Structure and Composition of Major Arbuscular Mycorrhiza (MA) under Different Farmer Management of Coffee and Pine Agroforestry System

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ABSTRACT

Utilization of Arbuscular Mycorrhiza (MA) as beneficial soil microbes is expected to support nutrient demand for improving crop performance. However, under the agroforestry system that facing a problem on light, water, and nutrients competitions, the role of MA is becoming unclear. The purpose of this research was to examine how far different management in Coffee Pines Agroforestry System (CPAS) affects MA structure and compositions. The relationship between soil parameters (e.g. pH, soil organic C) and MA activities was also being evaluated. The selected plot according to existing management practices were chosen as follows: (1) Low management (LC); (2) High management which then compared to (3) Business As Usual (BAU) plot in which were repeated in triplicate. ANOVA and multivariate analysis were employed to determine the effect of the treatments. The result showed that there were significant differences (p<0.05) in the structure and composition of the MA, in terms of the total number of MA spores and the abundance of *Glomus* sp. under the coffee tree sampling point, while the lowest number was detected in *Gigaspora* sp. genera. The more intensive land management resulted in a higher abundance of MA biomass which then leads to increased soil P and uptake-P along with MA infection.

INTRODUCTION

Arbuscular mycorrhiza (MA) is one of the promising options for examining indicators of the successfullness of sustainable land management of farming systems to reduce the level of rapid soil degradation and losses of soil fertility functions. The relationship between land use and mycorrhizal abundance has become an interesting subject for many researchers (Chen, Liu, Bi, & Feng, 2014; Jung, Martinez-Medina, Lopez-Raez, & Pozo, 2012; Koorem et al., 2017) as this microorganism living under a schematic symbiotic relationship between the host plant and the fungus (Jung, Martinez-Medina, Lopez-Raez, & Pozo, 2012; Songachan & Kayang, 2011). The scope of research by the MA association is very sensitive to changes in environmental conditions that have been reported by Chen, Liu, Bi, & Feng (2014) and Suamba, Wirawan, & Adiartayasa (2014). Land management that varies from intensive practices that damage soil quality to practices for conservation and maintaining soil processes mediated by soil organisms will also affect the presence of MA in the soil (Chen, Liu, Bi, & Feng, 2014; Neuenkamp, Zobel, Lind, Gerz, & Moora, 2019). It is very important to calculate mycorrhizal populations in landscape conditions as a result of being managed by farmers to monitor its degradation as an impact of anthropogenic activities (Moora et al., 2014). Reducing the number of MA can cause irreversible damage to habitats that will later impact the wider ecosystem services (Chen, Liu, Bi, & Feng, 2014; Solaiman, Abbott, & Varma, 2014). The study of the diversity and function of MA species is very important considering the impact of changes in land use on an ecosystem can reduce land productivity (Moora et al., 2014; Neuenkamp,
Zobel, Lind, Gerz, & Moora, 2019; Soka & Ritchie, 2016).

Many previous findings on changes in structure and communities of MA only focus on the impact of land-use changes (Chen, Liu, Bi, & Feng, 2014; Suamba, Wirawan, & Adiartayasa, 2014), environmental degradation (Garcés-Ruiz, Senés-Guerrero, Declerck, & Cranenbrouck, 2017) or even on selected cropping system (Trevizan Chiomento et al., 2019; Zhu, Yang, Song, & Li, 2020), however, the information of impact of land management, particularly on small scale coffee pines farming agroforestry system is lacking, particularly on the issue of tree host preferences by MA. So far, the structure and composition of MA under coffee and pines agroforestry systems have never reported in detail. As the reason that most coffee and pines farming practices did not allow fertilizer as the main input for nutrients, the demand of those only relies on the activities and utilization of the existing MA for scavenging nutrients in far and deeper soil horizon.

