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Response of Different Coffee-Based Agroforestry Management on Microbial Respiration and Density

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ABSTRACT

Coffee agroforestry has become a land use system that provides both ecological and economic benefits, so it is managed in various ways. Pruning and fertilizer management is a combination that is applied for optimal production. However, understanding the effect of combined management on soil respiration and functional microbial populations remains unclear. This study aimed to determine the effect of combining pruning and fertilizer management on soil respiration and functional bacterial populations as well as to elucidate the relationship between tested parameters. The study was conducted in UB Forest. A factorial randomized block design consisting of three factors, i.e., coffee pruning, type of fertilizer, and fertilizer doses was used. The results showed that combining three factors affected the diazotrophic bacterial population and soil respiration, which is sensitive to management changes. Coffee pruning and mixed fertilizer (inorganic + organic) application affected soil respiration and microbial populations, while the dose affected each parameter differently. The conducted study suggests that pruning management with mixed fertilizer application can substitute inorganic fertilizer as more environmentally sustainable management in coffee-based agroforestry.

Keywords: coffee-based agroforestry, diazotrophic bacteria, fertilization, pruning, P-solubilizing bacteria, soil respiration.

INTRODUCTION

Coffee-based agroforestry has been recognized as a land use system that provides ecological and economic benefits (Waktola and Fekadu 2021). The system structures resemble natural forests; thus the potential for biodiversity and soil conservation and maintaining yield for food security in coping with climate change (Tesfay et al. 2022). Coffee-based agroforestry creates a suitable microclimate (humidity and temperature) as shading for coffee and for soil organism habitats (Asfaw and Zewudie 2021). Shading in coffee-based agroforestry increases coffee production compared to the coffee without shading (Mokondoko et al. 2022). These benefits make this system a sustainable management system for yield and environmental sustainability. Coffee-based agroforestry system provides favorable habitats for beneficial microbes that support soil function, such as nutrient cycling. The beneficial microbes, e.g., free-living or symbiotic microbes, increase nutrient availability and uptake (Singh et al. 2022). Fertile soil is related to higher beneficial microbe abundance (Wang et al. 2017). Bacterial diversity is influenced by the degree of habitat disturbance caused by variations in land-use management practices which affected soil properties, such as fertilizers application and pruning (Mhete et al. 2020). Pruning affects the organic carbon input as well as changes soil bacterial abundance and diversity (Zhang et al. 2023). Long-term pruning reduces the richness of soil microbes, whereas low-input farming systems promote higher abundance and diversity of soil microbes (Bickel and Or 2020).

The density of soil microbes can be assessed through soil respiration, since it describes the overall biological activity in the soil (Ebrahimi et al. 2019). Furthermore, soil respiration also predicts the diversity of soil microbes, mainly beneficial bacteria, such as diazotrophs and P-solubilizer (PSB), which supply plant nutrients (Batista and Dixon 2019; Yu et al. 2022). The activity of soil microbes changes depending on management systems (Furtak and Gadja 2018). Kabiri et al. (2016) and Liu et al. (2018) reported that soil respiration is a sensitive indicator responding to changes in management systems and is strongly affected by microbial composition, soil and plant properties, as well as climate condition.

Coffee-based agroforestry management is carried out in several ways, including coffee canopy and fertilization management. Besides affecting coffee production, the type and dosage of fertilizers can also influence the structure and function of soil microbes (Lazcano et al. 2013; Guo et al. 2020). Coffee canopy management is conducted by pruning unproductive branches (Dufour et al. 2019). Pruning can modify the environment so the microclimate does not fluctuate (Niether et al. 2018). Temperature stability helps provide a suitable environment for soil microbial activity. However, the effect of combined pruning and fertilizing management on soil respiration and microbial density remains unclear. Thus, a further study on the management system's effect on soil respiration in coffee-based agroforestry as a sustainable management system is essential. This study aimed: 1) to determine the effect of coffee canopy and fertilization management on respiration and beneficial microbial populations; 2) to elucidate the relationship between combined coffee canopy management and fertilization on the tested parameters.

MATERIAL AND METHOD

Study site

The study was conducted in October 2021 – April 2022, located in a coffee-based agroforestry on Universitas Brawijaya Forest (UB Forest) in Malang, East Java, with an altitude of 1,200 meters above sea level (m asl). UB forest is located on the southern slope of Mount Arjuno, with an average annual temperature of 22 °C and annual rainfall 2000 mm/year (Figure 1). The study was conducted on two different management types of coffee-based agroforestry, namely pruned (7°49'19.3" S, 112°34'48.1" E) and unpruned coffee (7°49'27.2" S, 112°34'41.0" E). The coffee trees used were Arabica coffee (*Coffea arabica*), aged between 8 and 10 years.

Experimental design and sampling method

This research used a factorial randomized block design with three factors (2×3×3) (Table 1). The first factor was pruning management (i.e., T1 – pruned coffee, T2 – unpruned coffee), the second factor was the type of fertilizer (i.e., O – organic fertilizer, I – inorganic fertilizer, M – mixed fertilizer (50% organic, 50% inorganic)), and the third factor was fertilizer dosage (i.e., D1 – dosage based on business as usual (BAU) of the farmers, D2 – dosage recommended (Wahyudi et al. 2016), D3 – dose based on harvested nutrients from coffee beans). The three factors were combined and repeated four times, so that there were 72 experimental plots in this study site (Figure 2). Each experiment plot was 2×2 m.

