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Soil respiration and microbial communities across different farming management of shaded coffee and pines on agroforestry system

CAHYO PRAYOGO[1,2,♥], NOVI ARFARITA[3,4,♥♥], FITRIANA LUTHFININGSIH[5], NURULITA FAJRINA[5], NAYLIL AULA SHOLIH[5]

[1]Department of Soil, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran No. 1, Malang 65145, East Java, Indonesia.
Tel./fax.: +62-341-565845, ♥email: c.prayogo@ub.ac.id
[2]Research Centre on Biodiversity and Food Security IKN, Universitas Brawijaya. Jl. Veteran No.1, Malang 65145, East Java, Indonesia
[3]Department of Agrotechnology, Faculty of Agriculture, Universitas Islam Malang. Jl. Mayjen Haryono No. 193, Malang 65144, East Java, Indonesia.
♥♥email: arfarita@unisma.ac.id
[4]Research Study on Food Security, Universitas Islam Malang. Jl. Mayjen Haryono No. 193, Malang 65144, East Java, Indonesia
[5]Undergraduate Student, Department of Soil, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran No. 1, Malang 65145, East Java, Indonesia

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Abstract. *Prayogo C, Arfarita N, Luthfiningsih F, Fajrina N, Sholih NA. 2026. Soil respiration and microbial communities across different farming management of shaded coffee and pines on agroforestry system. Biodiversitas 27 (2): d270205. https://doi.org/10.13057/biodiv/d270205*. Small-scale shaded coffee and pine agroforestry has been recognized to have some advantages in ecological but it is vulnerable to climate change and yields sustainability. This study aimed to examine the impact of contrasting farmer management on soil properties, microbial respiration, and microbial structure and activities. The design of the experiment was a nested Randomized Complete Block Design (RCBD) of four contrasting farming management systems (LC, MC, HC, and BAU plots), consisted of three blocks of different position of sampling (zona A: 0.5 cm from coffee, B: 0.5 cm from pine, and C: in between coffee and pine stand, treated as nested sample), was within the size of 20 x 20 m, with 3 replicates in separate blocks, resulting 36 plots in total. Variables were measured: (soil C, N, C/N ratio, MBC, total/functional bacteria, respiration, coffee and pine litter, tree population, coffee production, and litter input). Data were analyzed using ANOVA, followed by DMRT (5%), and multivariate PCA, which were employed to group the treatments based on selected variables. The results showed that the HC plot, with the highest coffee population, produced the highest canopy cover, litter input, and the lowest soil pH, but it provided the largest total bacterial population and greatest soil respiration and MBC, which was significantly different from other plots. The least pine population at BAU plots increases soil C and N, contains 2 x higher N-fixing bacteria than treatment, and produces the greatest coffee yields. PCA revealed that there was a strong relationship between soil respiration and total soil bacteria population, soil MBC, soil C/N ratio and pine litter, significantly separating the different farmer management.

Keywords: Agroforestry, bacteria, litter, soil microbial biomass, soil respiration

INTRODUCTION

Coffee is a vital, globally traded commodity that sustains millions of small-scale farmers. As a climate-sensitive perennial crop, it is highly vulnerable to the impacts of climate change (Pham et al. 2019). Tropical smallholder coffee farmers contribute little to greenhouse gas emissions but are highly vulnerable to climate hazards due to weak infrastructure and development constraints (Pacillo et al. 2021; Rahn et al. 2025), but it could improve farmer incomes (Evizal et al. 2012, Evizal et al. 2016; Lisnawati et al. 2017; Suhartono and Widiyanto 2020). Tropical mountain regions of Indonesia generally implement agroforestry systems where coffee (*Coffea arabica*) and pine (*Pinus merkusii*) are cultivated together under shade management (Supriadi and Pranowo 2015; Suhartono and Widiyanto 2020; Prayogo et al. 2021a; Firmansyah et al. 2023). This system was also adopted by farmer at Universitas Brawijaya (UB) Forest, Indonesia, as an alternative way to optimize land resources since they had limited access to natural forest nearby and to improve their income without disrupting the existing vegetation, contrast with the open-shade coffee systems practiced widely in Latin America (Jha et al. 2014; Avelino et al. 2015; Bacon et al. 2017), vulnerable to climate change (Venancio et al. 2020; Byrareddy et al. 2021; Kath et al. 2021; Bracken et al. 2023), susceptible to pest and disease outbreaks (Lopez-Bravo et al. 2012; Guido et al. 2020; Mustari et al. 2021).

Shaded agroforestry systems, conversely, are resilient to environmental disturbances, recognized as potential strategies for mitigating climate change impacts on Arabica coffee through various ecological benefits (Toledo and Moguel 2012; Prayogo et al. 2021b; Fitch et al. 2022; Prayogo and Arfarita 2022). These systems minimize erosion, sedimentation, and nutrient loss in tropical environments (Sepúlveda and Carrillo 2015; Fitch et al. 2022). Nonetheless, questions remain about their long-term effectiveness under changing climatic scenarios (Lin 2007; Camargo 2010; Läderach et al. 2017; Gomes et al. 2020). Poor land management can speed soil degradation, disrupt microbial habitats, and increase warming impacts, reducing soil respiration and altering microbial structure and community composition in smallholder systems (Rohadi et al. 2013; Ebisa 2014).

Shaded agroforestry enhances soil nutrient availability through continuous organic matter input (Jha et al. 2014; Lopez-Rodriguez et al. 2015; Prayogo et al. 2021b) while preserving soil microorganism biodiversity (Rohadi et al. 2013; Prayogo and Arfarita 2022). Optimal coffee productivity occurs at light levels between 40-70% (Sobari et al. 2012; Lisnawati et al. 2017; Prayogo and Arfarita 2022), depending on farmer management, which affects microclimatic conditions (Seyersted et al. 2022). Limited studies have investigated management interventions like pruning or trimming pine and coffee branches, which could maintain the ideal condition of coffee production (Charbonnier et al. 2013; Lisnawati et al. 2017; Rowe et al. 2022). Pine shade trees regulate light intensity for coffee growth need to be evaluated (Charbonnier et al. 2013; Lisnawati et al. 2017; Firmansyah et al. 2023). The adoption of such practices remains low, particularly as pine trees become mature (Prayogo et al. 2021a, 2021b; Prayogo and Arfarita 2022). Information about how variations in tree population, canopy cover, and pruning management affect soil microorganism structure and activity is scarce (Seyersted et al. 2022). This system could create a competition for water and nutrient demand (Sobari et al. 2012; Charbonnier et al. 2013). Soil microorganisms, which influence carbon emissions through respiration, play crucial roles in nutrient cycling and soil fertility (Bertini and Azevedo 2022; Wu et al. 2024). Little is known about how coffee-pine agroforestry management affects yield, microbial diversity and function, soil respiration, and microbial biomass (MBC).

