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The impact of different management on pine-based agroforestry system and litter accumulation on the population and activity of cellulolytic bacteria

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Abstract. *Arfarita N, Nuraini Y, Rinady MVP, Noerhayati E, Prayogo C. 2024. The impact of different management on pine-based agroforestry system and litter accumulation on the population and activity of cellulolytic bacteria. Biodiversitas 25: 924-936.* Agroforestry leads to the accumulation of organic matter, and the environmental conditions, litter quality, and decomposing organisms influence the rate at which litter decomposes. Litter decomposition in agroforestry systems is slow due to the high lignin and phenolic substances content, low light intensity, and high humidity under the canopy's shading condition. Therefore, a study was carried out to address this issue to isolate cellulolytic bacteria capable of breaking down plant litter using a qualitative cellulase activity testing method. The complete randomized block design and Tukey's test were used to determine the treatment's significance. Six agroforestry systems were examined: pine-coffee, pine-banana, pine-cardamom, pine-cardamom, mixed garden, and citrus. The PK (Pine-Coffee) plot had the highest canopy cover, litter density, and cellulolytic bacteria. The study identified three cellulolytic bacteria isolates (PK1, PK13, and PK10) from the 50 isolated bacteria of PK plot, producing the largest clear zones on CMC media. These identified isolates belonging to the Bacillus genus were Gram-positive bacteria with rod-shaped cells. The different types of litter in agroforestry systems affect the content of lignin, polyphenols, cellulose, and C/N ratios, which can influence the abundance of cellulolytic bacteria and their potential cellulase activity.

Keywords: Agroforestry, cellulolytic bacteria, litter decomposition, litter quality

Abbreviations: CMC: Carboxy Methyl Cellulose, PCo: Pine-Coffee Agroforestry System, PB: Pine-Banana Agroforestry System, PCa: Pine-Cardamom Agroforestry System, PV: Pine-Vegetable Agroforestry System Agroforestry, MG: Mixed Garden, CO: Citrus Orchard

INTRODUCTION

Agroforestry systems are vital in increasing agricultural production by improving soil fertility, retaining organic matter, and promoting nutrient cycling (Tsufac et al. 2019). This system will accumulate a significant amount of organic matter in the form of litter on the soil surface (Rinady et al. 2023; Garratt et al. 2018). The plant litter decomposition rate depends on its quality, the physical and chemical environment, and decomposer organisms (Jugran and Tewari 2022). The structure of the stand, tree species, internal habitat conditions, and changes in leaf litter composition also affect the growth and activity of microbes (Fu et al. 2021). Decomposing is a process that makes organic matter into simpler organic compounds through microbial activity and their products, which is crucial to the environment (Gusmawartati 2017). The substrate's chemical and physical properties significantly affect the litter decomposition rate, including the nitrogen and lignin content and the carbon-to-nitrogen and lignin-to-nitrogen ratio. Recent studies have found that temperature is a more crucial factor than climate in determining litter decomposition rates, previously considered the dominant factor in the tropics (Krishna and Mohan 2017).

Pine is a widespread and dominant species in agroforestry, with the Chir pine (*Roxburghii pine*) being the most extensive species in India (Bisht et al. 2016) and Sumatra pine (*Pinus merkusii*) is a famous tree in Indonesia (Sallata 2013); it is one of the major tree species in agroforestry systems. These trees produce a large quantity of litter with a complex lignocellulose structure, resulting in slow decomposition (Soong et al. 2015; Çömez et al. 2021). The accumulation of this material can pose a hazard to the biogeochemical cycle, ecosystem, and energy flows worldwide (Zhou et al. 2015).

Pine tree litter comprises a low amount of easily decomposable organic matter, sugars, and water-soluble nutrients. However, it contains more complex compounds like cellulose, hemicellulose, and lignin; lignin releases phenolic acids that prevent the growth of other plants.

(Duffy 2014). The primary plant residues in pine litter consist of three complex carbohydrate biopolymers - cellulose (30-50%), hemicellulose (20-40%), and lignin (10-30%) (Shamshitov et al. 2022). This material is crucial in biogeochemical processes that help incorporate carbon and other nutrients into the soil (Bradford et al. 2016). Their decomposition can impact microbial populations and activities because carbon is the primary energy source for microbial growth and development (Osman 2013).

Bacteria play an important role in soil nutrient cycling by decomposing organic matter and mineralizing organic compounds (Thatoi et al. 2013). As litter decomposition progresses, fungal activity decreases, and bacteria become more abundant. Bacteria can break down lignin decomposition derivatives, including aromatic compounds, and compete with fungi for sugar as an energy source (Kielak et al. 2016). In exchange for the carbon source released by fungal enzymes, bacteria provide limited nutrients like nitrogen and iron through nitrogen fixation or growth factors like vitamins (Hoppe et al. 2014). More research is needed on litter bacteria degradation to understand the community composition of cellulolytic bacteria to plant litter quality, temperature, and decomposer bacteria. Furthermore, cellulolytic bacteria can decompose plant litter containing cellulose (Soares Júnior et al. 2013). Cellulases comprised of endoglucanase, exoglucanase, and β-D-glucosidase are crucial for cellulolytic bacteria to break down cellulose-based plant litter (Kumar et al. 2019).

Cellulolytic bacteria are heterotrophic bacteria that can break down abundant cellulose into glucose monomers (Hapsoh et al. 2017), also called saprophytic bacteria. These bacteria are beneficial in organic matter decomposition due to their small size, which provides a larger surface area for contact with the substrate than fungi and actinomycetes (Gusmawartati 2017). Katiyar et al. (2018) showed that *Bacillus* spp., specifically *Bacillus cellulosilyticus, Bacillus subtilis*, and *Bacillus sphaericus*, produced a higher percentage of extracellular enzymes (cellulases) to break down cellulose; other cellulolytic bacteria include *Trichonympha*, *Clostridium*, and *Actinomycete*s (Gupta et al. 2012). Additionally, Handique et al. (2017) have isolated, identified, and characterized cellulolytic bacteria from the gut of white grub *L. mansueta* and reported a cellulolytic index of 2.14 in *Citrobacter* sp. Similarly, MsangoSoko et al. (2020) have isolated and characterized cellulolytic bacteria from different gut compartments of the third instar larva of *A. dimidiata*. It was observed that the gut bacterial isolates exhibited significant differences in their capacity to break down CMC, as determined by the enzymatic index. Danu et al. (2023) reported that among the 25 cellulolytic bacteria isolated from different compartments of selected white grub species, *B. stratosphericus* strain CBG4MG1, *B. cereus* strain CBG2FC1, *Bacillus* sp. CBG3MG2 and *Paenibacillus ginsengagri* CBG1FC2, isolated from various gut portions of four white grub species, recorded the highest cellulolytic index compared to all other cellulolytic bacteria isolated. The bacterial isolates, on the whole, showed high CMC degrading activity, indicating the remarkable ability of gut bacterial isolates of white grub to be an essential source of cellulose-degrading microflora (Danu et al. 2023). The other cellulolytic species which were identified from intestines of omnivorous mammals belongs to *Bacteroides*, *Enterococcus*, *Eubacterium*, *Clostridium*, *Ruminococcus* and *Fibrobacter* which are the playing important role of nutrient absorption processes (Froidurot and Julliand 2022)

