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The effect of land-management and organic matter inputs on bacterial population and soil nutrients across different types of agroforestry system

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Abstract. *Rinady MVP, Nuraini Y, Prayogo C, Arfarita N. 2023. The effect of land-management and organic matter inputs on bacterial population and soil nutrients across different types of agroforestry system. Biodiversitas 24: 1333-1345.* Different management of agroforestry systems changes crop composition and growth. This will affect organic matter inputs that become the source of food and energy for soil microorganisms. The soil organisms themself play an important role in amplifying soil biogeochemical processes and regulating soil reactions. This process allows the nutrients to be released into the soil and absorbed by the crop. This study aimed to examine the abundance of soil bacterial population and the changes in soil chemical properties (soil pH, total soil organic carbon (C), and total soil N (Nitrogen) under different land management and organic matter inputs. The determination of total organic C and total soil N was used by Walkey and Black and Kjedahl methods. Total Plate Count (TPC) techniques were employed to measure Total Bacteria Population (TBP) and Total Cellulolytic Bacteria (TCB). The Randomized Complete Block Design was used along with the 5% Tukey's test to examine the significant effect of the treatments. These treatments consisted of various agroforestry system as follow: (i) pine-coffee agroforestry system (PK), (ii) pine-banana agroforestry system (PPs), (iii) pine-cardamom agroforestry system (PR), (iv) pine-vegetable agroforestry system (PS), (v) mixed garden (KC), and (vi) citrus (LJ). Each treatment was repeated four times to obtain 24 experimental plots using a size of 20 m x 20 m. The results showed that the highest organic C and N content was obtained at the PK (agroforestry of pine at 41 years and coffee at 11 years old) plot, which was about 6.64% and 0.56%. Those parameters strongly correspond to the greatest soil bacteria population and soil cellulolytic bacteria population at the value of 1,71 x 10^5 CFU/g and 4,24 x 10^4 CFU/g, resulting from the greatest quantity of in-situ litter accumulation at PK plot to reach about 201.35 g. The dry weight value of litter in situ at the PK plot is greater than that of the PS and LJ plots by 81% and 87%. A power equation followed the relationship between soil organic C and the total bacteria population. A similar trend has been observed between total soil Nitrogen and total bacteria population. We concluded that changes in different management could affect soil chemical conditions and the changes in the total population of soil bacteria and cellulolytic soil bacteria.

Keywords: Agroforestry, land use change, organic matter inputs, soil bacteria

Abbreviations: CMC: Carboxy Methyl Cellulose, TBP: Total Bacteria Population, TCB: Total Cellulolytic Bacteria, TPC: Total Place Count

INTRODUCTION

Agroforestry ecosystems have been recognized to accumulate high levels of organic matter (Garratt et al. 2018), including greater litter production at the soil surface (Muchane et al. 2020). Litter is one of the major organic materials closely linked to biogeochemical processes to incorporate carbon and other plant nutrients into the soil (Bradford et al. 2016). The decomposition of plant litter affects the microorganisms' population and activities since carbon is becoming the major source of energy (Osman 2013). The quantity and quality of litter determine the rate of decomposition processes (Rahmadaniarti and Mofu 2020). Factors influencing the litter decomposition rate are soil properties and environmental conditions such as soil temperature and moisture (Schneider et al. 2012). The soil environmental conditions were also impacted by different land management practices, which then those activities modify the accumulation of litter surface (Giweta 2020).

Signors et al. (2018) examined the changes in soil nutrients (C, N, P, K, etc.) across different land use and management. Higher nutrient accumulation was detected under native vegetation than in pasture, annual crops, fruit trees, and horticulture (Signors et al. 2018). Vegetation types and land management are important factors in ecosystem sustainability to secure nutrient availability (Soleimani et al. 2019). The decomposition of plant residues is governed by microbial activity (Thoms and Gleixner 2013), and several available substrates are provided by different plantations for microbial growth, thereby providing different soil microbial functions (Jagadamma et al. 2014). The plant composition changes could affect microclimatic soil conditions (Gutiérrez and Vivoni 2013; Ivanov et al. 2018), such as soil temperature, air temperature, and soil moisture (Berame et al. 2021). Those soil conditions then altered the microbial population and their activities (Chapin III et al. 2016) due to the increasing tree leaves deposits and decomposing fine roots. Unfortunately, research on the effect of different agroforestry systems on soil microorganism population and soil nutrient release is very limited, especially in tropical ecosystems. The agroforestry system is a system that can be considered as an alternative that helps prevent land degradation through a continuous production of high litter production which could maintain a high population of soil microorganisms (Parra et al. 2017).

Radhakrishnan and Varadharajan (2016) mentioned that diverse organic matter inputs are expected to increase microbial community and population. The results show that the bacteria population (64%) is more dominant than fungi (13%) and actinomycetes (23%) in agroforestry systems than others. The addition of litter could modify the availability of soil C, and N. Litter production is influenced by vegetation composition, environmental condition (humidity, temperature), and internal composition of litter (lignin, polyphenol, and soil properties (i.e., soil pH and nutrients). Changes in soil chemical properties have been recognized as changes in fungal and bacterial communities and populations (Deng et al. 2016). Microbes in agroforestry play many roles in crop growth and development: optimizing nutrient regeneration, soil biogeochemical processes, litter decomposition, nitrogen-fixing, and dissolving available phosphorus. Microbial resilience was to be higher in soil agroforestry systems compared to conventional farming systems (Rivest et al. 2013). The soil microorganisms that play a role in decomposing soil organic matter are cellulolytic bacteria—for example, their cellulase enzyme activity. Organic matter containing lignin and cellulose becomes the main substrate for improving cellulolytic bacteria growth. Cellulolytic bacteria have the potential to decompose lignin as one of the components, which is often made difficult to be degraded by other microbes. Cellulolytic bacteria can be found in agricultural land, forests, composting material, decaying plants, or leaf litter (Woo et al. 2013). The ratio of cellulolytic bacteria to the total bacteria population is an important indicator of soil health regarding their contribution to litter decomposition. However, the cellulolytic bacteria dynamic compared to the total bacteria population in soil under different agroforestry systems has never been in a detailed report; for example, in tropical ecosystems.

Various land uses with different types of management may affect the cellulolytic bacteria population and dynamic since each management produces different quantities and quality of organic matter inputs. Based on this, given the importance of the role of soil bacteria involved in organic matter decomposition and soil nutrient availability, research on the effect of different management and organic inputs is important. Therefore, this research was conducted to address the sustainability of agroforestry systems in terms of soil microbe population and nutrients, which may affect the increasing crop production.

MATERIALS AND METHODS

Study area

Research activities were carried out from February to June 2022 at Forest Area with Special Purposes of Brawijaya University (KHDTK-UB Forest), located at Sumbersari Hamlet, Tawangargo Village, Karangploso Sub-district, Malang District, East Java Province, Indonesia. The research was conducted in various plots, which were differentiated based on their land use. Analysis of soil pH, C-organic, total N, lignin content, polyphenols, and C/N ratio was conducted at the Chemical Laboratory, Department of Soil, Faculty of Agriculture, Brawijaya University, Malang, Indonesia. In addition, samples for the total bacteria population and cellulolytic bacteria identification were collected at the Microbiology Laboratory, The University of Islam Malang (Figure 1).

