

The Effect of Organic Fertilisers on Arbuscular Mycorrhizal Fungi Diversity in the Rhizosphere of *Coffea arabica* Plants on the Napu Highland, Central Sulawesi, Indonesia

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Abstract: *Coffea arabica* plantations in Central Sulawesi are carried out on the Napu highland, where this area is dominated by ultisol or yellow red podzolic soils, which have problems of high soil acidity and low availability of macro nutrients. This study used a completely randomised design method consisting of four treatments namely; without organic fertilisers (control)/P0, *Leucaena leucocephala* leaf compost 3 Kg/tree (P1), *Samanea saman* leaf compost 3 Kg/tree (P2), *Tithonia diversifolia* leaf compost 3 Kg/tree (P3), Cow and goat manure 3kg/tree (P4). The results showed that there were 10 species of Arbuscular Mycorrhizal Fungi (AMF) associated with *Coffea arabica* plants namely *Glomus* sp1, *Glomus* sp2, *Glomus* sp3, *Glomus* sp4, *Acaulospora* sp1, *Acaulospora* sp2, *Acaulospora* sp3, *Gigaspora* sp1, *Gigaspora* sp2 and *Gigaspora* sp3. Furthermore, the higher density of FMA spores was found in the treatment without organic fertiliser application/control (P0) which was 31 spores/10 g soil, compared to the treatment of *Tithonia diversifolia* leaf compost treatment (P3) with 11 spores/10 g soil, cow and goat manure treatment (P4) with 3 spores/10 g soil, and *Samanea saman* leaf compost treatment (P2) with 2 spores/10 g soil. And in the *Leucaena leucocephala* leaf compost treatment (P1), no AMF spores were found. The difference in AMF spore density in the various organic fertiliser treatments mentioned above is related to the effect of improving soil chemical properties on the soil. The results of this study contribute to the understanding of the importance of soil amendments with organic fertilisers for the improvement of organic and sustainable arabica coffee production in the future.

Keywords: Arbuscular mycorrhizal fungi; *Coffea arabica*; Organic; Rhizosphere; Ultisol

Introduction

The Napu highland, Central Sulawesi, is one of the *Coffea arabica* plantation development areas in Indonesia. This area is at an altitude of >1000m above sea level, the soil type is dominated by yellow red podzolic soil or Ultisol with high rainfall intensity. Yellow red podzolic soils have chemical properties namely low soil pH, low

availability of nitrogen, phosphate and potassium. Low organic matter with high rainfall, with clay soil properties (low infiltration rate) causes high erosion that can remove nitrogen and potassium elements. Such soil characteristics are often a severe problem and constraint in crop cultivation and food production in the tropics. They include soil moisture stress (dry seasons lasting more than 3 months make crop production difficult

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throughout the year), low nutrient content, erosion risk, low pH with aluminium (Al) toxicity, high phosphorus (P) fixation, low soil organic matter, and loss of soil biodiversity (Khan et al., 2024; Premalatha et al., 2023).

The development of coffee plants on yellow red podzolic land provides promising hope, but must be balanced with the improvement of the chemical properties of the soil (Al-Shammary et al., 2024), through the input of organic materials that can detoxify Al^{3+} which can be exchanged to some extent by complexing it with organic acids (Ur Rahman et al., 2024), increasing the content of nutrients in the soil needed for plant growth and production, including increasing pH and CEC (Yang et al., 2024). The addition of organic matter also improves soil physical properties such as structure, texture, consistency, porosity and even soil erosion (Kang et al., 2022), soil biological properties, especially the population and activity of soil microorganisms (Chen et al., 2024). Organic matter improves soil biological properties through its role as a source of energy and food for soil microorganisms so as to increase the number and diversity of microorganisms that function as decomposers (Yu et al., 2022).

