



Full Length Article

The Effects of Various Stands in Sacha Inchi Bean (*Plukenetia volubilis*) Agroforestry on Mycorrhizal Spore Density

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Received 03 June 2024; Accepted 02 September 2024; Published online 07 December 2024

Editor: Arshad Javaid

Abstract

Sacha Inchi (*Plukenetia volubilis* L.) bean cultivation needs to be developed considering its enormous benefits and potential. One of the ways that can be done to develop the cultivation is by utilizing mycorrhiza. Research on the exploration of the distribution of mycorrhiza in various types of land such as corn, sugar, palm, and tobacco fields has been done, but research on the distribution of mycorrhiza in Sacha Inchi bean agroforestry with various stands has never been done. This research aimed to collect data on the density of mycorrhizal spores in Sacha Inchi bean agroforestry. The methods used were survey and laboratory analysis. Samples were taken from four types of agroforestry land based on soil depth, namely 0–10 cm, 11–20 cm, and 21–30 cm and each treatment were repeated 3 times. Analysis of mycorrhizal spore density was done by using wet filtration and centrifugation techniques. The results showed there were 242 spores 100 g⁻¹ of soil on teak and Sacha Inchi land, 77 spores 100 g⁻¹ of soil on teak monoculture land, 126 spores 100 g⁻¹ of soil on mixed land, and 53 spores 100 g⁻¹ of soil on Sacha Inchi monoculture land. Based on the density of mycorrhizal spores, it is known that the spore density on Sacha Inchi planted in monoculture is the lowest compared to the Sacha Inchi planted with other plants. Mycorrhizal species that dominated in the four types of agroforestry land were *Glomus* and *Acaulospora*.

Editor: Arshad Javaid

Keywords: Agroforestry; Mycorrhizal; Sacha inchi; Spore density

Introduction

Sacha Inchi bean is a promising crop commodity that has the potential to be developed. This plant is shaped like a green star which when ripe will turn blackish brown, contains dark brown oval seeds, is slightly enlarged in the center, and shrinks towards the edges (Arkha and Halimah 2023). Sacha Inchi nuts contain 9.6% omega 9, 36.8% omega 6 and 45.2% omega 3, which are very beneficial for the body (Nisa *et al.* 2017). The many benefits possessed by Sacha Inchi beans make it referred to as a superfood, so efforts are needed to support the cultivation of this plant, one of which is the use of mycorrhiza. Mycorrhizae that are naturally found in the soil can not only support plant growth, but also maintain environmental quality, especially soil structure. Mycorrhiza can help improve soil structure, carbon transportation in the roots, and overcome soil fertility degradation (Sittadewi 2021).

Banyurip Village is a place in Indonesia with a large forest area. This forest is utilized by the local Forest Farmer

Group (FFG) to cultivate Sacha Inchi beans through an agroforestry system as an effort to manage the forest sustainably. To obtain better quality Sacha Inchi beans, it is needed to improve the cultivation techniques, one of which is the use of mycorrhiza, so it is necessary to know the density of mycorrhizal spores in the Banyurip Village Forest through this research.

Mycorrhiza is an association between fungi that can form symbiotic association with plant roots (Javaid and Khan 2019). Mycorrhizae that are symbiotic with plant roots are able to increase water and nutrient uptake, help plant growth, protect plants, and increase plant yields (Javaid 2009; Basri 2018). Mycorrhiza plays a role in maintaining plant stability, maintaining plant diversity, and increasing productivity. Mycorrhiza has a long and fine hyphal structure that is able to travel into the soil to absorb water, and micro and macro nutrients (Purba and Prasetya 2021). In addition, mycorrhizal fungi also help in nitrogen fixation in legumes and phytoremediation (Javaid 2010, 2011). Previous research showed that the density of

To cite this paper: Rosariastuti R, VP Pratama, Supriyadi, S Hartati (2025). The effects of various stands in Sacha Inchi bean (*Plukenetia volubilis*) agroforestry on mycorrhizal spore density. *Intl J Agric Biol* 33:33104. <https://doi.org/10.17957/IJAB/15.2250>

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mycorrhizal spores in the rhizosphere of tuber plants under suren, bitti, candlenut, and teak was 1–22 spores 50 g⁻¹ of soil (Prayudyaningsih and Nursyamsi 2015). Another study on the exploration of spore density also showed that the density of mycorrhizal spores in coffee and durian agroforestry was 102.2 spores 100 g⁻¹ of soil (Nugroho and Prasetya 2023). Research on the distribution of mycorrhizas in Sacha Inchi bean agroforestry with various stands (teak and emponies) has never been done. Therefore, this research aimed to collect data on the density of mycorrhiza in Sacha Inchi bean agroforestry with various stands (teak and Sacha Inchi) where this data can later be used to help the development of Sacha Inchi bean cultivation.