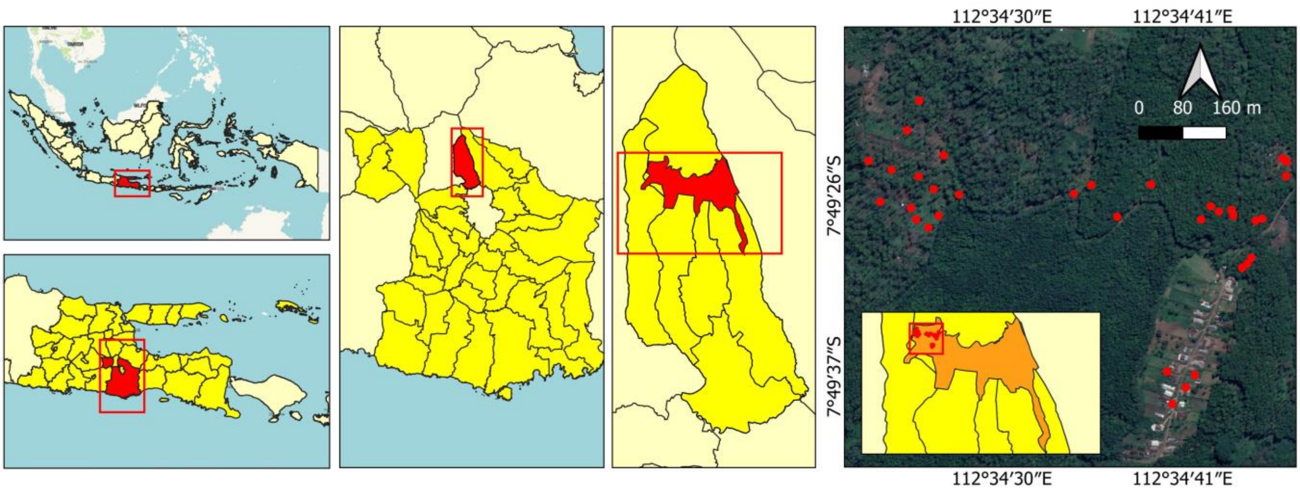

Figure 1. Location of Plot Point in UB Forest, Malang, Indonesia indicating the sampling sites

Table 1. Selected research plot

Plot code	Plot characteristics	Note
PK	Pines and coffee	Pines = 41 years old
PPs	Pines and banana	Coffee = 11 years old
PR	Pines and cardamon	
PS	Pines and vegetables (cabbage)	
KC	Mixed garden	
LJ	Citrus	

Experimental design

The research plot was determined through a preliminary survey. This is because the research was carried out on plants that had grown and had previously been producing. The survey method was carried out to determine the soil's research location and sampling point. The research design was a Randomized Complete Blok Design (RCBD) with six different land use types; (i) pine-coffee agroforestry system (PK), (ii) pine-banana agroforestry system (PPs), (iii) pine-cardamom agroforestry system (PR), (iv) pine-vegetable agroforestry system (PS), (v) mixed garden (KC) and (vi) citrus (LJ). The research plot measures 20 m x 20 m. The research plot is shown in table 1.

Soil sampling and analysis

Soil collection was carried out in a composite method, conducted at five different sampling points (1 was positioned in the center and four at diagonal points), at the area located between the tree stand (pine and coffee), collected from the soil at a depth of 0-20 cm. Soil samples were taken as much as 100 g at each sampling point to obtain 500 g of fresh soil from each plot. Soil samples for biological tests (total soil and cellulolytic bacteria) were collected in a cool box to maintain low-temperature conditions. This could guarantee that soil bacteria did not die before being stored in a fridge at a temperature of 4°C. Soil samples for chemical tests (soil pH, soil C-organic, total soil Nitrogen) were prepared by drying the soil sample at room temperature before grinding and sieving. The Walkey and Black Method (1934) was used to determine soil C-organic, while for examining total soil N measurements was determined through digestion, distillation, and titration step, following the procedures of the Kjeldahl method (1883). Soil pH measurements were carried out using H_2O as an extractor solution (1:1 soil: H_2O ratio) using a glass electrode of a pH meter (Ojo et al. 2015).

Litter sampling and analysis

Litter sampling was conducted in situ to determine the average amount of litter available in the field. The collection used a 50 cm x 50 cm litter metal frame. The litter samples taken are then separated into different classes of tree parts, such as twigs, leaves, and litter. Next, the dry weight of the litter was calculated by weighing the wet weight of the litter sample taken using balance. The fresh litter samples were then put into envelopes to obtain dry litter weight by placing them in the oven for 2 x 24 hours at 80°C. The Goering and Van Soest methods (1970) were used for litter lignin determination, and the Folin Denis method was used for polyphenol assessment (King and Heath 1967).

Soil microbes population analysis

The total cellulolytic bacteria population was measured using the Total Plate Count (TPC) method. Selective media for cellulolytic bacteria, namely CMC (Carboxy Methyl Cellulose), was used. CMC (Carboxy Methyl Cellulose) selective media was prepared by dissolving various ingredients, including 15 g agar, 10 g CMC, 0.02 g $FeSO_4$, 0.75 KNO_3, 0.5 g K_2HPO_4, 0.2 g $MgSO_4$, 7 H_2O, 1 g of glucose and 2 grams of Yeast Extract. These ingredients are then dissolved in 1 liter of sterile distilled water and heated to boiling. After the media has been prepared, it is sterilized in an autoclave. All the ingredients for the CMC selective media above are mixed and waited until warm, then platted in a petri dish of approximately 15-20 mL. The soil sample to be tested was taken as much as 5 grams and put into 45 mL of sterile distilled water. After that, the media was incubated and homogenized for 30 minutes in the incubator. Dilution series were made up to 10-2 and 10-3, pipette 100 µL of suspension from each level of dilution, then inoculated in CMC media that had been provided using the spread plate method and incubated for 2 to 5 days at room temperature. Incubation was carried out for 2 to 5 days at 37°C; the bacterial colonies were counted manually with a hand counter. Calculation of bacterial colonies is then calculated using the formula:

Total population of dry soil bacteria (CFU/g) = (number of colonies x fp)/(soil dry weight)

Where:
fp = Dilution factor on colonized Petri dish
Soil dry weight (g) = fresh weight x (1-water content)

Data analysis

Data analysis was performed using an Analysis of Variance (ANOVA) table with a level of 5%. If the data that has been processed shows significant results, then the analysis is continued using the Tukey test (5%) to determine the differences between treatments. Furthermore, correlation and regression tests are used to determine the closeness of the relationship between the observed parameters.

RESULTS AND DISCUSSION

Soil chemical properties of various land uses

The results obtained after analysis using ANOVA related to soil chemical properties in soil pH, soil C-organic, total soil nitrogen, and C/N ratio of soil in various land uses are presented in table 2. Changes in land use could affect soil organic C, total soil nitrogen, and soil C/N ratio.