Among soil microorganisms are arbuscular mycorrhizal fungi (AMF), which belong to the phylum Glomeromycota (Rajapitamahuni et al., 2023) and form a mutualistic symbiosis with the roots of most known terrestrial plant species, the benefits of which can enhance plant growth and health (Qiao et al., 2024), improve poor soil conditions (Ocampo-Alvarez et al., 2020), and improve the health of plants (Boadie-Ampong & Nishi, 2024) increase nutrient and water availability (Tartaglia & Aronson, 2024), and positively influence other soil organisms, including bacteria and other fungi and increase plant tolerance to environmental stress (Reghmit, 2023). The presence of AMF in coffee plants was first observed by Bagyaraj et al. (2015), who found highly mycorrhizal coffee plant roots from Java Island. Since then, several subsequent studies have verified the presence and importance of AMF symbiosis in coffee, especially in advanced weathered and low fertility soils (Murphy, 2024), as is the case in many tropical regions where the crop is widely cultivated.

The addition of organic matter to soil has been reported to have a positive effect on AMF external mycelial growth, especially in semi-arid regions. AMF colonises decaying leaves and its mycelium associates with organic matter particles in the soil. The mechanism responsible for this remains unclear. The growth of soil microorganisms is generally stimulated by the addition of organic matter, which is an important source of energy for them. In contrast, AMF are biotrophs and obtain energy from their host plants. No evidence has

yet been found that AMF can degrade carbon compounds on its own, and its outer mycelium cannot take up carbohydrates (Wahab et al., 2023). Lu et al. (2023), reported that organic fertiliser can increase AMF biomass and is less damaging to AMF richness than mineral fertiliser alone. The response of AMF to organic fertiliser is generally positive when AMF and host plants have a strong mutualistic symbiosis such as in phosphorus-deficient soils, drought and semi-drought areas, at low latitudes. Under other conditions, the response is generally negative. Organic carbon input, increased soil phosphorus and the ratio of N and P fertiliser together explained the effect of organic fertiliser on AMF emergence.

Many studies have shown how long-term fertilisation affects biochemical properties and microbial communities, but information on the response of AMF communities to long-term organic fertiliser application is still very limited. It is reported that FMA can regulate organism interactions, provide nutrients, and increase stress tolerance for host plants. However, the mutualistic symbiosis will change under high nutrient conditions, which allow plants to directly obtain sufficient nutrients, resulting in a decrease in soil AMF activity and/or diversity. Fertilisation significantly increases soil nutrient levels, which can trigger significant shifts in AMF community composition and diversity (Zimmermann et al., 2016), reported that there is synergy when *T. diversifolia* organic matter is applied together with AMF inoculation. Organic matter can improve soil physical properties and supply carbon for AMF survival, while AMF plays a role in increasing aggregate stability, porosity, and soil permeability. In addition, AMF also plays a role in decomposing organic matter and releasing high total P in the soil, making nutrients available to plants. However, research results are still inconsistent because they are influenced by many factors such as host plant, root system, AMF species, soil type, pH, nutrient content, climate and so on.

Organic matter can be obtained from various sources including plants that have high potential and nutrient content such as *Leucaena leucocephala*, *Samanea saman*, *Tithonia diversifolia* and animal manure. However, to our knowledge, no research has been reported on the effects of organic matter amendments on the diversity of AMF colonising the rhizosphere of *Coffea arabica* plants in yellow red podzolic soil. This study aims to determine the effect of applying several types of organic fertiliser on the diversity of arbuscular mycorrhizal fungi in the rhizosphere of arabica coffee plants in the Napu highlands, Central Sulawesi.

Method

Time and Research Location

This research was conducted from January to October 2022, in a 3-year-old *Coffea arabica* plantations, in the Napu highlands, Watutau village, Lore Peore sub-district, Poso district, Central Sulawesi, Indonesia (Figure 1). Analysis of soil chemical properties was carried out at the Soil Science Laboratory, Faculty of Agriculture, Tadulako University, Palu, while identification of species and density of arbuscular mycorrhizal fungi in *Coffea arabica* rhizosphere soil was carried out at the Forest Biotechnology and Bioremediation Laboratory of Biotech Centre, Bogor Agriculture University, Bogor, Indonesia.

