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Insights of symbiotic relationships and diversity of arbuscular mycorrhizal fungi in rubber-based agroforestry systems

Gusmiaty Gusmiaty[1,2], Mukrimin Mukrimin[1*], Nur Padli[1], Muhammad Restu[1,2], Syamsuddin Millang[1], Tutik Kuswinanti[2], Muh. Akhsan Akib[3], Syatrawati[4], Retno Prayudyaningsih[2,6]

[1] Department of Forestry, Faculty of Forestry, Hasanuddin University, Makassar, Tamalanrea Campus, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
[2] Research Collaboration Center for KARST Microbes BRIN-UNHAS, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
[3] Faculty of Agriculture, Hasanuddin University, Makassar, Tamalanrea Campus, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
[4] Study Program of Agrotechnology, University of Muhammadiyah Parepare, Indonesia
[5] Study Program of Plantation Crop Cultivation Pangkep State Agricultural Polytechnic, Pangkep, South Sulawesi, Indonesia
[6] Center for Applied Microbiology Research, National Research and Innovation Agency, Bogor, West Java, Indonesia
* Corresponding author's email: mukrimin@unhas.ac.id

ABSTRACT

Rubber agroforestry is a means of agricultural land management that can be widely utilised by farmers, with the potential to enhance the biodiversity of the surrounding forest. An important aspect of rubber agroforestry systems is the symbiotic relationship between rubber plants and arbuscular mycorrhizal fungi (AMF). AMF is a fungus that lives in the soil symbiotically with plant roots that provide many benefits to plants, including nutrition and plant growth sustainability. This study aims to identify the type and density of AMF in the soil, rhizosphere, and their associations in the roots of rubber plants in agroforestry patterns in Jojjolo Village, Bulukumpa District, Bulukumba Regency. Soil and root sampling was conducted on five 20 × 20 m plots per location, each 50 m apart, with 1.000 grams of soil per plot. Spore extraction was carried out through the filter pour method at hole diameters of 425 µm, 250 µm, and 45 µm. The ecstasy results were observed using a 4× magnification dissecting microscope. AMF roots are identified using the root staining method, and then the roots are observed with a compound microscope with 40× magnification. The results showed that the spores were found at three density levels. The density of rubber plants in agroforestry patterns is as many as six types of Glomus genus and 1 type of Acaulospora genus. AMF spore density was the highest, with an average of 88.97 per 50 grams of soil, found in rubber plantations with agroforestry planting patterns with sparse plant density intercropping of cloves and durian. Rubber plants in plantations with agroforestry cropping patterns have a moderate AMF colonisation percentage. This study reveals the community structure of AMF in agroforestry patterns and potential for soil fertility and chemical input reduction, particularly in Indonesian rubber agroforestry.

Keywords: agroforestry, AMF, rubber, roots, spores.

INTRODUCTION

Agroforestry is a land use system that combines agriculture and forestry, leading to positive ecological and economic interactions. Agroforestry systems put much emphasis on species diversity and the interactions between these different species. Agroforestry systems and their positive impact on the soil and the high erosion protection against wind, dehydration, and nutrient flushing have been widely recognised. From the perspective of land improvement, such use of trees is optimal for regenerating tired soils and for numerous ecosystem services, such as water filtration. This combination of tree rows and agricultural purposes is also interesting regarding biodiversity, soil fertility, yield, and ecosystem stability.

The rubber agroforestry system is one of the many agroforestry models developed in Bulukumba district. Rubber tree agroforestry is a means of managing agricultural land that can be widely used by local or rural farmers, with the potential for being widely used by local or rural farmers, with the potential to increase the biodiversity of the surrounding forest. Plants that grow randomly will form a layered canopy and root structure; another benefit of the agroforestry model is increased productivity and environmental protection. Rubber agroforestry is similar to a forest at the time it is established. Many factors affect crop productivity in agroforestry. In addition to selecting plants for agroforestry, the interactions between factors need to be considered. Organisms in the soil are important to observe because they play a significant role in land productivity, as the main target in cultivation.