Coffee pruning was carried out by manually cutting unproductive branches three times every year. Pruned coffee was kept at less than 150 cm (Figure 3a). The organic fertilizer used in this study was chicken manure (1.49% N, 2.91% P_2O_5, and 2.57% K_2O). Fertilizers were applied on the fertilizer hole around coffee stem (Figure 3b). The fertilizers were applied by spreading the fertilizer in the hole at 10 cm depth (50 cm from the stem). Soil sampling was carried out at four different points around the coffee tree, 30 cm from the stem. Soil sampling was carried out before fertilizer application and six months after the application of fertilizers. A soil auger was used to collect soil samples at two depths (i.e., 0–20 cm and 20–40 cm).

Microclimate data collection

Measurements of soil temperature and air temperature were used to evaluate the differences between pruned and unpruned coffee management. Measurements were done based on Rowe et al. (2022). The soil temperature sensor (HOBO MX2201) was placed at 5 cm from the topsoil. Air temperature was measured using Lascar-EL-USB-2. Manual measurements of soil and air

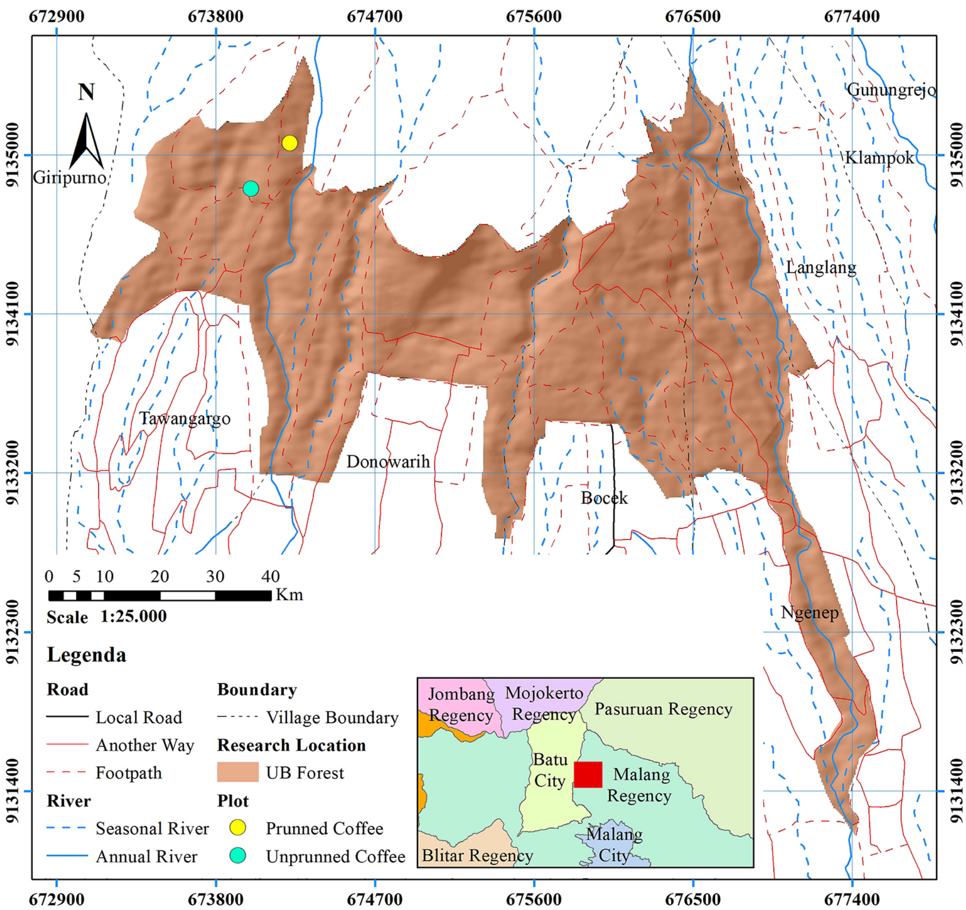


Figure 1. Study site

Table 1. Amount of fertilizer applied under different management

Fertilizer type and dose combination		Fertilizer requirement (kg/plant)			
		Chicken manure	Urea	SP-36	KCl
Organic fertilizer (O)	farmer-based dose (D1)	10.73	-	-	-
	recommendation dose (D2)	17.06	-	-	-
	harvested replacement based dose (D3)	0.89	-	-	-
Inorganic fertilizer (I)	farmer-based dose (D1)	-	0.48	0.87	0.46
	recommendation dose (D2)	-	0.75	1.38	0.84
	harvested replacement based dose (D3)	-	0.14	0.07	0.13
Mixed fertilizer (M)	farmer-based dose (D1)	5.36	0.24	0.43	0.23
	recommendation dose (D2)	8.53	0.37	0.69	0.42
	harvested replacement based dose (D3)	0.44	0.07	0.04	0.07