According to many studies (Danu et al. 2023; MsangoSoko et al. 2020; Kothari et al. 2013; Kumar et al. 2014; Van Dyk et al. 2009), *Bacillus* genera are capable of producing cellulolytic bacteria that can produce enzymes such as cellulase, CMCase, mannanase, pectinase, and xylanase Cellulose-degrading enzymes produced by cellulolytic bacteria are essential for the efficient and cost-effective decomposition of lignocellulosic biomass into biofuels (Jang and Kikuchi 2020). Furthermore, to obtain bacterial agents that can efficiently degrade cellulose and accelerate the pace of decomposition, it is essential to isolate these bacteria, particularly from pine-based agroforestry systems. This study conducted qualitative cellulase activity tests in various agroforestry systems to identify cellulolytic bacterial isolates with high cellulolytic activities. So far, cellulolytic bacteria have collected from winter wheat (*Triticum aestivum* L.), winter rape (*Brassica napus*), spring wheat (*Triticum aestivum* L.), spring barley (*Hordeum vulgare*), pea (*Pisum sativum*) soil (Shamsitov et al. 2023); Sago (*Metroxylon sago* Rott.) pith (Faizah et al. 2020); the feces of tropical endemic herbivores, including anoa (*Bubalus depressicornis*), banteng (*Bos javanicus*), muntjak (*Muntiacus muntjak*), and timor deer (*Rusa timorensis*) (Suharti et al. 2023); mangrove soil ecosystem include *Rhizophora* sp., *Avicennia marina*, and *Sonneratia alba* species (Dewiyanti et al. 2022). Unfortunately, the cellulolytic bacteria identified from agroforestry soil and ecosystems were rarely reported.

MATERIALS AND METHODS

Research location

The study was conducted from February to June 2022. Soil samples were collected from various agroforestry systems at KHDTK-UB Forest located at Sumbersari Sub-village, Tawangargo Village, Karangploso Sub-district, Malang District, East Java Province, Indonesia. The area is situated at the foot of Mount Arjuno, which covers an area of 544 Ha at an altitude of 1,200 meters above sea level (masl). This area has a 5 to 30% slope and is geographically located at 7°53'35"S, and 112°53'41"E. Plots based on different land use systems were selected across various agroforestry systems (Table 1). Soil and litter analysis was conducted at the Biology and Chemistry Laboratory, Soil Department, Faculty of Agriculture, Universitas Brawijaya, Malang, Indonesia. At the same time, cellulolytic bacteria were isolated and morphologically identified at the Microbiology Laboratory of the Universitas Islam Malang, Indonesia (Figure 1).

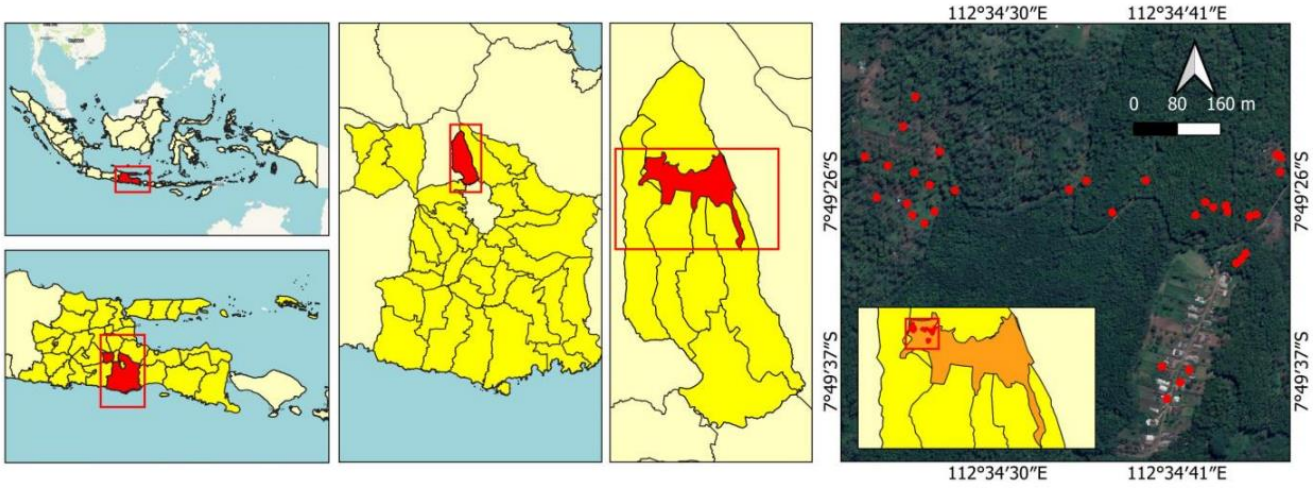

Figure 1. Location of study at KHDTK UB Forest, Malang, Indonesia

Table 1. Research plots

Plot code	Characteristics	Notes
PCo- PB- PCa PV MG CO	Pine and coffee agroforestry Pine and banana agroforestry Pine and cardamon agroforestry Pine and vegetable agroforestry Mixed garden (pine, coffee and avocado) agroforestry Citrus orchard	Pine = 41-year-old; Coffee = 11-year-old; Banana = 1 to 2-year-old; Cardamon = 1-year-old; Cabbage = 1 to 3 month; Mustard = 1 to 3 month; Avocado = 1-year-old; Citrus = 1-year-old

Research experimental design

A Complete Randomized Block Design (RBD) was employed which consisted of six different types of land use: (i) Pine-Coffee Agroforestry System (-PCo), (ii) Pine-Banana Agroforestry System (-PB), (iii) Pine-Cardamon Agroforestry System (PCa-), (iv) Pine-Vegetable Agroforestry System (PV-), (v) Mixed Gardens (MG-) and (vi) Citrus Orchard (CO-). The plot was 20 m x 20 m in each land use type, which was replicated 3 times, resulting in 18 plots in total.

Soil sampling

The soil was collected using the composite method, carried out at five different sampling points (from the middle and four on the diagonal position) within the areas between tree stands of pine and coffee, and taken at 0-20 cm soil depth. Soil samples were taken as much as 100 g at each sampling point; therefore, 500 g of fresh soil was obtained from each plot. Soil samples were labeled before being placed into the refrigerator at the temperature of 4°C.

Litter sampling

Moreover, litter sampling was collected from each of the six land use types. A litter metal frame within 50 x 50 cm was used to determine the average litter amount in each sampling site. The litter samples were then grouped into several classes (twigs, leaves, and branches). The weight of the litter is determined by weighing it on a scale when it is fresh. Next, the fresh litter samples were put into envelopes to measure their dry litter weights by placing them in the oven for 48 hours at 80°C.