The relationship between shade-tree composition, microbial functional groups, and carbon sequestration is still unclear, and farmer practices alter microclimate and soil conditions that regulate microbial abundance (Saraswati et al. 2007; Yunus et al. 2017). Soil microbes are fundamental to ecosystem functioning, especially in carbon and nitrogen cycling (Balser and Firestone 2005; Bragazza et al. 2015; Yao et al. 2018) and plant health (Garbeva et al. 2004). Canopy management and shade-crop selection regulate soil biological processes (Kutos et al. 2014), in which microbial diversity is essential for controlling biochemical reactions. Their abundance and diversity reflect favorable soil conditions, including adequate organic matter, temperature, moisture, and nutrient availability (Chauhan et al. 2023; Liu et al. 2023; Chen et al. 2024). This study aimed to (i) assess soil microbial biomass and community composition, respond to different agroforestry management systems; (ii) identify soil and environmental factors influencing microbial community changes; and (iii) understand biogeochemical processes, in terms of the continuity of supporting nutrient demand for tree and crop, to sustain shaded coffee-pine agroforestry productivity.

MATERIALS AND METHODS

Study area

The study area is located within the Universitas Brawijaya (UB) Forest area, which is administratively included in the Tawangargo Village, Karangploso Sub-district, Malang, East Java, Indonesia (Figure 1) and the research plot is located in Sumbersari Hamlet. The coordinate locations of the observation points are shown in Table 1, and the visual characterization of the plot experiment is presented in Figure 2.

Table 1. Coordinate the location of the plot experiment

Plot	Agroforestry practices	Coordinate	Elevation (m asl)
1	LC1	07°49'34.65" S, 112°34'37.33" E	1206
2	LC2	07°49'36.31" S, 112°34'36.12" E	1205
3	LC3	07°49'37.94" S, 112°34'36.16" E	1204
4	MC1	07°49'40.57" S, 112°34'27.95" E	1201
5	MC2	07°49'42.08" S, 112°34'25.99" E	1200
6	MC3	07°49'40.87" S, 112°34'26.54" E	1199
7	HC1	07°49'45.47" S, 112°34'24.31" E	1195
8	HC2	07°49'45.25" S, 112°34'23.13" E	1198
9	HC3	07°49'47.26" S, 112°34'23.79" E	1190
10	BAU1	07°49'19.63" S, 112°34'49.25" E	1284
11	BAU2	07°49'19.17" S, 112°34'49.64" E	1290
12	BAU3	07°49'19.05" S, 112°34'48.01" E	1295

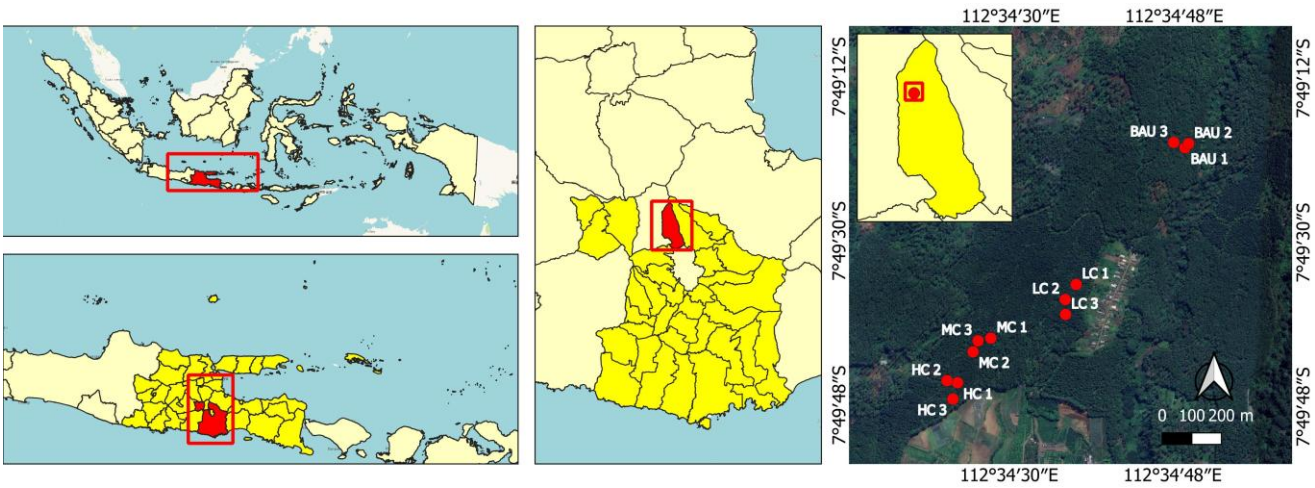

Figure 1. Study area in Tawangargo Village, Karangploso Sub-district, Malang, East Java, Indonesia

Figure 2. Farmer practice and management of coffee pines agroforestry system. A. LC (Low management of Coffee), B. MC (Medium management of Coffee), C. HC (High management of Coffee), D. BAU (Bussines As Usual), with low shaded of pines tree cover and high input coffee management, regular weeding and pruning)

In coffee and pine agroforestry in the UB Forest area of Sumbersari Village, farmers provide different management based on their interests. Soil particles in UB Forest were dominated by silt particles, which soil texture class of silty clay. The soil order in the UB Forest is classified as Inceptisol (indicated young soil), characterized by a cambic horizon with the dominant sub-group of Andic humudepts (74% of the entire UB forest). Soils formed from andic materials or volcanic ash generally have high silt content with abundant organic C, with low soil bulk density (<1 g cm^{-3}) (Kurniawan et al. 2019).

The average of monthly rainfall of the last 10 years in 2014-2024 (Figure 3) is categorized into C3 category according to the Oldeman Climate Classification, which has 6 months of wet months and 6 months of dry months. The average rainfall was 201.1 mm $month^{-1}$, equal to 2004.8 mm $year^{-1}$which was an ideal climatic condition for coffee to grow. Unlike smallholder coffee production in Toba region (North Sumatra), positioned closer to equatorial climate, which had received a greater amount of rainfall reached 2200 mm to 2676 mm of rain in a year (Dufour et al. 2019).