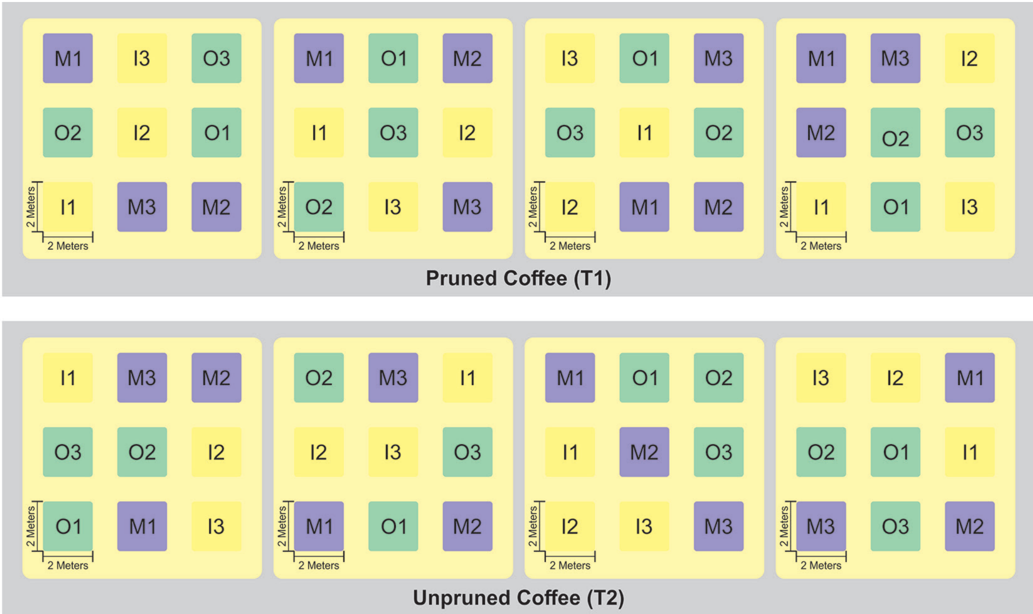
temperature were carried out using a thermometer to validate the results obtained from the sensor. The data obtained were then calculated for temperature fluctuations using the following formula:

$$\Delta T = T_{max} - T_{min} \quad (1)$$

where: ΔT – temperature fluctuation, T_{max} – maximum measured temperature, T_{min} – minimum measured temperature.

Soil respiration analysis

Microbial respiration analysis was measured using MicroResp™ with modification (Cameron 2007). A 0.35 g of soil was placed into the deep well plate, then covered using a 96-well microplate (Corning® 96-well EIA/RIA Clear Flat Bottom Polystyrene High Bind Microplate, Ref. 3590) containing agar (30 g/L) which was



***Fertilizer Dose:** O: organic fertilizer; I: Inorganic fertilizer; M: Mixed fertilizer (50% organic: 50% inorganic)
****Fertilizer Dose:** D1 : Dosage based on the farmer; D2: Dosage recommended (Wahyudi et al., 2016); D3: Dose based on harvested nutrient from coffee beans

Figure 2. Experimental plot design

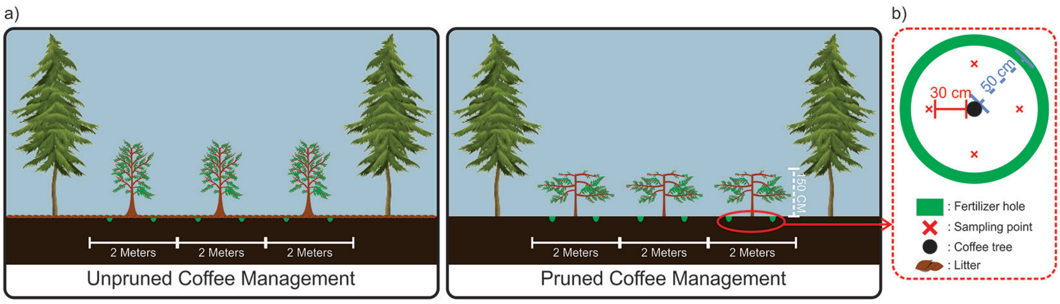


Figure 3. a) Plot condition under different pruning management, b) fertilizer hole and sampling point

amended with pH indicator (18.75 mg/L cresols red, 16.77 g/L KCl, 0.315 g/L $NaHCO_3$), the ratio was 1:2 (agar: indicator). The sample was incubated for 24 hours at 25 °C then the absorbance of the agar on the microplate was measured using a BMG Labtech Spectrostar Nano microplate reader with a wavelength of 570 nm. The absorbance data from the measurement results were then normalized (Ai) with the following formula:

$$Ai = \left(\frac{At_{24}}{At_0}\right) \times \text{mean } At_0 \quad (2)$$

where: At_{24} – the absorbance data 24 hours after incubation, and At_0 – the absorbance data before incubation.

The percentage of CO_2 was calculated after Ai was obtained using the following formula:

$$\%CO_2 = \frac{A+B}{1+D \times Ai} \quad (3)$$

where: $A = 0.2265$, $B = -1.606$, and $D = -6.771$.

CO_2 production was calculated using the following formula:

$$CO_2 \text{ rate } (\mu g/g/h \; CO_2 - C) = \frac{\left(\dfrac{\%\dfrac{CO_2}{100} \times \text{vol} \times \left(\dfrac{44}{22.4}\right) \times \left(\dfrac{12}{44}\right) \times \left(\dfrac{273}{273+T}\right)}{\text{sfw} \times \left(\%\dfrac{\text{sdw}}{100}\right)}\right)}{\text{Incubation time}} \quad (4)$$

where: *vol* – headspace volume in the well (μl), *T* – incubation temperature (°C), *sfw* = soil fresh weight/well (g), and *%sdw* = % of soil sample dry weight.