Materials and Methods

Time and place

Research was carried out from May to October 2023. Sampling was conducted on Sacha Inchi bean agroforestry land in Banyurip Village, Jenar District, Sragen Regency, Central Java Province, Indonesia. The types of agroforestry land used in this study were teak and Sacha Inchi agroforestry land; teak monoculture land; teak, Sacha Inchi, emponies agroforestry land and Sacha Inchi monoculture land. Laboratory analysis was carried out at the Soil Biology and Biotechnology Laboratory and Soil Chemistry and Fertility Laboratory, Faculty of Agriculture, Sebelas Maret University.

Materials and tools

Materials and tools used in the isolation of mycorrhizal spores are 100 g of soil samples, water, analytical scales, multistage sieves (45 μ m, 90 μ m and 120 μ m), pipettes, centrifuge tubes, centrifuge, 60% glucose solution, beaker glass, small funnel, filter paper, petridish, preparation glass and microscope.

Research method

The type of research conducted was exploratory descriptive research to determine the influence of the types of agroforestry and soil depth on the density of mycorrhizal spores. Quantitative observations were made using a factorial experimental design with the basic design was completely randomized design.

Soil sampling

Soil sampling was carried out using the Stratified Purposive Sampling method on four types of land, namely teak and Sacha Inchi agroforestry land; teak monoculture land; teak, Sacha Inchi, emponies agroforestry land and Sacha Inchi monoculture land based on soil depth, namely 0–10 cm, 11–

20 cm and 21–30 cm. Each depth was repeated 3 times so that 36 soil samples were obtained.

Isolation of mycorrhiza

Isolation of mycorrhizal spores was carried out by wet filtration (Gerdemann and Nicolson 1963) and centrifugation (referring to the centrifugation method of Brundrett *et al.* 1999). Isolation of mycorrhizal spores began with mixing soil and water samples in a ratio of 1:5 and then filtered in stages. The last filtered soil was then transferred into a centrifuge tube and given 60% glucose solution and then centrifuged at 3000 rpm for 5 min. Spores floating on the centrifugation results were taken and filtered using filter paper so that the spores would be left on the filter paper. Spores left on the filter paper were then counted for the density of mycorrhizal spores.

Soil analysis

Analysis of soil chemical properties includes pH using electrometric method (Rosariastuti *et al.* 2018), moisture content using gravimetric method (Wang and Shi 2019), organic carbon using Walkley & Black method (Rosariastuti *et al.* 2018), phosphorus availability using Olsen method (Effendy and Wijayani 2011), and potassium availability using extraction of NH₄OAc 1 N pH 7 method (Bilias and Barbayiannis 2019).

Data analysis

Data analysis was carried out statistically using the Analysis of Variance (ANOVA) to determine the diversity, if it showed a significant effect ($P \leq 0.05$) then continued with the Duncan's Multiple Range Test (DMRT) at the 5% level. The next data analysis was a correlation analysis to determine the relationship of chemical properties (soil pH, soil moisture content, soil organic carbon, soil phosphorus availability and soil potassium availability) to mycorrhizal spore density.

Results

Mycorrhizal spore density in several types of agroforestry land

The results of mycorrhizal spore isolation in Sacha Inchi bean agroforestry soil samples showed different amounts. ANOVA showed that type of agroforestry land had a very significant effect on mycorrhizal spore density ($P \leq 0.05$). Soil depth had no significant effect on mycorrhizal spore density and there was no interaction between type of agroforestry land and soil depth on mycorrhizal spore density.

Based on Fig. 1, it can be seen that the density of mycorrhizal spores from the highest to the lowest were in

teak and Sacha Inchi agroforestry land which was 242 spores 100 g^{-1} of soil; teak, Sacha Inchi, emponies agroforestry land which was 126 spores 100 g^{-1} of soil; teak monoculture land which was 77 spores 100 g^{-1} of soil; and Sacha Inchi monoculture land which was 53 spores 100 g^{-1} of soil.