Research design and soil sampling

The observation plots used in this study contained pine and coffee plants stands, with the age of 4 years and 25 years, respectively, at the time of sampling. The observation plots were selected based on different management practices that affect the level of plant density. Management differences on the agroforestry land plots selected in this study consisted of: (i) LC (Low management of Coffee) with moderately shaded pine tree cover, low coffee density and no input coffee management, no weeding or pruning, no fertilization. (ii) MC (Medium management of Coffee) with medium shade pines tree cover, medium coffee density, medium input coffee management, fertilization was applied once using organic manure (20 kg per tree), (iii) HC (High management of Coffee) with high shade pines tree cover, high coffee and pines density, medium input coffee management, fertilization was applied once using organic manure (10 kg per tree), and (iv) BAU (Bussines As Usual) , with low shaded of pines tree cover and high input coffee management, regular weeding and pruning. In terms of the BAU plot, recieve a optimum dosage of NPK fertilizer at 200 kg ha^{-1} and received organic manure application (20 kg per tree).

Design of the experiement was used a nested Randomized Complete Block Design (RCBD) of four contrasting farming management systems (LC, MC, HC, and BAU plots) consisted of three blocks of different position of sampling (zona A:0.5 m from coffee, B:0.5 cm from pine, and C: in between coffee and pine stand, treated as nested sample), was within the size of 20 m x 20 m, with 3 replicates in separate blocks, resulting 36 plots in total. Soil sampling was conducted at various distances (zones) between different plants. Soil sampling in each zone is done compositely by taking at four points based on the cardinal directions (Figure 4). Zone A is the zone which is close to coffee stand (0.5 m), whilst Zone B is the zone close to the pine stand (0.5 m). Zone C is the zone between coffee plants and pine stand. Soil sampling collection was conducted to determine soil nutrient status (pH, C, N), soil biological properties (soil microbial biomass carbon (MB-C), soil respiration, and total/functional soil bacteria: (i.e., N fixing and soluble P).

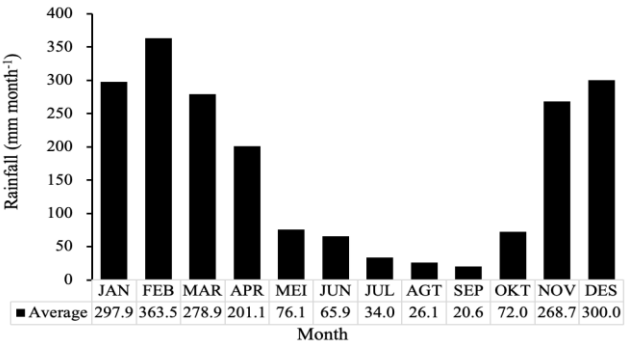

Figure 3. Average of monthly rainfall within 10 years (2014 to 2024) (Sources: bmkg.go.id)

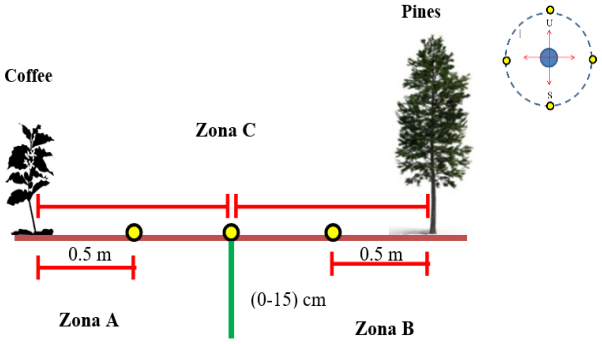

Figure 4. Design experiment of soil sampling location. : Point of soil sampling. Zona A: 0.5 m from coffee, Zona B: 0.5 m form pines, Zona C: between coffee and pines

Characterization of vegetation and litter measurement

Measurement of vegetation characteristics in each observation plot was carried out by calculating the total population, percent canopy cover, and Basal Area (BA). The BA measurement reflects the density of the stand, which is calculated based on the number of trees and tree diameter (determined by measuring the Diameter at Breast Height: DBH). The measurement of tree diameter was carried out by wrapping the metre at a height of 1.3 m from the ground (DBH), following the specified rules, to obtain the circumference value. The total population of coffee and pines was calculated using visual and manual calculation, and percent canopy cover was employed using the Canopy App software, which could taking vertical photograph and in which will be converted into canopy cover (%). Each plot (20 x 20 m) consisted of 400 images which were combined together before taking the average value. Basal area (BA) was determined using a formula derived from tree diameter as follows: Basal Area (BA) (m^2) = 0.00007854 × DBH^2 (diameter at the breast height:DBH, approximately 1.3 m from the ground) (Bettinger et al. 2017).

Sampling of litter in-situ at each point of observation was carried out using a 50 x 50 cm metal frame (Figure 5). Estimates of litter biomass in each plot were taken using a litter trap that collected the fallen tree leaves. The tool used was a litter net measuring 1 x 2.5 m. Litter traps were installed at a height of ±50 cm from the ground. Each observation location was installed with as many as 3 litter traps. Litter collection was carried out for 8 weeks. Litter fall caught in the net was collected every week, separated according to groups (branches, twigs, coffee leaves, and pine leaves) (Malik and Naharuddin 2023). The litter that had been taken was then weighed by wet weight using an analytical balance, then put into a paper envelope and dried for 48 hours at 80°C.

The Litter productivity per plot of each observation was calculated using the equation as follows:

$$Xi = \frac{Xi\frac{n}{i=1}}{n} gr/m^2/mg$$

Where, Xi: Average litter production per plot per period (week), X: Litter production per plot each period, n: Number of traps. The result is then multiplied by 4000 to determine the weight of litter input in ha^{-1} (Malik and Naharuddin 2023).

Soil nutrient analysis and physical properties

Soil nutrient assesment whereas soil sample was air-dried before use as fully mixed, naturally air-dried, ground, and passed through a 2 mm sieve before the following tests. such as: Soil organic Carbon was determined using the Walkey and Black method, soil N-total by the Kjedahl method, soil pH was determined using pH meter. Soil bulk density and moisture content were measured by the ring method and drying method (gravimetric method), respectively. Soil bulk density values were determined using the ring method. Undisturbed soil using a stainless steel coring ring (50 mm internal diameter and 50 mm length) from each plot at 0-20 cm depths. Soil cores were oven-dried at 105°C for 24 hours (Nelson and Sommers 1996; Moebius et al. 2007). Bulk density was calculated by dividing the mass of oven-dried soil by the core volume and gravimetric moisture content was calculated as the mass of water in the soil sample per mass of the oven-dried soil (g g^{-1}). Soil particle density (cm^3 cm^{-3}) was obtained by multiplying gravimetric moisture content (g g^{-1}) by soil bulk density (g cm^{-3}). Soil texture was employed using the pipette method, collected at a depth of 0-20 cm. The procedure included air-drying, grinding and sieving the soil samples to <2 mm (fine earth), before 20 g of this fine earth was taken for analysis. Dispersion was done overnight after OM had been removed, using an end-over-end rotator with 25 mL dispersant and diluted with deionized water to reach 1 L. The soil particles <100 mm were transferred to a 1-L cylinder for subsequent pipette analysis. This portion was divided into four size classes of <2, 2-20, 20-53 and 53-100 mm, within which the former three were directly determined by pipette (Nelson and Sommers 1996; Gee and Or 2002; Moebius et al. 2007).

