Effect of land use type on arbuscular mycorrhizal fungi diversity in high altitude of Karo Highland

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Abstract. This study evaluated the spore density and diversity of arbuscular mycorrhizal fungi (AMF) in different land use type at Karo Highland. Sampling of soil AMF was conducted once as a material for trap culture by using Pueraria javanica as a host. The trap culture was used to observe the spore density and percentage of root colonization. The results showed that spore density and AMF colonization was not affected by different land use type, but rather by soil chemical properties. Soil acidity and phosphorus content had negative effect on root abundance and colonization, while carbon, nitrogen and potassium content gave positive effects. This study found 15 AMF spores from all types of land use dominated by Glomaceae followed by Acaulosporaceae and Gigasporaceae. The occurrence of AMF species was not specific to one type of land use but rather evenly dispersed in all types.

1. Introduction
Changes in land use as a result of deforestation, agricultural land expansion and intensification, and dry land degradation will lead to a decrease in soil fertility. The conversion of natural ecosystems into agricultural land pose a threat to the diversity of vegetation and animals on the land surface. The consequences will ultimately alter the structure and function of macro- and microbial communities in the soil [1].

Arbuscular mycorrhizal fungi (AMF) are a group of microorganism living in the soil prone to the changes of vegetation cover and soil physico-chemical as a consequence of deforestation and land degradation [2]. These fungi form multiple associations with more than 80% terrestrial plants and were able to promote plant growth through mechanism of effective nutrient absorption [2], increasing tolerance in dry environment [3], and improving host survivability towards pathogen infection and root nematodes [4].

The effect of changes in land use on AM fungal diversity and abundance has been studied in both temperate regions [5] and the tropics [6-8]. It was reported that changes in land use, soil plowing, fertilization and fungicide use [9], intensity of land use [5] and intensity of tillage [10] have reduced the AMF species richness, spore abundance and percentage of root colonization at different places in Central Europe and tropical ecosystems in Africa.

Verbruggen et al. [5] showed that the decline in AMF communities on Dutch agricultural land is mainly from the organic and conventional land in contrary to the natural ecosystem of grassland. However, some studies also reported that the diversity and richness of AMF species might be higher on organically managed lands and natural vegetation areas than conventionally managed lands [11]. The results then was assumed to be site-specific depending on the overall soil characteristics and
regions. It has also been reported that changes in vegetation cover from tree-based intercrops to monoculture systems may reduce the AMF species richness [12]. The richness was lower on fertile lands compared to natural ecosystems such as tropical forests. It is possible that intensive land use changes over a long period of time have reduced or changed the AMF species abundance and diversity in monocropping and intercropping systems.

This study aims to study whether AMF abundance and diversity were affected by land management systems such as monocropping and fertilizer use on agricultural lands. It is also important to understand the multiple role of AMF in land use systems, especially for an effective management of sustainable agricultural production through AMF technology in the future.

2. Materials and methods

2.1. Sampling method
Soil samples were collected at a depth of 0-10 cm and 10-20 cm using a ground drill. Five sampling points for each type of land use, in a total of 20 soil samples were collected and mixed as 1 kg composite. Soil sampling was conducted only once in March 2019. Soil samples were used for soil physico-chemical analysis and as materials for trap culture and spore extraction.

2.2. Trap cultures
Trap cultures were established since some AMF species may not produce spores in soil, while considered as a reliable method for producing viable spores in a taxonomical study of AMF. Trap cultures was made by mixing 150 g of soil and 75 g of sand by using Pueraria javanica as a host plant. The culture was maintained in a greenhouse for 4 months and spore extraction was subsequently performed. The spores extracted from trap cultures were used to determine the composition and abundance of AMF species. Whereas the root of P. javanica was used to determine the percentage of root colonization [13] and the length of the colonized root [14]

2.3. Spore extraction
The method used to extract AMF spores was wet-sieving by Pacioni [15] followed with centrifugation by Brundrett [16]. AMF spores were identified on the basis of morphological characteristics, including the shapes, sizes and colors under light-compound microscope. Furthermore, the spores were matched with the database described in the INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi) by Schenk and Perez [17].