Soil chemical characteristics in several types of agroforestry land

Soil pH: Laboratory analysis results showed soil on the four types of agroforestry land at each depth had a neutral to slightly alkaline pH. ANOVA showed that type of agroforestry land and soil depth had a very significant effect on soil pH ($P \leq 0.05$), but there was no interaction between the two on soil pH.

Based on Fig. 2, it can be seen that soil pH from the lowest was in teak and Sacha Inchi agroforestry land was 7.51; teak, Sacha Inchi, emponies agroforestry land was 7.64; teak monoculture land was 7.69 and Sacha Inchi monoculture land was 7.71. All of the lands are classified as suitable and can support the growth of Sacha Inchi beans because Sacha Inchi bean plants can grow optimally on soils with neutral pH. Based on Fig. 3, it can be seen that soil pH from the lowest was at the depth of 11–20 cm (7.58), 1–10 cm (7.59) and 21–30 cm (7.74). Based on the depth of the soil, the pH at a depth of 21–30 was 16% higher than 1–20 cm.

Soil moisture content: The analysis showed that soil moisture content for the four types of agroforestry land was in the range of 6–10%. ANOVA showed that type of agroforestry land had a very significant effect on soil moisture ($P \leq 0.05$), but soil depth had no significant effect on soil moisture content so that differences in soil depth did not produce significant differences in soil moisture content. There was no interaction between type of agroforestry land and soil depth on soil moisture content.

A high percentage of soil moisture content indicates that the available water in the soil was also high. Based on Fig. 4, soil moisture content in teak dan Sacha Inchi agroforestry land and teak, Sacha Inchi, emponies agroforestry land were not significantly different. The highest percentage of soil moisture content was in Sacha Inchi monoculture land (10.39%), followed by teak and Sacha Inchi agroforestry land (8.73%); teak, Sacha Inchi, and emponies agroforestry land (8.31%) and teak monoculture land (6.06%).

Organic carbon: ANOVA showed that type of agroforestry land and soil depth had a very significant effect on soil organic carbon ($P \leq 0.05$). In the analysis of soil organic carbon data, there was an interaction between type of agroforestry land and soil depth on soil organic carbon ($P \leq 0.05$).

Based on Fig. 5, soil organic carbon in teak and Sacha Inchi agroforestry land (L1) and teak, Sacha Inchi, emponies agroforestry land (L3) were higher than the

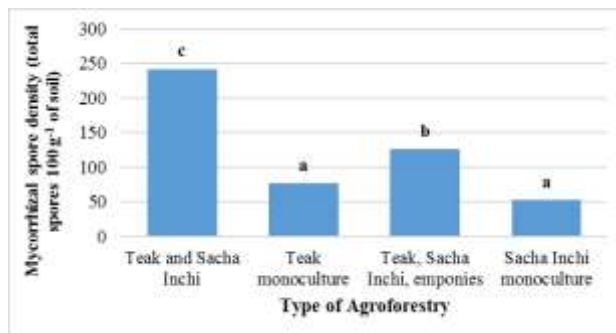


Fig. 1: Mycorrhizal spore density in the four types of agroforestry land. Bars with different letters show significant difference as determined by DMR test ($P \leq 0.05$)

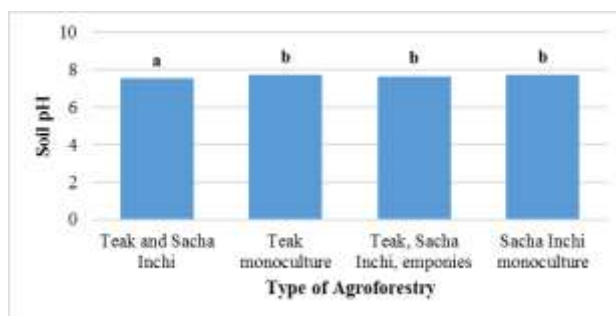


Fig. 2: Soil pH in the four types of agroforestry land. Bars with different letters show significant difference as determined by DMR test ($P \leq 0.05$)