Soil microbial biomass measurement

Soil microbial biomass was calculated by the Chloroform Fumigation Incubation (CFI) method. Soil samples were dried to 40% field capacity and sieved using a sieve with a pore size of <2 mm. Maintaining soil water Field Capacity (FC) during soil incubation requires consistent moisture monitoring, by adding distilled water weekly to compensate for evaporation, sealing containers with plastic wrap to minimize water loss, and periodic weighing of samples to calculate and replace lost water (Vance 1987; Franzluebbers et al. 1996). 10 g of aerated soil subsamples were fumigated for 24 hours with 30 mL of alcohol-free $CHCl_3$ in a desiccator. After the incubation period, the $CHCl_3$ was removed from the desiccator and defumigated for 24 hours, repeated 3 to 4 times. Soil samples were then extracted with 50 mL of 0.5 M K_2SO_4. The same was done for the same soil subsample, but without fumigation and defumigation treatments. The soil extract was filtered with Whatman paper no. 45. The total microbial biomass carbon (MB-C) in the extract of the soil subsample was calculated by the wet titration method (dichromate), at the same time with the same sample, soil C-Organic was also determined (Vance 1987; Franzluebbers et al. 1996). The soil microbial

biomass-C per gram of soil of each observation was calculated by the equation as follows:

$$S,C = Organic\ C\ (S,C) \times (\frac{V}{B})$$

$$Microbial\ biomass\ C\ (\mu g\ g^{-1}) = \frac{S-C}{0.038}$$

Where, S: Value of Organic-C extract sample with chloroform, C: Value of Organic-C extract sample without chloroform, V: Volume of total extraction sample (mL), B: Weight of soil sample (g), 0.38: factor conversion of C to Microbial-C) (Vance et al. 1987).

Soil respiration measurement

Measurement of soil microorganism activity is done to determine how much soil microorganisms do respiration, which produces CO_2. The method used was the jar/chamber method, and was measured by the titrimetric method (Rochette and Hutchinson 2005; Mewhort et al. 2020). Respiration measurements were made using a PVC chamber with a diameter of 25 cm and a height of 30 cm, and then two small bottles containing 30 mL of 0.2 N KOH and 10 mL of distilled water. After that, the chamber was closed airtight and incubated at a temperature of about 28-30°C for 10 days.

At the end of the incubation period, the amount of CO_2 produced by the titration method was determined by adding 2 drops of phenolphthalein to the bottle containing KOH. Then, the solution was titrated with HCl until the red color disappeared. Record the volume of HCl used. Then, 2 drops of methyl orange indicator and titrated with HCl until the yellow colour turned pink. The volume of HCl used then was recorded. The amount of HCl used in the second stage of the titration is related to the amount of CO_2 produced by the microorganisms. The soil respiration per gram of soil of each observation was calculated by the equation as follows (Rochette and Hutchinson 2005; Mewhort et al. 2020):

$$r = \frac{(a-b) \times t \times 120}{n}$$

Where, a: mL HCl of the soil sample, b: mL HCl of the control, t: Normality of HCl, n: Time (days) for incubation, r: Number of C-CO_2 produced in each g of soil per day.

Analysis of soil bacteria population

The isolation was conducted by dispersal technique requires media as a place to grow microbes using the TPC (total plate count method) method. In this case, the media used are three types of media. James Nitrogen Free Malate Bromothymol Blue (JNFB) media, was used for nitrogen-fixing microbial screening media, and Pikovskaya was employed for screening P-solubilising bacteria (Ikhwani et al. 2021). The methods begin with serial dilutions. Composite soil that has been taken from each location was weighed at 50 g to be put into an Erlenmeyer, add distilled water was added until it reached 500 mL. Soil was shaken with a shaker for 20 minutes so that the particles are released in the water, then the suspension for 30 seconds. Before dilution, it is necessary to sterilise the tools and materials first using an autoclave (Alexander 1977; Dobereiner 1995; Wibowo 2012). The dilution series is divided into two, namely bacteria 10^{-5} and fungi 10^{-4} dilutions, namely 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. For the 10^{-1} series, a test tube was prepared that was filled with 9 mL of sterile water and then plugged with cotton wool and sterilized it in an autoclave. Suspense that has been precipitated is taken 1 mL to put it in a test tube containing 9 ml of water, and shaken. For the 10^{-2} series, 1 mL was taken from the 10^{-1} series and added it to a test tube containing 9 mL of water, and repeated so on until the 10^{-5} series (Alexander 1977; Dobereiner 1995; Wibowo 2012). To obtain the total population of soil bacteria the following formula was used:

$$Total\ population\ (CFU/mL) = \frac{No\ of\ bacteria\ colony \times Total\ dilution\ factor}{Volume\ of\ culture\ plate}$$

Statistical analysis

Data was checked for normality before the ANOVA was employed to determine the significant effect of the treatment (different farmer management), which became the factors for those analyses, following the DMRT test at the level of 5%. Sampling position (A, B and C) are nested variable in each treatment, while multivariate analysis of PCA and Biplot was used for clustering the impact of different agroforestry management based on selected variables (i.e soil C, N, C/N ratio soil N fixing bacteria and soluble P, bulk density, pH, MBC, respiration), processed using Genstat Software Ver 12.