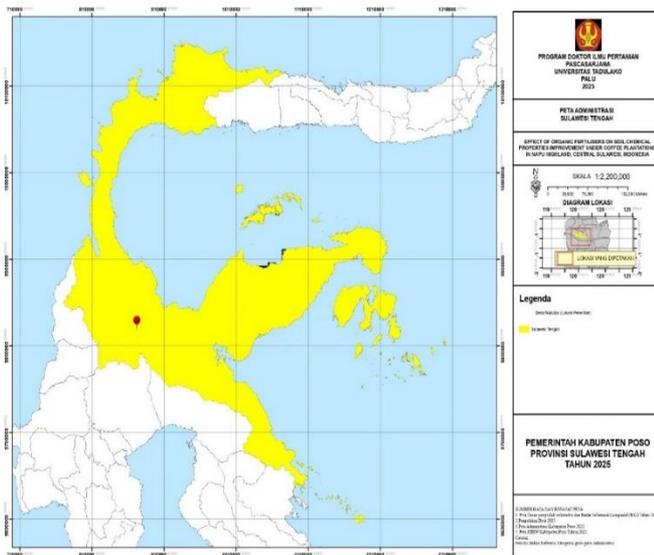


Figure 1. Research location map (red dot)

Fertilizers Application

In this study, four types of organic fertilizers were used, namely lamtoro leaf compost (*Leucaena leucocephala*), trembesi (*Samanea saman*), paitan (*Tithonia diversifolia*) and cow and goat manure. These organic fertilizers have been prepared in advance. Each type of organic fertilizer was applied at a dose of three kilograms per coffee plant. Fertilizer was given by dividing it evenly and putting it into four holes 10 cm deep made on the four sides of the coffee plant stem. The distance from the hole to the coffee plant stem is ± 30 cm. After the fertilizer was put in, the hole was covered again with soil.

Soil Sampling in the Rhizosphere of *Coffea Arabica* Plants

Soil samples were collected from the rhizosphere of *Coffea arabica* plants after 4 months of organic fertiliser application. Soil samples were taken from the soil surface to a depth of 20 cm. Soil samples were taken randomly from holes on the four sides of the coffee

plant, where each hole had been applied organic fertiliser. At each point, 125 g of soil samples were taken and then composited, resulting in a total of 500 g of soil samples from each tree. This was done in the same way for each tree in the same replicate in each treatment. Then the soil samples from the replicate trees in each treatment were composited together. A total of 1 kg of composite soil samples per treatment, so that there are 5 soil samples according to the number of treatments. Each soil sample was put into a plastic bag and coded according to the treatment. All soil samples were dried in the laboratory for the purpose of isolation and identification of arbuscular mycorrhizal fungi spores and for analysis of soil properties.

Isolation, Identification of AMF and Analysis of Soil Physico-Chemical Properties

The technique used for AMF spore isolation is the wet pour sieve technique. The working procedure of this pouring sieve technique begins with mixing 50 g of soil sample with 200-300 ml of water and stirring until the soil granules are crushed. It is then sieved in a set of sieves with sizes of 2 mm, 0.710 mm, 250 μ m, and 53 μ m, respectively from top to bottom. From the top of the filter, it is sprayed with tap water to make it easier for the filter material to pass. Then the top filter was removed and the second filter was again sprayed with tap water. After the second filter was discarded, the remaining soil left in the bottom filter was transferred to the centrifuge tube.

Spore extraction by the above method was then followed by centrifugation technique (Brundrett et al. 1996). The filter results in the centrifuge tube were added with 60% glucose placed at the bottom of the soil solution using a pipette. The centrifuge tube was sealed and centrifuged at 2500 rpm for 3 min. Next, the supernatant solution was poured into a 53 μ m sieve, washed with running water (tap water) to remove glucose. The sediment remaining in the above sieve was poured into a petri dish and then observed under a microscope to calculate the spore density and make preparations for identification of AMF spores present.

