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# Metagenomic Analysis of Soil Bacteria Community Planting Pattern Effect of Rubber-Ganyong Agroforestry System and Rubber Monoculture

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*Abstract* - **Plantation land with agroforestry is one alternative to reduce the adverse effects of reduced soil fertility quality and loss of microorganism biodiversity. The diversity and abundance of soil microbial communities leads to better nutrient availability in rubber-canna intercropping*g* fields than in rubber monoculture lands. Intercropping*g* planting patterns containing rhizosphere bacteria beneficial for fertility and availability nutrition and superior phytohormone promoters. Comparison of the presence of bacteria in intercropping*g* and monoculture soil soils using metagenomic analysis of 16S rRNA gene sequencing with Bioinformatics method. Analysis of the diversity and abundance of microorganisms using an alpha-diversity matrix of Chao1 and Shannon_E index types. Phylum Proteobacteria were found, which is one of the rhizosphere bacteria whose abundance according to alpha-diversity analysis data reached 49% in intercropping*g* and 27% in monoculture fields. Some bacteria of the phylum proteobacteria are: *Azospirillum sp., B. zhanjiangense, Phenylobacterium Pseudolabrys sp.* it is reported to play a role in the cycle of improving the quality of soil fertility. In the rubber-canna intercropping*g* system, the abundance and microbial communities available on the intercropping*g* land are higher in abundance and have specific functions compared to monoculture systems. Therefore the abundance of rhizosphere bacteria produces a more maximal mechanism.**

***Keywords*** *: Intercroppingg, Metagenomic analysis, Nutrient availability, Rhizobacteria.*

## INTRODUCTION

PT Perkebunan Nusantara (PTPN) VIII in Subang Regency manages a rubber plantation (*Hevea brasiliensis*) covering an area of 4,806 Ha, consisting of 295 Ha of community plantations, 68 Ha of large private plantations and 4,473 Ha of State-owned plantations (BPS, 2020). The conversion of land use from tropical forests to monoculture rubber plantations reduces soil organic carbon by about 50%, causing nutrient imbalances and reducing the composition of soil microorganisms resulting in a decrease in soil quality (Ali *et al.,* 2019; Liu *et al.,* 2021).

The use of intercellular crops in rubber plantations is a supporting effort to optimize the management of rubber farming businesses aimed at adding natural fertilizers that are beneficial to rubber plants. In addition, one of the alternatives that can be developed to increase the income of rubber farmers is through an integrated farming system with intercroppingg. Another advantage of intercroppingg is that it can function as a ground cover plant, so that it can be used to conserve rubber land and increase the productivity of rubber plants (Nugraha *et al*., 2019).

Canna (*Canna indica*) as an intermediate plant in agroforestry systems is reported to play an important role in soil conservation because rapid leaf growth helps protect the soil from direct rain influences, increasing soil fertility because it contains high macronutrients (Sasaerila *et al*., 2021). The plant is able to live in various levels of air humidity and adapts well to various dry or moist soil conditions and can live in the shade (Rahayu & Wijayanto, 2014; Sasaerila *et al.*, 2019). Shading provides benefits for canna because it is able to reduce 30-40% of light intensity and reduce the wind speed rate because canna plants cannot live in open land and cannot withstand strong wind gusts because cannabis have fragile stems (Karungamye, 2022). Therefore, canna plants are often used as intermittent plants among woody hard trees (Karungamye, 2022; Rahayu & Wijayanto, 2014). The intercroppingg system or often referred to as the agroforestry system is the joint planting of more than one plant at the same time on the same land (Singh *et al*., 2019).

Agroforestry systems are reported to increase the presence of nutrients (such as N and P), enzyme activity in the soil and increase crop yield productivity *(Zhang et al.,* 2017). Agroforestry is listed in the international organization document: *International assessment of Agricultural Knowledge, Science and Technology for Development*, it is assumed that it will have a positive effect on food systems and sustainable land use (Ollinaho & Kröger, 2021). The use of this system is one of the solutions to environmental problems such as carbon loss, soil fertility and soil degradation, as well as loss of diversity of soil microorganisms (Tang *et al*., 2019).

Soil as a medium for plants to grow is a complex and dynamic environment, where biologically most of the activity in the soil is influenced by the abundance and diversity of microorganisms (Chairani *et al*., 2016; Zhou *et al*., 2011). The diversity of soil microorganisms can be influenced by plants by releasing root exudate so as to cause physical and chemical changes in the rhizosphere and affect the diversity (diversity) of soil microorganisms (Zhou *et al*., 2022). The presence of abundant soil microorganisms leads to higher disease control because it creates stable conditions in the soil (Zhang *et al*., 2017).

The initial step to determine the abundance of rhizosphere microorganisms in the soil must pass the taxonomic identification stage of the microorganisms remaining in the soil. Identification of this diversity of microorganisms is commonly carried out by metagenomic analysis (Nazir 2016). The principle of metagenomic analysis is carried out on the basis of the isolation of DNA isolated directly from a community land or ecosystem. Metagenomic analysis is an appropriate technique to identify the abundance of soil microorganisms by exploring directly without passing through the cultivation stage of a single organism (Ghosh *et al.,* 2018).

Based on previous research by Aini, (2019) the results of the metagenomic analysis that has been carried out state that the agroforestry system of rubber and canna plants has differences in microbial abundance in the soil in the land pattern. Therefore, this study was conducted to compare bacterial communities that have the potential to provide good benefits to rubber plants for 4 years after being planted with cannabis.