RESULTS AND DISCUSSION

Soil characteristics

The results of the soil chemical analysis of 0 to 20 cm (topsoil) showed that the average percentage soil organic carbon in the four observation plots ranged from 4.62% to 6.69% and fell into the high and very high categories, and was significantly different across the plots ($p<0.05$). The highest average C-organic value was found in the BAU plot (6.53%), while the lowest was found in the MC plot (4.79%) (Table 1). The average N-total value in the observed plots ranged from 0.42% to 0.63%. The N-total values in the LC, MC, and HC plots were 0.42%, 0.44% and 0.46%, respectively, and were classified as medium, while the N-total value in the BAU plot (0.63%) was in the high category. The ratio of carbon and nitrogen in soil is expressed as the C/N ratio. Analysis of soil C/N ratio values in the four observation plots showed that the highest average soil C/N ratio was found in the HC plot (11.88), while the lowest was found in the BAU plot (10.42). The C/N ratio indicates the level of decomposition of organic matter. For comparison, soil organic content at a depth of 0-10 cm of the coffee agroforestry system in Lampung was 2.45 (Ramadhani et al. 2024), which was lower than the value of C-organic in this study. Meanwhile, soil C-organic at smallholder coffee plantations in Srimulyo Village, Dampit, was recorded at 1.59 to 2.93% (Khoirunnisak et al. 2023). In terms of total nitrogen content at 0 to 10 cm, the coffee agroforestry system only accumulates 0.19% which was 4 times lower to the value of total soil N in this

experiment, but the value of C/N ratio was in the opposite, 3 times lower than in Lampung (Ramadhani et al. 2024), indicated that soil organic material in this plot was much more decomposed. In general, soil texture in all the plots was classified as silty loam.

The average soil pH value was highest in the BAU plot (5.16), while the lowest was in the HC plot (4.98). The results of pH measurements on the four observation plots show that the pH of the observation plots is included in the acidic category (pH = 4.5 - 5.5). Another study showed that soil pH at 0-10 cm of AFS under various shading trees ranged between 5.54 and 5.83 (Colombo et al. 2023). The low pH value in the research plots can be caused by the presence of a thick layer of in situ pine leaf and coffee leaf at the HC plot, which reaches to be at 500 g m^{-2} and 16 g m^{-2}, respectively, causing the soil to become acidic. A thick layer of organic matter that undergoes decomposition causes acidification of the soil in the short term due to the production of organic acids, resulting in lower soil pH, because decomposition in the process releases functional groups and organic acid deposition that releases hydrogen in the soil. The release of H^+ that occurs in the soil causes the soil to become acidic. Soil pH did not differ significantly between organic and conventional agroforests in Costa Rica AFS (Tully et al. 2013). The highest soil C-organic at the BAU plot (6.53%) significantly reduced the value of soil bulk density to about 0.53 g cm^{-3}. The value of soil bulk density in this study was lower than that in various locations in Costa Rica, which ranged from 0.92 to 1.0 g cm^{-1} (Tully et al. 2013).

Tree population, basal area biomass, canopy cover, and coffee productivity

The total pine population showed that the LC plot had the highest tree population (946 tree ha^{-1}), which was significantly different from the others. The lowest number was found in the BAU plot (342 tree ha^{-1}), which was only 1/3 of the tree population at LC plot. On the other hand, the HC plot recorded the highest total coffee population (2679 tree ha^{-1}) while the MC plot had the lowest (1713 tree ha^{-1}) (Table 2). The high tree population was in accordance with total tree basal area, the lower density of pine, and a greater number of coffee population exceeded coffee production, in accordance with lower canopy cover. For comparison, a density of smallholder coffee farming (*C. arabica* var. Sigara Utang) in Toba region North Sumatra, which accounts for about 9.7% of Indonesian coffee export volumes (Susila 2005), reached 1666 plants ha^{-1} (2 m spacing between rows and 3 m between plants) (Dufour et al. 2019). This was comparable to the coffee density at LC and MC plot in this study, but lower than the coffee tree density at HC and BAU plot.

The results of canopy measurements showed that the HC plot had a canopy density of 78.2% (Table 2). The lowest canopy density was found in the BAU plot, which was 64.6%, which contributed to the higher coffee production. Canopy density in the observation plots can be caused by differences in the number of plant populations in each observation plot. Previous study on agroforestry system, which is dominated by the species of banana (*Musa* sp.), teak (*Tectona grandis*), and sengon (*Paraserianthes falcataria*) reported to have canopy cover ranged between 8% to 17.12%, which was 7 times lower than that of the canopy cover in this study (Hartoyo et al. 2020). Shade cover of coffee agroforestry system in Southern Columbia ranged from 3 to 67%, which had a dominant shade trees are the leguminous tree genera (Bosselmann et al. 2009), revealed different shade effects on coffee quality in the two areas. The high canopy covers above 60% with low management significant reduce coffee productivity at North Sumatra which remains low (1139 kg green coffee/ha/year) compared to that of Aceh (1568 kg green coffee/ha/year) (Saragih 2013). This productivity was comparable to those productivities at LC plot in this study (1.3 t ha^{-1}) (Table 2).

Table 1. Soil characteristics across different agroforestry practices

Types of agroforestry practices	Soil organic-C (%)	Soil total N (%)	Soil C/N ratio	Soil pH (H_2O)	Soil bulk density (g cm^{-1})	Soil particle density (g cm^{-3})	Soil texture
LC (Low management of Coffee)	4.96 a	0.44 a	11.23 ab	5.08 a	0.76 b	2.16 a	Silty loam
MC (Medium management of Coffee)	4.79 a	0.42 a	11.34 ab	5.02 a	0.77 b	2.1 a	Silty loam
HC (High management of Coffee)	5.48 ab	0.46 a	11.88 b	4.98 a	0.87 b	2.05 a	Silty loam
BAU (Bussines As Usual)	6.53 b	0.63 b	10.42 a	5.16 a	0.53 a	2.02 a	Silty loam

Note: Numbers accompanied by the same letter in the column show observations not significantly different at 5% DMRT

Table 2. Tree population, basal area biomass, canopy cover, and coffee productivity across different agroforestry practices

Types of agroforestry practices	Pines population (tree ha^{-1})	Coffee population (tree ha^{-1})	Pines basal area (m^2 ha^{-1})	Coffee basal area (m^2 ha^{-1})	Canopy cover (%)	Fresh weight of coffee production (t ha^{-1})
LC (Low management of Coffee)	946 c	1850 a	36.22 b	1.09 a	70.26 a	1.3 a
MC (Medium management of Coffee)	717 b	1713 a	29.04 a	1.46 a	71.74 ab	2.2 a
HC (High management of Coffee)	729 b	2679 b	32.40 a	1.74 a	78.20 b	4.5 b
BAU (Bussines As Usual)	342 a	2454 b	30.24 a	1.18 a	64.61 a	8.4 c

Note: Numbers accompanied by the same letter in the column show observations not significantly different at 5% DMRT