Utilization of MA as beneficial soil microbes is expected to support the absorption of nutrients needed for plants to increase their growth (Chen, Liu, Bi, & Feng, 2014; Ura’, Paembonan, & Umar, 2015). Besides being able to provide nutrients (Neuenkamp, Zobel, Lind, Gerz, & Moora, 2019), they also are able to fulfill crop water demand (Smith & Read, 2008), alleviated plant abiotic stress (Augé, Toler, & Saxton, 2016; Pozo, López-Ráez, Azcón-Aguilar, & García-Garrido, 2015), plant resistance to the pathogen (Jung, Martínez-Medina, Lopez-Raez, & Pozo, 2012). One important nutrient that potentially can be absorbed by MA is Phosphorus (P) (Jansa, Finlay, Wallander, Smith, & Smith, 2011). The P was not available for plants extracted from the soil by MA under a symbiosis scheme with the hosted plant (Chen, Liu, Bi, & Feng, 2014; Solaiman, Abbott, & Varma, 2014). The increasing number of MA spores provided rapid nutrients supplied by mycorrhiza to crop (Moora et al., 2014; Soka & Ritchie, 2016). According to Sukmawaty, Hafsan, & Asriani (2016), the presence of MA colonization in plant roots can increase drought tolerance. Ramadhan, Nihayati, & Sitawati (2017) concluded MA hyphae networking will increase water availability and nutrient supply for crops. Unfortunately, research on the interaction between MA and host crop and its mechanism under natural vegetation is rarely reported (Lekberg & Koide, 2013). Recently there was evidence that MA structure and communities were influenced by soil nutrients and light (Liu et al., 2014; Shi et al., 2014) as well as soil carbon content and its environmental condition, in particular, shading condition and climatic regime (Davison, Öpik, Daniell, Moora, & Zobel, 2011; Davison et al., 2012; Knegt et al., 2016). Under different environments such as shade-tolerant tree species, the structure and MA composition changes (Koorem et al., 2017; Shi et al., 2014). Plant performance is believed to give a major effect to MA (Hoeksema et al., 2010; Moora et al., 2014).

The growth of MA identically with the total number of MA spore in soil even cultivated MA outside their natural habitat is the association of the number of spores is correlated with types of host plants and has a close relationship with various environmental factors such as temperature, soil pH, soil moisture, carbon and phosphorus (Chen, Liu, Bi, & Feng, 2014; Knegt et al., 2016; Solaiman, Abbott, & Varma, 2014). All of those above factors affect the structure and composition of the MA (Shi et al., 2014; Suamba, Wirawan, & Adiartayasa, 2014). Under the forest ecosystem MA fungal communities are simultaneously shaped by host characteristics and canopy-mediated light availability (Koorem et al., 2017; Shi et al., 2014). The information on the influence of the level of land management in the current coffee pine agroforestry system at the farmer level is lacking. One disadvantage of the agroforestry system is the competition for light regime, water requirement and nutrition balance; therefore the role of the MA in this system is vital. So far, research on the changes in the structure and composition of MA in this system is very rarely reported in detail particularly in agroforestry systems involving many species of tree which may also influence organic matter input as a source of crop nutrients and energy for MA. The effect of different levels of land management by farmers became the focus of this study. Under shaded tolerant crops, it was reported that they have the ability on maintaining carbon supply to MA (Gommers, Visser, Onge, Voesenek, & Pierik, 2013; Shi et al., 2014). The problem of light, water, and nutrition competition is also seen in the UB Forest education and research forest area (Prayogo, Sholehuddin, Putra, & Rachmawati, 2019). This area is an area of coffee pine agroforestry system with very minimum inputs (fertilizers, pesticides, etc.); therefore the supply
of nutrients for these plants also depends on the structure and composition of the existing MA in soil.

The purposes of this study were: (1) to analyze the effect of differences in the management of pine and coffee agroforestry on the structure and composition of MA along with characterizing plot condition, and (2) to examine the relationship between the structure and composition of MA with some soil and land characteristics (plot conditions).

MATERIALS AND METHODS

Study Location

The research was conducted from November 2018 until February 2019 at Universitas Brawijaya (UB) education forest, particularly at Sumbersari sub-village, Tawangargo-Karangploso, Malang-East Java, Indonesia. This site’s geographical position is at 7°49’35.58” S and 112°34’42.57” E with an elevation of 1000 m above sea level. The soil in the study developed from the southwestern part of Mount Arjuno volcanic activity and can be classified as Andisols with the slope ranging from 15 to 30%.

There were 3 plots selected with pine trees at the age range between 20-25 years and 6-8 years for coffee with different levels of management. (Fig. 1).

The plot size was at the size of 60 m x 40 m with 3 replications as described as follows:

a. Plot 1: Low Management (LC) has no inputs on weeding, trimming, or pruning on the coffee tree or understories with the lowest density of coffee population and no application of organic manure or inorganic fertilizer even the use of pesticide. The distance of pines was at 2 m x 3 m.

b. Plot 2 High Management (HC) has high intensive management with weeding, trimming, or pruning on the coffee tree but has dense canopy cover and coffee density. The organic manure was used only in the first period of planting. The distance of pine tree was similar to Plot 1.

c. Plot 3 Business as Usual (BAU) or known as Best Farmer Practices (BFP) has similar management to recommended monoculture coffee farming with inputs on weeding, trimming or pruning on coffee tree or understory under application of organic and inorganic manure were use several times, this plot is used as a control plot. The distance of pines was at 2 m x 6 m, whereas the density of the pine was reduced to ideal condition for coffee:

Soil and MA sampling

Soil and MA samples were taken from each selected plot (LC, HC, and BAU or BFP) (Fig. 2) following a survey method, to examine the impact of different management of coffee pines agroforestry system at UB Forest which covers an area of about 514 ha. Sampling was carried out in the four cardinal points which positioned as North (U), South (S), East (T) and West (B) direction to be compiled following cardinal samplings points, described by Melo et al. (2019). Each corner is divided into three zones where zone A1 was located at 0.5 m from coffee/pines tree stands, whilst zone A2 and zone A3 was positioned 1 m and 1.5 m respectively. Samplings were performed at the depth of 0-20 cm of soil. Composite sample techniques were used resulted in 72 samples of core soil for MA identification.
Remarks: LC = low management, HC= High management, BAU = Business as Usual

Fig. 1. Location of the study and comparison of the plot condition according to different farmer management
Total Soil C, Soil P and Uptake P, MA Spore Abundance, and MA Infection

Total soil organic C were determined using the Walkley and Black method, total soil available P determined using Bray1 method and P uptake were detected by Spectro-photometrically using wet digestion methods (H\textsubscript{2}SO\textsubscript{4} and H\textsubscript{2}O\textsubscript{2} solutions), while soil pH was determined using digital pH meter (Bakhshipour, Kahneh, & Khah, 2009; Zhu, Yang, Song, & Li, 2020). Moreover, the analysis of the abundance of MA spore was using wet sieving method (Melo et al., 2019), along with the measurement of MA infection using blue staining technique (Melo et al., 2019). The structure and composition of MA were determined using their relative frequency value (Trevizan Chiomento et al., 2019). The classes of species according to the relative frequency as follows: dominant >50%, most dominant 31-50%, common 13-30%, and rare <13% (Trevizan Chiomento et al., 2019).

MA Root Infection, P Foliar, Tree Biomass Sampling and Canopy Cover

Plant roots were collected at the same position where soil samples were taken. A total of 50 g of root of coffee and pines were collected before it was identified in the laboratory. The identification of MA was performed to derive the percentage of MA infections. The 500 g of the foliar sample were air-dried from the upper part of mature leaves, ground and determined the P content using wet digestion procedure (H\textsubscript{2}O\textsubscript{2} solutions), while total lignin and polyphenol content of leaves tissue were determined using Acid Detergent Fiber (ADF) + H\textsubscript{2}SO\textsubscript{4} and Follins Dennis Reagent (FDR) (Ceccarelli et al., 2010). By multiplying foliar P content and tree biomass, the value of P uptake can be estimated. Biomass value was estimated using non-destructive sampling by measuring the diameter of coffee and pines at the breast height (DbH). The biomass can be derived using a general allometric equation following a formula of $Y$ (dry weight kg/tree) = 0.0417 $D^{2.6576}$ (Suprayogo et al., 2020). The use of allometric for estimating biomass has been recommended by previous researchers (Al Qassam & Prayogo, 2018; Herianto, Kusuma, Nihayati, & Prayogo, 2019; Prayogo, Sari, Asmara, Rahayu, & Hairiah, 2018). Canopy cover was measured using “CanopyApp” software in which the picture
of tree canopy was recorded using a handphone camera before the image then transferred into the percentage of canopy cover based on the logical programming of the software.

Data Analyses
The data of the observations were analyzed using ANOVA in licensed Genstat 20.00 (Brawijaya University license) software (VSNI-UK) with a 95% level of significances. If the effects of treatment were significant, the mean comparison between treatments was performed based on the Least Significant Different (LSD) (α ≤ 5%). In order to establish a relationship between parameters, correlation and regression techniques were employed. A multi-varied analysis (CVA or Biplot) were used for clustering the treatments based on various observed parameters as suggested by Garcés-Ruiz, Senés-Guerrero, Declerck, & Cranenbrouck (2017) and Zhu, Yang, Song, & Li (2020).

RESULTS AND DISCUSSION

Soil pH and Organic C
There is a significant difference of soil pH (p<0.05) across the treatments of different farmer management but there was no significant difference of soil pH between tree zones (distance from the tree stem), therefore the average soil pH were taken into account (Table 1a and Table 1b). The pH is slightly increased with the raising of the level of management, whereas the higher soil pH was detected in HC plots which had the highest soil organic content. The soil pH of the HC plot was significantly different from those values of BAU or LC (Table 1).