Litter characteristics and composition

The Goering and Van Soest (1970) method was used to determine lignin on the litter of each species, and the Denis Folin method assessed polyphenols content (King and Heath 1967). Walkey and Black were employed to determine litter C-organic content, while Kjedahl was used to examine N-total. C/N ratio.

Soil and air temperature and humidity

The HOBO sensor for measuring soil temperature and humidity was installed 10 cm deep into the soil while the sensor for air temperature and humidity was placed at 100 cm above soil surface. The average morning temperature was 06.00-08.00 AM, 11.00 AM to 01.00 PM and 03.00 to 04.00 PM for measuring average midday temperature and evening temperature, respectively.

Canopy cover

Canopy covers were determined using direct measurements, which were carried out using the Canopy App tool. Observation of the canopy cover was pictured through a cellphone camera positioned in the middle to determine the canopy's density. Several picture positions under the tree canopy were photographed to produce an average canopy cover.

Total population of soil cellulolytic bacteria

The Total Plate Count (TPC) method was used to measure the cellulolytic bacteria population. A selective medium for cellulolytic bacteria, CMC (Carboxy Methyl Cellulose), was

also used. The CMC media was prepared by dissolving various ingredients, including 15 g agar, 10 g CMC, 0.02 g FeSO4, 0.75 KNO3, 0.5 g K2HPO4, 0.2 g MgSO4, and 1 g of glucose and 2 grams of yeast extract in 1 liter of sterile distilled water. The mixture was then heated to a boiling point and sterilized in an autoclave. After reaching a warm temperature, the CMC media ingredients were mixed and poured into a petri dish of approximately 15-20 mL.

Next, to begin the process, 5 grams of soil sample was mixed with 45 mL of sterile distilled water, incubated, and homogenized for 30 minutes in the incubator. A series of dilutions were made up to 10^{-4}-, and 100 µL of suspension was pipetted from each level of dilution. The suspension was then inoculated on CMC media using the spread plate method and incubated for 2 days at room temperature. The incubation was conducted at 37°C. Bacterial colonies were manually counted using a hand counter for the next 3 following days, and the colony count was determined using a formula:

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

Note: fp = Dilution factor on colonized Petri dishes; soil dry weight (g) = fresh weight x (1 water content)

Isolation and characterization of soil cellulolytic bacteria

The cellulolytic bacterial colonies' characteristics were analyzed using macroscopic and microscopic methods. The macroscopic analysis involved observing the colony characters, like each colony's shape, size, edges, color, and elevation as it grew in the culture medium. At the same time, the microscopic analysis involved using the Gram staining method to determine whether the bacteria were Gram-positive or Gram-negative.

Isolates with different morphology were identified and separated according to Bergey's Manual of Determinative Bacteriology classifications. After purification, each isolate was inoculated onto new CMC agar media using the quadrant streak method and incubated for 2 days. This process was done to identify each isolate and obtain a single colony from each isolate. Finally, the single colonies were incubated with a loose needle onto the CMC agar medium obliquely as a bacterial stock. The Gram staining method was used to identify the physiological characterization of each isolate through a microscope by observing the color produced, with Gram-positive bacteria appearing purple and Gram-negative bacteria appearing pink.

Qualitative cellulolytic activity test

Moreover, to identify the ability of cellulolytic bacteria to degrade cellulose, the formation of clear zones was measured in each pure culture of bacterial isolates found in various areas of agroforestry system at KHDTK UB Forest with differing land cover. The isolates were streaked on CMC agar plates and incubated at 30°C for 5 days. After that, the CMC medium was flooded with 1% Congo red reagent for 15 minutes and washed with 1 M NaCl for 3 to 5 minutes (Liang et al. 2014). Qualitative cellulolytic activity testing of bacterial isolates was conducted by staining Congo-Red and forming clear zones using by Murtyaningsih and Hazmi (2017) method. The clear zone on CMC media indicates hydrolytic activity by cellulase enzymes secreted by bacteria. The reducing sugar level formed from the enzymatic reaction between the CMC (Carboxy Methyl Cellulose) substrate and the cellulase enzyme extract determines the qualitative cellulolytic activity. The Cellulolytic Index (IS) value is categorized as low if it is ≤1 cm, moderate at 1-2 cm, and high if it is ≥2 cm (Choi et al. 2005). The formula to calculate the cellulolytic activity index is given by Arifin et al. (2019).

Cellulolytic Index (IS) = Clear Zone Diameter/Colony Diameter

Data analysis

The current study data was analyzed using an analysis of variance (ANOVA) with a 5% significance level. If the data showed a significant effect, the Tukey test (5%) was used to determine the differences between treatments. Additionally, correlation and regression tests were used to determine the strength of the relationship between the observed parameters. PCA biplot multivariate analysis was employed to cluster and distinguish the relationship between the parameter/variable and that direction or magnitude. The treatment can be separated using the X and Y axes, and the overlapping treatment indicated no significant value among the selected parameter/variable.

RESULTS AND DISCUSSION

Canopy cover and soil microclimate

According to the ANOVA analysis, changes in land use had a significant impact on the canopy cover ($P>0.01$). The study revealed that the average canopy cover in each plot ranged from 61 to 82%. The PCo plot showed the highest canopy cover, with a value of 82.80%, which was not significantly different from the MG and PB plots, with respective values of 78.35 and 76.58%. The CO plot had the lowest value of canopy cover compared to the other plots. However, its value was not significantly different from the PCa and PV plots, with respective values of 61.12, 67.58, and 67.80% (Table 2).

The highest canopy cover of 82.80% was in the PCo plot, while the CO plot had the lowest cover value of 61.12%, which was not significantly different to PCa and PV plot (Table 2). Each plot's different canopy cover values were due to varying land management practices. At the PCo plot, management was relatively low compared to other plots, leading to a high canopy cover. The CO plot, on the other hand, had a low crown cover density value because it was a monoculture plot where only citrus trees were planted and managed by farmers. Sari et al. (2018) reported that the canopy density in the UB Forest area was generally in the medium category (50-100%). Canopy density is categorized into 4 groups: very dense (>201%), moderate (51-100%), low (12-50%), and very low (<12%) (Sari et al. 2018). The PCo PK plot had the highest canopy density compared to the other plots. The different plots had varying canopy

shapes, and the tree branch shapes could affect the shape of the crown. Canopy shape, in turn, could affect microclimate changes in the soil and air temperature in KHDTK UB Forest.

The weekly average soil temperature in UB Forest from June to July 2022 was 20-21°C, while its air temperature was 19-25°C (Table 3). The highest average of soil temperature was in May at 21°C (2022) and dropped to 20°C in August (2022), while the air temperature could reach 25°C in July 2022. The soil moisture in the research plots ranged from 80.22 to 95.38% which the highest soil moisture was found at PCo plot. Factors such as solar radiation, vegetation, and conditions above the ground surface influenced the temperature volatility. Canopy cover with a high percentage of lush vegetation could reduce hot temperatures, while a vast canopy could provide low temperatures and high humidity (Saroh and Krisdianto 2020).