Soil microorganisms, especially beneficial microbes like arbuscular mycorrhizal fungi (AMF), which act as key components of the soil microbial community, contribute to the development of healthy soils and agricultural sustainability (Bender et al., 2016; Guzman et al., 2021; Jeffries et al., 2003). AMF is one group of fungi that has been found scattered in a variety of habitats (Bordoloi et al., 2015; Souza, 2015). The AMF form the most important group in the agroforestry systems. AMF are mutualistic associations between the roots of plants and certain soil fungi. This symbiotic relationship comes with the benefits of increasing plant productivity, soil structure, pathogen resistance, and phosphorus (P) uptake. AMF are well known to form symbioses with widespread plant species and have been used as biofertilizers to help host plants cope with abiotic stress (Marro et al., 2022; Tedersoo et al., 2020)

A study conducted by Qiao et al. (2022) showed that AMF contributes to crop growth in agroforestry and is a predictor of plant growth in agroforestry systems. However, according to network analysis, AMF diversity and network complexity decrease with increasing stand age. Thus, standard age, as well as the trade-offs among soil function, productivity, biodiversity, and economic benefits, must be considered when establishing an agroforestry system. Although AMF research has been widely reported, research on AMF in rubber plants, especially in agroforestry patterns, is still very limited. Each soil type and environment will have a different AMF community, and the dominant AMF species in rubber plants will vary according to location and growth. Therefore, a study is needed to identify the type and density of AMF in the rhizosphere, as well as their association with the roots of rubber plants in agroforestry patterns in Jojjolo Village, Bulukumpa District, Bulukumba Regency. This study aims to reveal the structure of AMF communities in agroforestry patterns and their potential for soil fertility and chemical input reduction, particularly in Indonesian rubber agroforestry. Exploration and identification of AMF in rubber agroforestry patterns is expected to identify appropriate AMF types at the density level and intercropping types of rubber agroforestry systems that can serve as a reference in developing rubber agroforestry.

METHODOLOGY

Study area

Soil and root samples were collected from three Agroforestry rubber plantation sites in Jojjolo Village, Bulukumpa sub-district, Bulukumba regency at coordinates 5° 35’0” N – 5° 30’ 0” N and 120° 25’ 20” E – 120° 25’ 20” E. 120° 30’ 0” BT3. AMF was identified at the Microbiology Laboratory of BPSI KLHK and the PKR Microbiology Laboratory Karst Microbes (Figure 1).

Procedures

Sampling

Plant rhizosphere sampling in agroforestry rubber plantations is divided into three levels of density, namely agroforestry, a sparse density of intercrops with a total of 27 intercrops, Agroforestry B density of intercrops medium with a total of 108 intercrops, and Agroforestry C dense intercrops with a total of 253 plants (Figure 2).

Sampling of soil and roots by making observation plots based on the method used by the International Center for Research in Agroforestry (ICRAF) (Ervayenri, 2005). The plot size used was 20 × 20 m. Each location/area type consisted of 5 plots, each with a distance of 50 meters between plots. Soil sampling was done at 0–20 cm

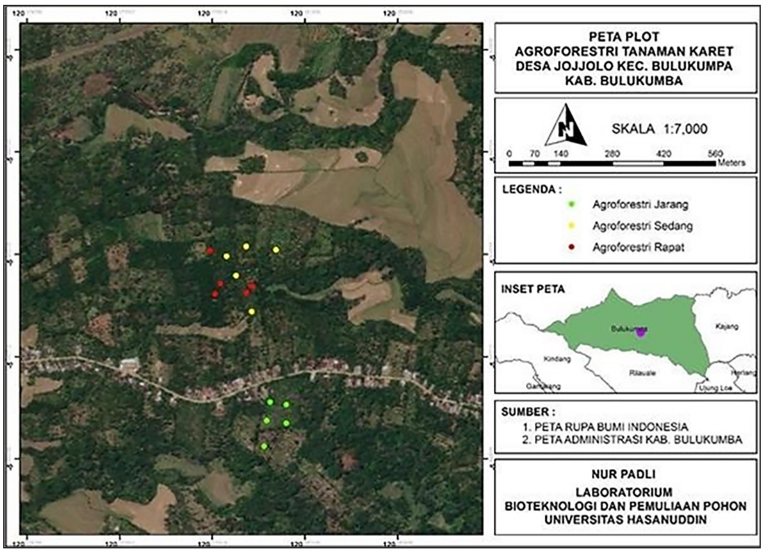

Figure 1. Location of sampling plot in rubber plantation area

Figure 2. Agroforestry area sparse (A), medium (B), dense (C)

depth from 5 points per plot. Because the weight of the soil at each point is 200 grams, the total soil sample taken for each analysis is 1000 grams. Soil samples for each point in one plot are mixed in one plastic sample bin until homogeneous to represent one plot (Figure 3).