## METHODOLOGY

### Time and Place

This research was carried out from September 6, 2021 to March 9, 2022 at the Molecular Biology Laboratory of Al Azhar University Indonesia, Jalan Sisingamangaraja Kebayoran Baru, South Jakarta. Soil sampling was carried out at PTPN VIII Rubber Plantation, Jalan Raya Kalijati Timur Km 7, Subang District, Subang Regency, West Java.

### Tools and Materials

The tools used in this study were plastics, shovels, cool boxes, knives, analytical scales, sudip, micropipettes, microtube racks, Erlenmeyer, heaters and magnetic stirrers, electrophoresis devices, Polymerase Chain Reaction (PCR), UV transimulator, cup cups, stirring rods, vortex. The materials used in this study were 30 grams of soil samples of intercroppingg land and rubber monocultures, zymoBIOMICS DNA Miniprep Kit research, microtube 2.5 mL, microtube 0.5 mL, tip micropipette, aquades, Topvision agarose, Buffer TAE 1x, Tris base, glicial acetic acid, EDTA, parafilm paper, 1 µL 0.25 mm primer mix (27F and 1492R), 12.5 µL myTaq Red Mix, 8.5 µL free nuclear water, GoTaq green DNA dye, loading dye, and 1kb HyperLadder.

## Research Stages

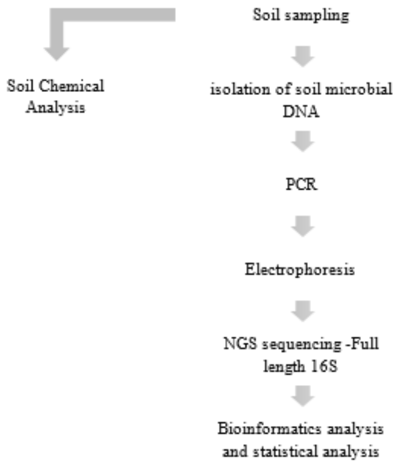


**Figure 1**. Flow chart of the stages of research.

## Soil Sampling

Soil sampling was taken from PTPN VIII plantation, Subang Regency, West Java. Samples were taken from 2 different treatments, soil on rubber fields planted with cannabis as a sideline crop since 2017, and soil on rubber fields not planted with cannabis. Samples were taken with 3 repetitions with sample marking: intercroppingg-1, intercroppingg-2, intercroppingg-3 soil on rubber fields planted with cannabis (Figure 2) and monoculture-1, monoculture-2, monoculture-3 soil on rubber fields not planted with cannabis (Figure 3). Soil samples were taken with a depth of ± 20 cm from above the surface around the plant, the soil was put into ziplock plastic ±1 kg, used for soil microbial isolation as much as 0.25 mg and the rest was used for soil chemical analysis. Then put in a *cool box* and taken to the molecular biology laboratory of Al Azhar University Indonesia.

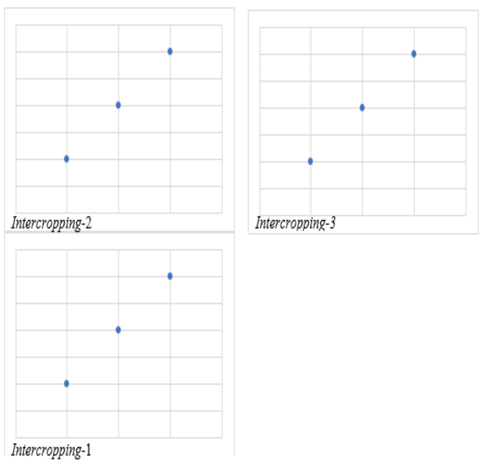


**Figure 2**. Diagram of soil sampling points on a rubber plot planted with canna with 3 repetitions (intercroppingg-1, intercroppingg -2, intercroppingg -3) with a distance of 1 m between sampling points.

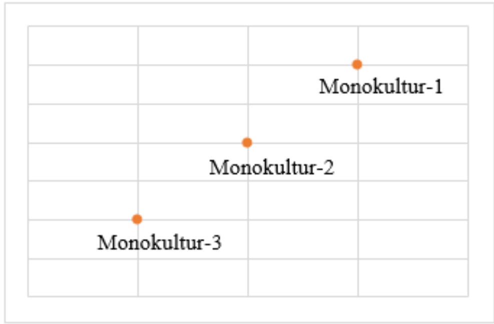


**Figure 3.** Diagram of soil sampling points on rubber plots not planted with canna with 3 repetitions (monoculture-1, monoculture -2, monoculture -3) with a distance of 1 m between sampling points.