Table 2. Soil chemical properties of various land use

Land uses	Soil chemical properties			
	Soil pH	Soil organic carbon (%)	Total soil nitrogen (%)	C/N
PK	5.4 a	6.64 b	0.56 bc	11.97 c
PPs	4.9 a	5.14 b	0.45 b	11.44 bc
PS	4.7 a	5.10 b	0.58 bc	8.88 a
PR	5.3 a	5.36 b	0.51 bc	10.44 abc
KC	4.9 a	6.41 b	0.61 c	10.64 abc
LJ	5.4 a	2.65 a	0.29 a	9.2 ab

Note: Numbers followed by different letters in the same column are significantly different at Tukey's 5% test. Land use type: PK (pine at 41 years and coffee at 11 years), PPs (pine at 41 years and banana at 1-2 years), PS (pine at 41 years and cabbage at 1-3 months), PR (pine at 41 years and cardamon at 1 year), KC (mixed garden: pine at 41 years, coffee at 3-4 years, avocado at 1 year, citrus at 1 year and mustard at 1-3 months) and LJ (citrus at 1 year).

Soil pH

The ANOVA analysis showed that land use changes did not significantly affect soil pH (P>0.05). The highest soil pH value was found in the PK plot but was not significantly different from the other plots, while the PR plot had the lowest soil pH value and was not significantly different from the other plots. Soil pH values in the PK, PPs, PS, PR, KC, and LJ plots were 5.4; 4.9; 4.7; 5.3; 4.9; and 5,4, respectively (Table 2).

Soil organic carbon

The results of the ANOVA analysis showed that changes in land use significantly affect the C-organic content of the soil (P<0.01). The PK plot (pine aged 41 years and coffee aged 11 years) was not significantly different (P<0.05) from the PPs, PS, PR, and KC plots, but the five plots were significantly different from the LJ plot (oranges 1-year-old). The highest soil C-organic content was found in the PK plot at 6.64%, while the LJ plot had the lowest organic C content of 2.65% (Table 2). The C-organic content of the soil in the PK plots decreased significantly by 60.1% compared to the LJ plots. The C-organic content in each field has a different value; this shows that land cover and organic matter input affect the amount of carbon in the soil.

Total soil nitrogen

The results of the ANOVA indicated a significant effect of changes in land use on total nitrogen in the soil (P<0.01). Soil nitrogen content in KC plots (mixed garden: pine aged 41 years, coffee aged 3-4 years, avocado aged 1 year, orange aged 1 year, and mustard aged 1-3 months) had the highest nitrogen content and was significantly different from the other plots, while the LJ plot (1-year oranges) had the lowest nitrogen content. The total soil nitrogen content in the KC plot was 0.61%, while in the LJ plot, it was 0.29%. There was a decrease in total soil nitrogen in the KC plot compared to the LJ plot by 52.45% (Table 2). Changes in land cover and management practices carried out by farmers cause the total soil nitrogen value to differ for each land use. The existence of organic and inorganic fertilizers on KC plots can increase the total nitrogen in the soil.

Soil biology properties of various land uses

Population of soil bacteria and soil cellulolytic bacteria

The results of the ANOVA analysis showed that changes in land use on the population of soil bacteria in June 2022 had a significant effect (P<0.01). In the first sample in June, the soil and cellulolytic bacteria populations in the PK plot (pine aged 41 years and coffee aged 11 years) had the highest values . They were significantly different from the other plots but not significantly different from the KC plot (mixed garden: pine aged 41 years, coffee aged 3-4 years, avocado aged 1 year, oranges aged 1 year, and mustard aged 1-3 months), while the LJ plot (citrus plants aged 1 year) had the lowest value and was significantly different from PK and KC plots but not significantly different from the PPs, PS and PR plots.

The results of the ANOVA analysis showed that changes in land use on the soil and cellulolytic bacteria population in July 2022 had a very significant effect (P<0.01). In the second sample of July, the population of soil bacteria in the PK plot (pine aged 41 years and coffee aged 11 years) had the highest value. It was significantly different from the other plots, while the LJ plot (citrus plant aged 1 year) had the lowest value and was significantly different from the PK plot. In the first sample in June, the highest overall bacterial population and cellulolytic bacteria at 10^{-2} dilution were found in the PK plot of 1.71 x 10^5 CFU/g and 4.24 x 10^4 CFU/g, respectively. While the populations of soil bacteria and cellulolytic bacteria, the lowest in June, were found in the LJ plot of 0.125 x 10^5 CFU/g and 1.35 x 10^4 CFU/g, respectively (Table 3). In the second sample in July, the highest soil and cellulolytic bacteria population at 10^{-2} dilution were found in the PK plot, respectively 1.81 x 10^5 CFU/g and 3.96 x 10^4 CFU/g. While the populations of soil bacteria and cellulolytic bacteria, the lowest in July, were found in the LJ plot of 0.125 x 10^5 CFU/g and 1.43 x 10^4 CFU/g, respectively (Table 3). The different populations of soil bacteria in each land use are influenced by organic matter input and the quality of the C/N ratio of soil and litter on the land. Soil organic matter, soil C/N ratio, and litter quality affect soil bacterial populations.

In situ litter fresh and dry weight

Measurement of the wet weight and dry weight of litter in situ was measured to know the average amount of litter on the land as the main source of organic matter, improving the soil's physical, chemical, and biological properties and providing nutrients. The results of the ANOVA analysis showed that changes in land use on the average wet weight and dry weight in situ had a very significant effect (P<0.01).

Table 3. The population of soil bacteria and cellulolytic bacteria in June and July 2022

Plot	Soil bacteria population on June 2022 (10^5 CFU/g)	Soil cellulolytic bacteria on June 2022 (10^4 CFU/g)	Soil bacteria population on July 2022 (10^5 CFU/g)	Soil cellulolytic bacteria on July 2022 (10^4 CFU/g)
PK	1.71 b	4.24 c	1.81 d	3.96 b
PPs	0.205 a	1.58 ab	0.238 ab	1.70 a
PS	0.271 a	1.33 a	0.183 ab	1.60 a
PR	0.339 a	2.39 ab	0.271 b	2.03 a
KC	1.57 b	2.90 bc	1.38 c	2.60 ab
LJ	0.125 a	1.35 a	0.125 a	1.43 a

Note: Numbers followed by different letters in the same column are significantly different at Tukey's 5% test. Land use type: PK (pine at 41 years and coffee at 11 years), PPs (pine at 41 years and banana at 1-2 years), PS (pine at 41 years and cabbage at 1-3 months), PR (pine at 41 years and cardamon at 1 year), KC (mixed garden: pine at 41 years, coffee at 3-4 years, avocado at 1 year, citrus at 1 year and mustard at 1-3 months) and LJ (citrus at 1 year).