In addition, soil and root sampling were conducted on three selected plant samples (Host Targets) at each plot by making a hole on each side at a distance of ¾ of the outer crown. Soil and root samples were taken simultaneously at a soil depth of 0–20 cm. and a hole diameter of 15 cm at a distance of ¾ from the outer crown. Soil and roots were taken randomly with a hoe in the soil. around the root area at four replicate points (Figure 4).

FMA spore extraction

Spore extraction is done to separate the spores and soil, which will then be identified. AMF spores were extracted by weighing 50 grams of soil samples and then putting them in a glass cup. A 1.000 ml beaker was filled with water to a volume of 1 liter. The suspension was stirred for ± 2 minutes until homogeneous, and the soil aggregate was broken so that the spores were free from dirt and soil. The suspension is allowed to stand for ± 15 minutes until the spores are free. After that, the suspension is poured into a graduated sieve with a diameter of holes 425 μm, 250 μm, and 45 μm (this procedure was repeated 3 times). The residue of each filter was rinsed with

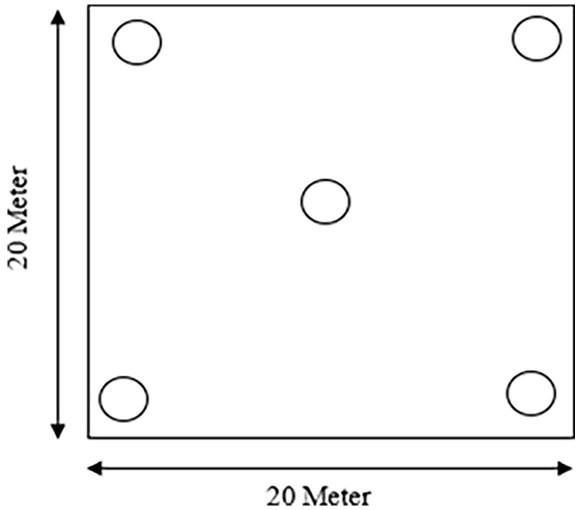

Figure 3. Sketch of soil sampling plot

a water tap to ensure that all small particles were removed. Filter residue with a size of 250 μm poured into a 45 μm sieve, then poured into a 15 ml centrifuge tube with the help of a spray bottle. After that, it was put into a centrifuge and spun at 2000 rpm for 5 minutes. Removed impurities that floated in the supernatant liquid, then filled with a sugar solution of 2 times the amount of water in the supernatant liquid. The centrifuge tube, and then put it back into the centrifuge, which is spun at 2000 rpm for 1 minute. After that, pour into a 45 μm sieve washed with running water, and the resulting sieve was poured into Petri dishes with a spray bottle to observe the spores under a microscope (Figure 5).

Counting the spore population

The filter results were transferred to Petri dishes. Spores were observed and counted under a dissecting microscope. with 4X magnification. Then, transfer the spores based on their morphotypes to filter paper with vertical lines and horizontal lines in a 60 × 15 mm cup for easy observation.

Spore observation

Preparation of spore preparations using PVLG and Melzer's solution separately on one prepared glass. Spores that have been counted and separated per morphotype were placed on a glass plate; the left side was dabbed with a solution of PVLG, and the right side was dabbed with Melzer's solution, and then each was covered with a cover slip. Spores are solved by pressing the surface of the coverslip using a stick. Discoloration of spores in Melzer's solution is one of the indicators to determine the type of spores present (Figure 6).

Figure 4. Soil sampling process (A, B) and root sampling process (C, D)

Figure 5. Weighing soil samples (A), filtering soil samples (B), and centrifuging of the soil filtrate (C)

Infection observation and identification of mycorrhizal species

The root hairs were carefully cleaned and stored in 50% alcohol, which was used to preserve the root hair samples from the study trees. After that, put the root hair pieces into a test tube. The root hair samples were soaked in 10% KOH solution for 24 hours. The role of KOH is to clean the cytoplasm of the root nucleus so that the dye is more easily absorbed. If the color of the roots still does not turn lighter, then the root sample is soaked in 10% H_2O_2 solution after the 10% KOH solution is removed. Furthermore, if the root color turned lighter, the root samples were washed under running water and then immersed in a 2% HCl solution for 24 hours for the neutralization process. Next, root samples will be stained using a staining solution. Immersion of root samples in the staining solution for 24 hours. After that, the staining solution is replaced by the staining solution (Figure 7).