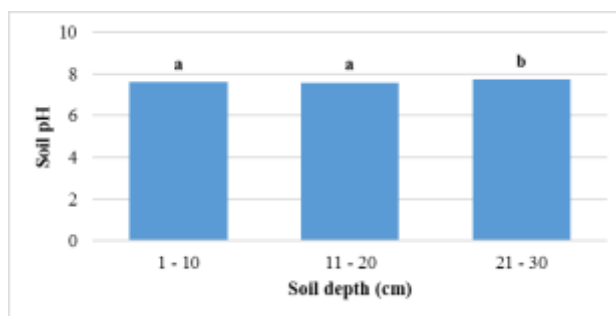


Fig. 3: Soil pH at each soil depth. Bars with different letters show significant difference as determined by DMR test ($P \leq 0.05$)

monoculture land. The highest percentage of organic carbon was found in teak and Sacha Inchi agroforestry land at 1–10 cm (L1K1) which was 2.46% and in teak, Sacha Inchi, emponies agroforestry land at 1–10 cm (L3K1) which was 1.84%. The lowest percentage of organic carbon was found in Sacha Inchi monoculture land at 21–30 cm (L4K3) which was 0.52% and in teak monoculture land at 21–30 cm (L2K3) which was 1.01%. Based on the result, the organic carbon in the four land type was very low till medium (Balittanah 2009).

Soil phosphorus availability: ANOVA showed that type of agroforestry land and soil depth had a very significant effect

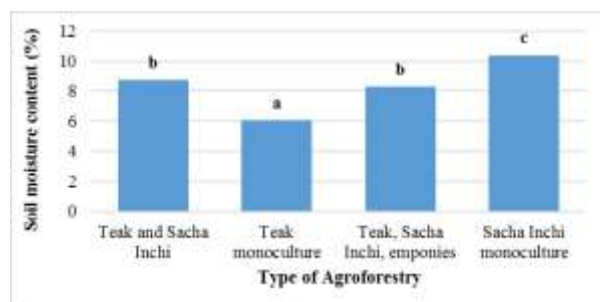


Fig. 4: Soil moisture content in the four types of agroforestry land. Bars with different letters show significant difference as determined by DMR test ($P \leq 0.05$)

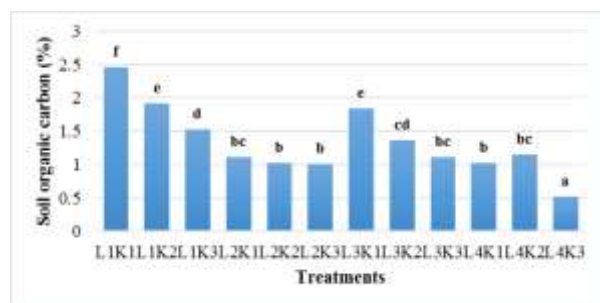


Fig. 5: Interaction between type of agroforestry land and soil depth on soil organic carbon across the four types of agroforestry land. Bars with different letters show significant difference as determined by DMR test ($P \leq 0.05$). L1 = teak and Sacha Inchi; L2 = teak monoculture; L3 = teak, Sacha Inchi, emponies; L4 = Sacha Inchi monoculture; K1 = 1-10 cm; K2 = 11-20 cm; K3 = 21-30 cm

on soil phosphorus availability ($P \leq 0.05$). In the analysis of soil phosphorus availability data, there was an interaction between type of agroforestry land and soil depth on soil phosphorus availability ($P \leq 0.05$).

Based on Fig. 6, the lowest phosphorus availability was found in teak and Sacha Inchi land at 21–30 cm (L1K3) which was 1.11 ppm (very low). In Sacha Inchi monoculture land (L4), the phosphorus availability at depths 1–10 cm (K1) and 21–30 cm (K3) was not significantly different. The highest soil phosphorus availability was found in Sacha Inchi monoculture land at 1–10 cm (L4K1) which was 14.45 ppm (medium).

Soil potassium availability: ANOVA showed that type of agroforestry land and soil depth had a very significant effect on soil potassium availability ($P \leq 0.05$). In the analysis of soil potassium availability data, there was an interaction between type of agroforestry land and soil depth on soil potassium availability ($P \leq 0.05$).