The average wet weight of litter in situ in the PK plot (pine aged 41 years and coffee aged 11 years) has the highest value. It is significantly different from the other plots (P>0.05), while the LJ plot (citrus plants aged 1 year) has the lowest value and is significantly different from the other plots. However, the dry litter weight in situ in the LJ plot was not significantly different from the PS plot. The values for wet weight and dry weight of litter in situ in the PK plot were 439.8 g and 201.35 g, respectively, while the mean wet weight and dry weight of litter in situ were found in the LJ plot at about 36.6 g and 25.25 g (Figure 2). The average wet weight value of litter, which shows different results for each plot, indicates the influence of different land cover and the level of litter input in a field. The dry weight value of litter in situ in the PK plot is greater than that of the PS and LJ plots by 81 and 87%. The existence of different land cover affects the input of litter on the land, so the average value of the dry weight of litter in situ in each plot is different.

Litter quality (C/N ratio, lignin and polyphenols)

Table 4 shows that each land cover has a different value of C/N ratio, lignin, and polyphenols of litter. The following results from a laboratory analysis of the C/N ratio of litter for each land cover contained in several plant leaf litters, including coffee, banana, cabbage, cardamom, mixed leaves, citrus, and pine in several selected lands in the UB Forest area. The results of the litter analysis showed that pine had the highest C/N ratio and was significantly different compared to other plant leaf litter, which was 32.95. The leaves of the cabbage plant had the lowest C/N ratio and were significantly different from those of other plants, which was about 9.25. The results of the ANOVA analysis showed that the difference in litter quality to lignin had a very significant effect (P<0.01). The results of the litter analysis showed that pine had the highest lignin and was significantly different compared to other plant leaf litter, namely 45.28. The leaves of the cabbage plant had the lowest lignin and were significantly different from those of other plants, around 4.33. Also, the results of the ANOVA analysis showed that the difference in litter quality to polyphenols had a very significant effect (P<0.01). The results of the litter analysis showed that citrus had the highest polyphenols and was significantly different compared to other plant leaf litter, which was around 7.73%. The leaves of the cardamon plant had the lowest polyphenols, at 0.37%.

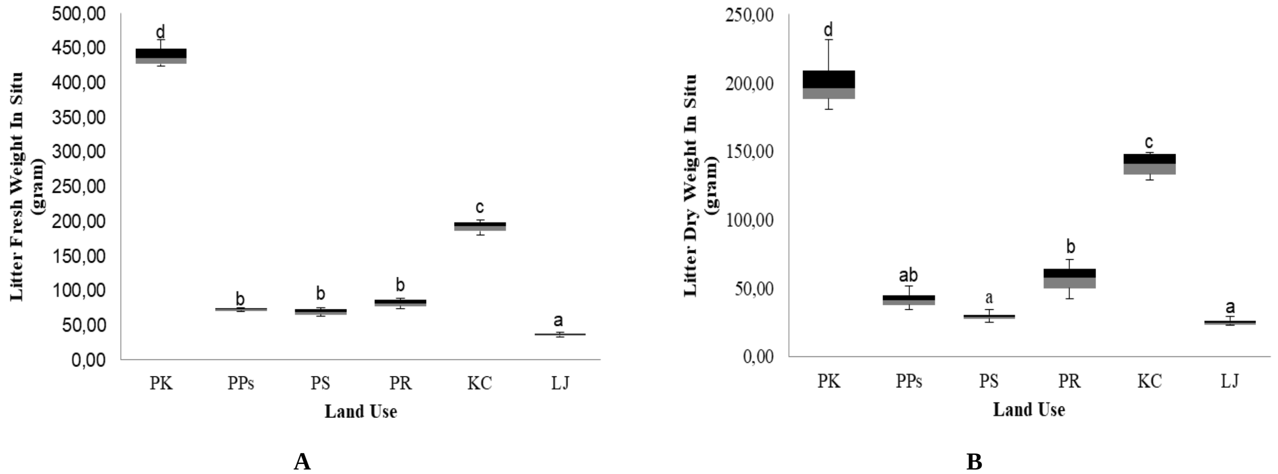

Figure 2. The average wet (A) and dry weight (B) of litter in situ on different land uses. Note: Numbers followed by different letters in the same column are significantly different at Tukey's 5% test. Land use type: PK (pine at 41 years and coffee at 11 years), PPs (pine at 41 years and banana at 1-2 years), PS (pine at 41 years and cabbage at 1-3 months), PR (pine at 41 years and cardamon at 1 year), KC (mixed garden: pine at 41 years, coffee at 3-4 years, avocado at 1 year, citrus at 1 year and mustard at 1-3 months) and LJ (citrus at 1 year)

Table 4. Value of C/N ratio on leaves of coffee, banana, cabbage, cardamom, mixed leaves, citrus and pine leaves

Leaves	C/N ratio	Lignin (%)	Polyphenols (%)
Coffee	15,66 bc	33.66 c	0.82 a
Banana	22,51 de	16.37 b	0.61 a
Cabbage	9,25 a	4.33 a	0.81 a
Cardamon	26,36 e	9.98 ab	0.37 a
Mixed litter	12,95 ab	14.31 ab	3.65 b
Citrus	20,47 cd	16.79 b	7.73 d
Pines	32,95 f	45.28 d	6,09 c

Note: Numbers followed by different letters in the same column are significantly different at Tukey's 5% test. Litter is classified as high quality if it has a C/N ratio < 25, lignin content < 15%, and polyphenols < 4% (Rahmadaniarti and Mofu 2020).

Figure 3 shows the regression results between the dry weight of litter in situ and the C-organic content of the soil are written with the following equation: y = 0.0149x + 3.98989, where x is the average dry weight of litter in situ and y is the C-organic content soil. The coefficient of determination (R^2) obtained was 0.48.

The results showed that the C-organic content of the soil had a positive correlation with total N, with an r-value of about 0.87. Figure 4 shows that the regression results between soil C-organic content and total soil N are written with the following equation: y = 0.0719x + 0.1187, where x is soil C-organic content and y is soil total N. The results of the correlation analysis showed that the soil C-organic content had a positive and strongest relationship with total soil nitrogen (R^2 = 0.76).

The carbon content in the soil affects the soil bacterial population. The results showed that the soil's C-organic content had a positive correlation with the population of soil bacteria in June, with an r-value of about 0.64, and in July, the r-value was 0.67. Figure 5 shows that the regression results between soil C-organic content and the bacterial population in June 2022 are written with the following equation: $y = 0.0149\ x^{2.0814}$, where x is the soil C-organic content and y is the first sample of the bacterial population in June 2022. The results of the correlation analysis showed that the soil C-organic content had a positive and strongest relationship with the bacterial population in June 2022 (R^2 = 0,47). Figure 5 shows the regression results between soil C-organic content and the bacterial population in July 2022 are written with the following equation: $y = 0.0217x^{2,1023}$, where x is the soil C-organic content and y is the second sample of the bacterial population in July 2022. The results of the correlation analysis showed that the soil C-organic content had a positive and strongest relationship with the bacterial population in July 2022 (R^2 = 0.49). The highest populations of soil bacteria in June and July were found in PK plots, respectively, which were 1.71 x 10^5 CFU/g and 1.81 x 10^5 CFU/g with a soil organic C content of 6.64%.

