests is low [3]; it is due to poor P nutrients and too dry soil conditions.

Table 2. Some soil properties in three types of land covers in the UNS educational forest.

| Land cover     | C organic (%) | Level  | Available P (ppm) | Level | Soil Moisture (%) | pH     | Level               |
|----------------|---------------|--------|-------------------|-------|-------------------|--------|---------------------|
| Mahogany       | 3.25 b        | High   | 3.06 a            | Very Low | 7.01 b           | 6.15  b  | Slightly acid       |
| Pine           | 2.76 b        | Medium | 4.82 b            | Very Low | 7.38 b           | 5.6 a  | Slightly acid       |
| Pine replanting| 1.81 a        | Low    | 4.89 b            | Very Low | 5.16 a           | 6.2 b  | Slightly acid       |

Note: Numbers in the same column followed by the same letter are not significantly different based on Duncan’s multiple range test with a 95% confidence interval. The level is determined based on the scoring from the Indonesian Soil Research Institute [19].

3.1 Spore density and diversity

3.1.1 Density and distribution of AMF spores. The differences in several types of land covers in the UNS educational forests have no significant effect (p > 0.05) on the density of AMF spores. The average density of spores in the three land covers is 744 spores/100 g of soil (Figure 1A). The number of spores is not much different from the number of spores found in dry Afromontane forests in Northern Ethiopia, with an average of 599 spores/100 g with a range of 86–1460/100 g of spores depending on the forest extensibility [6]. Birhane's research results also reveal that the high spore density is influenced by specific plant communities as AMF hosts, low soil attachments and the density of fine roots from understorey at the soil surface.

In the three land covers, three types of spores are found, namely Gigaspora sp., Glomus sp. and Archaeospora sp. (Figure 1A), but among the three types of spores, Gigaspora sp. and Glomus sp. are the two dominant spore types in all land covers. The results of this study indicate a lower number of spore types if compared to the seven types of spores found in the forests of Rarowatu Utara District, Bombana Regency, Southeast Sulawesi. Among the seven types of spores found, Glomus sp. 1 is the dominant species in the forests [17]. Glomus is found in a wide range of habitats because it is a genus that is adaptive and tolerant of various environmental conditions [22]. Both types of dominant spores can be developed as a source of bio-fertilizer inoculum in the nursery of various types of trees in UNS educational forests.
Figure 1. Density and distribution of spore species on the three land covers in the UNS forests (A) and examples of spore variety found (B), including Gigaspora sp., Glomus sp. and Archaeospora sp.

3.1.2 Some mycorrhiza index. To describe the conditions of spore diversity found on the three land covers in the UNS educational forests, an assessment was carried out on the Shannon-Wiener diversity index (H'), the Simson dominance index (D) and the Evenness index (E). The greater H' value indicates that the diversity and density of types or species on a land is getting higher. The Simson Dominance Index (D) closer to one indicates that the distribution of species diversity in a land is getting lower. Evenness index (E) describes the size of the number of individuals between species in a community, the more evenly the number of individuals between species, the more balanced the ecosystem [21].

The results of the assessment of the Shannon-Wiener diversity index (H') show that the covers of pine replanting (H' = 1.00) > mahogany (H' = 0.88) > pine (H' = 0.63) (Figure 2). The H' value on these three land covers is in the low category. The richness of spore types in the three land covers are the same, namely 3 types (Gigaspora sp., Glomus sp. and Archaeospora sp.). But the evenness of the number of individuals of each type is different (Figure 1) resulting in a different H' value. This result is similar to that found in the forests in Bombana Regency, namely H' = 0.59 ± 0.17 [17] which is in the low category. Thus, although the richness of spore types in the area is higher, namely 7 types, the H' produced is lower. It implies that the evenness of the number of individuals is not evenly distributed.

The low H' on the three types of land covers is also reinforced by the measurement results of the Simson dominance index (D), namely land covers of pine replanting (D = 0.39) < mahogany (D = 0.45) < pine (D = 0.61) (Figure 2). The value of D closer to 1 means that the diversity of types on the land covers is getting lower and there is the dominance of types or species [21]. This means that the diversity of AMF spore types in the pine replanting area is higher than that of mahogany and pine land covers. The Simson index in the forests of Bombana Regency is 0.26 ± 0.13 which indicates that the diversity of types in the forests is better than in the UNS educational forests [17].