Observation of FMA colonization

Observations of FMA colonization were made systematically by taking pieces of root hairs, 1 cm long, randomly, ten pieces of root hair, and arranging them on a microscope slide, then observing them with a compound microscope. One tree species was studied using one microscope slide

Figure 6. Laboratory activities, including (a) transferring and counting spores using a micropipette, and (b) observation of AMF spores

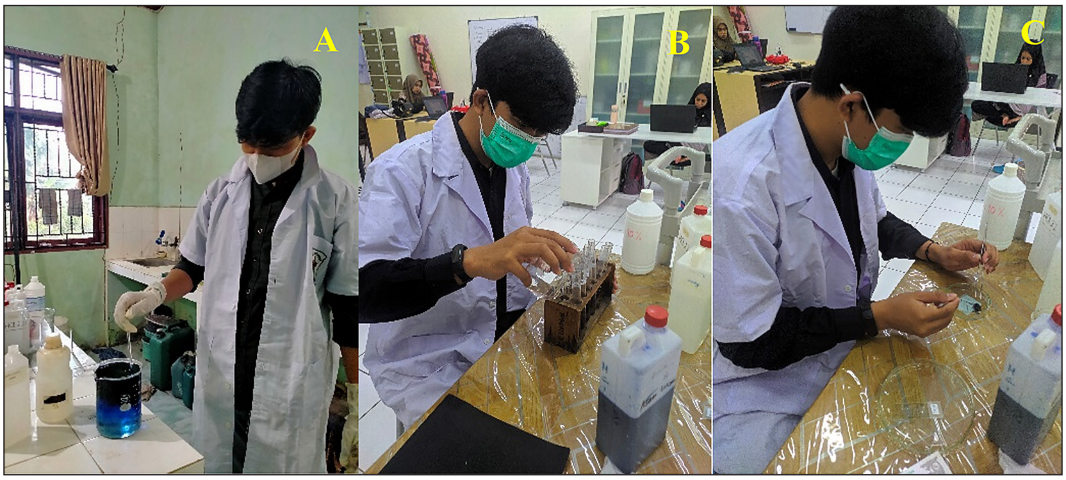

Figure 7. Preparation of root staining solution (A), application of the solution onto the root sample (B), and root sample preparation process (C)

containing ten root haircuts with six fields of view. Microscope slides that showed signs of colonization were counted. The percentage of colonized roots can be calculated using the formula developed by Brundrett et al. (1996) as follows:

$$\text{Percentage of FMA colonization} = \frac{\text{The number of colonized fields of view}}{\text{Total field of view}} \cdot 100\% \quad (1)$$

Table 1. Classification of the number of root infections

Percent colonization (%)	Category
0–5	Very low
6–25	Low
26–50	Medium
51–75	High
76–100	Very high

Data analysis

Data from observations in the laboratory were analyzed using a randomized complete block design (CRD). The results of the variance that have a significant effect are tested using the Tukey HSD distance test to make it easier and more efficient. Excel software was used to speed up data analysis. The amount of root FMA colonization is classified into several categories, as can be seen in Table 1.

RESULTS AND DISCUSSION

Soil analysis results

Soil chemical properties at each plant density level were observed based on several variables, including pH (H_2O), organic C (%), organic N (%), C/N, P_2O_5 (ppm), Mg, K, and CEC (cmol/kg). Soil analysis was conducted at the Laboratory of Soil Chemistry and Fertility, Department of Soil Science, Faculty of Agriculture, Hasanuddin University, with results based on the criteria outlined by *Balit tanah*. The results of soil analysis can be shown in Table 2.