3. Results and discussion

3.1. Abundance of AMF species from different types of land use
Based on the morphological characteristics of AMF spores, there are 15 morphotypes collected in this study. Analysis of variance resulted in no significant differences between spore abundance and among land use types (Table 1), but the mean number of AMF spores was slightly lower in the natural sites. Likewise for AMF colonization, no significant differences were obtained among types of land use. However, the relationship between AMF spore abundance and root colonization was negatively correlated (Figure 1).
Table 1. AMF spore abundance per 100 g of air-dried soils

| Land Use Type     | Total | Mean | Jacknife estimate |
|-------------------|-------|------|-------------------|
| Coffee            | 632   | 70.4 | 1123              |
| Citrus            | 438   | 68.6 | 846               |
| Maize             | 572   | 72.6 | 1068              |
| Natural forest    | 463   | 54.9 | 883               |

F-probability 1.27
P-value 0.33

Grand mean 66.6

Figure 1. AMF spore abundance and root colonization at different types of land use

The mean number of AMF spore abundance (66.6 spores per 100 g of soil) was lower than the results obtained in Cassuarina equisetifolia –structured natural site [18] in the Secanggang coastal forest area, Langkat Regency, North Sumatra. The stand of C. equisetifolia was populated by 128.2 spores per 50 g of soil. This difference might be influenced by climatic factors and the differences in soil characteristics among study sites [19]. As it was already known that AMF will develop and grow optimally on infertile soils with no record of any intensive fertilization. The use of intensive fertilizers, especially P fertilizer, tend to reduce the spore abundance compared to the untreated and un-fertilized land sites.

From Figure 1, it was shown that AMF spore abundance in coffee, citrus and maize was higher than natural sites, although a higher colonization percentage was observed from the natural sites. This was assumed to be related to soil conditions where natural forest soils have higher carbon and phosphorus content compared to the other three types of land use. High soil phosphorus content can suppress the growth and development of AMF.
3.2. AMF species composition from different types of land use

Based on the INVAM database by Schenk and Perez [17], the AMF spores in this study were morphologically differentiated into 15 morphospecies (Table 2). Most of the spores obtained were classified in Glomaceae (10), Acaulosporaceae (4) and Gigasporaceae (1). This result is almost similar with the AMF diversity in *C. equisetifolia* [18] located in the coastal forest area which was also dominated by Glomaceae (10), followed with Acaulosporaceae (3) and Gigasporaceae (1). Members of Glomaceae was regarded as the dominant AMF species in all studied ecosystems. This result was supported by many studies which reported the dominance of Glomaceae in various ecosystems and vegetation [20, 21]. Two other AMF species namely Acaulosporaceae and Gigasporaceae were generally recorded in smaller numbers with limited distribution. Other studies also reported that Gigasporaceae produced fewer spores than Acaulopsoraceae and Glomaceae [22].

There was a varying in relative abundance among AMF species recorded in this study. The highest relative abundance was observed from *Glomus* sp.9 followed with *Glomus* sp.3 and *Glomus* sp.6, while *Gigaspora* sp. had the lowest relative abundance. The four species of *Acaulospora* spores had almost the similar relative abundance, ranging from 3.85–5.72. The distribution of AMF spores from each type of land use showed that *Glomus* sp.9 was dominant in all types explaining its highest relative abundance. Maize plants had the higher AMF abundance than the other land types, while in contrary, the natural sites had the lowest abundance of AMF spores.

| No. | Morphospecies of AMF | Relative Abundance of AMF | Spore abundance |
|-----|----------------------|---------------------------|-----------------|
|     |                      |                           | Coffee | Citrus | Maize | Natural Forest |
| 1   | *Glomus* sp.9        | 13.76                     | 17.9   | 12.6   | 17.9  | 8.3            |
| 2   | *Glomus* sp.3        | 12.48                     | 13.1   | 8.1    | 11.3  | 9.8            |
| 3   | *Glomus* sp.6        | 9.45                      | 8.5    | 7.7    | 15.1  | 6.4            |
| 4   | *Glomus* sp.7        | 7.75                      | 6.7    | 6.4    | 8.5   | 5.1            |
| 5   | *Glomus* sp.1        | 7.69                      | 4.6    | 5.8    | 8.3   | 4.3            |
| 6   | *Glomus* sp.4        | 7.32                      | 3.6    | 5.8    | 6.5   | 4.5            |
| 7   | *Glomus* sp.8        | 6.79                      | 5.6    | 4.2    | 4.8   | 3.6            |
| 8   | *Glomus* sp.2        | 5.76                      | 4.8    | 6.2    | 5.8   | 3.6            |
| 9   | *Acaulospora* sp.2   | 5.72                      | 1.6    | 0.5    | 5.3   | 0.0            |
| 10  | *Glomus* sp.10       | 5.29                      | 3.1    | 4.3    | 5.4   | 2.6            |
| 11  | *Acaulospora* sp.4   | 4.30                      | 4.2    | 2.5    | 4.7   | 0.0            |
| 12  | *Acaulospora* sp.1   | 4.18                      | 2.4    | 0.0    | 5.3   | 3.8            |
| 13  | *Acaulospora* sp.3   | 3.85                      | 2.1    | 2.6    | 3.8   | 2.4            |
| 14  | *Glomus* sp.5        | 3.62                      | 2.5    | 2.7    | 3.0   | 2.1            |
| 15  | *Gigaspora* sp.      | 2.04                      | 0.0    | 2.4    | 2.8   | 1.4            |