Based on Fig. 7, the highest soil potassium availability was found in Sacha Inchi monoculture land with a depth of 21–30 cm (L4K3) which is 0.77 meq 100 g⁻¹. These results indicate that the potassium availability in L4K3 is high. The lowest potassium availability was found in teak and Sacha Inchi agroforestry land with a depth of 21–30 cm (L1K3) which was 0.28 meq 100 g⁻¹. These results indicate that the

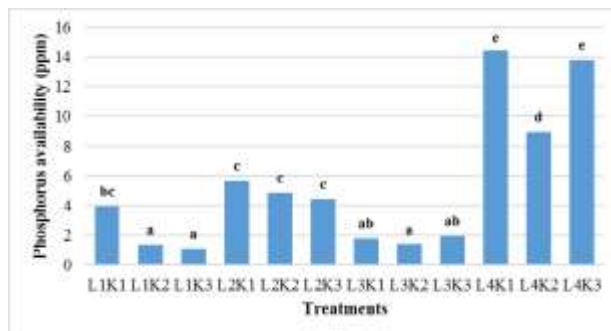


Fig. 6: Interaction between type of agroforestry land and soil depth on soil phosphorus availability across the four types of agroforestry land. Bars with different letters show significant difference as determined by DMR test ($P \leq 0.05$). L1 = teak and Sacha Inchi; L2 = teak monoculture; L3 = teak, Sacha Inchi, emponies; L4 = Sacha Inchi monoculture; K1 = 1-10 cm; K2 = 11-20 cm; K3 = 21-30 cm

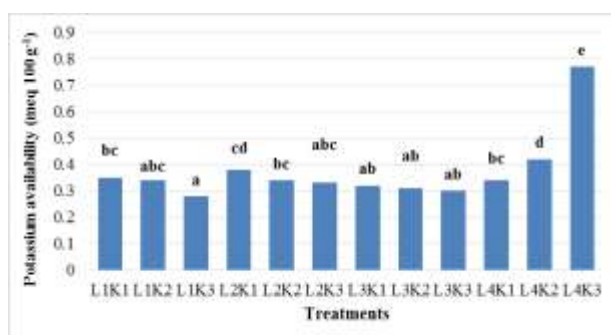


Fig. 7: Interaction between type of agroforestry land and soil depth on soil potassium availability across the four types of agroforestry land. Bars with different letters show significant difference as determined by DMR test ($P \leq 0.05$). L1 = teak and Sacha Inchi; L2 = teak monoculture; L3 = teak, Sacha Inchi, emponies; L4 = Sacha Inchi monoculture; K1 = 1-10 cm; K2 = 11-20 cm; K3 = 21-30 cm

potassium availability in L1K3 is low.

Discussion

Mycorrhizal spores with the highest density were found in teak and Sacha Inchi agroforestry land. Agroforestry has plants with more than one type of plant. Rooting areas in agroforestry land support the formation of mycorrhizal spores (Nugroho and Prasetya 2023). The combination of roots of various types of plants in agroforestry land will produce exudates that can trigger a variety of soil microorganisms (Qiao *et al.* 2022). These soil microorganisms include typical bacteria and fungi, including mycorrhizae, which are more abundant in agroforestry lands. The high density of mycorrhizal spores in a field can help plant growth to be better because one of the roles of mycorrhiza is to increase the content of nutrients available to plants, especially P nutrients. P nutrients stored