Spore type of arbuscular mycorrhizal fungi

Observations of AMF spores grouped based on morphotypes obtained two types of AMF genus, namely Glomus and Acaulospora. The results of spore identification in the rhizosphere of

Table 2. Results of soil analysis at each density level in the agroforestry pattern of rubber plants

Plant density	Soil properties							
	pH (H_20)	Organic C (%)	Organic N (%)	C/N	P_2O_5(ppm)	Mg (cmol/kg)	K (cmol/kg)	KTK (cmol/kg)
Sparse	6,48 SA	1,69 L	0,13 L	13 H	9,93 L	1,19 M	0,51 M	20,48 M
Medium	6,71 SA	2,61 M	0,25 M	10 M	12,26 M	1,82 M	0,54 M	25,54 H
Dense	6,08 S	1,77 L	0,19 L	9 M	15,12 M	0,77 L	0,51 M	22,35 M

Note: SA – slightly acidic; S – sour; L – low; M – medium; H – high.

Table 3. Results of identification and characterization of AMF spore types on rubber plants in agroforestry patterns

No.	Morphotype	Characteristics	Melzer's	Genus
1	SRB	Round shape, small size, black color, rough surface texture, no *hyphal attachment*	Not reacting	Glomus 1
2	LRB	Round shape, size large, dark brown color, rough surface texture, no *hyphal attachment*	Not reacting	Glomus 2
3	SRB	Round shape, size small, dark brown color, rough and mottled surface texture, no *hyphal attachment*	Not reacting	Glomus 3
4	LOB	Oval shape, large size, brown color, texture smooth surface, no *hyphal attachment*	Not reacting	Glomus 4
5	SBO	Oval shape, size small, brown color, rough surface texture, there is *hyphalattachment*	Not reacting	Glomus 5
6	YRS	Round shape, size small, yellow color, rough and mottled surface texture, no hyphal *attachment*	Not reacting	Glomus 6
7	CRS	Spherical shape, small size, bright yellow color, rough surface texture, and thorn-like structure, no there is *hyphalattachment*	React	Acaulospora 1

Note: Small round black (SRB), large round brown (LRB), small round brown (SRB), large oval brown (LOB), small brown oval (SBO), yellow round small (YRS), clear round small (CRS).

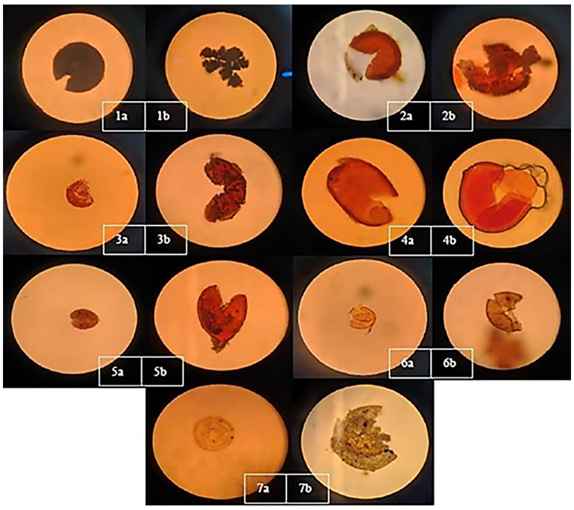

Figure 8. Spore types at 40× magnification that are dripped solution with PVLG (a) and Melzer's (b): genus glomus (1,2,3,4,5,6), genus acaulospora (7)

rubber plants in agroforestry systems are presented in Table 3 and Figure 8.

FMA spore density

AMF spore density in the soil in rubber plantations with agroforestry patterns with different levels of plant density can be seen in Table 4.

Colonization of AMF on plant roots in agroforestry planted rubber plantations AMF association on rubber roots

The percentage level of root colonization of agroforestry rubber plants can be seen in Table 5.

AMF association in intercrops

The level of AMF colonization on the roots of clove, coffee, durian, and porang plants, which are intercropping species in Rubber plantations in this research location, are presented in Table 6 and Figure 9.

Discussion

Soil analysis results

Soil acidity (pH) indicates how acidic or alkaline the soil is. This variable plays an important role in determining whether plants easily absorb nutrients. In general, plants absorb nutrients well at a pH of neutral. This study obtained two types of soil pH at three density levels, where the agroforestry density level was sparse, and the soil pH level was low. Medium has the same type of soil pH, which is slightly acidic with

Table 4. Spore density at three density levels

No.	Plant density	∑ average number of spores	∑ genus
1	Sparse	88.97a	7
2	Medium	75.15a	7
3	Dense	60.67a	7

Note: Values followed by the same letter indicate that they are not significantly different at the 95% test level.