Spore preparations using Melzer's dye and polyvinyl lacto glycerol (PVLG) preservative were placed separately on a glass slide. AMF spores obtained from the extraction results after counting were placed in Melzer's and PVLG solutions and the types of AMF spores present in both solutions were the same. Then the spores were carefully broken by pressing the cover slip using the tip of a stick. Healthy spores were selected and stored on slides that had been given PVLG and Melzer's solution and then covered with cover glass. Identification of AMF spores was done by observing morphology (shape, size, colour, carrier hyphae, wall layer, spore ornamentation, spore mother cell, and

bulbus suspensor). AMF spore nomenclature follows the latest nomenclature.

Identification of mycorrhizal fungus spores was carried out by observing the morphology of colour, shape, size, hyphae, spore ornamentation, and spore reaction to melting solution. The colour change of spores in Melzer's solution is one of the indicators to determine the type of spores present. After that, AMF spores were manually identified based on the description of existing AMF species (International Culture Collection of Vesicular-Arbuscular Endomycorrhizal Fungi. The colour change of the spores in Melzer's solution is one of the indicators to determine the type of spores present. After that, AMF spores were manually identified based on the description of existing AMF species (International Culture Collection of Vesicular-Arbuscular Endomycorrhizal Fungi at <http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html>). Spores were observed under a stereo microscope and spores were identified using an Axio Imager microscope at 200x magnification. Spore density was calculated per 10 grams of soil by directly counting the number of spores present in each soil sample. AMF species richness was defined as the number of AMF species present per soil sample.

Selected soil physico-chemical properties were analysed using standard protocols. Organic C was quantified using the Walkley-Black method. Total N was analysed using the Kjeldahl method. Total phosphate (mg/100g) was determined using 25% HCl extraction, and total potassium was determined using 25% HCl extraction. Soil pH was determined using a digital pH meter and soil texture using a pipette method.

Research Methods

This study used a completely randomised design (CRD) method consisting of four treatments, namely; without organic fertilizers (control)/P0, *Leucaena leucocephala* leaf compost 3 kg/tree (P1), *Samanea saman* leaf compost 3 kg/tree (P2), *Tithonia diversifolia* leaf compost 3 kg/tree (P3) and Cow and goat manure 3 kg/tree (P4). Each replicate and experimental plot consisted of 4 plants. And each treatment was repeated three times, so there were 15 experimental plots or 60 *coffea arabica* plants.

Data Analysis

Data analysis in this study used descriptive analysis by calculating the average value of each replicate on the same organic fertiliser treatment. The average value of soil chemical properties is adjusted to the standard and classification according to (Al-Soghir et al., 2022). Micromorphology of each species of arbuscular mycorrhizal fungi was also described based on its characteristics

according to the literature. The results of data analysis will be presented in the form of tables and graphs.

Result and Discussion

Arbuscular Mycorrhizal Fungi Associated with Coffea Arabica Plants

The results showed that there were 10 species of Arbuscular Mycorrhizal Fungi (AMF) associated with arabica *coffea arabica* plants in Watutau Village, East Lore District, Poso Regency, Central Sulawesi, Indonesia. There were 5 species of AMF associated with *Coffea arabica* plants in the treatment without organic fertiliser application (control), 1 species of AMF in the treatment of *Samanea saman* leaf compost, 2 species in the treatment of *Tithonia diversifolia* leaf compost and 1 species in the treatment of cow and goat manure mixture, while in the treatment of *Leucaena leucocephala* leaf compost none of the AMF species were found. The species and description of each AMF associated with *Coffea arabica* plants in the four compost treatments can be seen in Figures 2, 3, 4 and 5.

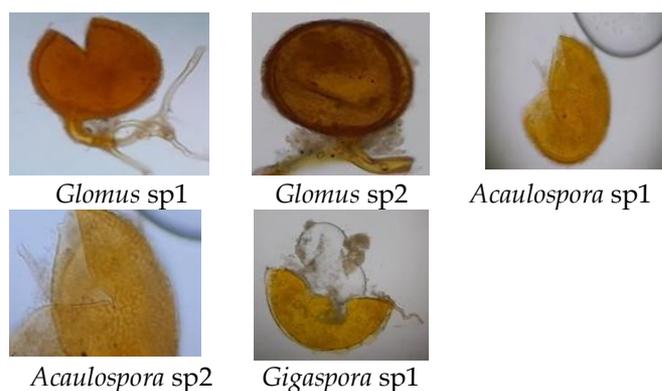


Figure 2. Micromorphology of Arbuscular Mycorrhizal Fungi associated with *Coffea arabica* Plants in without organic fertilizers treatment (control/P0)