## DNA isolation

The sample was weighed as much as 0.25 grams, then put into the ZR BashingBead Lysis tube (0.1 mm and 0.5 mm) and added 750 µL of Zymo Biomics lysis solution to the tube and closed tightly. ZR BashingBead Lysis tube is homogenized with vortex for 20 minutes, then centrifuged to separate component particles into two phases, namely pellets and supernatants at a rate of 10,000xg for 1 minute. Then as much as 400 µL of supernatant was transferred into a Zymo-spin III-F filter with a collection tube and centrifuged at a rate of 8,000xg for 1 minute. Then the Zymo-spin III-F filter was removed while the collection tube added 1,200 µL ZymoBIOMICS DNA binding buffer and homogenized using a micropipette. Furthermore, 800 µl was taken from the ZymoBIOMICS DNA binding buffer product and inserted into the Zymo-spin IICR column and the collection tube was centrifuged at a rate of 10,000xg for 1 minute, the flow through was discharged and repeated. Then inserted 400 µL ZymoBIOMICS DNA wash buffer 1 into the Zymo-spin IICR column and a new and centrifuged collection tube at a rate of 10,000xg for 1 minute and the flow through was disposed of. ZymoBIOMICS DNA wash buffer 2 of 700 µL was inserted into the Zymo-spin IICR column and the collection tube was centrifuged at a rate of 10,000xg for 1 minute and the flow through was disposed of. Furthermore, 200 µL ZymoBIOMICS DNA wash buffer 2 was inserted into the Zymo-spin IICR column and the collection tube was then centrifuged at a rate of 10,000xg for 1 minute. The Zymo-spin IICR column was transferred to a 1.5 mL microtube and centrifuged at 10,000xg for 1 minute, then 55 µL of ZymoBIOMICS DNase free water (an enzyme that protects nucleic acids from degradation) was added to the column matrix and incubated for 1 minute and then centrifuged at a rate of 10,000xg for 1 minute. Dna isolate results in microtubes are stored in a -4°C freezer until used for PCR (*Polymerase Chain Reaction*).

DNA isolation was obtained from the taking of 3 soil plot points of intercroppingg land samples (Figure 5) and 3 points of monoculture land sample soil plots (Figure 6) with a depth of 0-20 cm. The isolation results obtained were then carried out electrophoresis tests using a primer of 16S 27F – 1429R which resulted in an amplification product of about 1,500bp.

**PCR Reaction Test**
DNA isolate results were used for the PCR reaction stage using a thermal cycler (BIOMETRA) device with a final volume of 25 uL (Table 1).

**Table 1**. Volume composition for PCR

| No | Final volume composition 25 µL | (µL) |
|---|---|---|
| 1. | MyTaq | 12.5 µl |
| 2. | primers 27F and 1492R | 6.5 µL |
| 3. | $ddH_2O$ | 2 µl, |
| 4. | DNA template | 2µL |

First, $ddH_2O$ is inserted into the microtube and then the primer mix is inserted, then the MyTaq Red Mix reagent and the last template DNA are inserted into the same microtube sequentially. Furthermore, the microtube is inserted into the thermal cycler device with a PCR reaction pre-healing denaturation process of DNA tamplate 1 minute with a temperature of 95 °C. Followed by the healing process where two DNA threads are physically separated using a temperature of 95 °C for 15 seconds, then the annealing process is a primary attachment process to the DNA template from the healing process for 15 seconds with a temperature of 55 °C and the elongation process where a single thread of DNA is read by DNA polymerase by adding dna bases complement then the DNA fragments can be propagated exponentially for 20 seconds with a temperature of 72 °C. This reaction lasts as many as 35 cycles. PCR product results were visualized by electrophoresis and visualization under UV using the 1.5 µL PeqGreen dye.

**Electrophoresis**

Preparation

The manufacture of stock 10X TAE solution in 500ml of aquades was made using 24.25 grams of tris base, 5.7 mL of glicical acetic acid, 3.7 EDTA and 500 mL of aquades. Then dilute TAE 10X into TAE 1X in 500 mL with 50 mL TAE 10X stock plus 450 mL aquades for use in the electrophoresis process. Furthermore, the manufacture of 1% agarose gel in 30 mL, Agarose is weighed as much as 0.3 grams and mixed with 30 mL of aquades, then homogenized on a magnetic stirrer until dissolved and the solution becomes clear. The agarose solution is cooled and inserted 1.5 µL of peqgreen, homogenized with a magnetic stirrer and poured into an electrophoresis mold.

Electrophoresis Process
The compacted agarose is put into the electrophoresis device container and soaked TAE 1X until it sinks. Parafilm paper is prepared and placed on the workbench, then a 1 µL loading dye is affixed on a parafilm of 7 points and each point is filled with a sample of 5 µL and a ladder of 1Kb is used as a DNA marker of 5 µL, then homogenized using a micropipette. An already homogeneous sample is put in the *well.* Next the electrophoresis device is ignited with 70 volts for 25 minutes and the PCR product is visualized under the UV transimulator.

**Bioinformatics Analysis**
Six DNA isolate samples were sent to the laboratory of PT Genetika Science Indonesia on February 22, 2022, for a Full Length 16S Barcoding bioinformatics analysis test using Oxford Nanopore technology. DNA concentrations were determined using a NanoDrop spectrophotometer and a Qubit fluorometer. GridION data sorting using MinKNOW software version 20.06.9. The Base calling process uses Guppy software version 4.0.11 and FASTQ is visualized using NanoPlot to read DNA classification with centrifuge 1.0.3, then downstream analysis process by krona tools.

**Data Analysis**
The results obtained from the laboratory of PT Genetika Science Indonesia were then analyzed using Ms.Excel and graphs, to distinguish the abundance of bacterial species that have the potential to benefit the soil planted with the rubber-canna agroforestry system and the rubber monoculture system.

## Soil Chemical Analysis

Six soil samples were sent to the PT UNILAB PERDANA Laboratory on November 11-23, 2021 for soil analysis tests using several soil chemical parameters on rubber-cannabis agroforestry and rubber monoculture lands.

# RESULTS AND DISCUSSION

## Isolation of 16S ribosomal DNA sequencing (16S rRNA)

The electrophoretic agarose gel showed a uniform amplicon band size in each sample of ±1,500Bp (Figure 4). Good amplification results can be caused by specific primary suitability and PCR process optimization. The corresponding primer causes the amplification of specific regions in the genome. And the optimization of the PCR process produces a specific PCR so that a thick DNA band is formed.