The management of diverse farmers in each plot caused changes in the microclimate of UB Forest, specifically in soil temperature. Such changes directly affected the overall quality of soil organic matter and microbial populations. From May to August 2022, the average weekly soil temperature in UB Forest ranges from 20-21°C, as shown in Table 2. In May, June, and July, the soil temperatures in the plot were 21, 20.8, and 20°C, respectively. In June, the PCo, PB, and PV plots had the same soil temperature of 20.25°C. However, the PCa plot had a soil temperature of 20.5°C and the MG plot of 20.75°C. The dense vegetation canopy and litter production on the PCo floor plot also contributed to the low soil temperature and the soil temperature and organic matter availability affected the amount of bacteria and its activity. The forest canopy is vital in changing the tree stand structure and renewing the forest. The gap size of the canopy affects the environment, such as temperature and humidity, which in turn could influence nutrients and living soil microbes. Land with high canopy management caused unstable amounts of carbon and low soil respiration rates due to a disrupted environment (Yang et al. 2017). Natural gap formation processes caused by farmers' management could lead to temperature, humidity, and litter input changes, affecting organic matter dynamics, nutrient cycling, and microbial community composition. The lower the canopy cover percentage, the lower the total microbes; the low amount of microbes resulted from low soil organic matter, decreasing litter input. Bacteria showed higher populations with small canopy cover gaps with a more suitable environment for soil microbial communities (Yang et al. 2017). The research showed that the soil moisture levels of UB Forest from May to August 2022 were between 80.22 to 93.50% (Table 4). Within this period the soil moisture at PCo plot is relatively higher than the other plot followed by MG, PCa, PB, PV plot and CO plot was the lowest. Moreover, air moisture at PCo plot is also relatively higher than the other. The increase of soil moisture was correspond to the raising of air moisture (Table 4). This may correspond to the low canopy cover and low density of litter at CO plot which was only 30% compared to MG plot (Table 5).

Solar radiation, vegetation, and conditions above the soil surface affected the volatility of temperatures. Compared to the CO plot, the PCo plot (pine aged 41 years and coffee aged 11 years) had a lower light intensity value. Therefore, the air humidity value in the PCo plot was high, reaching 99.68% in June and 99.71% in July. The amount of solar heat absorbed directly by the soil is affected by the low intensity of sunlight that enters the PCo plot, which affects soil temperature. In addition, the density of the vegetation canopy and the amount of litter produced on the floor of the PCo plot also contribute to the low soil temperature. The number and activity of bacteria are affected by soil temperature and soil organic matter availability (Susilawati et al. 2013).

Table 2. Canopy covers various agroforestry systems

Agroforestry system	Canopy cover (%)
PCo	82.80 b
PB	76.58 b
PCa	67.58 a
PV	67.80 a
MG	78.35 b
CO	61.12 a

Note: Numbers followed by different letters in the same column indicate that the treatment is significantly different in the 5% Tukey follow-up test

Table 3. Weekly soil and air temperature across different agroforestry systems

Agroforestry system	Weekly Soil and Air Temperature (°C)							
	May 2022		June 2022		July 2022		August 2022	
	Soil	Air	Soil	Air	Soil	Air	Soil	Air
PCo	21.00	23.00	20.25	25.00	20.80	22.00	20.00	20.00
PB	21.00	23.00	20.25	25.00	20.80	22.00	20.00	20.00
PV	21.00	23.00	20.25	25.00	20.80	22.00	20.00	20.00
PCa	21.00	23.00	20.50	25.00	20.80	22.00	20.00	20.00
MG	21.00	23.00	20.75	25.00	20.80	22.00	20.00	20.00
CO	21.00	23.00	20.50	25.00	20.80	22.00	20.00	20.00

Table 4. Soil and air moisture across different agroforestry systems

Agroforestry system	Weekly soil and air moisture (%)							
	May 2022		June 2022		July 2022		August 2022	
	Soil	Air	Soil	Air	Soil	Air	Soil	Air
PCo	93.50	97.83	95.24	99.68	95.24	99.71	95.38	96.00
PB	82.76	85.37	82.82	85.15	82.59	86.00	82.90	86.01
PV	81.82	82.02	81.91	82.04	82.01	82.82	82.04	82.39
PCa	84.12	84.80	84.28	85.07	84.33	85.94	84.97	85.36
MG	91.62	92.87	91.73	93.40	91.76	93.53	91.69	93.27
CO	80.22	82.14	80.25	82.18	80.81	82.50	80.90	82.27

Table 5. Litter Thickness and dry weight across different agroforestry systems

Agroforestry system	Litter thickness (cm)	Litter dry weight (g/m^2)	Canopy cover (%)
PCo	2.25 d	201,25 d	82,80 b
PB	0.88 bc	47.50 ab	76,58 b
PCa	0.58 ab	58.60 b	67,58 a
PV	0.28 a	29.20 a	67,80 a
MG	0.98 c	147.60 c	78,35 b
CO	0.30 a	25.35 a	61,12 a

Note: Numbers followed by different letters in the same column indicate that the treatment is significantly different in the 5% Tukey follow-up test

Canopy cover, litter accumulation and quality

The percentage of canopy cover impacts the thickness of leaf litter, soil temperature, and soil microbes (Liu et al. 2014). Among the plots studied, the PCo plot had the highest canopy cover value of 80.82%, and the CO plot had the lowest value of 61.12%, which was not significantly different to PCa and PV (Table 5). Different management on agroforestry system is significantly affected the litter thickness ($p<0.01$), ranging from 0.30 to 2.25 cm. The PCo plot had the highest litter thickness of 2.25 cm, significantly different from the other plots. The PV plot had the lowest litter thickness, which was not significantly different from the CO plot. The greater the percentage of canopy cover, the more litter production is produced (Rinady et al. 2023; Azizah et al. 2023). Leaves reduce the intensity of sunlight exposure, slowing wind speed and rainfall rate. Therefore, even the leaves are not directly exposed to the ground, and the litter density on the soil surface positively impacts the microclimate, which supports all living things, especially soil microbes (Saroh and Krisdianto 2020). Trees are essential in maintaining and restoring soil fertility by producing litter (Safriani et al. 2017; Azizah et al. 2023). The litter production size can estimate the relevant organic matter source for soil fertility in forest ecosystems. Litter production is influenced by stand density, understorey density, tree age, and the quality of the tree-growing area. Fallen litter contains soil nutrients that plants cannot directly use (Prayogo et al. 2021a; Prayogo and Arfarita 2022). Therefore, the litter must first be decomposed by soil microbes (bacteria), which greatly affect the availability of nutrients, and these nutrients can create fertile soil conditions (Sudomo and Widiyanto 2017; Rinady et al. 2023). The litter layer protects plants from direct rainwater, sunlight penetration, and temperature changes while facilitating water and air circulation. It is a layer bridging the soil and the atmosphere and providing a barrier against external factors. The litter biochemical decomposition process can be impeded and increasing soil carbon content, which serves as the primary food chain of microbes (Prayogo et al. 2021a; Prayogo and Arfarita 2022; Azizah et al. 2023).