Table 5. Percentage of AMF colonization in rubber plant root

No.	Density level	Type of intercrop	∑ average colonization (%)	Category
1	Sparse	Clove, Durian	45.49b	Medium
2	Medium	Coffee, Porang, Clove	28.3a	Medium
3	Dense	Coffee	38.06a	Medium

Note: Values followed by the same letter indicate that they are not significantly different at the 95% test level.

Table 6 Percentage of AMF colonization on roots of intercrops in rubber plantations

No.	Density level	∑ colonization (%)	Category
1	S^{C}	14.42	Low
2	S^{D}	31.11	Medium
3	M^{CF}	12.78	Low
4	M^{PR}	84.44	Very high
5	M^{C}	34.83	Medium
6	D^{CF}	48.97	Medium

Note: C (clove), D (durian), CF (coffee), PR (porang).

levels of 6.48 and 6.71, while agroforestry meeting obtained an acidic soil pH type with a level of 6.08. In strongly acidic soils, certain ions (Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+}) rise to levels toxic for the majority of plants (Silva, 2012). Additionally, acidic soils have high cation exchange capacity and promote the leaching of nutrients, resulting in soil unfavorable for plant growth (Johnson, 2002). At the other extreme, alkaline soils tend to be unfavorable for plant growth, with iron, manganese, and phosphate deficiency creating an unfavorable condition for plant growth, in alkaline soils, boron can rise to phytotoxic

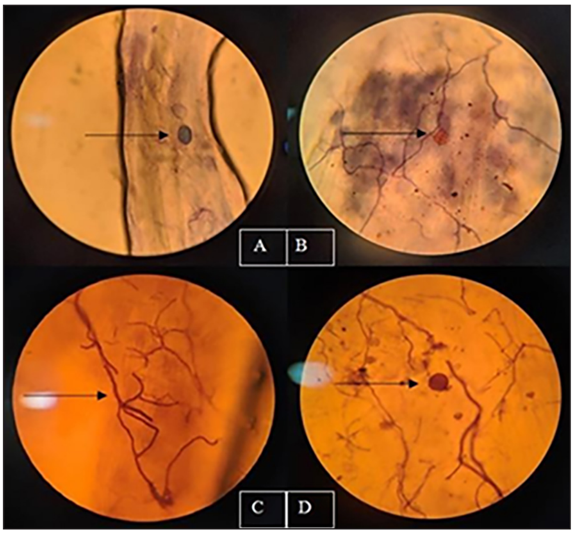

Figure 9. Types of AMF colonization in agroforestry rubber plants: vesicles (A), microcellotia (B), hyphae (C), spores (D).

concentrations (Marschner, 2012). Phosphorus uptake by the plant is optimal at soil pH ranging from 6 to 6.5 because below pH 6, aluminum (Al^{3+}), iron (Fe^{2+}; Fe^{3+}), manganese (Mn^{2+}), calcium (Ca^{2+}), and magnesium (Mg^{2+}) dominate cation exchange capacity, and phosphorus blocking becomes important (Oteino et al., 2015). The uptake of P is in the form of primary (H_2PO^{4-}) or secondary (HPO_4^{2-}) orthophosphates, which, unfortunately, are only present in very low concentrations in most tropical soils (Van Der Wal et al., 2007). Under these conditions, the use of organic matter or chemical fertilizers can be essential to enhance P uptake. However, arbuscular mycorrhizae can extract phosphorus from the soil, make it available to crops, and increase yield (Soti et al., 2015). Soil organic matter (BOT) has an important role in improving soil's physical, chemical, and biological properties and will directly affect soil fertility; besides that, the content of organic matter is also one factor that affects soil fertility. C-organic content in sparse and dense agroforestry densities was low, with levels of 1.69% and 1.77%, while the medium density was classified as medium, with 2.61%. Soil C-organic content on rubber land is in the low category. This shows that there is still a lack of additional materials for the organic specialty of the crop. Organic matter improves all aspects of the soil (chemical, biological, and physical). Therefore, its availability is critical. AMF and organic matter are two components that always influence each other. According to Gude et al.

(2012) fresh organic input drives soil organic matter dynamics. Through this process, nutrients (carbon, phosphate, and nitrogen) can be available for plants and organisms in the soil ecosystem. As we know, nutrients can be used by AMF to develop together with the host plant, and then we can find the organic matter quantity using the AMF spore number. In agroforestry, the roots of trees and crops are intermingled, and their interactions together with the production of exudates could alter both bulk soil and rhizosphere environments, such as the soil organic matter content and pH (Duchene et al., 2017).