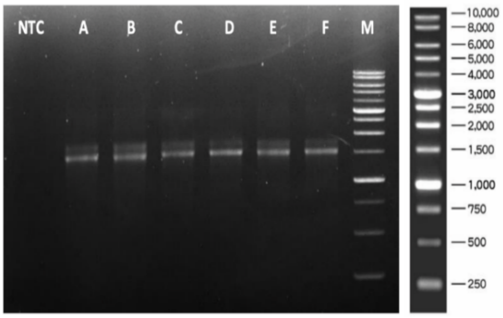


**Figure 4.** Electrophoresis of 16S rRNA sequencing PCR results.

## Diversity and Abundance of Taxonomic Units

Analysis of microbial species diversity is one of the important characteristic indications for *phytosociology*, namely the reciprocal relationship between plants and the diversity of microbial species in the soil (Thukral, 2017). The analysis using the diversity index is calculated from the number of species groups, the number of different species in each sample or habitat of the microbial community residing in an ecosystem. This study used several types of matrices to analyze the abundance and diversity of microbial community activities derived from the soil of the rubber-canna interCroppingg system and rubber monoculture system in Subang Regency, one of which is the alpha-diversity matrix. Alpha-diversity matrix analysis is commonly used because the average defines microbial diversity across different habitats on a local scale. There are two types of alpha-diversity involved in the analysis of the diversity of microbial communities in this study, namely the Chao1 Index and the Shannon_E Index (Table 2).

Table 2. Indices of diversity and abundance of bacterial communities resulting from alpha-diversity analysis on rubber-canna intercroppingg land and rubber monoculture land.

| Sampel | Chao1 | Shannon_E |
|---|---|---|
| Intercroppingg-1 | 1567 | 5.02 |
| Intercroppingg-2 | 1725.9 | 5.15 |
| Intercroppingg-3 | 1434.6 | 4.83 |
| x̄ ± σ | 1567 ± 145.9 | 5.00 ± 0.16 |
| Monokultur-1 | 2028.9 | 4.92 |
| Monokultur-2 | 1711.4 | 4.54 |
| Monokultur-3 | 1596 | 4.88 |
| x̄ ± σ | 1778.7 ± 224.2 | 4.78 ± 0.21 |

Shannon_E Index is measured based on two factors, namely the number of species and the average species and is used to show the diversity of an individual species. The higher the index value, the higher the diversity (Sarma, 2015). Based on the results of the Shannon_E index analysis, the highest index value obtained in the intercroppingg-2 sample on intercroppingg fields was 5.15, with the average index value being 5.00 ± 0.16 in intercroppingg ± fields and 4.78 ± 0.21 in monoculture lands (Table 2). The results of the Shannon_E index analysis in this study showed that soil microbial diversity was higher in the intercroppingg system than diversity in the monoculture system. These results are in line with previous findings, where species that have the same number of individuals from different species will have a higher diversity index, compared to communities dominated by one or more species (Thukral, 2017).

In this study, an analysis was carried out using a venn diagram to see the amount of diversity of species units based on the amount of species diversity found between the land of the rubber-cannabis intercroppingg system and the rubber monoculture. The number of species diversity found only in rubber-cannabis intercroppingg fields is 531 (Figure 5a) while the number of speceis found only on rubber monocrop lands is 375 (Figure 5b) and the number of diversity found in both rubber-canna intercroppingg lands and rubber monocultures is 2221 (Figure 5). Based on the results of both (Shannon_E index and venn diagram) shows that the diversity of soil microbial communities is higher in the rubber-canna intercroppingg system.

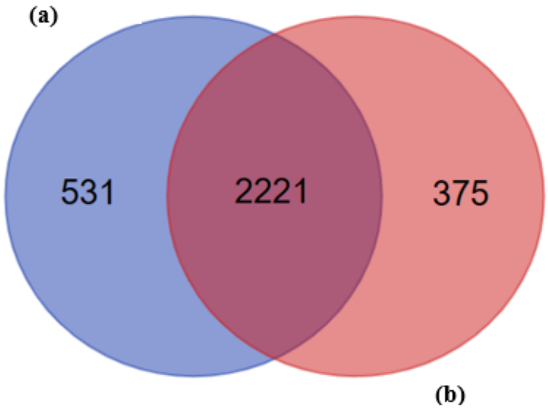


**Figure 5.** Venn diagram of the number of species diversity in rubber-canna intercroppingg land (a) and rubber monoculture (b).

The Chao1 Index is used to identify the abundance of an individual in a particular sample. In this study, the results of the Chao1 index showed that the intercroppingg-3 sample had the lowest index value of 1434.6 with the average value of abundance in intercroppingg land was 1567 ± 145.9 while in monoculture land it was 1778.7 ± 224.2 (Table 2). The low abundance of certain groups in the intercroppingg system is thought to be caused by the dominance of certain microbial groups in the intercroppingg system, thus causing the abundance index of microorganisms in one field to be low, while in monoculture fields it tends to have a uniform abundance in each sample, resulting in a higher Chao1 index value (Wu *et al*., 2018). The abundance of microbial species is calculated based on the quantity of the number of individuals per unit area per sample and the presence of species in the area (Meng *et al*., 2020). To determine whether there is dominance of certain groups of bacteria in the second sample of the planting system, a krona visualization analysis was carried out to describe the abundance of bacteria to review the amount of soil microbial abundance between the rubber-canna intercroppingg system and the rubber monoculture.