Pinus spp. litter contains low concentrations of labile organic matter and nutrients, such as sugar and water-soluble nutrients. However, it contains a larger proportion of compounds with large molecular weights, such as cellulose, hemicellulose, and lignin. Furthermore, lignin releases phenolic acids, which can be allelopathic (Duffy 2014).

The results of the ANOVA analysis revealed that the different types of litter significantly impacted the lignin and polyphenol content ($p<0.01$). The litter analysis indicated that cabbage litter had the lowest lignin content, which differed significantly from other plant litter (4.82%). Conversely, pine litter had the highest lignin content, which significantly differed from other plant litter (47.04%), as shown in Table 6.

Furthermore, the ANOVA analysis demonstrated that the litter quality variation greatly affected polyphenols ($p<0.01$). The analysis highlighted that cardamom plant litter had the lowest polyphenol content (0.40%). On the other hand, citrus litter had the highest polyphenol content (7.80%), and it was significantly differed different to the other litter (Table 6). The analysis also indicated that the different types of litter is significantly impacted the percentage of cellulose ($p<0.05$). The litter analysis revealed that coffee litter had the highest cellulose content (14.35%), which significantly differed from other plant litter except with the litter of banana, cardamom, citrus and pine. In addition cabbage litter had the lowest cellulose content (9.74%) and it significantly differed significantly from other plants. Except with the mixed litter (Pine+Coffee and Avocado) and Citrus.

Table 6. shows the total population of soil cellulolytic bacteria

Agroforestry system	Cellulolytic bacteria population * (x10^4 CFU/g)
PCo	4,03 c
PB	1.62 a
PV	1.40 a
PCa	2.07 b
MG	2.77 b
CO	1.41 a

Note: Numbers followed by different letters in the same column indicate that the treatment is significantly different in the Tukey 5% follow-up test

The ANOVA analysis revealed that different types of litter significantly impacted the lignin+polyphenol/N ratio ($p<0.01$). Pine litter had the highest value of lignin+polyphenol/N ratio (46.65%) and was significantly different from other plant leaf litter. In comparison, cabbage litter had the lowest ratio of 3.41%, substantially different from other litters. Coffee, Banana, Cardamom, Mixed, and citrus litter had lignin+polyphenol/N ratios of 12.74, 12.45, 6.76, 10.82, and 16.73%, respectively. Additionally, each type of litter significantly affected the C/N ratio of litter ($p<0.01$). Pine litter had the highest C/N ratio value of 31,37 and was significantly different from other plant leaf litter. This material contained lignin almost 4 times higher than those lignin content on. Meanwhile, cabbage litter had the lowest C/N ratio and was significantly different from the leaves of other plants (9.93). This was due to the low carbon content of Coffee and banana litter. Table 6 shows the C/N ratio of litter for each land cover contained in several plant litters, including coffee, bananas, cabbage, cardamom, mixed litter, citrus, and pine in several land uses in the KHDTK UB Forest area was determined their decomposition processes. The material that has a higher C/N ratio and lignin content, it will be slower to decompose. Coffee litter was mainly composing PCo and Mixed Garden plots and Banana contributed to PB plot. Moreover, Cardamon, Cabbage, Cabbage and Citrus litter contributed to the litter deposition of PCa, PB, and CO plots, respectively. Pines litter was found in most of all plots except the CO plot.

The analysis of litter tissue revealed that coffee plant litter had a polyphenol content of around 0.88%, while pine litter had a relatively high content of 6.17%. Regarding lignin content, coffee litter had about 29.67% lignin, while pine litter had approximately 47,04% which was higher than other litter. Based on the lignin analysis results, the two organic materials were classified as low-quality because they were not easily decomposed. Halle and Abay (2015) stated that plants are classified as high quality if they have a lignin content of less than 15% and a polyphenol content of less than 4%. Organic plant materials with high nitrogen content, low lignin, and polyphenols can release nitrogen faster than the higher ones. Therefore, it can be concluded that this study's plant material with high lignin and polyphenol content is resistant to microbial decomposition. Table 6 shows that Cabbage and Cardamon litter litter fall under the high-quality category with a ratio value of (lignin + polyphenols)/N of less than 10 which easier to degrade by soil microorganism. On the other hand, Pine litter had the highest ratio of (lignin+polyphenol)/N of more than 10 compared to other litters along with the greatest C-organic, Lignin, Polyphenolic, and C/N ratio. Thus, the involvement of soil microbe that could be decomposed that material is necessary. The lignin content in litter affects the population of soil cellulolytic bacteria. In this study, pine and coffee had the highest lignin value compared to other leaves. High lignin and polyphenols can affect the presence of degrading bacteria. Moreover, high-quality litter significantly affected the soil bacterial community composition response. Bacteria are essential in litter decomposition (Zeng et al. 2017). Various studies on litter decomposition have been carried out on leaf litter where high N levels could affect lignin degradation by microbes, increasing the perception of lignin as a recalcitrant. Lignin is the most abundant organic component in forest ecosystems and is essential to plant litter input. Lignin also influences ecosystem dynamics (Rahman et al. 2013). Furthermore, its effect of soil microclimate and soil organic matter on soil cellulolytic bacterial communities.

The C-organic content of litter ranged from 16.63% (Cabbage) to 36.44% (Cardamom). The total nitrogen content of litter ranged from 1.14% (Pine) to 2.42% (Coffee). Based on organic C and total N content, the C/N ratio of organic matter ranges from 9.93 (Cabbage) to 31.37 (Pine). Sismiyanti et al. (2018) classified a high-quality organic matter with a total N content of <2.5%, <15% lignin, <4% polyphenols, and a C/N ratio value of <25. A litter with a nitrogen content greater than 2.5% will be immobilized, and litter with a nitrogen content ranging from 1.5% to 2.5% may facilitate nitrogen mineralization (Khoirunisa et al. 2020). Therefore, based on the nitrogen content values, this study employed all types of organic matter with different rate of decomposition and it will affect mineralization processes. At the same time, based on the litter overall analysis, it was found that coffee and pine litter possess high-quality C/N ratios of 14.30 and 31.37, respectively. Which could be classified as low quality of litter, but at the same time can provide a larger quantity of Carbon to soil and it could be as a source of energy and food for soil organism (Prayogo et al. 2021b; Sudharta et al. 2022; Mardiani et al. 2022). The C/N ratio is a crucial indicator for high-quality organic crop residue, enhancing soil fertility since the nutrient can release quickly, for example Cabbage litter which was found mainly at PV plot. Banana and cardamon litter which has higher C/N ratio than Coffee was due to less content of Nitrogen which was also one of the macronutrients for crop since this nutrient was involving on crop photosynthetic activity (Azizah et al. 2023; Rinady et al. 2023).

