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Temporal Dynamics of Soil Carbon Stocks and Mineralization Rates in *Coffea arabica* Agroforestry Systems

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Abstract: The increasing demand for agricultural land is driving deforestation, which contributes to rising greenhouse gas emissions—a primary driver of climate change. Agroforestry systems present a valuable alternative approach for mitigating these emissions. This study investigates the influence of various agroforestry systems on key parameters for assessing soil carbon dynamics, including soil carbon stock, microbial biomass carbon (MBC), and microbial respiration (MR) in northern Thailand. The study compared different agroforestry systems, such as coffee monoculture (CA), coffee intercropped with forest trees (CF), coffee intercropped with persimmon trees (CP), and natural forest (NF), as reference areas. Soil samples were collected from three depths (0–20, 20–40, and 40–60 cm) across three seasons (rainy, cold, and summer). Results showed that soil carbon from land use changes from NF to coffee agroforestry systems led to a 12.10% increase in CF and an 11.89% decrease in CP. The CA system showed a non-significant 9.06% decrease compared to the natural forest. MBC levels were higher in the agroforestry coffee areas (CF and CP), comparable to those in NF. However, CA exhibited significantly lower MBC ($p < 0.05$) compared to other treatments. While MBC and MR showed a positive correlation, higher MBC did not necessarily indicate increased microbial activity or significant dissolved organic carbon (DOC) accumulation. Environmental factors, particularly seasonal variations, significantly influenced the results across all three investigations. This study demonstrates that both agroforestry coffee systems (CF and CP) more effectively mitigate deforestation impacts and enhance soil fertility compared to coffee monoculture. For carbon stock enhancement specifically, CF emerged as the optimal choice.

Keywords: agroforestry; coffee system; soil carbon stock; mineralization rates; SDGs15

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1. Introduction

Northern Thailand, a region known for its dense forests and rich biodiversity, has experienced significant deforestation in recent decades, primarily driven by agricultural expansion. Local farmers, often using slash-and-burn practices, clear vast areas of forest to meet the growing demand for cash crops like maize, rubber, and coffee. While these agricultural activities contribute to the local economy, the environmental costs are profound [1]. Deforestation in this region not only threatens local ecosystems and biodiversity [2,3] but also plays a significant role in exacerbating climate change. As stored carbon is released into the atmosphere in the form of carbon dioxide, deforestation for agriculture has become a critical concern for both regional and global climate initiatives [4–6].

Arabica coffee has become a valuable economic crop in northern Thailand, particularly in the highland regions, where cool temperatures and fertile soil create ideal conditions for its cultivation. Initially introduced as part of efforts to promote sustainable livelihoods and replace opium cultivation, Arabica coffee quickly gained importance for its economic benefits to local farmers and its potential to thrive in mountainous areas [7,8]. However, its expansion posed challenges, as monoculture practices often led to deforestation and environmental degradation. Agroforestry systems that integrate Arabica coffee with native trees and other crops have emerged as a solution to balance economic growth and environmental preservation. Agroforestry not only improves coffee quality and yields but also helps reduce deforestation, restore soil fertility, and protect biodiversity by maintaining forest cover [9]. This shift toward more sustainable agricultural practices underscores the role of Arabica coffee as both a key economic driver and a tool for environmental conservation in northern Thailand.

Agroforestry is thus a representative of a forest in which plants can use carbon dioxide in the photosynthesis process to create organic carbon and accumulate plant biomass.

When components of plants descend to the ground and are decomposed by soil microbes, organic carbon is sequestered in the soil. This contains plant root-exuded organic matter, dead microbial cells, and chemicals synthesized by microbes. The process of storing carbon in the soil is known as carbon sequestration. This is considered one of the carbon management approaches to reduce carbon dioxide emissions in the atmosphere by storing carbon in the soil. The primary carbon stores are soil, air, and water [10]. Forest areas store more carbon in the soil than general agricultural areas [11], especially in the part of the fine roots and small soil fragments. This represents long-term stable carbon storage and the restoration of soils in degraded areas [12] and includes agroforestry as well [13,14]. Soil fertility encompasses more than merely plant nutrition. However, this involves the living microorganisms in the soil systems. The respiration of plant roots, benthic animals, and soil microorganisms that decompose organic matter all control the production of nutrients in the soil [15–17].