in the soil can be dissolved by phosphatase enzymes produced from mycorrhizal activity so that the P-available content in the soil can increase (Khairuna *et al.* 2015). Mycorrhiza also play a role in raise the ability of roots to absorb water and nutrients, increasing plant resistance to drought stress and enhance soil aggregates (Megasari *et al.* 2022). Number of mycorrhizal fungal spores is related to the level of root infection by mycorrhiza where the more mycorrhiza there, the higher the mycorrhizal infection of plant roots (Permanasari *et al.* 2016). The higher percentage of mycorrhizal infection will increase roots' ability to absorb water and nutrients because the root absorption area is wider (Musafa *et al.* 2015). Based on the results of Nugroho and Prasetya (2023) research, mycorrhizal spore density in agroforestry land with coffee and durian plants is 102.2 spores 100 g⁻¹ of soil, the percentage of mycorrhizal infection in coffee plants is 42.29% and in durian plants is 42.77% where both are included in the medium category. Based on Alayya and Prasetya (2022) research results, mycorrhizal spore density in agricultural land with rice plants is 26.5 spores 100 g⁻¹ of soil and in cassava plants is 70 spores 100 g⁻¹ of soil with the percentage of mycorrhizal infection in rice plants is 45.52% and in cassava plants is 50.76% where both are included in the high category. The density of mycorrhizal spores in teak and Sacha Inchi agroforestry land and teak, Sacha Inchi, emponies agroforestry land was 242 spores 100 g⁻¹ of soil and 126 spores 100 g⁻¹ of soil where these results were higher than the density of mycorrhizal spores in coffee and durian agroforestry land in research by Nugroho and Prasetya (2023) so it can be concluded that mycorrhizal spore infection in the roots of teak, Sacha Inchi, and emponies is higher than in the coffee and durian agroforestry land. Similarly, the density of mycorrhizal spores in Sacha Inchi monoculture land which is agricultural/cultivation land, is 53 spores 100 g⁻¹ of soil where this result is higher than the density of mycorrhizal spores in agricultural land with rice plants in Alayya and Prasetya (2022) research so it can be concluded that mycorrhizal spore infection in the roots of Sacha Inchi bean plants is higher than in the roots of rice plants. High mycorrhizal infection in plant roots will increase roots' ability to absorb water and nutrients so that plant growth will be better.

Sacha Inchi bean plants can grow optimally on soils with neutral pH. Soil pH on the four types of land is in the range of 7.51–7.71. Teak and Sacha Inchi agroforestry land and teak, Sacha Inchi, emponies agroforestry land have a lower pH value than the other two fields. Both of these lands are agroforestry lands where there is more than one type of plant. The variety of plant species indicates that the number of roots in the soil is also increasing. Plant roots are capable of producing metabolite compounds or root exudates such as organic acids, amino acids and nucleotide compounds (Prayudyaningsih *et al.* 2015). Exudates that are intensively released by the roots into the soil can attract microorganisms to grow in and around the roots (Widyati

2017). The microorganisms are then able to decompose organic matter where the results of the decomposition process are organic acids. Organic acids are produced from the break down of organic matter cause a decrease in soil pH (Sari and Sudarsono 2017). Based on the depth of the soil, the pH at a depth of 21–30 was 16% higher than at a depth of 1–20 cm. Increased soil pH as well as increased soil depth may be due the presence of bases leached to deeper layers (Tomo and Prasetya 2021). A lower soil pH will increase number of mycorrhizal fungal spores present. Number of mycorrhizal spores will be more in soil with a lower pH (Yusriadi *et al.* 2018). This is because mycorrhiza has acidophilic properties or likes acidic conditions so that more mycorrhiza will be found in soils with low pH (Putra and Jilil 2015). The results of the research conducted are in accordance with this statement where the highest density of mycorrhizal spores is found in teak and Sacha Inchi agroforestry land with the lowest soil pH, namely 7.51. While the lowest density of mycorrhizal spores is in the Sacha Inchi monoculture land with the highest soil pH of 7.71.

The percentage of soil moisture content for teak and Sacha Inchi agroforestry land was 8.73% and teak, Sacha Inchi, emponies agroforestry land was 8.31%. These results are quite high because both lands are agroforestry lands with more than one type of plant. The more types of plants in agroforestry land cause high litter in the land (Darmayanti and Solikin 2019). High levels of litter in a field can reduce evaporation so that the water content in the soil does not decrease quickly. Piles of debris in the soil have the ability to retain water content so as to prevent soil water evaporation (Liu *et al.* 2022). The moisture content in the teak monoculture land is 6.06%, was the lowest compared to the other fields. This could be due to the lesser thickness of the leaf litter. Another factor that causes low soil moisture content in teak monoculture land is low organic matter. Low organic matter content is visible on low carbon organic percentage. Water availability in the soil is influenced by organic matter because it can bind the water well (Hasibuan 2015). Organic matter causes an increase in the amount of micropores and macropores in the soil through the gluing of soil particles so as to increase soil water holding capacity (Bot and Benites 2005). Sacha Inchi monoculture land has the highest percentage of soil moisture content (10.39%) even though this land was cultivated with one type of plant. The high soil moisture percentage in this field was due to its high canopy density. Canopy density have a significant impact on the increase of soil porosity (Leonika *et al.* 2021). The dense canopy makes the soil moist because sunlight is blocked from going directly to the soil. The dense canopy was able to prevent direct sunlight so that soil moisture is maintained (Saroh and Krisdianto 2020). Teak and Sacha Inchi agroforestry land and teak, Sacha Inchi, emponies agroforestry land have a high percentage of moisture content at 8.73 and 8.31%, respectively, and these two fields have a higher density of mycorrhizal spores than other