Organic materials with a higher C/N ratio tend to be more difficult to decompose; in contrast, low nitrogen content with high lignin and polyphenol indicates low organic matter quality and slower nutrient absorption. High-quality litter is identified by a C/N ratio <25, which results in faster decomposition. The lignin/N ratio is a reliable predictor of the decomposition rate for some litter classes, while the C:N ratio seems to be a better parameter

in the decomposition process (Rahmadaniarti and Mofu 2020; Chen 2014). About 35-50% of plant dry weight of cellulose is related to hemicellulose and lignin cellulose. Hemicellulose and lignin constitute 20-35% and 5-30% of the plant's dry weight, respectively (Chen 2014). Cellulose is a linear polysaccharide consisting of monomeric glucose units linked together by b-1,4-glycosidic bonds (Behera et al. 2016; Chen 2014). As a plentiful source of glucose in Indonesia, cellulose needs to decompose rapidly by utilizing cellulolytic bacteria, which may speed up organic matter decomposition (Hapsoh et al. 2017).

The ratio of (polyphenol+lignin)/N in litter quality has the greatest impact on releasing soil NH_4^+ and NO_3^- formation (nitrification) compared to lignin content, polyphenols, or the C/N ratio. High-quality litter is indicated by a C/N content of less than 25, polyphenol content of less than 3%, and lignin content of less than 15%, or a ratio of (lignin + polyphenols)/N of less than 10 (Andita Sari et al. 2020). Moreover, low-quality litter will inhibit decomposition rates because its high polyphenol content can be highly inhibitive.

The ANOVA analysis revealed that different types of litter quality significantly affected the total population of soil cellulolytic bacteria ($p<0.01$). As shown in Table 7, PCo plots with litter types of pine and coffee leaves had the highest values for the total population of soil cellulolytic bacteria (4.03 $x10^4$ CFU/g). These values were significantly different from those of other plots. On the other hand, the CO plot with lime leaf litter type had the lowest soil cellulolytic bacteria population values (1.41 $x10^4$ CFU/g), which was also significantly different compared to other plots. Fifty bacterial isolates were obtained from various land covers in UB Forest, which were able to grow on a solid cellulolytic medium containing CMC. These isolates included 16 isolates from the PCo plot, 7 isolates from the PB plot, 6 isolates from the PV plot, 8 isolates from the PCa plot, 9 isolates from the MG plot, and 4 isolates from the CO plot (Table 8). Farmers' land management practices can significantly impact the study plots' canopy cover which then affects soil and microclimatic environtment. There ara 50 isolate of cellulolytic bacteria which has been collected in this study. The differences on the number of cellulolytic bacteria maybe due to the differences on canopy cover and soil microclimatic factor such as: soil temperature and humidity.

The canopy cover across all study plots was classified as moderate density, with the PCo plot having the highest value at 82.80% and the CO plot having the lowest canopy cover at 61.12% (Table 5) which contribute for maintaining soil/air temperature and soil/air moisture volatility. The soil temperature at all plot were ranged between 20.00 to 21°C. while the soil humidity was from 80,25 to 99,71%. The microclimate changes caused by different land management practices, such as soil temperature and humidity, can alter the soil microbial communities and ecosystem functions. The changes in land management practices involve the canopy cover has been observed at across two contrasting timberline ecotones in southeast Tibet. European Journal of Soil Science (Yuan et al. 2015). The differences in microclimates and microbial communities are influenced by changes in vegetation cover and organic matter quality in an alpine forest-tundra ecotone was also reported recently (Chen et al. 2018). The various environmental conditions stimulate different soil cellulolytic bacteria in different plots. The differences in ecological conditions caused by farmers' management practices trigger changes in microclimatic conditions (soil temperature and humidity), affecting the microbial community. The microclimate and vegetation types of land cover affect the soil microbial community. Schoenborn et al. (2022) have suggested that microclimate, especially soil temperature, humidity, and the types of land cover vegetation, can produce different litter quality, which can affect microbial communities, such as soil cellulolytic bacteria. Soil temperature, soil moisture, and the type of vegetation encourage the existence of a microbial community (Yuan et al. 2015; Chen et al. 2018).

The effect of litter quality and soil cellulolytic bacterial community on litter decomposition

The KHDTK UB Forest has various research plots, each dominated by a different agroforestry management system vegetation. The most common vegetation in the area is Pine trees species, which provide shade to other plants such as coffee, bananas, vegetables (cabbage), spices (cardamom), and mixed plants which was planted underneath. Additionally, there is also a limited monoculture crop except for citrus orchard plot which usually act as a cash crop for fulfilling farmer daily income.

Table 7. The total population of soil cellulolytic bacteria across different agroforestry systems

Agroforestry system	Cellulolytic bacteria population * ($x10^4$ CFU/g)
PCo	4,03 c
PB	1.62 a
PV	1.40 a
PCa	2.07 b
MG	2.77 b
CO	1.41 a

Note: Numbers followed by different letters in the same column indicate that the treatment is significantly different in the Tukey 5% follow-up test

Table 8. Cellulolytic Bacterial Isolate across different agroforestry systems

Agroforestry system	Cellulolytic bacteria isolates
PCo	16
PB	7
PV	7
PCa	8
MG	8
CO	4
Total cellulolytic bacteria isolates	50

Table 9. Cellulolytic activity of bacteria isolates across different agroforestry systems

Isolates	Diameter of bacterial colony (mm)	Clear zone of CMC (mm)	Cellulolytic Index (IS)
PCo1	0.38	0.76	1.11
PCo2	0.50	0.00	0.00
PCo3	0.23	0.00	0.00
PCo4	0.20	0.00	0.00
PCo5	0.50	0.80	0.60
PCo6	0.20	0.00	0.00
PCo7	0.23	0.00	0.00
PCo8	0.20	0.00	0.00
PCo9	0.35	0.00	0.00
PCo10	0.33	0.60	0.82
PCo11	0.25	0.00	0.00
PCo12	1.00	0.00	0.00
PCo13	0.23	0.43	0.90
PCo14	1.00	0.00	0.00
PCo15	0.30	0.00	0.00
PCo16	0.30	0.00	0.00
PB1	0.30	0.39	0.30
PB2	0.18	0.00	0.00
PB3	0.60	0.30	0.10
PB4	0.10	0.00	0.00
PB5	0.10	0.00	0.00
PB6	0.10	0.00	0.00
PB7	0.10	0.00	0.00
PV1	0.10	0.00	0.00
PV2	0.48	0.70	0.48
PV3	0.30	0.00	0.00
PV4	1.20	1.50	0.25
PV5	0.15	0.00	0.00
PV6	0.20	0.00	0.00
PV7	0.20	0.00	0.00
PCa1	0.20	0.30	0.50
PCa2	0.20	0.30	0.50
PCa3	0.00	0.00	0.00
PCa4	0.00	0.00	0.00
PCa5	0.50	0.80	0.58
PCa6	0.53	0.60	0.15
PCa7	0.13	0.00	0.00
PCa8	0.10	0.00	0.00
MG1	0.50	0.00	0.00
MG2	0.23	0.00	0.00
MG3	0.43	0.63	0.47
MG4	0.40	0.60	0.50
MG5	0.40	0.60	0.51
MG6	0.60	0.65	0.08
MG7	0.60	0.33	0.86
MG8	0.20	0.00	0.00
CO1	0.43	0.45	0.07
CO2	0.43	0.53	0.26
CO3	0.73	1.00	0.38
CO4	1.31	0.00	0.00