Functional bacteria population analysis

Diazotrophic bacteria and P-Solubilizing bacteria (PSB) population were tested to determine management effect on functional bacteria density in the soil. The standard plate count method was used to determine the population of each type of functional bacteria. Nitrogen-free bromothymol blue (NFB) medium was prepared (0.2 g/L $MgSO_4.7H_2O$, 0.1 g/L NaCl, 0.015 g/L $FeCl_3.6H_2O$, 0.5 g/L K_2HPO_4, 4.8 g/L KOH, 5 g/L malic acid, 0.05 g/L yeast extract, 1 mL/L bromothymol blue (BTB) and 15 g/L agar) for diazotrophic bacteria enumeration (Ustiatik et al. 2022). PSB population were isolated using pikovskaya medium (0.5 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4.7H_2O$, 0.001 g/L $MnSO_4$, 0.001 g/L $FeSO_4$, 0.2 g/L NaCl, 0.2 g /L KCl, 10 g/L glucose, 5 g/L $Ca_3(PO_4)_2$, 0.5 g/L yeast extract, and 15 g/L agar) (Purnomo et al. 2021). A 25 mg/L nystatin was added into the medium to inhibit fungal contamination (Mahgoub et al. 2021).

Soil analysis

Soil pH was measured using Eutech PC 700 Meter With pH Electrode. A 10 g of air-dried soil that passes through a 2 mm sieve was mixed with an extractor in the form of 10 mL H_2O, and then shaken for one hour at 150 rpm. Soil organic C (SOC), Total N, P, K (TN, TP, TK, respectively), available P (AP), and exchangeable base (K, Na, Ca, Mg) were carried out to determined initial soil characteristics. Organic C was analyzed using Walkey and Black method, N total was using Kjeldahl Method. HCl 25% extraction was used to determine soil total K and P. The spectrophotometry method with Bray-1 Extractant was used to determine soil available P and was read using Hitachi U-1100 spectrophotometer. Exchangeable base was determined using NH_4OAc pH 7 extraction method and read using the Perkin Elmer Analyst 200 Atomic Absorption Spectrometer.

Data analysis

Data analysis was performed using R studio. The obtained data were tested for normality using Shapiro-Wilk test. Three-way ANOVA with a 5% confidence level was performed to determine the effect between treatments, followed by Fisher's Least Significant Difference (LSD) test on parameters that were significantly different using the "agricolae" packages. Principal component analysis (PCA) was used to analyze the relationship between management and the tested parameters. The "ggplot2", "corrplot", and "factoextra" packages were used on PCA.

RESULTS AND DISCUSSION

Initial soil characteristics

At 0–20 cm depth, according to Indonesia Soil Research Institute, the pruned and unpruned coffee plots were characterized by low soil pH, high SOC, high TP and AP, and high TN. However, exchangeable bases (K, Ca, Mg, and Na) were low to moderate in the pruned plot than in the unpruned plot. At 20–40 cm depth, the plots had low soil pH, high organic C, high total N, P, and available P. Despite that, K, Na, and Ca were low, even though Mg was high at the pruned compared to the unpruned plot (Table 2). The high SOC in all plots and depths indicated high organic matter (OM) input, particularly from litterfall of pine

Table 2. Soil properties before application of fertilizer

Soil characteristics	Pruned-coffee		Unpruned-coffee	
	0–20 cm	20–40 cm	0–20 cm	20–40 cm
Soil pH H_2O	5.09 ± 0.06	5.41 ± 0.09	5.24 ± 0.12	5.58 ± 0.07
Soil pH KCl	4.65 ± 0.06	4.60 ± 0.05	4.55 ± 0.06	4.51 ± 0.07
Soil organic C (g $100g^{-1}$)	7.83 ± 0.94	8.79 ± 0.67	6.88 ± 0.43	6.67 ± 0.34
Total N (g $100g^{-1}$)	0.60 ± 0.01	0.56 ± 0.00	0.50 ± 0.02	0.39 ± 0.02
Total P (mg kg^{-1})	944.5 ± 76.2	788.9 ± 26.8	687.1 ± 100.5	529.5 ± 69.9
Available P (mg kg^{-1})	11.65 ± 2.88	16.74 ± 2.06	14.98 ± 3.28	19.35 ± 6.29
Soil exchangeable K (me $100g^{-1}$)	0.48 ± 0.04	0.26 ± 0.04	0.87 ± 0.23	1.94 ± 0.98
Soil exchangeable Ca (me $100g^{-1}$)	9.98 ± 0.77	9.57 ± 0.49	19.77 ± 0.89	12.43 ± 1.62
Soil exchangeable Mg (me $100g^{-1}$)	4.81 ± 1.09	4.94 ± 1.06	11.63 ± 1.31	1.14 ± 0.25
Soil exchangeable Na (me $100g^{-1}$)	0.32 ± 0.02	0.26 ± 0.01	0.42 ± 0.01	0.39 ± 0.04

Note: mean ± standard error of difference.

and coffee, organic fertilizer, and understory. In addition, high SOC may support microbial activities in the soil as soil SOC provides a source of energy and nutrients for microbes, when SOC levels are high, microbial activity is also high, because there is more food and energy available for microbes to use (Kästner et al. 2021). The study site is an agroforestry land with high input of organic materials, specifically from litterfall, thus increasing organic C and the increasing other nutrients are due to large trees acting as nutrient pumping from deeper layers of the soil (Sarvade et al. 2019).