The soil pH was a range between 5.15 to 5.45 in which this value was comparable to soil pH under Clotalaria anagyroides, Eupatorium adenophorum and Hedycium coronaroium plot colonized by various MA in Meghalaya-North India ranged between 5.81 to 6.20 (Songachan & Kayang, 2011). This study classified the pH value into slightly acid, due to the accumulation of organic material in soil surface in the form of pines needle and coffee leaves. The consequences of acidic soil condition the level of soil acidity due to the provision of organic material depends on the level of maturity of the organic material supplied the expiration limit of the organic material, and the type of soil. If the addition of immature organic matter will cause a slow process of increasing soil pH because organic matter is still not well decomposed and still releases organic acids (Atmojo, 2003). Humic and Fulvic acids producing from organic matter decomposition processes are releasing H⁺ to the soil but organic matter structure is highly variable and largely amorphous and contains varying carboxylic and phenolic groups. These groups dissociate H⁺ to produce negatively charged, hydrophilic sites to bind hydrophilic compounds like nutrient cations, while also containing lipophilic sites to bind hydrophobic compounds Soil under A horizon pH was 5.5 ± 0.1 in pines stands and 5.9 ± 0.1 in hardwood stands, while mean B horizon pH was 4.2 ± 0.09 in pines stands and 4.56 ± 0.08 in hardwood stands in Hoosier National Forest (Duffy, 2014). Soil pH has been shown to have a greater influence on AM fungi than plant host and acidic soils reportedly have reduced diversity of AM fungi (Dumbrell, Nelson, Helgason, Dytham, & Fitter, 2010). In this study, the reduction in soil pH influenced the decreasing of the total number of MA spore. This was due to the fact, that an increase of pH was determined by the bigger accumulation of litter as the main sources for soil organic matter. The deposit of coffee and pines litter impacted the higher content of soil organic matter accumulation.

Table 1a. The analysis of variance (p<0.05) under Coffee tree system to the level of soil pH, soil organic C, Uptake P, and soil P

| Plot | Soil pH | Soil Organic C (%) | Uptake P (g P/tree biomass) | Soil P (mg/kg) |
|------|---------|--------------------|-----------------------------|---------------|
| BAU  | 5.15 a  | 7.12 a             | 4.94 a                      | 7.33 a        |
| LC   | 5.18 a  | 5.99 a             | 4.85 a                      | 6.05 a        |
| HC   | 5.38 b  | 11.01 b            | 4.00 a                      | 11.05 b       |

Remarks: BAU = Bussiness as Usual, LC = low management, HC = High management
Soil organic C status had a similar pattern to soil pH in which a significant difference had been observed among the treatments (p<0.05) (Table 1a and Table 1b). Since there is no effect of zones (distance from the tree stem), the average value from those positions was taken into accounts and pooled together (Table 1). HC plot had the highest soil organic C since this plot had the highest tree population, resulted in the accumulation of surface litter before it being decomposed as soil organic material and however LC plots were in the opposite, however, there is no significant difference on soil organic matter between those plots. Soil organic C under pines system was about 5.9 to 11.0. This value was within the range of soil organic carbon under pines system ranged from 2.4 to 5.6, with a mean of 3.6 ± 0.1 compare to 4.6 ± 0.2 in ridge hardwood stands (Duffy, 2014). The provisioning of organic matter will lead to an increase in soil C-organic content. Soil organic C is a food source of soil microorganisms, so the presence of C-organic in the soil will affect microorganism activities. The litter layer can act as a bridge layer between soil and the atmosphere creating a condition that is protected from a direct drop of the rainfall, solar light penetration, buffering the changes in temperature, providing water and air circulation. This then affects the number of MA and biochemical process of litter decomposition in the soil in which caused the raising level of soil carbon as the main sources of food energy for microbe (Aučina et al., 2007). In this study, the increase in soil C was in the line with the greater number of Glomus sp. spore and Acaulospora sp. Unfortunately, litter from Pinus spp. contains low concentrations of labile organic substances and nutrients, such as sugars, and water-soluble nutrients, but contains larger proportions of large molecular weight compounds, such as cellulose, hemicellulose, and lignin (Duffy, 2014). Lignin releases phenolic acids, which can be allelopathic. It has been reported that aliphatic organic acids are common for many other Pinus species, including Pinus elliottii Engelm. (slash pine), Pinus taeda L. (loblolly pine), and Pinus palustris Mill. (longleaf pine). Approximately, 60-80% of litter extracts of Pinus ponderosa Lawson & C. Lawson (ponderosa pine), were made of oxalic, mallic, gallic, and protocatechuic acid, with oxalic acid dominating pines litter (Duffy, 2014). The results of the leaves tissue analysis showed that coffee leaves had polyphenol levels of about 5.56%, while pine leaves were at 6.76% which were classified as high. In terms of lignin content, coffee leaves had lignin at about 24.76%, while pine leaves were about 29.42%. The three results of lignin analysis are classified as low quality of organic matter since both materials were not easily to be decomposed.