The soil in this area mostly contains sufficient level nutrient such as ; C-organic and Nitrogen. As the result of decomposing organic material from litter or dead plants (Prayogo et al. 2021b; Sudharta el al., 2022; Mardiani et al. 2022). This material is important for supporting the growth of cellulolytic bacteria and other soil microbe. Soil moisture is also critical for bacterial growth, and different soil types will produce different bacterial communities (Ibrahim et al. 2018). To measure the activity of these bacteria, the researchers measured the clear zone on the CMC substrate for each isolate. Then, they selected the best isolate for each substrate by measuring the clear zone. The formation of a clear zone indicated that the isolate could degrade the cellulolytic component. Selected figures of these isolates collected from PCo plot were presented in Figure 2.

Therefore, to evaluate the ability of bacterial isolate to degrade certain cellulose substrates, their cellulase activity was determined on CMC media with Congo red. The three isolates (PCo1, PCo13, and PCo10) showed the highest Cellulolytic Index (IS) values compared to other bacterial isolates, producing an average clear zone of 1.11 mm, 0.90 mm, and 0.82 mm, respectively. These three isolates with high degradation on cellulose sources were PCo1, PCo13, and PCo10. The clear zone produced by the three bacterial isolates, caused by the reaction of Congo red, has a strong interaction with the β-1,4-glycosidic bond in a CMC solid medium (Arifin et al. 2019), making it possible to analyze their cellulase activity. The complete information of Cellulolytic Index (IS) was presented in Table 9. Figure 2 shows the Congo red isolates analysis of PCo1, PCo13, and PCo10 was successfully diluted cellulolytic material and became a good candidate for isolate which has an ability to degrade cellulose.

These bacteria contain endoglucanase and β-glucosidase cellulases that could degrade CMC, as it is soluble cellulose (Balla et al. 2022). Cellulose, a polysaccharide type, is a carbon component found in soil. Soil microbes contain cellulase, an enzyme that can convert cellulose into oligosaccharides. The potential for cellulase enzyme production in cellulolytic bacteria can be detected by forming clear zones around bacterial colonies on CMC selective media. The cellulolytic index is the ratio between the clear zone's diameter and the colony's diameter used to test cellulase activity (Faizah et al. 2020; Dewiyanti et al. 2022). The greater the cellulolytic index, the higher the potential for cellulase enzyme production. Microbes' ability to degrade cellulose is determined by their genetic potential and the different environmental conditions in which they grow and develop. In the tropics, cellulose is the most common organic polymer and a major source of glucose. However, its use as an energy source requires biodegradation, which the size of the clear zone can determine in a growth medium. Previously, six potential isolates consisted of A1E, A1K, A1D, A2A, A1I, and B1A with cellulolytic index were 1.13, 0.97, 0.93, 0.88, 0.82, and 0.82, respectively, were collected from Sago pits (Faizah et al. 2020) were comparable to the cellulolytic index of this study.

The test results of gram staining test showed that not all isolates tested were gram-positive bacteria, while some isolates were gram-negative bacteria. Gram-positive bacteria are characterized as having a purple color, which indicates that these bacteria can bind crystal violet. In gram-positive bacteria, the cell walls contain less fat, so they become dry due to alcohol treatment, the pore size and permeability of the cell walls are reduced and crystal violet is not washed away, which causes positive bacteria to

remain purple (Yusnia et al. 2019). Selected Cellulolytic from PCo which has high activity were follow gram staining test and the result were presented in figure 3. There was an indication that these strains belonging to *Bacillus* spp. based on their morphological characterization and cell shape.

Gram-negative bacteria are characterized by red morphology. This is caused by gram-positive bacteria which contain thicker peptidoglycan compared to gram-negative bacteria. Gram-positive bacteria have a blue color because this type of bacteria can form complex bonds with crystal violet dye because it contains thick peptidoglycan. The peptidoglycan of gram-positive bacteria is 90 µn, the rest is teichoic acid, while the peptidoglycan of gram-negative bacteria is around 5-20 µn, while the rest is polysaccharides (Ismail et al. 2017).

Moreover, the biplot method was utilized to analyze and interpret data from microclimate tests, canopy cover, litter thickness, c-organic soil, total soil nitrogen, litter quality, and total population of soil cellulolytic bacteria (Figure 4). Two vectors were used to form an angle which revealed the correlation between those variables. If the angle is less than 90 degrees, the correlation is positive, while an angle of 90 degrees indicates no correlation, and an angle greater than 90 degrees indicates a negative correlation. The proximity of parameters indicates that they share similar characteristics or a stronger correlation. This method helps understand the relationships between various parameters and their environmental effects. The PCA revealed that for the first axis (X) represented 44,8% of the varian and the second axis (Y) was 24,1% (Figure 4).