***Krona Visualization***

Krona visualization analysis was also carried out in this study to complement the visualization of metagenomic analysis by creating a clearer depiction of the estimated taxonomic abundance of microorganisms (Ghosh *et al*., 2018).. The results of the krona analysis showed that the abundance of Phylum Proteobacteria was most commonly found in the intercroppingg system with an average abundance of 49% (Figure 5a), while in monocultures the average abundance was 27% (Figure 5b). The phylum Proteobacteria consists of four classes, namely alpha-proteobacteria, beta-proteobacteria, delta-proteobacteria and gamma-proteobacteria. Two orders of alpha-proteobacteria (*Rhizobales, Rhodospirllales, sphingomonadales),* four orders of beta-proteobacteria (*Buhkolderiales, Nitrosomonadales, Neisseriales, Rodocyclales*), five orders of delta-proteobacteria (Mysococcales, Desulfobacterales, *Syntrophobacterales, Desulfovibrionales, Delsulfuromonodales, Bradymonadales*), eleven orders of gamma-proteobacteria ( *Chromatiales, Nevskiales, Enterobacterales, Xanthomonadales, Legionellales, Pseudomonadales, aeromonadales, Oceanospirillales, Thiotrichales, Cellvibrionales, Alteromonadales*) were identified in all observed samples with varying amounts of abundance.

The presence of higher dominance of Phylum Proteobacteria was identified in soil samples of intercroppingg systems than in monoculture systems. Figure 5a shows a representative chronological diagram showing the dominance of Phylum Proteobacteria, which is about 49% dominating the soil bacteria group in the intercroppingg system sample, while in the monoculture system soil sample, the composition of the Phylum Proteobacteria bacteria group is about 27% of the total soil bacteria identified in this study (Figure 5b).

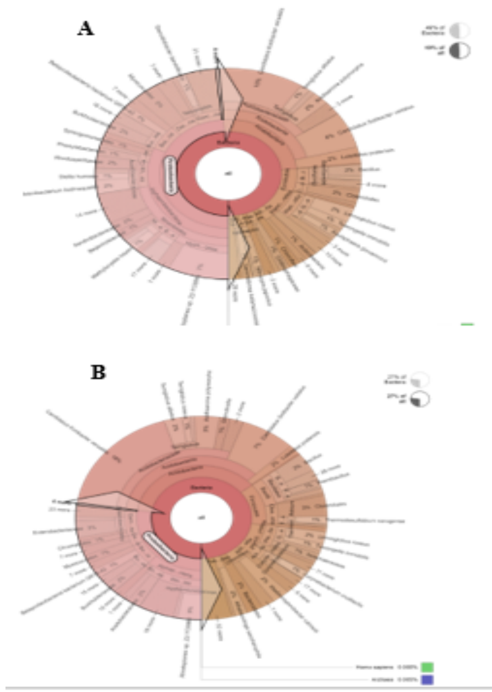


**Figure 6**. One of the results of the krona visualization analysis shows the abundance of Proteobacteria in rubber-canna intercroppingg land (A) and rubber monoculture (B) with abundance marked with black arrow blocks and the percentage value is at the top right of the krona visualization.

**Species Diversity and Abundance**

To find out in more detail the relationship between the abundance, dominance and functional predictions of the Phylum Proteobacteria group in both planting systems, especially at the species level, further analysis was carried out by looking at the soil microbial species whose abundance was highest in both planting systems. Of the top 10 microbial specificities of each land treatment both intercroppingg and monoculture, seven microbial species were found more in the intercropping*g* system fields (Figure 11).

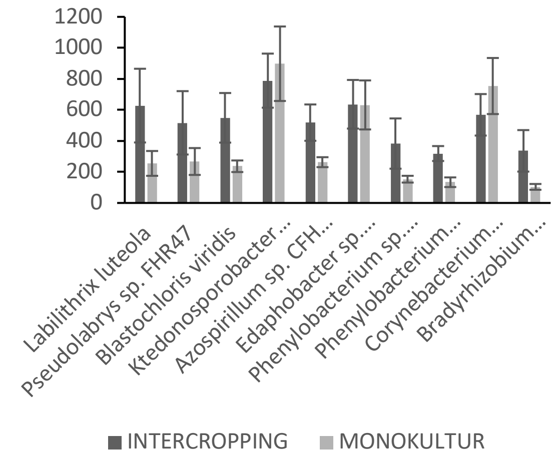


**Figure 7.** The number of species level abundances taken from the top 10 orders of each land treatment both intercroppingg and monoculture.

Interestingly, of the 10 most species found, 7 out of 10 species are from the Phylum Proteobacteria namely *Labilithrix luteola, Pseudolabrys sp., Blastochloris viridis, Azospirillum sp., Phenylobacterium sp., P. zucineum, B. zhanjiangense.* While the rest are like, *K. rubrisoli* from the phylum Chloroflexi, *Edaphobacter sp.* from the phylum Acidobacteria, and *C. crudilactis* from the phylum Actinobacteria (Figure 7).