Microclimate

The effects of pruning on soil and air temperature were different (Table 3). The average air temperature was higher than the soil temperature. The average daily soil temperature for the unpruned plot was higher than for the pruned plot. However, the plots showed no differences in ∆Temperature (temperature difference between the highest and lowest temperature). This finding revealed that pruning does not create fluctuations in soil temperature. However, the study recorded that pruning impacts air temperature fluctuations. The pruned plot had a higher average air temperature than the unpruned plot. This finding aligns with ∆Temperature. The pruned had higher air temperature fluctuation than the unpruned plot. The finding highlights that pruning can significantly impact air temperature, but not soil temperature, because pruning removes leaves, which are responsible for absorbing sunlight and converting it into heat. Without leaves, the plant can less regulate its temperature, and the air temperature around it is higher than in unpruned areas (Huang et al. 2023).

Pruning branches reduces the canopy cover and increases land openness so that more sunlight enters the system (Niether et al. 2018). Despite this fact, coffee pruning also helps in microclimate regulation within the system. However, for arabica coffee, the species is susceptible to temperature changes (Vinci et al. 2022). Coffee tree have optimal growth temperatures between 18–23 °C, with a temperature tolerance up to 30 °C (Martins et al. 2016). The obtained data showed that both managements have the optimal temperature for coffee growth. However, temperature optimization, e.g., by pruning management, is also essential for microorganisms in the soil as they play an important role in nutrient cycling, plant growth, and soil health. They are also sensitive to temperature changes, and slight temperature changes can significantly affect their activity. For example, soil microbial respiration is sensitive to temperature, reflecting the temperature sensitivity of microbial growth and metabolism (e.g., enzyme activity and C utilization efficiency) (Wang et al. 2021).

Soil pH

The study finding highlighted that coffee-based agroforestry management significantly affected soil pH ($p < 0.05$), both at topsoil (0–20 cm) and subsoil (20–40 cm) (Table 4). However, there were no significant differences in the soil pH of pruning management at 0–20 cm depth ($p > 0.05$); in contrast, the study detected significant differences of soil pH in the deeper layer (20–40 cm depth) ($p < 0.05$). In addition, the research found significantly different soil pH at 0–20 cm depth due to applying various fertilizer types. The deeper layer of the study plot had higher soil pH than the shallow layer (0–20 cm depth) on both management pruning and different fertilizer types. The highest soil pH was found in organic fertilizer with the recommended dose (OD2). In contrast, organic fertilizer application with nutrient replacement doses (OD3) had the lowest value. Applying organic fertilizer using the recommended doses increases soil pH by 8.11% at 0–20 cm depth. However, at 20–40 cm depth,

Table 3. Microclimate condition under different pruning management

Management	Average soil temperature (°C/day)	∆ Soil temperature (°C)	Average air temperature (°C /day)	∆ Air temperature (°C)	% Canopy cover*
Pruned coffee (T1)	20.87	3.5	22.61	9.5	64.68
Unpruned coffee (T2)	21.98	3.5	22.35	6.0	69.40

Note: *data obtained from Research Group of Tropical Agroforestry, Universitas Brawijaya; ∆ temperature is the difference between max. and min. temperature.

Table 4. Soil pH under different management (pruning and fertilizer) of coffee-based agroforestry systems

Management		Soil pH					
		0–20 cm depth			20–40 cm depth		
		D1	D2	D3	D1	D2	D3
Pruning management	T1	5.6 ± 0.05	5.67 ± 0.06	5.6 ± 0.04	5.8 ± 0.04a	5.81 ± 0.05a	5.76 ± 0.03ab
	T2	5.51 ± 0.06	5.62 ± 0.1	5.35 ± 0.04	5.47 ± 0.04d	5.71 ± 0.05bc	5.65 ± 0.03c
Fertilizer type	O	5.7 ± 0.06ab	5.84 ± 0.08a	5.41 ± 0.06d	5.73 ± 0.08	5.88 ± 0.03	5.73 ± 0.04
	I	5.48 ± 0.07cd	5.45 ± 0.05cd	5.52 ± 0.08cd	5.6 ± 0.11	5.64 ± 0.07	5.69 ± 0.04
	M	5.49 ± 0.04cd	5.6 ± 0.09bc	5.5 ± 0.06cd	5.62 ± 0.05	5.76 ± 0.04	5.69 ± 0.04

Note: mean ± standard error of difference following; means with different letters show significant differences based on Fisher's LSD test at a 5% level;. Factor of the study: 1. Pruning management (i.e., T1 – pruned coffee, T2 – unpruned coffee), 2. Fertilizer dosage (i.e., D1 – dosage based on the farmer, D2 – recommended doses, D3 – dose based on nutrients replacement of harvested coffee beans), 3. Fertilizer type (i.e., O – organic, I – inorganic, M – mixed: organic+inorganic).

pruning increased soil pH by 3.22% compared to unpruned coffee.