The results showed that total soil nitrogen positively correlated with soil bacterial populations in June and July 2022, with r-value = 0.45 and 0.48, respectively. Figure 6 shows that the regression results between total soil nitrogen and bacterial population in June 2022 are written with the following equation: $y = 1.9508x^{2.1043}$, where x is total soil nitrogen and y is the first sample of the bacterial population in June 2022. The results of the correlation analysis showed that the soil C-organic content had a positive and strongest relationship with the bacterial population in June 2022 (R^2 = 0.35).

Figure 6 shows that the regression results between total soil nitrogen and the bacterial population in July 2022 are written with the following equation: $y = 1.7163x^{2.0156}$, where x is total soil nitrogen and y is the second sample of bacterial populations in July 2022. The results of the correlation analysis showed that the soil C-organic content had a positive and strongest relationship with the bacterial population in July 2022 (R^2 = 0,32).

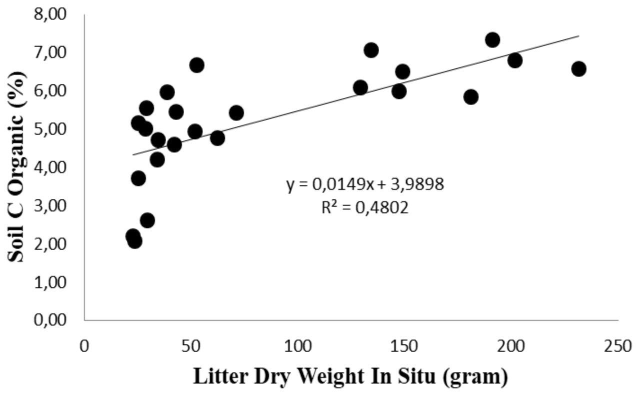

Figure 3. Relationship between litter dry weight in situ and soil C-organic content

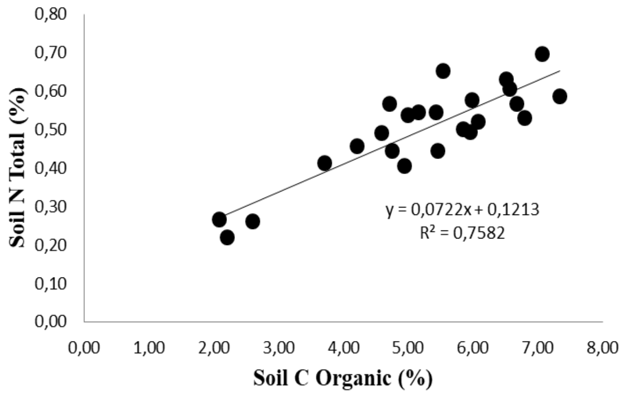

Figure 4. Relationship between soil C-organic content and soil total N

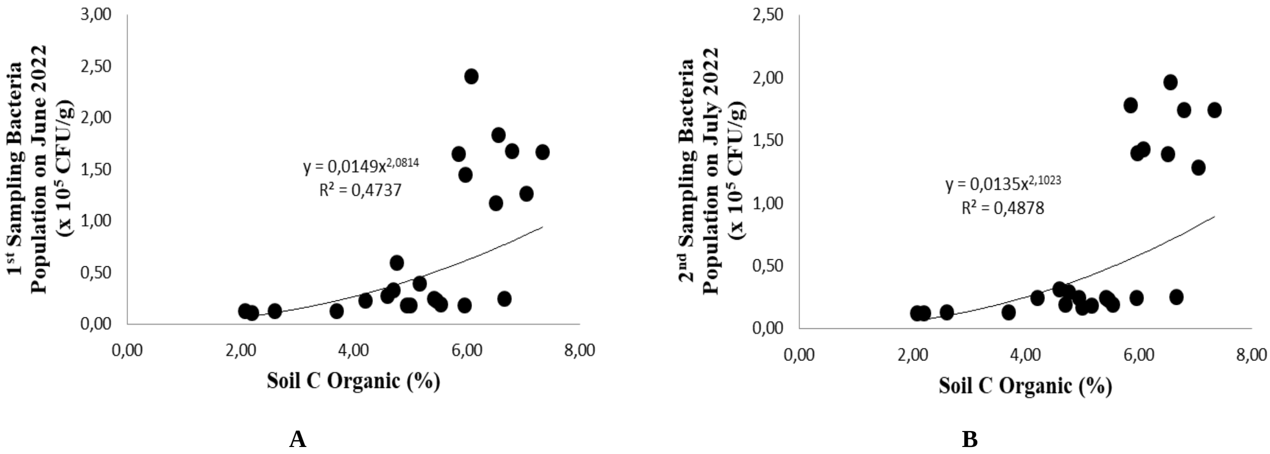

Figure 5. Relationship between soil C-organic content and soil bacterial population: A. June 2022, B. July 2022

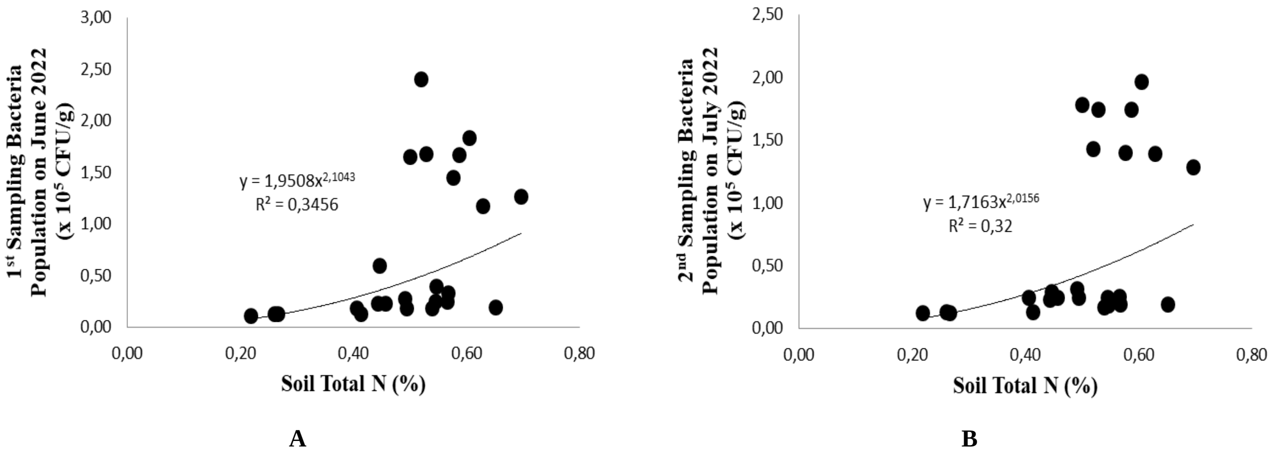

Figure 6. Relationship between soil total N content and soil bacterial population: A. June 2022, B. July 2022