The highest coffee production was recorded at the BAU plot at 8.4 t ha^{-1}, and the lowest was detected at the LC plot at 1.3 t ha$^{-1,}$ which was 6.5 times lower than BAU yields (Table 2). For comparison, during the 2017/2018 growing season in Brazil, coffee beans grown in full sun yielded the highest production at 3576 kg ha^{-1}, agroforestry systems (AFS) with *Moringa oleifera* and *Croton floribundus* produced intermediate yields of 3006 kg ha^{-1} and 2128 kg ha^{-1}, respectively, while AFS with *S. macranthera* had the lowest yield at 1881 kg ha^{-1}. A strong positive correlation exists between solar radiation and coffee productivity, suggesting that denser shading adversely affects grain production (Colombo et al. 2023). This coffee production was within the range of coffee productivity in this study. In terms of shading condition, the ideal canopy cover was around 50%, so that the condition of all plot in this study was beyond that ideal range and facing various consequences for achieving optimum yield. Soto-Pinto et al. (2000) find negative effects when shade density is above 50%. The benefits of shade are explained primarily by a reduction of heat-induced stress in the plant and a lengthening of the maturation period of coffee berries. Coffee with shading is reported to produce yield at 2.47 t ha^{-1} which was higher than without shading system in Aceh Tengah (1.86 t ha^{-1}), the farmer's plant *Citrus maxima*, *Leucaena leucocephala*, and *Mangifera indica* as shade trees of coffee. In the production phase, 30% of coffee plant growth is influenced by genetic traits, while 70% is influenced by environmental factors, mainly light intensity (Samsuri et al. 2021). Coffee shade is a strategy to adapt with the variability of environtmental and global climate changes. Shade trees protect the coffee from the high temperature on coffee understorey. maintaining water availability, nutrient demand and microbial habitat.

Litter input

There was a significant ($p<0,05$) impact of type of agroforestry management and position of sampling (zone A, B and C) on pine and coffee litter, along with weekly input. The highest pines litter and weekly input of litter was found in the HC plot, which was significantly different from the other plots, while the lowest input of litter was found in the LC plot (Table 3). The lower pines litter at the BAU plot was due to less pines population and lowest canopy cover.

In agroforestry systems, nutrients are sourced from rainfall, fertilizers, and organic residues, which accumulate in shade trees, crops, soil, or litter. Additionally, significant interactions occur among crop layers through processes such as residue deposition and mineralization (Aldana et al. 2019). Farfan and Urrego (2007) provided evidence in their study of *Cordia alliodora, Pinus oocarpa*, and *Eucalyptus grandis* within Colombian coffee agroforestry systems, indicating that nitrogen and organic carbon concentrations were higher in the shade coffee system. *Cordia alliodora* exhibited the highest concentration of nitrogen (C/N) ratio: 18,1, whereas *P. oocarpa* had the highest organic carbon content but the lowest nitrogen levels, resulting in a higher C/N ratio (73.9), meaning that the decomposition process was not fully realized in the Pines environment. The C/N ratio of the litter in this study was between 10 and 11, which means that those value was lower than optimum bacterial growth, whereas the C/N ratio should be in the range of 20:1-30:1 with a fixed ratio of 25:1 (Panigrahi and Brajesh 2019).

Total soil bacteria, N fixing and soluble P

Measurement of total bacterial abundance was conducted to determine the total number of bacteria in soil with different agroforestry practices. The results of the total bacteria analysis are shown in Table 4. The ANOVA analysis results showed that there was a significant ($p<0.05$) effect of different management on total soil bacteria. The highest total number of bacteria was found in the HC plot at zone A with 4.89 CFU x 10^5, while the least was found in the LC plot zone C with 1.56 CFU x 10^5. This was due to the HC plot consisting of higher canopy cover (78%) and greater pine and litter accumulation, along with higher total weekly litter input. In general, the higher population of soil bacteria was obtained from locations close to the pine and coffee stand (Zones A and B) compared to Zone C. Higher accumulation of litter provides an ideal condition for soil microorganisms to grow better.

Table 3. Litter input across different agroforestry practices

Types of agroforestry practices	Sampling position	Dry weight of Pines litter (g m^{-2})	Dry weight of Coffee litter (g m^{-2})	Dry weight of branches (g m^{-2})	Litter fall input (g ha^{-1} week^{-1})
LC (Low management of Coffee)	Zone A	223.4 b	7.7 ab	30.1 a	527.5 ab
	Zone B	228.8 b	6.5 ab	36.7 a	481.5 a
	Zone C	202.2 ab	3.7 a	45.5 a	486.7 a
MC (Medium management of Coffee)	Zone A	164.0 ab	13.7 ab	58.9 a	504.9 a
	Zone B	203.6 ab	17.0 ab	58.0 a	694.0 b
	Zone C	230.1 b	15.7 ab	84.8 ab	566.0 ab
HC (High management of Coffee)	Zone A	458.1 c	20.9 ab	48.8 a	984.4 c
	Zone B	544.1 d	16.3 ab	44.3 a	1063.0 c
	Zone C	428.6 c	23.8 cd	96.9 ab	1017.0 c
BAU (Bussines As Usual)	Zone A	132.4 a	27.3 b	83.5 ab	524.6 ab
	Zone B	197.3 ab	16.9 ab	141.6 b	677.95 b
	Zone C	150.1 ab	15.1 ab	107.7 ab	665.4 b

Note: Numbers accompanied by the same letter in the column show observations not significantly different at 5% DMRT

Table 4. Structure and population of total soil bacteria, N fixing and soluble P

Types of agroforestry practices	Sampling position	Total soil bacteria (CFU x 10^5)	Total soil N-fixing bacteria (CFU x 10^5)	Total soil soluble P bacteria (CFU x 10^5)
LC (Low management of Coffee)	Zone A	2.31 b	1.17 a	1.0 ab
	Zone B	3.20 bc	1.27 ab	1.2 ab
	Zone C	1.56 a	1.32 ab	0.74 a
MC (Medium management of Coffee)	Zone A	2.10 b	1.22 ab	2.28 b
	Zone B	3.9 bc	1.53 ab	1.37 ab
	Zone C	1.77 a	1.36 ab	2.23 b
HC (High management of Coffee)	Zone A	4.89 c	1.75 ab	1.44 ab
	Zone B	2.87 b	1.83 ab	1.18 ab
	Zone C	3.18 bc	1.50 ab	1.48 ab
BAU (Bussines As Usual)	Zone A	2.00 b	2.32 b	2.42 b
	Zone B	2.86 b	2.22 ab	1.54 ab
	Zone C	1.97 ab	1.96 ab	1.68 ab

Note: Numbers accompanied by the same letter in the column show observations not significantly different at 5% DMRT

ANOVA revealed that total nitrogen-fixing bacteria showed significant difference ($p<0.05$). Based on Table 4, it can be seen that the total nitrogen-fixing bacteria in the BAU plot at zone A have the highest value (2.32 CFU x 10^5), while the LC plot at zone A has the lowest value (1.17 CFU x 10^5). The highest total soluble P bacterial results were in the BAU plot zone A at 2.42 CFU x 10^5, which was significantly different from other plots ($p>0.05$), while the lowest bacteria were in the LC plot zone C at 0.74 CFU x 10^5. It seems that there was an indication that functional bacteria, such as the nitrogen-fixing group, prefer a condition which highly associated with greater soil nitrogen content and a lower soil C/N ratio.