The study proved that pruning and fertilizer application (dose and type) influence soil pH; however, the effect varies at different soil layers. Unpruned coffee management provides additional organic materials (OM) from coffee and pine leaves in the form of pine and coffee litter that falls to the soil surface. Fresh OM are typically high in C and low in N, thus decrease in the decomposition rate (C must be mineralized before plants can use N). Moreover, fresh OM can increase the soil pH, as OM releases alkaline compounds into the soil (Adeleke et al. 2017). This result aligns with this study that pruned has a higher soil pH than unpruned plots. The applied organic fertilizer that has gone through a decomposition process; thus, it impacts on the increasing soil pH quicker than the OM input from fresh litterfall, such as in the unpruned plot. Moreover, OM release base cations (e.g., K, Ca, Mg) during decomposition and mineralization, which is OM is decayed into constituent parts, resulting in increased soil pH, as base cations are alkaline (Kawahigashi et al. 2011; Butterly et al. 2013). Aldrich-Wolfe et al. (2020) reported that organic fertilizers increased soil pH by 13.05% compared to inorganic fertilizers. Moreover, Cooper et al. (2020) reported that the higher the amount of organic fertilizer added to the soil will significantly impact on increasing soil pH.

Soil respiration

The results showed that the combination of pruning and fertilization (i.e., type and doses) significantly affected soil respiration ($p \leq 0.05$), both at 0–20 cm and 20–40 cm depths (Table 5). Pruned coffee combined with organic fertilizer application based on farmer application dose (T1OD1) had lower soil respiration than other treatments, and the highest soil respiration was found at the application of mixed fertilizer (organic and inorganic) with nutrient replacement dose (T1MD3). At 0–20 cm depth, soil respiration was strongly affected by the type of fertilizer. Inorganic fertilizer increased soil respiration by up to 14.25% compared to organic fertilizer application. This study revealed that pruning significantly increased the respiration rate at 0–20 cm depth. The finding is aligned with Montejo et al. (2021) who stated that pruned management gave highest soil respiration rates at topsoil. This is because pruning increases the surface area of the soil, which exposes more of the soil to oxygen that is essential for aerobic respiration (e.g., microorganisms break down OM and release carbon dioxide and heat). The increased respiration rates at topsoil can lead to a number of benefits, including: increased nutrient cycling, improved soil structure, reduced soil compaction, and increased plant growth (Cui and Holden 2015; Zhao et al. 2018; Sheng et al. 2022).

Furthermore, the initial soil properties (Table 2) showed that all plot had a high SOC as a food source of soil organisms. Therefore, application of inorganic fertilizer released nutrients (i.e., N, P, K) faster than organic fertilizer; those nutrients are used as an energy source for soil microorganism to decompose OM and resulted in an increased in soil respiration rate (Spohn and Schleuss 2019). Comeau et al. (2016) stated that fertilizer application increases the soil respiration rates more than in an unfertilized area, because it provides more

Table 5. Soil respiration rates under different management of coffee-based agroforestry

Fertilizer type	Fertilizer dosage	Soil respiration rate (μg CO_2-C/g/h)			
		0–20 cm depth		20–40 cm depth	
		T1	T2	T1	T2
O	D1	3.96 ± 0.29c	4.31 ± 0.46bc	2.52 ± 0.27def	1.26 ± 0.08ghi
	D2	4.13 ± 0.03c	4.37 ± 0.07bc	2.78 ± 0.27cde	4.87 ± 0.07a
	D3	4.15 ± 0.38c	4.32 ± 0.25bc	2.25 ± 0.44ef	5.14 ± 0.35a
I	D1	4.61 ± 0.24bc	4.4 ± 0.27bc	0.59 ± 0.08i	5.14 ± 0.36a
	D2	4.44 ± 0.16bc	5.33 ± 0.31a	0.86 ± 0.04ghi	3.41 ± 0.14cd
	D3	4.42 ± 0.31bc	5.44 ± 0.17a	0.52 ± 0.03i	3.56 ± 0.03bc
M	D1	4.18 ± 0.28c	4.4 ± 0.32bc	0.68 ± 0.07hi	4.62 ± 0.21ab
	D2	5.05 ± 0.63ab	4.96 ± 0.05ab	1.92 ± 0.36efg	3.25 ± 0.4cd
	D3	5.55 ± 0.08a	4.3 ± 0.34bc	1.76 ± 0.44fgh	2.44 ± 0.22def

Note: mean ± standard error of difference following; means with different letters show significant differences based on Fisher's LSD test at a 5% level;. Factor of the study: 1. Pruning management (i.e., T1 – pruned coffee, T2 – unpruned coffee), 2. Fertilizer dosage (i.e., D1 – dosage based on the farmer, D2 – recommended doses, D3 – dose based on nutrients replacement of harvested coffee beans), 3. Fertilizer type (i.e., O – organic, I – inorganic, M – mixed – organic+inorganic).