Soil P level under HC plot in which it was significantly different (p<0.05) and it’s almost double compare to LC and BAU plots both under coffee and pines tree, however, based on zones no impact on changes of soil P (Table 1a and Table 1b). There is a strong positive correlation between soil P and soil organic matter level. Sari, Sudarsono, & Darmawan (2017) explains that the addition of organic matter can increase the availability of P in the soil. The effect of organic matter on the availability of P can be directly through the mineralization process or indirectly by assisting the release of fixed P. The result of decomposition of organic material in the form of organic acids can form chelation bonds with Al and Fe ions so that it can reduce the solubility of Al and Fe ions, thus the availability of P increases. Organic acids produced from the decomposition of organic matter can also release P that is absorbed so that the availability of P rapidly increases. In terms of P uptake, only under the Pines tree in which a significantly different across the treatments (p<0.05) were found. As comparison, it can be seen that an increase in soil organic C (%) was in the line with the soil P status in soil was followed by the raising of the total number of MA spore.

| Plot | pH     | Soil Organic C (%) | Uptake P (g P/tree biomass) | Soil P (mg/kg) |
|------|--------|--------------------|-----------------------------|---------------|
| BAU  | 5.24 a | 7.337 a            | 563.6 a                     | 7.13 a        |
| LC   | 5.23 a | 6.053 a            | 396.1 a                     | 6.95 a        |
| HC   | 5.45 b | 11.05 b            | 806.0 b                     | 8.29 b        |

Remarks: BAU = Business as Usual, LC = low management, HC = High management
Coffee and Pines Biomass and Tree Canopy Cover

In general coffee tree biomass is about 10% lower compared to pines tree biomass. Those values range between 1.56 kg dry weight/tree (HC) to 1.93 kg dry weight/tree (BAU) for coffee tree biomass, whilst for pines were about 142.5 kg dry weight/tree (LC) in which, this is significantly (p<0.05) 30% lower than those values of HC plot at about 185.4 kg dry weight/tree. (Fig. 3). Coffee and pine tree biomass were directly affected by different land management. The contribution of coffee and shade trees to carbon stocks in agroforestry systems varies considerably over time because of differences in land use and farmer practices (Herianto, Kusuma, Nihayati, & Prayogo, 2019; Prayogo, Sari, Asmara, Rahayu, & Hairiah, 2018; Suprayogo et al., 2020).

The results from this study confirmed that the average level of the canopy in each plot has a value ranging from 63% - 70%. The highest canopy cover was found in the LC plot of 70.42%, while in the BAU and HC plots had an average value of 63.75% and 68.79%, respectively canopy density even it does not have a difference in each plot but lower management caused the raising of canopy cover and resulted in lowering light intensity coming into the system. LC has the highest value of canopy density because the plot is not treated at all which indicates there is no human intervention. Whereas, the lowest density value found in BAU was due to the coffee trimming.

Remarks: LC=Low management, HC=High management, BAU = Business as Usual

Fig. 3. Tree biomass measurement and canopy cover across different farmer management
Total Arbuscular Mycorrhiza (MA) Spore, Relative Frequency, and MA Infection

There were three major types of spores obtained during the study, namely *Glomus* sp., *Acaulospora* sp., and *Gigaspora* sp. (Fig. 4).

The highest number of MA under coffee and pine trees are in the HC plot in which it was significantly different (p<0.05) to either LC or BAU plot. The number of MA at HC plot on average was 25% higher than LC and BAU. The most common MA under different management of coffee and pine trees agroforestry system in this study was *Glomus* sp. In terms of location sampling with different zones (distance from the tree stem) there is no significant different (p<0.05) to the number of MA per 100 g of soil (Table 2a and Table 2b). The changes in the number of MA in each plot are caused by differences in organic matter and soil pH. The distribution of mycorrhizae is influenced by many factors including soil type and structure, soil P, and N available in the soil, water availability, pH, and soil temperature (Hartoyo, 2012). *Glomus* sp. is the most dominant genus in different types of ecosystems (Sukmawaty, Hafsan, & Asriani, 2016). This showed that *Glomus* sp. has a high level of adaptation to the different environments both to those of acidic and neutral soil or even if any differences in crop management. Miska, Junaedi, Wachjar, & Mansur (2016) showed that the results of the extraction of MA from various locations, *Glomus* sp. generally have relatively high spore densities compared to other genera. Since there was clear evidence that difference management affect those genera, therefore *Glomus* sp. can be as one of soil quality indicator and the effect of changes in land management. In contrast, the dominant type of MA under native shrubs (*Crotalaria anagyrodes, Eupatorium adenophorum, Hedychium coronarium*) were *Acaulospora* sp. which is higher than those to *Glomus* sp., while MA infection/colonization of *Acaulospora* sp reached about 66 to 71%. The *Acaulospora* sp. and *Glomus* sp. has been known to those widely abilities for competing to other types of MA (Songachan & Kayang, 2011). Gao & Guo (2010) reported that *Acaulospora* sp. were associated with acidic soil and forest ecosystem. However, in comparison to the result of this study *Glomus* sp. became the most abundant MA. Each spore genus has different characteristics and sizes. Mycorrhizal *Gigaspora* sp. has a yellowish spore with a boulbus suspensory which has a short, thin spore-wall and the terminal wall with a size of 240 x 240 µm. Spores of the genus *Acaulospora* sp. are yellow with orange in the spore-wall in the form of a small hole evenly distributed. *Acaulospora* sp. spore size namely 73 x 78 µm. *Glomus* sp. has more than one spore wall, *Glomus* spores which are found have round shapes, yellowish spore walls, slightly reddish yellow, to brown with a size of 113x114 µm (Fig. 4).