Discussion

Soil chemical properties of various land uses

Changes in land use that cause changes in land cover affect litter input. Soil organic carbon can be influenced by various factors such as weather and climate, parent material and texture, soil pH, vegetation, and land use (Ali et al. 2017). The results showed that the PK plot (pine aged 41 years and coffee aged 11 years) had a soil C-organic content of 6.64%, while the LJ plot, namely forest land converted to citrus cultivation, had a relatively low C-organic content, i.e., 2.65%. The change in the land has decreased significantly by 60.1%. Ghimire et al. (2018) explained that soil on forest land has higher soil organic carbon than agricultural land and degraded land. Agricultural land is highly disturbed by tillage and excessive use of chemical fertilizers, while forest land is less disturbed by adequate vegetation cover. Conventional farming systems can reduce soil organic carbon. Forest soil organic carbon stocks are associated with higher organic inputs from fallen litter and lower soil disturbance than croplands. Soil pH in tropical forests is in acidic conditions for the most part at the level of 4.5 to 5.5. Soil pH affects all biological, chemical, and physical soil properties, the growth of certain organisms, soil microbial biomass, and microbial activity. Several factors affect the overall soil pH, such as parent material, living organisms, climate, time of day, topography, plant growth, native vegetation, rainfall, organic matter, temperature, and human activities (Mohd et al. 2014).

The PK plot has a high C-organic soil value. It is significantly different from the LJ plot because it has a land management system that allows fallen litter to remain on the soil surface, and there is no tillage, so litter decomposition takes place naturally with the help of soil microorganisms. While in LJ plots with land conditions that are not dense and the presence of tillage resulting in low carbon content in the soil. In this plot, the input of organic matter from fallen litter is low compared to other plots. Planting citrus plants in monoculture can reduce litter input to the land. Land use and vegetation type, influence soil erosion and C dynamics through their effect on soil organic carbon content (Ghimire et al. 2018).

Latifah et al. (2022) found a strong positive correlation between litter biomass and SOC (R=0.88) at a significance level of 0.05. This study shows that plant leaf litter is important in maintaining SOC. The plant leaf litter biomass in this agroforestry location (2 tons/ha) was produced between the monoculture sites (1.2 tons/ha) and the secondary forest locations (4.48 tons/ha). Setyastika et al. (2022) stated that agroforestry land had a relatively high organic C content, although it was not significantly different from 5-year-old cassava, especially in the 0-20 cm soil layer. Planting is carried out continuously every year by removing stubble, biomass, or burning and accelerated by tillage which often results in loss of organic matter. Intensive tillage activities cause the loss of nutrients and SOC to produce annual crops (Chen et al. 2019). Soil organic carbon is considered a key indicator of soil health, a universal proxy for various ecosystem services, and an important driver of agricultural sustainability (Lal 2015; Palmer et al. 2017; Rutgers et al. 2012). Increasing plant litter in agroforestry systems can increase soil organic C status (Rodrigues et al. 2015). Prayogo et al. (2021) that C-organic soil is a food source for soil microorganisms, so the activity of microorganisms will be affected by the presence of C-organic in the soil. The contribution of coffee and shade trees to carbon stocks in agroforestry systems varies greatly over time due to differences in land use and farmer practices (Prayogo et al. 2018; Suprayogo et al. 2020).

Changes in land cover or land use (for example, from forest to agricultural land) can change vegetation, remove biomass and disturb the soil, resulting in loss of soil carbon and other nutrients, changes in soil properties and biodiversity, both above and below ground changes (Smith et al. 2016). Kurniawati et al. (2019) explained that providing a carbon source through soil organic matter would increase the role of functional microbes in the soil. Several factors exponentially affect the optimal environment to increase microbial growth and reproduction, including humidity, oxygen availability, temperature, C/N ratio, and soil pH. Nutrients are important for plant growth in addition to the element carbon (C), namely nitrogen (N). The results showed that KC plots (mixed garden: pine aged 41 years, coffee aged 3-4 years, avocado aged 1 year, orange aged 1 year, and mustard greens aged 1-3 months) had the highest nitrogen content in the soil and was significantly different from the others with 0.61%, while the LJ plot (1-year oranges) had the lowest nitrogen content and was significantly different from the other plots of 0.29%. Farmers' land management and tillage practices, applying organic and inorganic fertilizers, cause the nitrogen content in the soil in each plot to have different values. Kurniawan et al. (2019) that several factors affect the nitrogen content in the soil, including the decomposition of organic matter, rainfall, and management practices carried out by farmers such as fertilization. Wubie and Assen (2020) say that agroforestry has a higher soil total N content than cassava cultivation. Continuous deforestation and planting could cause the soil's organic matter content and total nitrogen (N) to decrease. The research results by Setyastika et al. (2022) showed that the total N content at 10 or 15 years of cassava cultivation was lower compared to 5 years of age; this indicated that continuous cassava cultivation tended to reduce the total N. Intensive land management with a continuous cropping system can deplete soil organic matter and total N (Tanimu et al. 2013). This occurs due to the removal of soil nitrogen during cassava harvesting, which is 1.51 kg N ha^{-1} $harvest^{-1}$ (Sumithra et al. 2013) to 1.71 kg N ha^{-1} $harvest^{-1}$. Agroforestry contributes to greater soil inorganic N availability than treeless agriculture. N availability can largely control net primary production, management, and natural ecosystem functions. Soil acidity caused by parent material and soil acidification is recognized as an important limitation of agriculture (Muchane et al. 2020).

The KC plot has the highest nitrogen content in the soil compared to other plots because this land has the practice of managing land by applying organic fertilizers, namely chicken manure and inorganic fertilizers in the form of pearl urea and *phonska* (NPK) fertilizers. The nitrogen content in the soil in the PK plot was lower compared to the KC plot, which was 0.56%, because there was no land management in the PK plot, such as applying fertilizer optimally as in the KC plot. Different land uses lead to different cultivation systems depending on the type and amount of vegetation that grows and their relation to what is given to the soil. The addition of nitrogen sources to the soil can be done through the applying inorganic and organic fertilizers, such as chicken manure. The availability of N in the soil is affected by the soil pH value. Nitrogen becomes a nutrient available to plants as nitrate when the soil pH increases to 5.5. The low pH in each plot can be caused by inaccurate land management, such as the amount of weeds and grass allowed to grow on the land and the decomposition process of organic matter (Wibowo and Kasno 2021). In this study, the PK plot has the highest soil pH compared to other plots. Soil pH in agroforestry was higher than in cassava cultivation for 5, 10, and 15 years. This happens because of the continuous cultivation of seasonal crops, which causes soil acidification (Tanimu et al. 2013). FAO and ITPS (2015) state that soil acidity caused by parent material and soil acidification is recognized as an important limitation of intensive agriculture. Soil acidity can lead to nutrient deficiencies and toxicity, negatively impacting beneficial microorganism activity, organic matter decomposition, nutrient mineralization, and plant uptake. Living organisms are affected by environmental conditions such as geochemical (pH and salinity) and physical (temperature, pressure, and radiation) factors. Both factors suppress microorganisms' diversity (Soares et al. 2012).