Nitrogen (N) has an important role in plant growth. Nitrogen can be absorbed by plants from the soil in the form of NH_4^+ and NO_3. Nitrogen availability in Sparse and dense plant density falls into the medium category with levels of 0.13% and 0.19%. Medium plants are included in the low category at a density of 0.25%. According to Hardjowigeno (2007), the C/N ratio is helpful as a marker of the ease of organic matter breakdown and activities of soil microorganisms, most of the energy required to maintain a functioning soil population and support the continuity of processes in the soil is so much derived from the conversion of organic carbon to carbon dioxide, but if the C/N ratio is too wide, it means the availability of C as an energy source. excessive in comparison to the availability of N for microbial protein formation, the activity of microorganisms will be inhibited, so data on C-Organic content and soil C/N ratio are very important to know. The C/N ratio in medium and dense plant densities is classified as medium, while in the density of the plant, the C/N ratio is low and rarely belongs to the high category. The ratio of carbon to nitrogen in the soil, which averages between 10 and 12 C/N, is useful as a marker of the ease of organic matter breakdown and soil microorganism activity.

P_2O_5 content in medium and dense plant densities is classified in the medium category with levels of 12.26 ppm and 15.12 ppm. The sparse density is categorized as low, with 9.93 ppm. Soil P_2O_5 availability. Low P content indicates low organic matter content and poor P-containing minerals, leading to low soil P-total content. In addition, there are very low levels of phosphorus in the soil solution as it leaches away, thus removing phosphorus from the soil little by little.

Mg content in sparse and medium plant density is classified as medium, with levels of 1.19 cmol/kg and 1.82 cmol/kg. While Mg in dense plant density is classified in the low category with levels of 0.77 cmol/kg. When viewed from the Mg content at three plant density levels, the soil conditions at the three locations are less fertile, so action needs to be taken to encourage the increase of the element Mg. Elegance potassium (K) content in the soil at three density levels is classified in the medium category with each level. 0.51, 0.54, and 0.51 cmol/kg, respectively.

Cation exchange capacity (CEC) is one of the soil testing parameters that shows the ability of soil to exchange cations to hold exchangeable cations. CEC affects soil structural stability, nutrient availability, pH, and reaction to fertilizers. CEC is the ability of soil to absorb and exchange cations. The analysis results show that the CEC in Sparse and dense plant density is classified as moderate with 20.48 cmol/kg and 22.35 cmol/kg. Meanwhile, the medium plant density is classified as high, with levels of 25.54 cmol/kg.

Identification and characterization of spore types of arbuscular mycorrhizal fungi (FMA)

Based on Table 3, AMF spores in the rhizosphere of rubber plants in agroforestry patterns are grouped based on morphotypes, which were then identified based on the genus. In this study, the genus of AMF was determined based on the following characteristics: shape, color, size, and reaction with Melzer's solution on spores.

The results of the study found two genera, namely the genus Glomus and Acaulospora. The Glomus genus has the largest distribution found in rubber plants in agroforestry patterns, with as many as seven Glomus species. Glomus has the widest distribution area and is the most tolerant of soil salinity conditions. The previous study by Musdalifah et al., (2025) revealed that the fungal community inhabiting the organs of *H. brasiliensis* was identified, with 101 species and ten genera. Based on the morphological identification by Suharno et al. (2017), AMF found in the *B. precumbens* rhizosphere were identified as genus Glomus, Scutellospora, Acaulospora, and Claroideoglomus. Chanda et al. (2014) also found dominance of the Glomus genus in Assam (India), lying in the Eastern Himalayan region. A study was conducted by Summuna et al. (2019), that Glomus species was the most common and predominant AM fungus, with a frequency occurrence of 60.75%, followed by

Acaulospora spp. (22.82%), *Scutellospora* spp. (8.05%) and *Septoglomus* spp. (4.69%). It can be concluded that the high rate of spread of glomus is due to its ability to be more adaptive to various environmental conditions compared to other genera. In addition to adaptation factors, the soil texture also affects the development and growth of spores.