Table 2a. The analysis of variance (p<0.05) under Coffee tree system to the number of spore of *Glomus* sp., *Acaulospora* sp., *Gigaspora* sp., total number of spora (100 g of soil), relative frequency and MA infection

| Plot | Number of spore *Glomus* sp. | Number of spore *Acaulospora* sp. | Number of spore *Gigaspora* sp. | Total number of spore (100 g soil) | Relative frequency (%): (Glomus sp., Acaulospora sp. and Gigaspora sp.) | Total Mycorrhizae infections (%) |
|------|-----------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------------|----------------------------------|
| BAU  | 36.33 b                     | 28.59 a                           | 3.83 a                           | 68.75 ab                          | 53 41 6                                 | 12.83 a                          |
| LC   | 31.83 a                     | 25.6 a                            | 2.75 a                           | 60.18 a                           | 53 42 5                                 | 11.42 a                          |
| HC   | 38.92 b                     | 30.2 a                            | 3.75 a                           | 72.87 c                           | 53 41 6                                 | 11.67 a                          |

Remarks: BAU = Business as Usual, LC = low management, HC = High management
Table 2b. The analysis of variance (p<0.05) under Pines tree system to the number of spore of *Glomus* sp., *Acaulospora* sp., *Gigaspora* sp., total number of spora (100 g of soil) and MA infection

| Plot | Number of spore *Glomus* sp. | Number of spore *Acaulospora* sp. | Number of spore *Gigaspora* sp. | Total number of spore (100 g soil) | Relative frequency (*Glomus* sp., *Acaulospora* sp. and *Gigaspora* sp.) (%) | Total Mycorrhizae infections (%) |
|------|-----------------------------|-----------------------------------|---------------------------------|-----------------------------------|--------------------------------|---------------------------------|
| BAU  | 36.58 b                     | 27.0 a                            | 3.42 a                          | 67.00 ab                          | 55                             | 40                             | 5  | 3.17 a |
| LC   | 32.00 a                     | 26.2 a                            | 2.58 a                          | 60.78 a                           | 53                             | 43                             | 4  | 2.92 a |
| HC   | 39.75 b                     | 30.3 a                            | 2.92 a                          | 72.97 c                           | 54                             | 41                             | 5  | 2.17 a |

Remarks: BAU = Business as Usual, LC = low management, HC = High management

Remarks: Magnification 400 x, LC = Low management, HC=High management, BAU = Business as Usual

Fig. 4. Types of spores obtained during the study from each MA genera
It also reported that the *Glomus* sp. spore was dominated the diversity of major spore types found in various ecosystem conditions because this type of AMF has a broad host range (Wanda, Yuliani, & Trimulyono, 2015). Hartoyo, Gulamahdi, Darusman, Aziz, & Mansur (2011) reported that the diversity of AMF in the *Centella asiatica* rhizosphere shows that of the total 14 species found, 10 species belong to *Glomus* types. The results of observations on the percentage of root colonization, number of spores, and species diversity showed that the combination of inoculum from *D. heterocarpon* with the three host plants also showed the highest value, namely *S. vulgaris* 95.5% (very high), 105 spores with 7 types spores (*Glomus* 6 types and Acaulospora 1 type), *P. javanica* 48.9% (moderate), 114 spores with 5 types of *Glomus* sp. spores and *D. ovalifolium* 57.8% (high), 65 spores with 6 types of *Glomus*, Acaulospora sp., Gigaspora sp., total number of spore (100 g of soil) and MA infection (Muryati, Mansur, & Budi, 2016). In this study, the relative frequency for representing structure and composition of MA (Trevizan Chiomento et al., 2019) was recorded at above 50% for *Glomus* sp., 45% for Acaulospora sp., and 5% for Gigaspora sp. respectively.

There is no significant difference (p<0.05) to those number of spore in either Acaulospora sp. or Gigaspora sp., number of spores of Acaulospora sp. in coffee and pines trees are lower 25% than *Glomus* sp. but it was 10 times higher than those value of Gigaspora sp. This is in accordance with research Anggreiny, Nazip, & Santri (2017), of the six genera found, *Glomus* sp. and Acaulospora sp. were the genus with the highest number of spores found in all sample plants, and Gigaspora sp. was only found in *A. cadamba* plants. For comparison the total number of MA spore in soil were at about 36.8 spore per 20 g of soil and those value had a significant correlation to the percentage of sand in soil (Bakhshipour, Kahneh, & Khah, 2009). 23 MA species were isolated (Songachan & Kayang, 2011) and have less number of species in subtropical China which confirmed that Acaulospora sp. is the major species (Yang, Lu, & Zhang, 2011).