The ratio of N-fixing bacteria to total soil bacteria was highest in BAU plot zone A at 11.58% and lowest in HC zone A plot at 3.59%. The ratio of soluble P bacteria to total soil bacteria was highest in the MC zone C plot at 12.60 and lowest in the HC zone A plot at 2.95%. Based on these results, it can be seen that the highest total bacteria are found in the BAU plot zone A . This can occur because the soil chemistry in the BAU plot is more favourable for bacterial growth, both nitrogen-fixing bacteria and phosphate-solubilising bacteria. In addition, zone A is a soil sampling point that is 50 cm away from the coffee stand, which indicates that the zone has a lot of root tree biomass, root exudate, and fertilizer application. Fertilisation in the BAU plot has been used regularly using a combination of organic manure (chicken/goat manure and NPK) and regular weeding. Very limited information of fungal and bacterial population under shaded coffee system, if any, there was a study in Western Ghats, India which inform that of the two species of coffee, Arabica occupied more AM fungi, bacterial population, N fixers, P solubilizers and cellulose decomposing organisms while Robusta coffee were dominated by fungi and actinomycetes, coffee grown under two shade tree species supported higher population of all microorganisms (Bagyaraj et al. 2015). So, the role of the shaded coffee system is important for maintaining soil microbes structure and composition and at the same time it conserves soil nutrients.

Soil respiration and soil microbial biomass-C

Based on ANOVA, there was a significant impact of different farming management ($p<0.05$) on soil respiration. Soil respiration at HC plot ranged between 579.34 and 730.27 kg C-CO_2 ha^{-1} day^{-1}), which was more than 2 times higher than BAU plot, as the plot which produced the lowest soil respiration. This was due at the BAU plot received the lowest pine litter accumulation, leading to a low amount of soil bacteria population and low soil respiration. Generally, the greater quantity of soil respiration was identified under the zones A and B, whereas this zone was under the canopy of coffee or pines tree.

The highest average result of microbial biomass-C was found in HC plot with an average of 1021.16 µg C g^{-1} and significantly different ($p<0.05$) from the other three observation plots, while the lowest microbial biomass-C was found in LC plot with an average of 588.74 µg C g^{-1} (lower 50% than HC). The effect of sampling location on the four locations of the observation plots did not make a difference to the microbial biomass-C (MBC). The highest microbial Biomass-C was found in zone B (50 cm close to the pine tree) with an average in all plots of 820.07 µg C g^{-1}, while the lowest was in zone C, which was 553.66 µg C g^{-1}. When described further, in zone A, the highest microbial biomass-C was found in plot HC (1221.67 µg C g^{-1}) and the lowest mean was found in plot LC (545 µg C g^{-1}). In Zone B, the highest microbial biomass-C was found in the HC plot (1015.72 µg C g^{-1}) while the lowest was found in the LC plot (650.59 µg C g^{-1}). In Zone C, the microbial biomass-C was highest in the HC plot (826.10 µg C g^{-1}) and the microbial biomass-C was lowest in the BAU plot (553.15 µg C g^{-1}).

Table 5. Soil Respiration and soil microbial biomass

Types of agroforestry practices	Position of sampling	Soil respiration (kg C-CO_2 ha^{-1} day^{-1})	Soil microbial biomass C (µg C g^{-1} $soil^{-1}$)
LC (Low management of Coffee)	Zone A	368.60 bc	535.3 ab
	Zone B	422.41 c	650.5 b
	Zone C	456.67 cd	570.3 ab
MC (Medium management of Coffee)	Zone A	333.71 b	657.8 b
	Zone B	363.92 bc	995.6 d
	Zone C	333.36 b	479.8 a
HC (High management of Coffee)	Zone A	584.10 d	1221.0 e
	Zone B	730.27 e	1015.0 d
	Zone C	579.34 d	826.1 c
BAU (Bussines As Usual)	Zone A	254.44 a	567.1 ab
	Zone B	265.43 a	676.0 bc
	Zone C	282.81 ab	519.7 ab

Note: Numbers accompanied by the same letter in the column show observations not significantly different at 5% DMRT

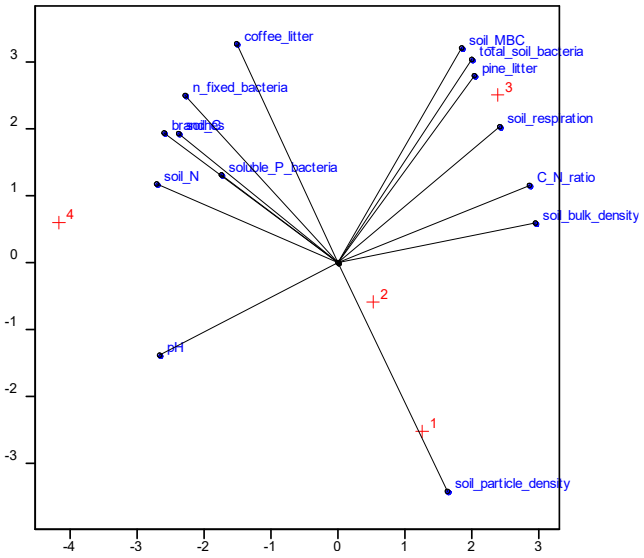

Figure 5. Multivariate PCA and biplot across different agroforestry management

Microbial biomass is a vital source of soil organic matter necessary for microbial respiration. In the agroforestry system, the tree provides soil protection, keeping the soil temperature more stable and at milder and more suitable levels for the growth of microorganisms in the soil. In contrast, from the previous study, regardless of the sampling site (0.7 m or 1.0 m away from the tree stand), there was no difference in soil respiration (BSR). Possibly, there was the influence of weeds in the coffee inter-row in all treatments, which provides greater nutrient cycling through the addition of organic material, greater protection of the soil from solar radiation, increasing biological activity, and soil resilience (Colombo et al. 2023). Furthermore, with high litter production and decomposition rates, elevated levels of soil organic carbon (SOC) and microbial biomass are observed, resulting in improved microbial activity. Coffee agroforestry systems significantly enhance soil characteristics by promoting the diversity and functional structure of microbial communities, but those consequences of high microbial activity will be followed by greater soil respiration, which lead to potential for the increasing global carbon emissions, but it will give a benefit for improving below ground carbon pool when those soil microbes die.