nutrients for microorganisms to break down OM. However, the effects of fertilizer application on soil respiration rates varies depending on the type of fertilizer and the amount of fertilizer applied (Huang et al. 2021; Zhou et al. 2021). At 20–40 cm depth, unpruned plots combined with organic fertilizer application with nutrient replacement doses (T2OD3) significantly increased soil respiration, 8.8 times higher than application of all fertilizer types and doses in the pruned plot (Table 5). The conducted study recorded that soil respiration in all fertilization management in the pruned plots were lower (112%) than in the unpruned plots. Also, pruning management with nutrient replacement doses of mixed fertilizers led to an increase in soil respiration at 0–20 cm depth, while unpruned plot with nutrient replacement doses of organic fertilizer significantly increased soil respiration at 20–40 cm depth. Huang et al. (2021) explained that inorganic fertilizer affects autotrophic respiration; in contrast, organic fertilizer affects heterotrophic respiration. This study focused on the total soil respiration rate, explaining why different management gave different soil respiration rates at each depth. Applying inorganic fertilizers mixed with organic fertilizers and the temperature changes due to pruning heavily influencing soil respiration, specifically autotrophic respiration. Applying N fertilizer (inorganic fertilizers) increases root biomass, even in a small dose, and triggers autotrophic respiration (Chen et al. 2017), due to root exudates secretion and microbial activity in the soil to decompose OM (Chen et al. 2017, 2019). Heterotrophic respiration is suspected as a main driver, which determines soil respiration due to the given management at 20–40 cm depth. A similar finding was reported by Lai et al. (2017), organic fertilizers increase soil respiration. The presence of OM from organic fertilizer and coffee litter is also suspected to increase the respiration rates at 20–40 cm depth. (Kurniawan et al. 2021) reported that organic fertilizers increase the SOC and soil C storage at 20–40 cm soil depth. The high organic C on the layer is a suitable environment for microorganisms so that the soil respiration rate increases.

Functional bacterial population

The combination of management, pruning, and fertilizers type and dosage significantly affected the population of diazotroph bacteria population ($p < 0.05$) at both tested depths (Table 6). At 0–20 cm, coffee pruning combined with inorganic fertilizer application based on the farmer's dose (T1ID1) was 6.8 times higher than the combination of nutrient replacement dose of inorganic fertilizers (T1ID3). The treatment T1ID3 was the lowest diazotrophic bacterial population compared to other managements. At 20–40 cm depth, the pruned coffee combined with the recommended dose of mixed fertilizer (T1MD2) had the highest diazotrophic population compared to other managements. In contrast, the unpruned coffee with a combination of nutrient replacement doses

Table 6. Diazotrophic bacteria population on different coffee-based agroforestry management

Fertilizer type	Fertilizer dosage	Diazotrophic bacteria population (×10^6 CFU/g)			
		0–20 cm depth		20–40 cm depth	
		T1	T2	T1	T2
O	D1	1.92 ± 0.02a	1.54 ± 0.06bc	0.78 ± 0.21fgh	1.4 ± 0.17b
	D2	0.4 ± 0.03hi	0.48 ± 0.14ghi	1.16 ± 0.11bcd	0.73 ± 0.13fgh
	D3	0.27 ± 0.07i	1.26 ± 0.11d	1.21 ± 0.07b	1.12 ± 0.16bcde
I	D1	1.95 ± 0.32a	0.75 ± 0.21fg	0.72 ± 0.06fgh	1.16 ± 0.17bc
	D2	0.71 ± 0.15fgh	0.72 ± 0.19fgh	1.15 ± 0.08bcd	0.65 ± 0.04gh
	D3	0.25 ± 0.08i	1.27 ± 0.05d	0.91 ± 0.08def	0.35 ± 0.03i
M	D1	1.77 ± 0.04ab	1.29 ± 0.07cd	0.92 ± 0.02cdef	0.86 ± 0.04efg
	D2	0.96 ± 0.09ef	1.13 ± 0.03de	1.78 ± 0.08a	0.49 ± 0.08hi
	D3	0.31 ± 0.08i	1.24 ± 0.04d	0.76 ± 0.08fgh	0.73 ± 0.15fgh

Note: mean ± standard error of difference following; means with different letters show significant differences based on Fisher's LSD test at a 5% level;. Factor of the study: 1. Pruning management (i.e., T1 – pruned coffee, T2 – unpruned coffee), 2. Fertilizer dosage (i.e., D1 – dosage based on the farmer, D2 – recommended doses, D3 – dose based on nutrients replacement of harvested coffee beans), 3. Fertilizer type (i.e., O – organic, I – inorganic, M – mixed: organic+inorganic).

of inorganic fertilizers (T2ID3) had the lowest diazotrophic population compared to other treatments. T1MD2 increased the bacterial population by 4.09 times than T2ID3 (the lowest diazotrophic population in this study). Many factors affect the population of diazotrophic bacteria, including soil nutrients. Diazotrophic bacteria need various nutrients to grow, including N, P, and K. They are most abundant in environments rich in these nutrients (Tang et al. 2017). This study proved that fertilizer application, both organic and inorganic, increased the soil nutrients that are required for bacterial growth; thus, the population drastically increased. This result contradicts a report by Chen et al. (2021) that inorganic fertilizers reduce the abundance of diazotrophic bacteria. This study revealed that coffee-based agroforestry management did not affect the PSB population ($p > 0.05$) at 0–20 cm; in contrast, the treatments significantly affected the PSB population ($p < 0.05$) at 20–40 cm depth (Table 7). Organic fertilizer based on farmers' dose (OD1) and recommended doses (OD2) had the highest population of PSB at 0–20 cm and 20–40 cm depth, respectively. The results of the conducted study also revealed that pruning with the application of inorganic fertilizers based on farmers' dose, which had the highest PSB and diazotrophic bacteria population, was similar to pruning with the application of farmers' doses of organic and mixed fertilizer. The result proved that organic fertilizer could substitute inorganic fertilizer in terms of creating environmental conditions suitable for functional bacteria. Then, suitable environmental conditions support the growth and development of functional bacteria as a part