MA colonies are the number of infections (colonization) of mycorrhizae in plant roots. The highest percentage of mycorrhizal colonies of coffee trees was in the BAU plot with an average number of 12.83% per tree and the lowest was in the LC plot with an average number of 11.42% per tree (Table 2a). However, there was no significant different (P<0.05) in term of those colonization both under pines or coffee tree host. These results indicate the level of mycorrhizal colonies in coffee trees is relatively low which was only 4 times lower than of mycorrhizal colonies in pines trees. The highest pine mycorrhizal colonies are in the BAU plot with an average number of 3.17% per tree and the lowest mycorrhizal colonies are in the HC plot with an average number of 2.17% per tree. In contrast to the number of mycorrhizae, from study, the percentage of mycorrhizal colonies did not significantly different between plots and zones (distance from the tree), but it was mainly caused by tree host preference. These results indicate that high spore density does not always indicate a high percentage of root colonies. The result was in the opposite to the study of MA infection by Sarah and Ibrar (2016), who found that the number of MA spore was determined MA infection. Based on these results, the management level can increase the total number of spores, spores of *Glomus* sp., Acaulospora sp., and Gigaspora sp., to about 10% of MA spores per 100 g of soil. The level of MA infection under the pines tree system was 3 times lower than the coffee tree system. In contrast, MA infection under pines seedling can be neared 100% (Aucina et al., 2007), while root AM fungal colonization was reported tend to be higher when the canopy of the forest canopy was removed (Koozem et al., 2014; 2017). This result was relevant with the finding of this study which confirms that under BAU plot when the canopy cover only at about 62% it has higher MA infection compare to LC or even HC plot which had greater canopy cover (65 to 70%).

Since there is no significant difference base on the sampling position, then the average value was taken into account and pooled together. It was clearly seen that MA prefers to colonize Pines tree instead of the coffee tree. The correlation table between mycorrhizal spores and mycorrhizal colonies of coffee and pine trees are presented in Table 3a and Table 3b. In terms of the coffee tree system, a strong correlation between no of MA and uptake P and between soil P and soil organic C were detected resulted in the value of coefficient correlation at r=0.81 and r=0.83, respectively. Unlike coffee tree system, under pines system, not only the strong correlation between no of MA and soil P but also Uptake P and soil organic matter which produce a coefficient correlation at the value
of $r=0.68$; $r=0.95$ and $r=0.76$ respectively (Table 3a and Table 3b). Moreover, under this system, there was also a strong correlation between soil P and Uptake P and soil organic C with coefficient values at $r=0.73$ and $r=0.82$, while the relationship between soil organic C and uptake P resulted at about $r=0.76$. In contrast to this, MA abundance typically was detected under the soil with low organic carbon source (Jung, Martinez-Medina, Lopez-Raez, & Pozo, 2012).

Mycorrhizal spores are able to assist plants in absorbing nutrients that contribute importantly to soil fertility by increasing the ability of plants to absorb nutrients (Chen, Liu, Bi, & Feng, 2014; Koorem et al., 2017; Songachan & Kayang, 2011). MA is able to help plants absorb elements nutrients both macro and micro, especially in the form of bonding with other elements and they are not available to plants. That influence most popular of MA is plant absorbing phosphorus in the soil, crops grow faster than those of crops that do not contain MA. In addition to phosphorus, hyphae also transporting other nutrient sources to host plants such as ammonium, calcium, sulfur, potassium, zinc, and copper (Indriani, Mansyur, Susilawati, & Islami, 2011; Bücking, Liepold, & Ambilwade, 2012). Therefore, uptake P nutrients will increase with the help of mycorrhizae which relevant to this study finding, whereas the increase of uptake P was strongly correlated to the raising of the number of total MA. Hidayati, Faridah, & Sumardi (2015) explained that in general mycorrhizal inoculation increased the average biomass growth of *Mangium* seedlings by 54.40% compared to *Mangium* seedlings without mycorrhizal inoculation. Daras, Trisilawati, & Sobari (2013) reported that mycorrhizal inoculation can increase the growth of the Robusta coffee seedlings planted on podzolic soil in greenhouses. Results from Ibiremo, Daniel, Oloyede, & Iremiren (2011), giving information that mycorrhizae can increase P uptake of coffee plants in the soil with low P content. Mycorrhizae have a good symbiosis with coffee plants, especially on low nutrient soils (Lebrón, Lodge, & Bayman, 2012). This matter caused by the extra-radical hyphae formed and serves as a nutrient provider. This hypha is massive able to absorb nutrients from the non-rhizosphere region, which is not usually reached by plant roots (Daras, Sobari, Trisilawati, & Towaha, 2015; Jehne & Lee, 2014).