Soil MBC values from different AFS shading trees in Londrina, Brazil, was between 299 µg C g^{-1} to 352 µg C $g^{-1,}$ sampling at 0.7 m from the coffee stand, and when samples were collected from a position of 1 m from the coffee stand, the MBC ranged from 301 to 419 µg C g^{-1}. It can be that soil microbial carbon biomass was higher ($p<0.05$) at 0.7 m than at 1.0 m (Colombo et al. 2023). This phenomenon is attributed to the closer root systems and increased self-shading of coffee trees, which create milder temperature conditions favoring microbial growth. The microbial biomass of nitrogen is unaffected by shading or soil location, while full-sun coffee trees consistently show lower microbial biomass carbon than shaded ones (Guimarães et al. 2017). In the coffee-araucaria agroforestry system, shading does not impact microbial group distribution, microbial biomass carbon, microbial activity, or metabolic quotient (Melloni et al. 2018). The trees in this system provide vital soil protection, maintaining a stable temperature that promotes the growth of soil microorganisms. In this study, soil MBC was significantly ($p<0.05$) lower under the pines stand compared to the coffee stand, and the lowest was identified from the sample collected in between. Based on the results of soil respiration analysis, the highest respiration value was obtained in the HC zone B plot with a value of 730.27 kg C-CO_2 ha^{-1} day^{-1}, while the lowest value was in the BAU zone A plot, which was 254.42 kg C-CO_2 ha^{-1} day^{-1}. When linked to total bacteria, the respiration value is directly proportional. High total bacteria cause soil respiration to increase. This is because soil respiration is obtained from the activity of microorganisms in the soil, including bacteria. The BAU plot had low respiration values, but high total N-fixing bacteria and P-solubilizing bacteria. This could be because respiration is not only influenced by bacteria, but also by other microorganisms. The presence of N-fixing bacteria is 11.58% and P-solubilizing bacteria is 12.08% of the total bacteria, so it does not have a significant effect on the amount of respiration.

Relationship amongst the variables

Multivariate PCA and Biplot were adopted for clustering and positioning across the treatments based on selected variables, based on the angle position, magnitude and similar direction of those parameters, and the result was presented in Figure 5. PC1 represented 76.5% and PC2 occupied 23.5% to separating the impact of different farming management in coffe pines agroforestry system. In terms of soil C and N, the variable that had a strong relationship is the amount of coffee litter, which also provides a good environment for nitrogen-fixing bacteria and soluble P bacteria to nurture and be well adapted to this condition, On the other hand, pine litter was in accordance with the rise of total soil bacteria, exceeded soil microbial biomass and soil respiration along with the greater C/N value. It also can be concluding that the type of agroforestry was separated one to another, in which the LC plot (no 1) was positioned in lower far below, under the MC plot (no 2), whilst the HC plot (no 3) was located in the right above which is in the opposite of the BAU plot (no 4) (Figure 5). Previously, Canonical Correspondence Analysis (CCA) technique was used to identify patterns of association between environmental variation and species distribution within the agroforestry ecosystem, evaluate the relationships between bird and coffee insect species with environmental factors (Kurnianto et al. 2025) and it providing valuable insights into ecosystem dynamics within agroforestry (Jiang et al. 2014). Principal component analysis (PCA) biplot methods has been successfully to distinguish the distribution of 150 coffee accessions based on agro-morphological parameters, bioactive compounds, and antioxidant activity, with accessions grouped by geographic origin base on selected variable, in which PC1 (26.7%) represented a morphometric axis, separating accessions with larger and heavier beans (negative PC1) from those with smaller seeds. PC2 (17.0%) captured a functional axis, with strong positive loadings for total phenolics, total flavonoids, and antioxidant activity, indicating higher bioactive levels in accessions with elevated PC2 scores (Cueva-Carhuatanta et al. 2026)

In conclusion, the different agroforestry practices modified soil properties and litter input accumulation, which then affected on soil respiration as an indicator of soil microbial activity. The HC plot, which was predominantly by Coffee population at 2679 tree ha^{-1}, resulted the highest canopy cover (78%). The greatest amount of weekly litter accumulation (1021,46 g ha^{-1} $week^{-1}$) which was obtained from this plot, led to the lowest soil pH (4.98). However, this condition creates a largest total bacterial population (3.65 CFU x 10^5), soil respiration (631.23 kg CO_2 ha^{-1} day^{-1}), and soil microbial biomass carbon (1020 µg C g^{-1} $soil^{-1}$). In contrast BAU plots which less pine stand, accumulated almost 2 x higher N-fixing and P-solubilizing bacteria than other plots and produced the highest coffee yield, under the canopy cover at 64.6%. Sampling location under canopy cover received more organic input which affect soil respiration, microbial biomass and functional microbe. Soil respiration and microbial biomass were determined by the position of sampling, in which the location under the tree canopy was indicated to have a higher population of total and functional bacteria (N-fixing and soluble P). The PCA analysis showed a significant grouping amongst the treatment. The choice of the agroforestry tree composition and structure will determine all biogeochemical processes and regulate the sustainability of this system, in terms of the continuity of supporting nutrient demand for tree and crop, at the same time providing a better niche for soil microbe which regulates the rate of soil respiration. Higher coffee population at HC plot creates unfavorable conditions such as: a dense canopy cover, lowering soil pH and functional N-fixing and soluble P bacteria, which affect reduction on coffee productivity by 50% lower than BAU plot. Controlling highest soil respiration at HC plot by pruning and trimming either on coffee or pines even reducing the number of their population could reduce global C emission and at the same time protect the environment and creating a better habitat for soil microorganisms particularly soil bacteria functional group, which improves belowground soil carbon sequestration. Unfortunately, this study still has some limitations due to the single-site study design, no true control without pine, potential spatial autocorrelation, and a lack of microbial community composition beyond CFU approaches.

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