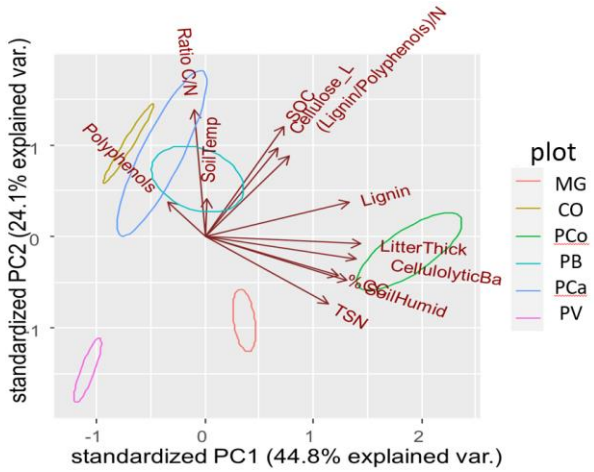

Figure 4. Principal component analysis

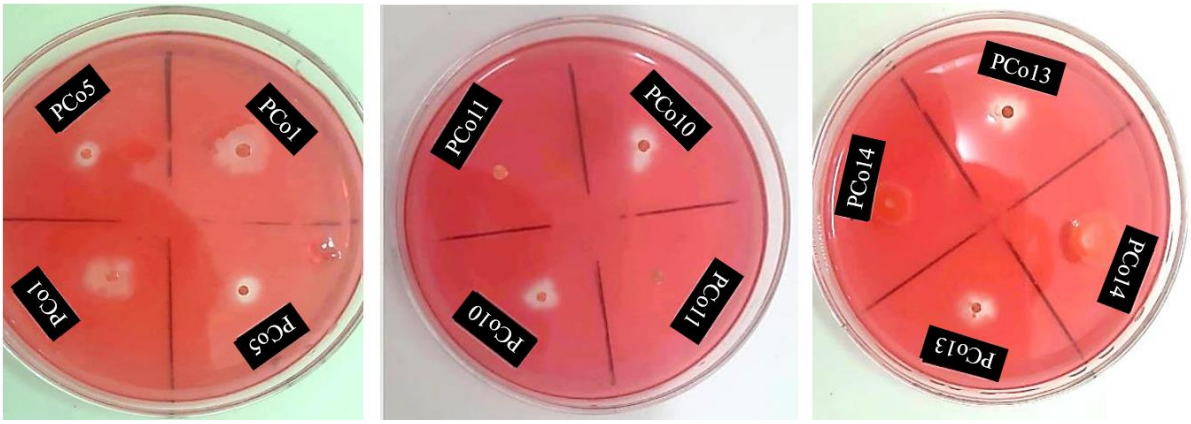

Figure 2. Selected soil cellulolytic bacteria isolates collected from PCo plot

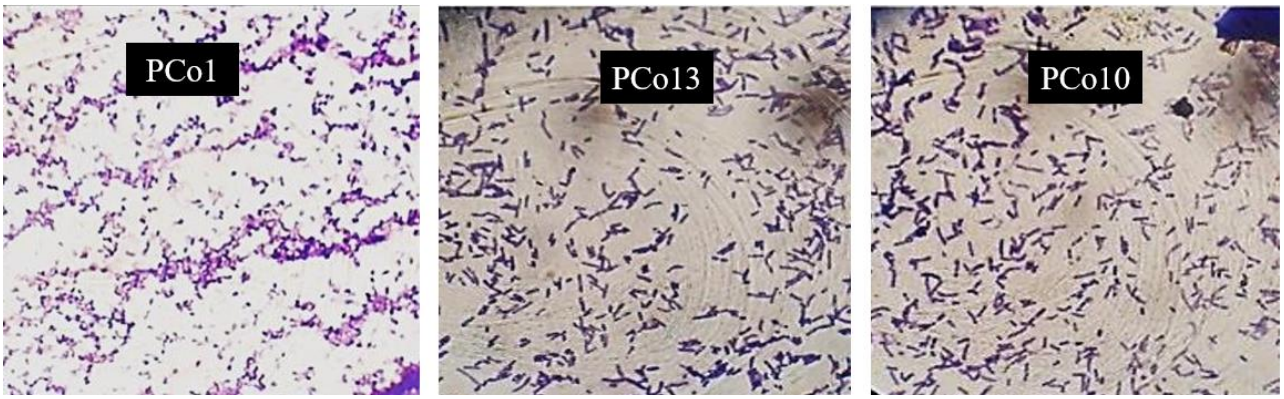

Figure 3. Selected gram staining test of cellulolytic bacterial isolates from PCo plot

The study found a positive correlation between soil temperature and litter quality, specifically polyphenols, (lignin+polyphenols)/N, and C/N ratios, with soil organic C (Figure 4). The higher soil temperatures and high-quality litter could increase soil organic C. It was also found that soil temperature was closely related to land use at the study site, with cultivated areas having higher soil temperatures than natural areas, which can impact soil organic carbon content (van Strateen et al. 2015). Moreover, the total soil cellulolytic bacteria population was positively correlated with soil moisture, crown cover, total soil nitrogen, and litter thickness (Yuan et al. 2015; Chen et al. 2018). Litter serves as a source of energy for soil microbes, and its accumulation can increase organic matter content, which in turn increases C accumulation on the soil surface (Azizah et al. 2023; Rinady et al. 2023). The study also found that land use with high sunlight intensity and soil temperature could slow decomposition; microclimate differences, particularly soil moisture, significantly impacted litter decomposition (Lee et al. 2014). PCo plots related to various microclimate datasets (soil temperature and humidity), lignin, canopy cover, litter thickness, total soil nitrogen, and total soil cellulolytic bacteria population, analyzed using PCA multivariate approaches are positioned on the middle far right and it was not overlapping with another plot. (Figure 4). In addition, PCa and PB overlap each other, indicating that there is no significant difference among them based on the above-selected parameter. In contrast, PV was located in the lower far right position, while the CO plot was found at far above position. Figure 4 shows that lignin has a strong relationship with lignin and the population of soil cellulolytic bacteria since they have almost similar directions and magnitude and the angle between those parameters was less than 90 degrees. Moreover, the data suggests that lignin has a positive correlation with all the observed parameters, including microclimate (soil temperature and humidity), litter quality (cellulose, polyphenols, (lignin+polyphenols)/N and C/N ratio), soil chemical properties (soil C-organic and total soil nitrogen), and total soil cellulolytic bacteria population. The soil C-organic was closely correlated with cellulose and the ratio of (Lignin/Poliphenol)/N, while the Polphenol has a strong correlation with C/N ratio. Notably, the decomposition of lignocellulosic plant materials in high-temperature environments and low humidity levels is relevant for the presence of microbes. Therefore, microclimate and litter quality are crucial factors that may influence lignin degradation. A recent study found that farmers' different land management practices can impact the canopy cover density, affecting the microclimate and soil microbial community. The type of litter in agroforestry systems can also impact the content of lignin, polyphenols, cellulose, and C/N ratios, which can affect the abundance of cellulolytic bacteria and cellulase activity in the process of litter degradation.

In conclusion, litter quality plays a crucial role in the microbial population and activity, which, in turn, impacts litter decomposition. The vegetation type in an area can affect litter quality, including parameters such as lignin, polyphenols, (lignin+polyphenols)/N, and C/N ratios. The community and activity of soil cellulolytic bacteria are also affected by these parameters, which, in turn, impact organic carbon in the soil. Finally, lower litter quality may inhibit the existence and activity of cellulolytic bacteria, leading to low soil carbon content. The type of litter in agroforestry systems can also impact the content of lignin, polyphenols, cellulose, and C/N ratios, which can affect the abundance of cellulolytic bacteria and cellulase activity in the process of litter degradation. Pine litter, for instance, is classified as low-quality because it has high lignin, polyphenol, cellulose, and C/N ratios. Therefore, to accelerate the pine litter decomposition process, three bacterial strains (PCo1, PCo13, and PCo10) were tested to degrade cellulose substrates by producing cellulase substrates on CMC media with Congo red. The highest IS (Cellulolytic Index) value was observed in these strains belonging to *Bacillus* spp. based on their morphological characterization and cell shape. Therefore, these three strains are suitable as bioconversion candidates for plant litter decomposition.

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