fields. Land with a high number of mycorrhizae was known to be able to maintain and enlarge available water in the soil. Mycorrhiza plays a role in increasing porosity because its hyphae are able to expand the surface of plant roots (Puspitasari and Winarsih 2023). Hyphae in mycorrhiza act as a bridge connecting the roots with soil moisture (Ramadhan *et al.* 2017).

The highest soil organic carbon content was found in teak and Sacha Inchi agroforestry land at 1–10 cm (L1K1) which was 2.46% and the lowest percentage of organic carbon was found in Sacha Inchi monoculture land at 21–30 cm (L4K3) which was 0.52%. Organic carbon levels in agroforestry land are greater than monoculture land because organic matter inputs from stands and roots are higher (Handayani and Suryadarma 2022). In monoculture fields, organic carbon content is relatively lower than in agroforestry fields. The low organic carbon content in Sacha Inchi monoculture land was due to the fact that the plants were still in the early phase of planting so that not much litter was produced and the organic matter was low. Agroforestry land is able to increase organic carbon compared to monoculture land because there are several types of plants in agroforestry land (Saputra *et al.* 2020). Organic carbon content in monoculture land is lower than in land with more than one plant due to the lack of litter or plant residues in monoculture land (Ferreira *et al.* 2020). Mycorrhizal spore density is high when the organic carbon content of the soil is high. Organic carbon is needed by mycorrhiza to mineralize and provide nutrients for plants (Nurhalimah *et al.* 2014). This is in accordance with research results where the highest density of mycorrhizal spores was found in teak and Sacha Inchi agroforestry land and teak, Sacha Inchi, emponies agroforestry land also the highest organic carbon content was also found in these lands. Agroforestry has more organic matter that produces more root exudates, hence the higher density of mycorrhizal spores (Huang *et al.* 2023). The high organic carbon content in the same direction as the high density of mycorrhizal spores can also be caused by mycorrhiza being able to increase carbon reserves in the soil. Increased soil carbon reserves due to the presence of mycorrhiza is because mycorrhizal colonization helps plants increase photosynthetic efficiency by up to 30%, thus adding more carbon to the soil (Birhane *et al.* 2020).

The highest soil phosphorus availability was found in Sacha Inchi monoculture with a depth of 1–10 cm (L4K1) which was 14.45 ppm. The high soil phosphorus availability content in L4K1 can be caused by the high soil pH in L4 (7.71), while the pH in L1 is the lowest (7.51) so that soil phosphorus availability is also low. Phosphorus availability in soil is influenced by pH (Rosariastuti *et al.* 2024). In soils with low pH, P will bind Fe and Al and form Fe-P and Al-P, which are compounds that are not available to plants (Camila *et al.* 2023). Another factor that supports the high phosphorus availability of soil in L4 is the input of P nutrients through fertilization activities because L4 is a

monoculture Sacha Inchi cultivation field. Application of P fertilizer on agricultural land can increase the P concentration in the soil (Panday *et al.* 2019). Soil phosphorus availability in L1, L2 and L4 at a depth of 1–10 cm (K1) was higher than at depths of 11–20 cm (K2) and 21–30 cm (K3). Only in L3 was the soil phosphorus availability content in K3 higher than K1, but the difference was not significant. The higher soil phosphorus availability in K1 could be due to the higher amount of organic matter in the topsoil compared to the layer below so that the ability to decompose organic matter is also higher. The process of decomposing organic matter will produce organic acids that can bind Al and release P bound by Al so that it will be present in the soil (Punuindoong *et al.* 2021). The high and low phosphorus availability is also implicated to the number of mycorrhizal spores found. The number of mycorrhizae is lower if the phosphorus availability in the soil is high (Purwati *et al.* 2019). High levels of P in soil reduce the permeability of root cell membranes and reduces the amount of root exudation, thereby preventing the growth of mycorrhizal fungi (Liang *et al.* 2021). This statement is consistent with the research results where the highest phosphorus availability was found in L4 and the lowest mycorrhizal spore density was also found in L4 (53 spores 100 g⁻¹ of soil). In contrast, the lowest soil phosphorus availability was found in L1 and the highest spore density was also found in L1 (242 spores 100 g⁻¹ of soil).