The evenness index (E) for the three land covers in the UNS educational forests ranges from 0.91 in the pine replanting land covers to 0.57 in the pine land covers (Figure 2). The value of E close to 1 indicates that the distribution of the number of individuals between types is more evenly distributed; it implies a better balance of ecosystem conditions [21]. In the pine replanting area, the distribution of the number of individuals between the three types of mycorrhiza spores is more even than in the other two land covers (Figure 1A). The results of the evenness index assessment in the forests of Kab. Bombana are lower, namely E = 0.37 ± 0.10 [17]. It implies that the distribution of the number of individuals between species in the forests is more uneven and there is dominance of types or species.
Figure 2. Various values of mycorrhizal index in three types of land cover (H' = Shannon-Wiener diversity index; D = Simpson diversity index and E = evenness index).

The three indices assessment result shows that three types of land covers in the UNS educational forests have similar classification value as low in spore diversity. However, the distribution of population numbers per species is more even in the pine replanting land covers than in mahogany and pine. It hopefully gives beneficial impacts to land conditions that are open and prone to climate change. AMF can increase plant tolerance towards abiotic stress associated with climate change and help plants be more tolerant of rainfall variability [23].

3.2 Factors affecting spore density

The results showed that the density of spores in the three types of land cover in UNS educational forests correlated with several parameters, including: canopy density (r = -0.55*), light intensity (r = 0.50*), soil temperature (r = 0.56*), standing litter thickness (r = -0.55*), and soil C-organic (r = -0.68**). The correlation results showed that the lower canopy density, the higher the spore density. Canopy density shows negatively correlated with light intensity (r = -0.88*) and soil temperature (r = -0.95**). This correlation indicates that the lower canopy density or, the more open area receives higher light intensity, leads to higher soil temperature, and increases AMF spore density (Figure 3). The light intensity has a significant effect on AMF activity [24]. The higher light intensity promotes the formation of AMF spores [25].

In UNS educational forests, on pine replanting area with 16.5% canopy density receive 48910.50 lux of light intensity and has a soil temperature of about 32 °C. While in mahogany and pine area with an approximately 85% canopy density has lower soil temperature, i.e., 25 °C, the intensity of light received is also lower, about 14500 lux (Table 1). The higher soil temperature leads the soil in the pine replanting area to become more temperature stress, increasing the density of AMF spores because AMF spores will form when the environment is abiotic or biotic. Spores are a resistant form of AMF propagule and remain alive in unfavorable conditions [15]; the higher the degree of stress, the more spores are formed. The research result in three types of plantations in Bilaspur, India, also showed that increasing soil temperature due to decreasing soil moisture affects more AMF spore density [26]. The
resulting study in the UNS forest also showed that soil temperature was negatively correlated with soil moisture \( (r = -0.45^{**}) \). Several other studies have revealed that the changing seasons also affect the dynamics of AMF spore density in various habitats \([26],[8]\).

This study showed that AMF spore density negatively correlated with soil organic C content \( (r = -0.68^{**}) \). This study's results contradict the results of research \([17]\), which showed that the density of mycorrhizal spores had a significant positive correlation with soil organic C. However, the UNS educational forest research results similar to the research results \([8]\) in the Chilean Mediterranean-type ecosystem showed that mycorrhizal spore density significantly negatively correlated with soil organic-C content.

The part of mycorrhizae involved in the formation of soil organic matter is hyphae. As known, mycorrhizal hyphae function to channel and spread glomalin into the soil. Glomalin is a carbohydrate compound that functions as an adhesive material for soil particles in the soil aggregation process. The distribution of decomposed glomalin and hyphae is a factor that increases the levels of soil organic matters. Spores are devices or organs that function as maintenance organs for the survival of mycorrhizae. Mycorrhizal spores form when environmental conditions are not favorable. Therefore, if there are more spores, it means that there are less hyphae that survive in the soil. It is thus very possible that the less organic matter the more spores present in the soil. The low condition of organic matters, such as in coastal soils, indicates that environmental conditions are not conducive for the development of hyphae so that the hyphae survive by forming as many spores as possible \([27]\).

![Figure 3. The relationship between AMF spore density, canopy density, and soil temperature on three land cover type in UNS educational forest.](image)

4. Conclusion
AMF is an important soil fungus in the UNS education forest, as shown by the finding of AMF spores in the three types of land covers studied, which means that all vegetation is connected to the AMF hyphal network. Three types of AMF spores were found, namely Glomus, Gigaspora, and Archaeospora, where the first two types of spores are the dominant spore types. The increase in soil temperature due to more open land cover is a determining factor for the high number of spores in the UNS education forest. The increasing AMF spores affect the reduced C-organic content of forest soils.

Climate change can be a threat and a challenge if an increasing number of open land areas in UNS educational forest. The choice of forest management strategy needs to focus on increasing the soil organic matter content. It will improve the soil's physical, chemical, and biological properties; hopefully, the improvement of soil properties leads to the mycorrhization process and show the healthy performance of vegetation in the UNS educational forest.
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