The presence of bacterial communities in the soil does not entirely have a good impact on soil health, sometimes there are some bacteria that are detrimental as pathogen transmission agents. However, many of them also have an impact on improving soil quality. Like bacteria from the Phylum Proteobacteria some species have been reported to have functional benefits on the soil (Akob *et al*., 2022; Billah *et al*., 2019). Dang et al (2020) in their study that analyzed the impact of the intercropping*g* system on the composition and microbial structure of the rhizosphere on land planted with legume groups, showed an increase and dominance of the composition of the bacterial group of taxa phylum Protobacteria. Interestingly, this increase in the Protobacteria Phylum group was followed by an increase in N content in the soil and in legume plants when compared to fields that were not treated by the intercroppingg method.

In this study, bacterial species from the Phylum Proteobacteria had a higher abundance in the intercroppingg planting pattern, namely *Azosprillum sp.* with an average abundance of 518 ± 174 in intercropping*g* fields while in monoculture lands it was 63 ± 32; *Pseudolabrys sp.* 517 ± 237 in interceroppingg land while in monoculture land it is 267 ± 86; *Phenylobacterium sp*. 383 ± 156 in intercroppingg land while in monoculture land it is 153 ± 21; and *P. zucineum* 319 ± 162 in intercroppingg fields while in monoculture lands it is 134 ± 31.

The difference in the number of species abundance (Figure 7), is influenced by several factors, namely, (1) the availability of organic matter formed by dissolved organic carbon in the soil; (2) *the* intercroppingg system changes the physicochemical properties of the soil such as temperature and humidity and causes the soil environment to be conducive to the microbial activity of the rhizosphere (Wang *et al*., 2021). The characteristics of the rhizosphere microbial community and the activity of soil enzymes are influenced by plants by releasing root exudate. Intercroppingg systems can alter soil microecology as demonstrated by the increased abundance of microbial diversity on agricultural land (Tang *et al*., 2020).

**Soil chemical analysis**

In the soil *enzyme* activity intercroppingg system, the amount of microbes and nutrient content of the soil was found to be higher under the intercroppingg system than under the monoculture. Soil microbes play an important role in the cycle of nitrogen, phosphorus, calcium and other soil nutrients, due to their contribution to soil structure formation, decomposition of organic matter, nitrogen fixation, and removal of toxins (Zhou *et al*., 2011).

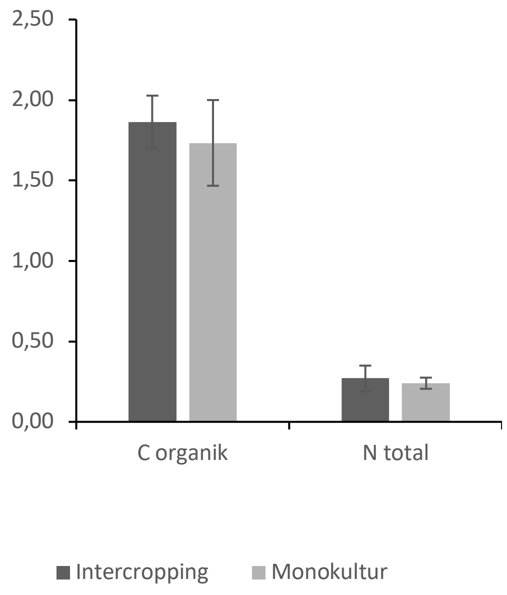


**Figure 8.** Graph of comparison of soil chemical analysis for the presence of organic C and total N in rubber-canna intercroppingg and rubber monoculture (value n = 3). With organic C values (x̄ ± Sd) : 1.86 ± 0.16 in the intercroppingg land system and 1.73 ± 0.08 in in the rubber monoculture system. And the value of (x̄ ± Sd) N total: 0.03 ± 0.27 in the rubber-canna intercroppingg system and 0.2 ± 0.03 in the rubber monoculture land.

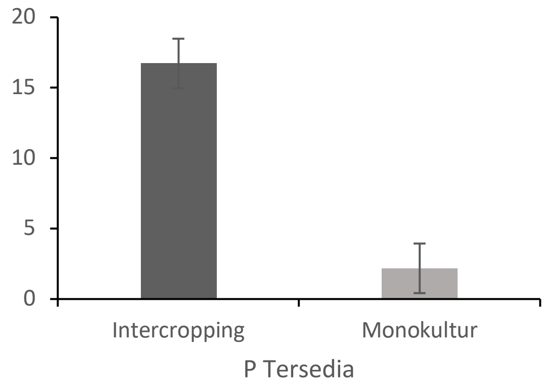


**Figure 9.** Graph of comparison of chemical analysis of P availability in soil on rubber-canna intercroppingg land and rubber monoculture land (n=3). With a value of (x̄ ± Sd) 16.72 ± 11.2 in rubber-canna intercroppingg land and 2.18 ± 1.8 in rubber monoculture land.

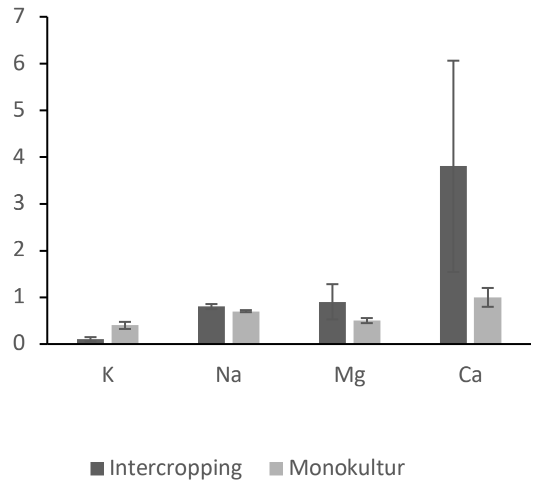


**Figure 10.** Graph of soil chemical analysis of soil cation composition on intercroppingg intercroppingg and monoculture land with a sample repetition value of n=3. With values (x̄ ± Sd), K : 0.1 ± 0.05 intercroppingg rubber-canna fields and 0.4 ±0.08 rubber monoculture fields. Na : 0.08 ± 0.1 rubber-canna intercroppingg land and 0.7 ± 0.02 rubber monoculture land. Mg : 0.9 ± 0.4 intercroppingg land and 0.5 ± 0.1 rubber monoculture land. Ca : 3.8 ± 2.3 rubber-canna intercroppingg land and 1.0 ± 0.2 rubber monoculture land.