**Table 3a.** The relationship between number of MA spore, MA infection, soil P, Uptake P and soil organic matter under coffee tree

| No of MA  | MA infection | Soil P | Uptake P | Soil organic C |
|-----------|--------------|--------|----------|----------------|
| No of MA  | 1            |        |          |                |
| MA infections | 0.13         | 1      |          |                |
| Soil P    | 0.04         | -0.27  | 1        |                |
| Uptake P  | 0.81*        | 0.05   | -0.08    | 1              |
| Soil organic C | 0.15        | -0.48  | 0.83**   | -0.13          |

**Table 3b.** The relationship between number of MA spore, MA infection, soil P, Uptake P and soil organic matter under pines tree

| No of MA spore | MA infection | Soil P | Uptake P | Soil organic C |
|----------------|--------------|--------|----------|----------------|
| No of MA spore | 1            |        |          |                |
| MA infection   | 0.28         | 1      |          |                |
| Soil P         | 0.68’        | 0.21   | 1        |                |
| Uptake P       | 0.95*        | 0.30   | 0.73’    | 1              |
| Soil organic C | 0.76”        | -0.47  | 0.82”    | 0.76”          |
Though the principal components analysis (PCA) Biplot was adopted (Fig. 5), it was verified that the first principal component analysis (PCA1) respond to 97.01% of the total variable (total no of MA spore, soil pH, organic soil C, Soil P, and MA infection). A total number of MA spore/100 g of soil, uptake soil P and mycorrhiza infection (%) while the second principal components analysis (PCA2) was compounded by 2.98% of the total variance, the joint of both PCA1 and 2 results in 100 % of the total variance. Total soil organic C and soil P correlated positively, and similar conditions appeared for the relationship between uptake P and mycorrhiza infection (%). PC1 axis was accounted mostly for by total soil organic C, soil P, soil pH, and the number of MA spores while the second PC2 axis was Uptake soil P and MA infection (%). The soil C and soil P were in the same direction and magnitude (bottom right), while Uptake and MA infections (%) was chosen the other position (upper middle) (Fig. 5)

Fig. 5. Biplot multivariate analysis across different farmer management based on the selected parameter (soil pH, soil organic C, soil P, total no of MA spore, and MA infection)
In more detail assessment PCA Canonical Variate Analysis on number of MA species (number of *Glomus* sp. spore, number of *Acaulospora* sp. spore and number of *Gigaspora* sp. spore were with the total number of spores in 100 g of soil) were employed. The result revealed a group distribution of the effect of the treatments to those above parameters. For soil under the coffee tree, two plot were close and overlapping one to another, means that there is no significantly (p<0.05) different of those plots in term of no of *Glomus* sp. spore, no of *Acaulospora* sp. and no of *Gigaspora* sp. were with total no of spore in 100 g of soil). The first principal component (PC1) axis for Coffee tree sampling points was accounted for 92.41% and the second (PC2) axis accounted for 7.59% of the variance (Fig. 6). The biplot of PCA clearly identified two groups BAU and HA were bound together and LC which separated from previous groups. Similarly, the pattern of CVA was identified in sampling points under the pines tree (Fig. 6). However, the value of the first principal component (PC1) axis for pines tree sampling points was accounted for 94.71% and the second (PC2) axis accounted for 5.29% of the variance. In this study, soil properties which were influenced the number of MA and MA infection was soil P, similar to those result reported by Melo et al. (2019) using a CCA plot. However, in other study soil pH, SOC, soil N, and P were determined communities and structure of MA using multivariate NMDS assessment (Zhu, Yang, Song, & Li, 2020).

**Fig. 6.** CVA multivariate analysis across different farmer management based on the selected parameter (number of *Glomus* sp. spore, number of *Acaulospora* sp. spore, number of *Gigaspora* sp. spore and the total number of MA spore (100 g/soil)
CONCLUSION

Structure and community of MA in Coffee and Pines agroforestry system are determined by different farmer management and host (tree types) instead of those position of sampling (distance from the main tree) while Glomus sp. is the most common species in each plot compared to Acaulospora sp. and Gigaspora sp. The percentage of mycorrhizal colonies did not significantly different between plots and zones (distance from the tree), but it was mainly caused by tree host preference. These results indicate that high spore density does not always indicate a high percentage of root colonies while under the coffee tree system those infections were more effective compare to the pines tree system. There was a strong relationship between soil organic C and soil P to increase uptake-P which had a strong correlation with no of MA spore while multivariate analysis can be distinguished and clustered the effect of treatments based on selected parameters.

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