The description of each species of arbuscular mycorrhizal fungi found in rhizosphere soil samples of *Coffea arabica* plants in the control treatment (without organic fertiliser application) is as follows: *Glomus* sp. 1, morphological description; The spores are brown in colour, measuring 150 μm in diameter and characterised by germination pathways on the subtending hyphae. The spores of this genus only form spore-walls. *Glomus* sp. 2, morphological description; brownish coloured spores with a diameter of 120 μm . Distinctive characters are seen in the germination path of the hyphal stalk. Spores only form spore-walls. *Acaulospora* sp. 1, morphological description; the spores are yellow in colour and about 110-120 μm in diameter. Spores consist of spore-wall and germinal-wall. There is also a distinctive character in the form of ornamentation on the

spore-wall. *Acaulospora* sp. 2, morphological description; the spores are clear to yellowish in colour with a diameter of 190-230µm. Spores consist of 2 walls, namely spore-wall and germinal-wall. There are also ornaments on the spore-wall. *Gigaspora* sp. 1, morphological description; the spores are clear to yellowish in colour with a diameter of 190-230µm. Spores consist of 2 walls, namely spore-wall and germinal-wall. There are also ornaments on the spore-wall.



Glomus sp3

Figure 3. Micromorphology of Arbuscular Mycorrhizal Fungi associated with *Coffea arabica* Plants in *Samanea saman* leaf compost treatment (P2).

Description of arbuscular mycorrhizal fungi species found in rhizosphere soil samples of *Coffea arabica* plants in the *Samanea saman* leaf compost treatment (P2) is as follows: *Glomus* sp3, morphological description; brown spores, diameter size 150µm. Spores only form spore-walls.



Acaulospora sp3

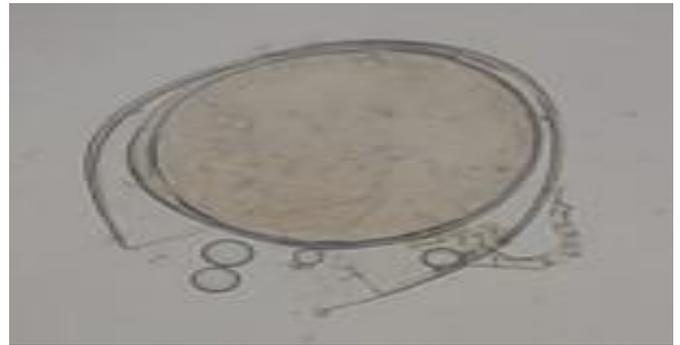
Gigaspora sp2

Glomus sp4

Figure 4. Micromorphology of Arbuscular mycorrhizal fungi associated with *Coffea arabica* Plants in *Tithonia diversifolia* leaf compost treatment (P3)

Description of each species of arbuscular mycorrhizal fungi found in rhizosphere soil samples of *Coffea arabica* plants in the *Tithonia diversifolia* leaf compost treatment (P3) is as follows: *Acaulospora* sp. 3, morphological description; clear coloured spores, 170-200µm in diameter. On the spore-wall there are ornaments typical of this genus. *Gigaspora* sp. 2, morphological description; yellow coloured spores with a diameter of 460µm. Spores and hyphae are brightly coloured with typical bulbous ornamentation. *Glomus* sp. 4, morphological description; yellow-coloured spores

with a diameter of 170µm. The spore consists of the spore-wall only.