FMA spore density

Based on Table 4, the level of plant density has no significant effect on spore density. The average number of the highest spore counts was obtained at sparse plant density, with an average of 88.97/50 grams of soil. Higher density spores in rubber plantation areas with agroforestry plantation patterns with sparse plant density are thought to be caused by pH slightly acidic, C-Organic, N-Organic, and P_2O_5 low, C/N high, Mg, K, and CEC moderate. Other research conducted by Prihastuti (2007) also obtained similar results, namely the highest spores with a total of 311 found in areas with soil chemical properties of acid pH (5.15), very low N-Organic (0.05%), P_2O_5 availability was low (4.28 ppm), K content was low (0.03 cmol/kg), and CEC was moderate (19.2 cmol/kg).

FMA colonization of plant roots in agroforestry planted rubber plantations, FMA association on rubber roots

Based on Table 5, the value of AMF colonization of rubber plants at medium and high plant density levels was significantly different from AMF colonization in the roots of rubber plants of the sparse plant density agroforestry pattern. However, the level of AMF colonization with rubber plant roots at 3 levels of plant density is included in the medium category, and the highest colonization (45.49%) was obtained at a sparse plant density. The existence of colonization by AMF on rubber roots is characterized by vesicles, microsclerotia, hyphae, and spores . Other research conducted by Suharno et al. (2017) found that the percentage of root colonization ranged from 65.3 to 85.3% with an average of 73.6%. The value was categorized as high and showed a good cooperation of bio-symbion and myco-symbion. Carballar-Hernández et al. (2013) observed colonization of AMF on Agave potatorum that ranged from 20–83%. The percentage of colonization on plant roots was associated with the ecological system of both AMF and host plants in their habitat.

FMA association in intercrops

The level of AMF colonization on the roots of intercrops in rubber plantations varies from low to very high. The availability of P_2O_5 in the soil is the nutrient element that has the greatest effect on AMF colonization. The level of P_2O_5 availability in the soil is inversely proportional to the level of AMF colonization. The results of research on AMF colonization of sparse-density cloves with low category obtained low P_2O_5 content, and AMF colonization of medium-density cloves obtained a medium category with medium P_2O_5 content. Colonization AMF from durian plant roots is categorized as moderate with low P_2O_5 content. Colonization on medium and dense densities obtained from coffee intercrops is classified as low and medium with P_2O_5 content. The highest colonization was found in *porang* intercrops, with a medium density level obtained by the content of P_2O_5 being moderate.

Observations of AMF colonization in agroforestry pattern rubber plants revealed four types of colonization in the form of vesicles, microsclerotia, hyphae, and spores. Vesicles are spherical, oval, or irregularly shaped structures that act as storage organs for food reserves such as lipids. The main function of vesicles is to play an important role in nutrient storage since their cells contain high levels of lipids and glycogen, but in some cases, they might assume a reproductive function because vesicles may form spores that act as props gules. The vesicle formation process depends on the AMF species. For some species, vesicle formation occurs very early after symbiotic phase establishment, while for others, it occurs at the same time as sporulation or even after the formation of the first arbuscules (Souza, 2015). Microsclerotia are compact, multicellular structures produced by some fungi as survival structures living in the soil. This structure is important for the survival of the fungus in poor environmental conditions, such as drought or low nutrient availability. Microsclerotia are formed from aggregations of hyphae and are usually dark in color, hard, and resistant to environmental stress (Song, 2018). Spores are one of the AMF propagules that can colonize the host plant's roots. The source of inoculum, which can cause root infection by AMF, consists of hyphae, and infected parts of the root (Silvana et al., 2020).

CONCLUSION

This study effectively examined the diversity and composition of Arbuscular AMF in rubber-based agroforestry systems, identifying two primary genera, *Glomus* and *Acaulospora*. The results indicated that *Glomus* species were the most dominant, demonstrating their ability to thrive in various environmental conditions. Furthermore, the study found notable variations in AMF spore density and root colonization rates across different plant densities, with sparse agroforestry systems exhibiting higher spore density and colonization compared to medium and dense systems. By addressing a significant knowledge gap regarding AMF in Indonesian rubber agroforestry, this study provides valuable insights into their contribution to soil fertility and plant productivity. The findings contribute to a better understanding of how AMF can be optimized within agroforestry systems for sustainable land management and pave the way for future investigations into their ecological roles and potential benefits for improving soil health and biodiversity.

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