Chemical analysis of soils derived from rubber-canna intercroppingg fields had a comparison of the availability of different amounts of P with soils from rubber monocultures (Figure 9). Viewed in interceppingg fields the presence of P detected is very high, ranging ±16.7% compared to soils from monoculture lands ranging ±2.2% (Figure 9). A comparison of the differences in the amount of availability is also seen in the organic C content (Figure 7). In intercroppingg fields, organic C ranges from ±1.86%, while in monoculture lands it is ±1.73%. The total N content differed only ± 0.1%, namely in intercroppingg land ±0.8% and monoculture land ±0.7% (Figure 8). And chemical analysis of cation arrangements in soils showed a large extent higher in rubber-cannabis intercroppingg fields than in rubber monoculture fields (Figure 14).

## GENERAL DISCUSSION

In this study based on the results of metagenomic analysis of the abundance and diversity of soil microbial communities, in terms of the Shannon_E and Chao1 index values (Table 2), it was observed that the diversity of soil microbes in the rubber-cannabis intercroppingg system land was higher in diversity with an average Shannon_E index value of 5.00 ± 0.16 in intercroppingg land compared to an average of 4.78 ± 0.21 in monoculture land (Table 2) ± . The increased diversity of soil microbial communities on intercroppingg lands is thought to be influenced by land pattern systems (Yang *et al.,* 2016). Intercroppingg system land patterns encourage an increase in the population of rhizosphere microbial communities from the secondary metabolism of plant roots (exudate) that stimulate and sort to maintain or eliminate the presence of certain microbial populations (Zhou *et al*., 2022). The existence of soil microbial communities is reported to play a very important role for the nutrient cycle, contributing to C storage and nutrient storage supply (Zhang *et al*., 2017).

Meanwhile, based on the abundance in terms of the Chao1 index value, the abundance value of microbial communities in the rubber-canna intercroppingg field is lower with the average value of abundance being 1567 ± 145.9 compared to 1778.7 ± 224.2 on rubber monoculture land (Table 2). This happens because of the presence of dominant individuals, causing the Chao1 index value to be low (Wu *et al*., 2018).

The low value of the Chao1 index is illustrated by visualization in the presence of dominance of the abundance of bacteria Phylum Proteobacteria. The abundance of proteobacteri bacteria is 22% superior in the rubber-canna intercroppingg system compared to the rubber monoculture system, based on metagenomic analysis of krona visualization (Figure 6).

The abundance of proteobacteria indicates that the soil nutrient condition status of the rubber-canna intercroppingg field is better than that of monoculture land, the difference in the amount of the higher Proteobacteria Phylum abundance results in better nutrient content in intercroppingg land than in *monoculture* land. This is in accordance with a study conducted by Dang *et al*., (2020) which showed that the group of proteobacteria is relatively abundant in soils using intercroppingg systems. Furthermore, Dang et al (2020) explained that in the soil the intercroppingg system is richer in nutrients, including the nitrogen content in the soil increases, thus having an impact on increasing the yield of the main crop on the rubber-canna intercroppingg land. In addition to its presence as an indicator of good nutritional conditions in a field, several functional species of Phylum Proteobacteria are also reported to play a role in improving nutritional conditions in the soil in the proteobacteria habitat (Zhang & Liu, 2020).

From the analysis of the abundance and diversity of the top 10 species units, it is known that 7 of them come from the Proteobacteria phylum (Figure 7). Some of these bacteria have high abundance in intercropping systems and are reported to be related to biogeochemical processes in improving soil quality by creating nutrient-rich soil as an ideal condition for the development and growth of plants and rhizosphere bacterial communities (Akob et al., 2022; Billah et al., 2019; Wu et al., 2018). The bacteria known to play a role in biogeochemical processes in this study include:
(1) Pseudolabrys sp., which has the highest abundance and is ranked second in bacterial abundance (Figure 7), with 517 ± 237 (intercropping of rubber and ganyong) and 267 ± 86 (rubber monoculture), is reported to play a role in secreting naphthol-ASBI-phosphohydrolase, an enzyme needed to dissolve P so that it can be used by plants (Shen et al., 2021). In this study, the results of soil chemical analysis showed a very high P value in the intercropping of rubber and ganyong (Figure 9). It is suspected that the availability of dissolved P in the soil is related to the abundance of Pseudolabrys sp. which plays a role in the P solubilizer enzyme. Fu et al., (2019), which states

that the naphthol-ASBI-phosphohydrolase enzyme affects the shift in soil microbial community structure, thus correlating with increasing the availability of dissolved P in the soil (Fu et al., 2019). The presence of dissolved P in the soil will be absorbed by plants for their development and growth, as well as to suppress plant diseases and produce phytohormones such as Indole Acetic Acid (IAA), Gibberellic Acid (GA), Abscisic Acid (AA), and Salicylic Acid (SA) (Billah et al., 2019). In addition, the availability of P is also reported to be able to increase the absorption of Ca, so its presence also increases in the soil of intercropping of rubber and ganyong (Figure 10) (Li *et al*., 2016).