Gigaspora sp3

Figure 5. Micromorphology of Arbuscular Mycorrhizal Fungi associated with *Coffea arabica* plants in animal manure compost treatment (P4)

Descriptions of arbuscular mycorrhizal fungi species found in rhizosphere soil samples of *Coffea arabica* plants in the animal manure compost treatment (P4) are as follows: *Gigaspora* sp. 3, with morphological description; clear coloured spores with a diameter of 150-230 µm. Spores consist of spore-wall and germinal-wall, there is also a bulbous suspensor. According to (Asad et al., 2023; Urgiles-Gómez et al., 2021; Salamanca-Jimenez et al., 2017), that like many other plants, coffee plants are also reported to be symbiotic with AMF. The results of this study are similar to the study conducted by Lara-Capistran et al. (2021) where they have identified 22 AMF species in coffee rhizosphere soil from coffee production areas in Brazil; and the most commonly found AMF genera were *Acaulospora* and *Glomus*. The results of Ouhaddou et al. (2025), are similar to the results of this research where they also found three AMF genera symbiotic with several food crops in Malang, East Java, namely *Glomus* sp., *Acaulospora* sp., and *Gigaspora* sp. They also found that spore density had a very strong relationship with soil pH and available P and soil C-organic levels.

The genus *Glomus* includes species of arbuscular mycorrhizal fungi that often form abundant spores in soil and roots. It is morphologically characterised by spores developing at the tips of sporogenous hyphae or as intercalated subglobose swellings in the interior of sporogenous hyphae. Spores are usually attached to a single subtending hypha that is simple, straight, or curved, and rarely swollen (Błaszowski et al., 2019); (Niezgoda et al., 2024). The genus *Glomus* has a high level of adaptation to environmental conditions (Kartika et al., 2019), so *Glomus* is most commonly found in symbiosis with the roots of various plant species (Tian et al., 2019; Khaliq et al., 2022).

Spores Density

The results of arbuscular mycorrhizal fungi spore density analysis showed that the spore density in the treatment without organic fertiliser application/control (P0) was 31 spores/10 g soil, which was higher when compared to the treatment of *Tithonia diversifolia* leaf compost treatment (P3) of 11 spores/10 g soil, cow and goat manure treatment (P4) of 3 spores/10 g soil, and *Samanea saman* leaf compost treatment (P2) of 2 spores/10 g soil. And in the *Leucaena leucocephala* leaf compost treatment (P1), no AMF spores were found. Spore density in each organic fertiliser treatment is presented in Figure 6 below:

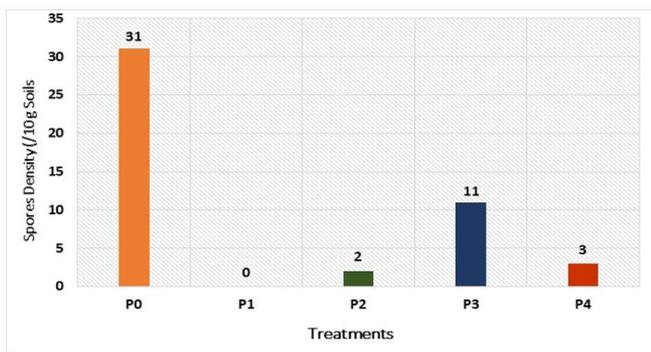


Figure 6. Spores density of arbuscular mycorrhizal fungi in several organic fertiliser treatments

The difference in the number of spores found in each treatment is thought to be caused by many factors such as the availability of nutrients in the soil. Arbuscular mycorrhizal fungi are a form of association between fungi and plant roots. Based on the results of research by Wahab et al. (2023), Owiny et al. (2024), showed that mycorrhizal colonisation on non-productive land or on nutrient-poor land is higher when compared to productive land. This occurs through the formation of hyphae on the root surface, especially in areas with nutrient-poor conditions, low pH and lack of water (Entry et al., 2002). Mycorrhizae can actually work in nutrient-poor soil conditions, while in fertile soil the role of mycorrhizae is not so obvious (Kuyper & Jansa, 2023). Furthermore, Malik et al. (2025), suggested that the highest number of AMF spores was found in soils with low pH, P, and C-Organic values. This condition is a characteristic of marginal or dry soil. Dry soil or marginal soil has acidity, low C-Organic, P, K, Ca, Mg, Na (Deru et al., 2023). Arbuscular mycorrhizal fungi will more easily colonise plants growing on land with limited nutrient content, there are certain components of the host plant root eskudat that can stimulate the development and germination of hyphae and increase when there is a lack of phosphorus (P) (Pang et al., 2024).