Soil bacteria population

Coffee leaves have a C/N ratio of 15.66. The use of vegetation with high decomposability properties (such as low C/N (19.71), low lignin concentration (4.18%), and low lignin/N (1.97)) is beneficial for increasing N release so that it has the potential to increase mineral availability N for the next crop regardless of farm management. However, agricultural management significantly impacts the availability of N minerals in the soil, which is greater because it is managed organically than conventionally managed soils. This can be attributed to N mineralization from soil organic matter and litter input (Garcia et al. 2021). Following the results of Prayogo et al. (2021) that the total bacterial population in the PK3 (Pine 35 years + coffee 4-5 years) plot is 10.1 x 10^6 CFU mL-1, while the lowest total bacterial population detected in the PS plot is around 1.4 x 10^6 CFU mL-1 which is only 10% compared to soil aquariums in PK plot 3. There was a significant decrease in the total bacterial population of 86.3%.

In the first sample in June, the highest soil and cellulolytic bacteria populations at 10^{-2} dilution were found in the PK plot, 1.71 x 10^5 CFU/g and 4.24 x 10^4 CFU/g, respectively. While the populations of soil bacteria and the lowest cellulolytic in June were found in the LJ plot, respectively, 0.125 x 10^5 CFU/g and 1.35 x 10^4 CFU/g. In the second sample in July, the highest population of soil bacteria and cellulolytic bacteria at 10^{-2} dilution was found in the PK plot, respectively 1.81 x 10^5 CFU/g and 3.96 x 10^4 CFU/g, while the population of bacteria and cellulolytic bacteria was lowest in July was found in the LJ plot of 0.125 x 10^5 CFU/g and 1.43 x 10^4 CFU/g, respectively. Bello et al. (2013) explained that soil supports diverse microbial communities that play an important role in ecosystem-level processes such as the decomposition of organic matter and nutrient cycling. The richness, abundance, and activity of microbial communities are susceptible to the influence of soil physical and chemical properties such as pH, moisture, organic matter content, and nutrient availability. Therefore, changes in soil's physical and chemical properties can cause changes in microbial communities, composition, and function. In addition, changes in land use significantly affect the balance and stability of biological commodities and ecosystem processes (Tin et al. 2017). True cellulolytic microorganisms play a critical role in sustainable energy production: degrade plant materials; produce a variety of chemicals through biomass combustion; provide genetic information for the production of cellulolytic and hemicellulolytic enzymes for applications in many biotechnological and industrial processes (Koeck et al. 2014).

The PK plot's highest soil and cellulolytic bacteria population significantly differed from the other plots. The highest average also supported this in situ dry litter weight of 201.25 g. The availability of food sources for soil bacteria from abundant litter input and high C-organic content in PK plots supports soil microbial populations and activities, especially soil bacteria. Yavitt (2021) explains that soil organic matter stocks are a long-term balance between the input of plant residues into the soil and the decomposition of these residues by soil microorganisms. The high population of soil and cellulolytic bacteria in the PK plot is also supported by the soil pH value of 5.4. The input of organic matter from litter and soil pH influences environmental conditions suitable for growing soil microbes, especially bacteria. Tripathi et al. (2018) that the optimum soil pH for most bacteria growth at 3 to 7.8. These results indicate that the assemblage of community bacteria is more clustered in soils that are more acidic and alkaline and phylogenetically less grouped in soils closer to neutral pH. This statement follows the soil pH conditions in the PK plot to support the population and activity of soil bacteria. However, limited information regarding bacterial communities across land-altering gradients substantially limits the ability to model and predict tropical ecosystem responses to current and future environmental changes (Filgueiras et al. 2015 and Lee-Cruz et al. 2013).

Bevivino et al. (2014) when forest ecosystems are converted to grasslands and grasslands to agricultural lands, there will be a sharp change from one type of soil microbial community to another. Because an ecosystem's ability to withstand serious disturbances may depend in part on its microbial component, both in terms of characterizing the composition and/or structure of the bacterial community can be used to assist in better understanding and manipulating ecosystem processes. Soils provide many ecosystem services, such as primary production and biogeochemical cycles. That includes: carbon storage, mineralization, and bioremediation of toxic compounds that can occur through microbial activity below the soil surface (Gleixner 2013). Various soil physicochemical factors, including organic matter content, pH, and bulk density, affect bacterial communities (Hartmann et al. 2012), regulated by climatic, geological, and land use factors. In addition, it has also been proven that the abundance and structure of different microbial groups are not affected by the same parameters (Birkhofer et al. 2012). Practices caused by land use changes result in unfavorable soil variables. Therefore, intensive land use can indirectly affect microbial biodiversity by modifying the soil environment (Bagella et al. 2014).

Dewi et al. (2017) explained that organic matter is important in soil's physical, chemical, and biological properties. In soil physical properties, organic matter supports aggregate stability, infiltration, percolation, and water retention. In soil chemical properties, organic matter can increase cation exchange capacity, soil buffer capacity, and nutrient supply, while its effect on soil biology is related to increased activity and diversity of soil organisms. The ratio of bacteria in the soil has been shown to correlate with environmental factors such as soil pH, plant substrate, and soil organic carbon content. Bacteria that can grow on CMC (Carboxyl methyl cellulose) media are cellulolytic bacteria that can degrade cellulose. CMC media contains pure cellulose substrate in amorphous form so that the activity of the endo-1,4-β-glucanase enzyme on the CMC substrate is the activity of the cellulase enzyme. Shorter cellulose chains or oligosaccharides are produced by endo-1,4-β-glucanase acting on the inner chain of CMC (Scheffer et al. 2022). Cellulose is the main constituent compound in agricultural waste, and in nature, this compound can be destroyed by microorganisms that grow on cellulose. Cellulolytic microorganisms are microbes that can hydrolyze cellulose. The soil's physical, biological, and chemical conditions can influence the presence of microbes. Cellulolytic bacteria are small (0.5-1.0 µm) and are prokaryotic with a single cell. Their small size provides an advantage in the decomposition process of organic matter; bacteria have a larger surface area for contact with the substrate than fungi and actinomycetes. Moreover, cellulolytic bacteria have a high reproduction rate. Consequently, they have rapid population growth and enzyme production (Gusmawartati et al. 2017). A complex process that requires the participation of microbial cellulolytic enzymes is required in the degradation of cellulose materials process (Irfan et al. 2012).

Different practices can change ecosystems in the soil, often leading to soil carbon depletion and loss of biodiversity, thereby affecting the structure of resident microbial communities (Lauber et al. 2013). Changes in land use that affect the input of litter in the land affect the C-organic content of the soil. The relationship graph between the dry weight of in situ litter and the C-organic content of the soil is presented in Figure 6. It has a positive correlation with the C-organic content of the soil, with an r-value of about 0.69. The highest soil C-organic content was found in the PK plot, which was 6.64%, with an in situ dry litter weight of 201.25 g. Schnecker et al. (2014) that the decomposition of soil organic matter (SOM) depends on extracellular enzymes produced by microorganisms. Microbes use enzymes to obtain carbon (C) or limiting nutrients and to target the most abundant substrates. Therefore, extracellular enzyme activity is often associated with the chemical composition of SOM, C, and nitrogen (N) content. Microbial communities that cannot adapt to the available substrates cause low microbial activity. The formation of soil organic matter (SOM) in conceptual and quantitative models has been described primarily as a function of plant and chemical inputs (Kallenbach et al. 2016). Biological and chemical soil variables under different organic coffee growing systems have shown highly significant correlation coefficients. It is assumed that there is an increase in microbial activity with an increase in total carbon content (Notaro et al. 2013).