The highest soil potassium availability was found in Sacha Inchi monoculture with a depth of 21–30 cm (L4K3) which was 0.77 meq 100 g⁻¹ (high), while the lowest was found in teak and Sacha Inchi land with a depth of 21–30 cm (L1K3) which was 0.28 meq 100 g⁻¹ (low). The high K content in soil can be due to harvest residues and the use of fertilizers for a long time to form K deposits in the soil (Jawang 2021). Increased soil potassium availability is because the soil can retain K and reduce the level of K leaching (Suseno *et al.* 2018). Just like soil phosphorus availability, soil potassium availability is also related to the density of mycorrhizal spores. An increase in K content in soil will lead to a decrease in the number of mycorrhizal fungal spores because high K can suppress mycorrhiza from developing properly (Nurhandayani *et al.* 2013). This statement is consistent with the research results where the highest potassium availability was found in L4 and the lowest mycorrhizal spore density was also found in L4 (53 spores 100 g⁻¹ of soil). Then, the lowest potassium availability content was found in L1 and the highest density of mycorrhizal spores were found in L1 (242 spores 100 g⁻¹ of soil).

Correlation analysis was performed to determine the relationship between observed parameters. According to the results of the correlation analysis, some of the analyzed soil chemical characteristics correlated with mycorrhizal spore density. Soil pH correlated very closely with mycorrhizal spore density ($r = -0.542^{**}$) and the relationship was negative. The negative correlation

indicates that the lower soil pH value, the higher the density of mycorrhizal spores in the soil. These results are supported by the research of Nongkling and Kayang (2019) which states that there is a negative relationship between soil pH and mycorrhizal spore density. In addition to soil pH, organic carbon and soil phosphorus availability have a very close correlation with mycorrhizal spore density. The correlation coefficient (r) for organic carbon with soil mycorrhizal spore density was 0.669** and the relationship was positive. The positive correlation indicates that the higher organic carbon value, the higher the density of mycorrhizal spores. This result is supported by the study of Salim *et al.* (2020) which states that organic carbon has a strong correlation with mycorrhizal spore counts. Organic carbon can attract the presence of mycorrhiza in the soil (Thirkell *et al.* 2016). The correlation coefficient (r) for soil phosphorus availability with mycorrhizal spore density was -0.582** and the relationship was negative. This negative correlation indicates that higher values of soil phosphorus availability will be followed by a decrease in the density of mycorrhizal spores in the soil. This result is consistent with the study of Alimi *et al.* (2021), where mycorrhizal spore density was negatively correlated with P content.

Conclusion

Mycorrhiza was found in all types of agroforestry land studied. The density of mycorrhizal spores from the highest to the lowest was in teak and Sacha Inchi agroforestry land with 242 spores 100 g⁻¹ of soil; teak, Sacha Inchi, emponies agroforestry land with 126 spores 100 g⁻¹ of soil; teak monoculture land with 77 spores 100 g⁻¹ of soil; and Sacha Inchi monoculture land with 53 spores 100 g⁻¹ of soil. Based on the density of mycorrhizal spores, it is known that the spore density on Sacha Inchi planted in monoculture is the lowest compared to the Sacha Inchi planted with other plants. Mycorrhizal spore density was strongly correlated with type of agroforestry land, soil pH, organic carbon, and soil phosphorus availability. High soil pH and soil phosphorus availability will reduce mycorrhizal spore density, while high organic carbon will increase mycorrhizal spore density.

Acknowledgements

The author acknowledges and thanks all parties, both internal and external, who have helped and facilitated this research so that it can be completed.

Author Contributions

RR and S conceived and designed this research. VPP performed the experiments, analyzed the data and drafted the manuscript. MRR, VPP, S, SH revised and finalized the manuscript. All authors have read and agreed to the

published version of the manuscript.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

Funding Source

The author would like to thank Sebelas Maret University for financially supporting the completion of this research through non-APBN UNS Funds with contract number 228/UN27.22/PT.01.03/2023

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