The beneficial effect of AMF on plant growth is often associated with the effect of uptake of unavailable

nutrients, especially phosphorus (P), on land that is high in nutrient content the number of available mycorrhizae is not so great. Colonisation of AMF in fertile soil spore germination is somewhat inhibited so that not many spores or hyphae are found. In nutrient-poor soils where P availability is very low, the role of mycorrhizae is greater in the soil and there are more of them. This phenomenon was found in this study, and is thought to be related to the improvement of soil chemical properties as a result of organic fertiliser application. Furthermore, (Liu et al., 2024; Silva et al., 2023), explained that high P concentrations in the soil can inhibit mycorrhizal colonisation. This opinion is also supported by the results of Schwalb et al. (2021), research which found that high P content in the soil reduces root exudation so that mycorrhizal colonisation in plants is inhibited. This is because when the P content is high in the soil and translocated as a result of photosynthate, the assimilated soluble carbohydrates are more aimed at the formation of new protoplasm and cell tissue in the shoot. As a result, soluble carbohydrates that are translocated and accumulated to the roots are low.

This will affect the development of AMF in the soil, as mycorrhizal hyphae require sufficient carbohydrate content for spore germination. The results of the analysis of soil nutrient content in *Coffea arabica* plantations before and after the application of organic fertiliser showed an improvement in soil chemical properties which can be seen in Figure 7 below:

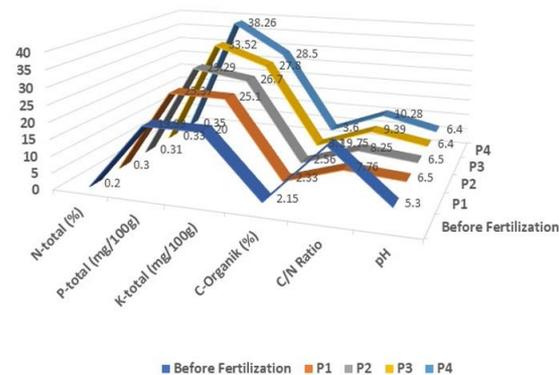


Figure 7. Soil chemistry in *Coffea arabica* plantations before and after application of organic fertilizer

Conclusion

The results showed that there were 10 species of Arbuscular Mycorrhizal Fungi (AMF) associated with *Coffea arabica* plants namely *Glomus* sp1, *Glomus* sp2, *Glomus* sp3, *Glomus* sp4, *Acaulospora* sp1, *Acaulospora* sp2, *Acaulospora* sp3, *Gigaspora* sp1, *Gigaspora* sp2 and *Gigaspora* sp3. The higher density of AMF spores was found in the treatment without organic fertiliser

application/control (P0) which was 31 spores/10 g soil, compared to the treatment of *Tithonia diversifolia* leaf compost treatment (P3) with 11 spores/10 g soil, cow and goat manure treatment (P4) with 3 spores/10 g soil, and *Samanea saman* leaf compost treatment (P2) with 2 spores/10 g soil. And in the *Leucaena leucocephala* leaf compost treatment (P1), no AMF spores were found. The difference in AMF spore density in the various organic fertiliser treatments mentioned above is related to the effect of increasing soil pH, C-Organic content of Nitrogen, Phosphorus, Potassium, CEC in the soil. The results of this study contribute to the understanding of the impact of agricultural management systems through soil amendments with organic fertilisers to be applied to organic and sustainable arabica coffee production.

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Author Contributions

A is the main researcher and a PhD student, and this article is part of his research. Y and W are the supervisors who directed the implementation of the research stages including data analysis, article writing and the student's dissertation. I.M and A.H are the student's dissertation examiners who also contributed to the improvement of research methods and the writing of this manuscript.

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Conflicts of Interest

The authors declare that there is no conflict of interest between the authors.

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