Wibowo and Kasno (2021) that functional processes in soil are greatly influenced by organic carbon content, such as the ability to store nutrients, especially nitrogen, water holding capacity, and aggregate stability. Soil nitrogen is also important in maintaining soil quality, crop production, and environmental protection. Land use plays a major role in the distribution and amount of soil organic carbon and total nitrogen. The relationship between soil organic carbon and total soil nitrogen content is that an increase will follow an increase in soil organic carbon content in soil total nitrogen content. Soil microbial communities are adept at decomposing various plant compounds and using carbon (C) to synthesize their biomass (Kallenbach et al. 2016). Kurniawan et al. (2019) explained that litter quality in all land use systems and organic fertilizer inputs influence soil carbon stores. Soil organic C content of various land covers is affected by litter input and manure input in all land usages, except protected areas. Soil organic matter (SOM) is a substrate (a source of energy and nutrients) and a product of soil microorganisms. A change in the composition of SOM will affect changes in the decomposer population (Hoffland et al. 2020). Litter availability causes a high population of soil bacteria as an abundant source of soil organic matter, so the population is also high. The conversion of forests into agricultural land can cause a decrease in soil fertility from both chemical and biological indicators. Microbes and C elements are generally more sensitive to land-use changes than physical properties (Prayogo et al. 2021).

In this study, pine and coffee had the highest lignin values compared to other leaves. The high lignin and polyphenols can affect the presence of degrading bacteria. Adding high litter showed significant results on the soil bacterial community composition response. Bacteria are important in litter decomposition (Zeng et al. 2017). Research on litter decomposition was carried out on leaf litter where high levels of N could affect microbial degradation of lignin, thus creating the image of lignin as a recalcitrant. Lignin in forest ecosystems is the most abundant organic component and is an important part of plant litter input. Lignin also affects ecosystem dynamics (Rahman et al. 2013). Halle and Abay (2015), based on the quality of the different plants, there are several groups, namely plants classified as high quality if they contain N at least 2.5%, lignin content <15%, and polyphenol content <4%. Plant materials containing N <2.5% are of low quality. Organic plant materials with high N content, low lignin, and polyphenols can release N faster than organic materials with a higher lignin and polyphenol content. Therefore, plant materials with high lignin and polyphenol content in plant materials should be resistant to microbial decomposition. The C/N ratio is also one of the most important indicators of the quality of organic crop residues to improve soil fertility. Organic materials that have a higher C/N ratio, the more difficult it is to decompose quickly. Conversely, organic matter quality is classified as low if the N content is small and the lignin and polyphenol content is high. In this condition, the absorption of nutrients tends to be slow and takes time. Litter is classified as high quality if it has a C/N ratio < 25, lignin content < 15%, and polyphenols < 4%, so it decomposes quickly (Rahmadaniarti and Mofu 2020).

In many cases, cellulose is present in its pure state, related to hemicellulose and lignin of plant dry-weight cellulose, which comprises about 35-50%. Hemicellulose and lignin comprise 20-35% and 5-30% of plant dry weight, respectively. Cellulose is a linear polysaccharide consisting of monomer glucose units linked together by b-1,4-glycosidic bonds (Behera et al. 2016). Excess N fertilization in the long term causes changes in community structure and the overall bacterial population. Therefore, understanding changes in the structure and composition of soil bacterial communities following long-term fertilization may have significant implications for the rational use of N fertilizers and sustainable agriculture. The effect of N application on the diversity of soil bacteria can be caused by a direct role of N as a nutrient or an indirect role in changing soil characteristics. Long-term increases in N fertilizer inputs negatively affect soil pH, leading to decreased bacterial activity and can cause soil acidification and reduce crop yields (Xu et al. 2020).

Banerjee et al. (2016) that land use change is one of the most significant factors impacting underground biodiversity. Agroforestry systems that occur concurrently with other land uses can serve as a model to address how soil microbial communities react to land-use changes and how we can best manage these responses. Agroforestry systems differ in plant cover and species composition, factors that directly affect the quality and quantity of organic matter inputs with subsequent effects on soil properties. While the relationship between plants and microbial diversity has been proposed for a long time. Agroforestry can provide continuous input of organic matter into the soil, especially in the deep roots of the forest component, increasing soil organic matter (SOM) stocks and increasing soil microbial biomass (Rodrigues et al. 2015). Agroforestry is an alternative system that accommodates plants and tree species to maintain water, biodiversity, and environmental aridity. This can be observed from the diversity of agroforestry, which is mostly dominated by single tree species such as Sengon *(Paraserianthes falcataria)* as a Promising Multipurpose Tree Species (MPTS) with a monoculture system, which can be harvested in a short time during cultivation (7-8 years) or with monoculture production forest systems such as Pinus *(Pinus merkusii*) or Mahogany *(Swetenia mahogany).* Each concealment system will greatly affect plant diversity and their capacity to absorb carbon from the atmosphere to become a plant biomass carbon store or store it as soil organic carbon stock (Prayogo et al. 2018; Hairiah et al. 2020). That efforts to increase biodiversity in a landscape will lead to an increase in species richness because they have the opportunity to grow simultaneously, but the impact is the emergence of competition between plants which will cause a decrease in tree growth and performance (Prayogo et al. 2021).

Based on this research, it can conclude that the highest soil organic carbon content was obtained in the PK plot (pine at 41 years and coffee at 11 years), which is associated with the highest soil bacteria in June and July 2022. On the other hand, the LJ treatment (citrus: orchard plot) has the lowest value of soil organic carbon content, total soil nitrogen, the lowest bacterial population, and low organic input in the form of litter production. These results indicate that changes in land management and soil organic matter input affect the soil bacterial population and nutrient availability. The decrease in litter input impacts soil microbial populations, especially soil bacteria because the primary source of organic matter and energy source for microbes in the soil is reduced. The dry weight value of litter in situ at the PK plot is greater than that of the PS and LJ plots by 81 and 87%. The KC plot (mixed garden: pine aged 41 years, coffee aged 3-4 years, avocado aged 1 year, orange aged 1 year, and mustard greens aged 1-3 months) had the highest total nitrogen due to the application of organic fertilizers, in the form of chicken manure and inorganic fertilizers which support total nitrogen in the soil. There was a significant relationship between total organic C and soil N and total soil bacterial population. This shows that the changes in land management in the agroforestry system improve soil's chemical and biological properties. These results show that a land management change into an agroforestry system obtains benefits more than a monoculture system (i.e., citrus: orchard plot).

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