Based on the results on different types of land use, the natural forests had relatively lower abundance of AMF spores compared to other land uses. While in maize land, the number of each spore species was higher compared to the others. In maize fields, where land conditions were relatively drier with higher temperature, it allowed AMF to grow and produce more viable spores than in more humid land. Besides that, the fibrous root of maize plants in the absence of lignification, was advantageous for the AMF colonization leading to optimal growth and sporulation.
3.3. Distribution pattern of AMF species in different types of land use

The soil physico-chemical characteristics were significantly different in each type of land use except for pH and potassium content (Table 3). The pH values in all types of land use tend to be the same in acidic conditions. The highest carbon content is obtained in natural forests followed by citrus, maize and coffee. The highest nitrogen content was obtained in citrus land while the highest phosphate content was obtained in natural forests. Meanwhile, the potassium content in the soil is relatively the same for all types of land use.

| Land Use Type   | pH  | C (%) | N (%) | P (ppm) | K (%) |
|-----------------|-----|-------|-------|---------|-------|
| Coffee          | 4.0 | 3.4   | 0.3   | 10.8    | 0.3   |
| Citrus          | 4.2 | 5.4   | 0.9   | 12.4    | 0.2   |
| Maize           | 4.3 | 3.7   | 0.4   | 16.1    | 0.3   |
| Natural forest  | 3.9 | 6.6   | 0.6   | 21.1    | 0.3   |

In addition, linear regression analysis resulted in no significant correlation between the soil chemical properties and spore abundance even though the data showed positive effects of carbon, nitrogen and potassium and negative effects of phosphorus and pH. The increasing phosphorus content and soil acidity (pH) will reduce AMF spore abundance and vice versa. Increases and decreases in carbon, nitrogen and potassium result in increases and decreases in spore abundance in sequence.

| Soil characteristics | pH | Carbon | Nitrogen | Phosphorus | Potassium |
|----------------------|----|--------|----------|------------|-----------|
| Intercept estimate   | 76.2| 67.5   | 73.7     | 81.4       | 62.4      |
| Coefficient estimate | -1.26| 1.87  | 2.49     | -0.58      | 54.6      |
| F-statistic          | 0.06| 0.32   | 0.03     | 0.35       | 2.58      |
| p-value              | 0.80| 0.56   | 0.83     | 0.54       | 0.15      |

Some AMF species in this study failed to form spores in the trap cultures. This can be explained from the differences in environmental conditions and in the composition of extant host plants [23,24]. Generally, AMF species was not exclusive to any types of land use only because one species can be found in all types of land use. An AMF species was commonly found in several types of land use where the difference was only recorded in the number of spores produced by that species. Spore formation was regarded as a strong response to environmental conditions, especially under limited access to water and nutrients availability, especially phosphorus (P). Many AMF species thrived in the stressful environment by forming abundant spores in response to the environmental to ensure high survival rates [25]. Thus, the number of AMF spores was an indicator of environmental factors that were less favorable for the host plants. Species of AMF that are capable of producing many spores may be more tolerant of interference from environmental factors. Other study reported that Glomaceae and Acaulosporaceae were adapted to changes in soil chemical properties which explained their dominant spore production in this study.
4. Conclusions
Different types of land use was found to have no direct impact on the AMF spore abundance. The difference in the abundance of AMF spores was more affected by soil physico-chemical properties. Phosphorus content and soil acidity (pH) negatively affect the AMF spore abundance, while the carbon, nitrogen and potassium content played a positive role to the abundance. The study results also showed that high AMF abundance and low root colonization occurred when environmental conditions were unfavorable to the growth and development of AMF species.

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