Table 7. P-solubilizing bacteria population under different coffee-based agroforestry management at different soil depths

Management	P-Solubilizing bacteria population (×10^3 CFU/g)					
	0–20 cm depth			20–40 cm depth		
	D1	D2	D3	D1	D2	D3
O	2.58 ± 0.22	1.92 ± 0.17	1.91 ± 0.05	0.26 ± 0.07b	0.42 ± 0.01a	0.26 ± 0.05bc
I	1.75 ± 0.14	1.92 ± 0.22	1.88 ± 0.31	0.26 ± 0.02b	0.17 ± 0.02d	0.29 ± 0.03b
M	1.83 ± 0.19	2.04 ± 0.1	1.88 ± 0.31	0.25 ± 0.03bc	0.31 ± 0.05b	0.18 ± 0.03cd

Note: mean ± standard error of difference following; means with different letters show significant differences based on Fisher's LSD test at a 5% level;. Factor of the study: 1. Fertilizer dosage (i.e., D1 – dosage based on the farmer, D2 – recommended doses, D3 – dose based on nutrients replacement of harvested coffee beans), 2. Fertilizer type (i.e., O – organic, I – inorganic, M – mixed: organic+inorganic).

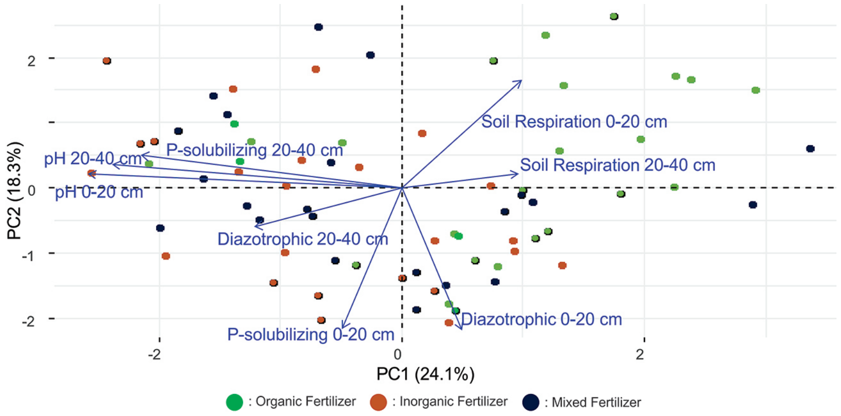


Figure 4. Principal component analysis among parameters

of provisioning environmental services (Parmar and Sindhu 2013).

The relationship between the parameters observed in the treatment

According to PCA analysis, among the tested parameters, soil pH, soil respiration, and diazotrophic bacteria population at the topsoil (0–20 cm depth) were sensitive to different management of coffee-based agroforestry. These parameters were strongly affected even with a slight change in the management, whether pruning, fertilizer types and dosage (Figure 4).

Principal Component Analysis results revealed that soil respiration, diazotrophic bacteria population, as well as soil pH are sensitive and can explain the effect of combined management better than all the tested parameters in this study. Xue and Tang (2018) reported that the changes in land-use cause the changes in soil temperature and water availability, thus affecting soil respiration. Respiration has also been reported to have high sensitivity in detecting changes in fertilization management (Iovieno et al. 2009; Sun et al. 2018). The strong sensitivity of soil respiration makes it easier to detect environmental changes, including the changes in soil temperature, air temperature, and organic matter input (Zhang et al. 2013; Rodtassana et al. 2021) .

The results showed that different management in this study affected the population and activity of soil microbes; both can be detected from respiration and population. The combination of pruning with mixed fertilizers provides suitable conditions for the bacteria, increasing the activity and population of functional soil bacteria. This finding aligns with Pramanik et al. (2018) that pruning improves soil bacterial populations. Sun et al. (2015) reported that combining organic and inorganic fertilizers increase the abundance of microbes that play an important role in the nutrient cycle.

The conducted research revealed that combining the three treatment factors affected the soil respiration rates and diazotrophic bacterial populations at all depths. However, the study results showed that each dose had a different effect on the parameters tested. Pruning with mixed fertilizer had a better impact than other combinations, but the optimal applied dosage remained unclear. Further research is needed to determine the best dosage for the soil to assess the impact of various fertilizer dosages on microbial activity and bacterial density. Long-term assessments will also help to understand how the interactions between management will affect the sustainability of the coffee-based agroforestry system.

CONCLUSIONS

The combination of pruning and fertilizer management affected the soil pH, diazotrophic bacterial population, and soil respiration rate at topsoil (0–20 cm depth). These parameters are sensitive to slight changes in the management of coffee-based agroforestry. The study suggested that coffee pruning is beneficial for the microclimate due to removing unproductive branches, thereby providing a more suitable living environment for microorganisms in the soil. Also, the conducted study suggests that pruning management with mixed fertilizer application can substitute inorganic fertilizer; thus, it can be considered more environmentally sustainable.

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