(2) Azospirillum sp. plays a role as a vibroid cell with a single polar flagellum and is reported to be very important as a plant growth promoter and a provider of N in the soil (Lin *et al*., 2016). The abundance of Azospirillum sp. is 518 ± 174 in the intercropping of rubber and ganyong and 263 ± 32 in the rubber monoculture, causing the total N content to be 0.3% in the intercropping of rubber and ganyong, higher than the total N content of 0.2% in the rubber monoculture (Figure 8).

(3) Bradyrhizobium zhanjiangense is also known to play a role as a nitrogen fixer and denitrifier involved in the N cycle. In addition, it has the Acetylene Hydratase (AH) enzyme which is sensitive to the presence of N, so it can catalyze the reduction of dinitrogen into ammonia (Zhou et al., 2022). The acetylene enzyme is quickly absorbed by the soil to improve soil health and enhance plant growth by providing biologically available N (Akob et al., 2022; Zhang & Liu, 2020).

(4) Phenylobacterium sp. has an abundance of 383 ± 156 in the rubber-ganyong intercropping system and 153 ± 21 in the monoculture land. Phenylobacterium sp. is reported to be related to metabolic pathways, one of which is flavonoid and flavonol which play a role as plant growth promoters, especially in root growth (Khalid et al., 2019), as well as indole alkaloids which are substrates needed to synthesize plant growth hormones, all of which are regulated by Phenylobacterium sp. in the soil that interacts with plant roots (Baumann et al., 2017). The higher abundance of Phenylobacterium sp. in the rubber-ganyong intercropping system causes an increase in the regulation of the interaction between Phenylobacterium sp. bacteria with the surrounding plant roots.

In the intercropping system, soil microbes indirectly stimulate plant growth because they play an important role in increasing fertility and productivity, as well as influencing the physical and chemical properties of the soil (Fahad et al., 2022). The soil chemical content is better in the rubber-ganyong intercropping system compared to monoculture systems. The soil in the rubber-ganyong intercropping system has higher levels of organic C, total N, Ca, Na, Mg, and soluble P, except for K content (Figure 10). Low cation content in K compounds is associated with higher nutrient removal through leaching, erosion, or biomass harvesting and less nutrient return to the soil (Setyastika et al., 2022).

In addition to the K content in the rubber-ganyong intercropping land, other soil nutrients such as organic C, total N, Ca, Na, Mg, and soluble P are better than the nutrient content in monoculture land due to the higher abundance and diversity of functional microbial communities in rubber-ganyong intercropping land (Figure 7). This is in line with Tang et al.'s statement (2019). Soil nutrient content increases along with the increase in diversity and abundance of microbial communities that encourage the improvement of soil nutrients in the intercropping system. Microbial communities are significantly influenced by biogeochemical processes, rhizosphere composition and structure, which can determine or modify the specificity of microbial community diversity and abundance due to the role of plants in producing exudates into the rhizosphere (Xiao et al., 2013).

Intercropping systems have been shown to positively influence soil microbial diversity and function, with the use of a cover crop or intercrop like ganyong in the case of rubber-ganyong intercropping system, altering the soil microbial community through the production of root exudates that invite beneficial microorganisms in the rhizosphere (Effendi et al., 2020). This results in higher abundance and more specific functions of the microbial community compared to monoculture systems. For instance, the abundance of bacterial species such as Azospirillum sp., B. zhanjiangense, Phenylobacterium sp., and Pseudolabrys sp. have been observed to positively impact soil quality by increasing the availability of soluble phosphorus in the soil, improving nutrient availability, acting as biofertilizers, and aiding in the production of plant hormones and growth regulator promoters for plant and root development. Soil microbes can also alter the soil microecology by enhancing nutrient cycling, organic matter decomposition, and suppressing soil pathogens. Therefore, the use of intercropping systems can create a favorable environment for the growth and diversity of soil microbial communities, leading to improved soil quality and agricultural productivity.

## CONCLUSION

Based on the results of the research, it can be concluded that the diversity of soil microbe species in a land is related to soil fertility which causes an increase in nutrient availability in the soil. The results of this study indicate that the intercropping system of rubber and ganyong can create a favorable environment for the growth of diverse soil microbe communities that symbiotically interact with root exudates, altering soil microecology and thus increasing the chemical cycles that occur in the soil. Soil microbe diversity in intercropping land of rubber and ganyong is higher compared to monoculture rubber land, indicated by the average Shannon_E index value of 5.00 ± 0.16 in intercropping land and an average of 4.78 ± 0.21 in monoculture land. Meanwhile, the Chao1 abundance index value in intercropping land of rubber and ganyong is lower with an average abundance value of 1567 ± 145.9 compared to 1778.7 ± 224.2 in monoculture rubber land, caused by the dominance of Proteobacteria Phylum in intercropping land of rubber and ganyong. Four bacterial species of the Proteobacteria Phylum, namely Azospirillum sp, Pseudolabrys sp., Phenylobacterium sp., B. zhanjiangense, which were observed as rhizosphere bacteria, have a high abundance in intercropping land of rubber and ganyong and are functionally suspected to contribute to improving soil quality, including increasing the availability of P nutrients that have an impact on improving soil quality in intercropping land of rubber-ganyong.

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