Although several studies have reported the benefits of agroforestry systems, there is still a gap remaining in the information on studying the land use change from forest to coffee plantations on the soil carbon dynamics. Additionally, research on how coffee agroforestry and monoculture coffee cultivation influence carbon sequestration in Thailand remains inadequately documented. Therefore, the objectives of this study are (i) to assess soil carbon stock in different coffee cropping systems compared with natural forest, (ii) to understand the carbon cycle and its mineralization under various coffee cropping systems, and (iii) to examine the seasonal changes across three distinct seasons to understand the short-term carbon dynamics. A systematic examination of land use alterations on soil carbon reserves across several agroforestry systems was assessed. Comparisons between traditional coffee cultivation and agroforestry adaptation were also examined to promote sustainable coffee production and mitigate greenhouse gas emissions.

2. Material and Methods

2.1. Study Area

The study area is located at the Nhong Hoi Highland Agricultural Research Station in Pong Yang Sub-district, Mae Rim District, Chiang Mai Province, Thailand (18°55′19.6″ N 98°48′55.0″ E) (Figure 1). The total land area is about 13 ha, with an elevation ranging from 850 to 900 m above the mean sea level. The average annual precipitation amounts to 1355 mm. The mean temperature is approximately 28.5 °C. The average slope is about 20%. Within the boundaries of the area lies an unclassified natural deciduous forest, devoid of any specific categorization of tree species. The primary agricultural crop testing area encompasses Arabica coffee (*Coffea arabica* L.), persimmon (*Diospyros virginiana* L.), plum (*Prunus domestica* L.), and avocado (*Persea americana*). The area was initially a pristine deciduous forest. Subsequently, it is designated as a research zone for the cultivation of crops suitable for highland environments. The development of Arabica coffee cultivars with various planting strategies is the primary goal. These coffee systems encompass the coffee monoculture system, coffee grown with forests system, and coffee grown with persimmons system.

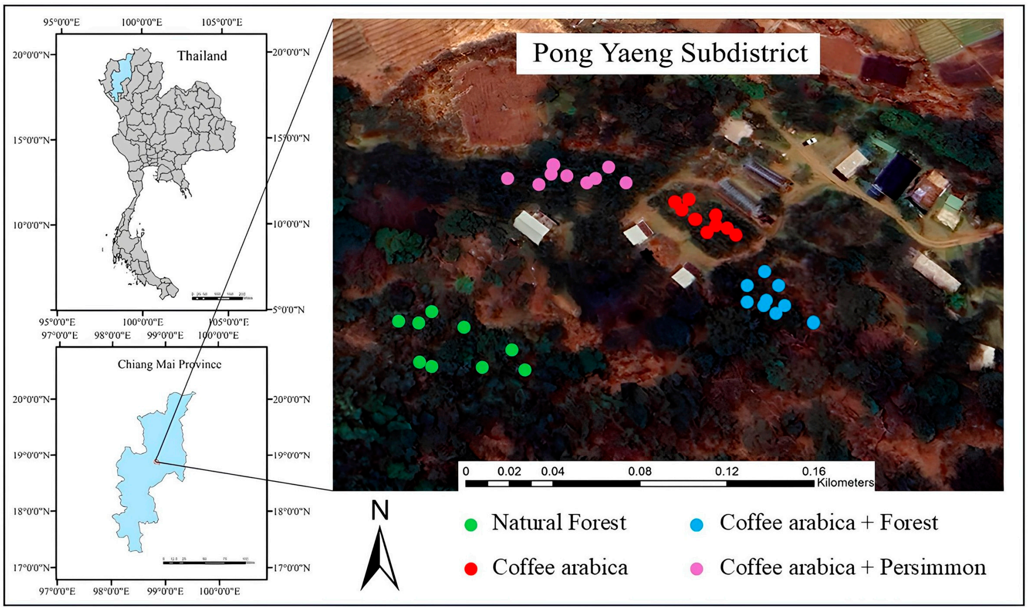

Figure 1. Location of the study sites and the distribution of the field sample sites in the Nong Hoi Highland Agricultural Station in Pong Yeang Subdistrict, Muang District, Chiang Mai Province, Thailand.

2.2. Data Collection

The sampling sites represented four distinct land use types, namely natural forest (NF), coffee monoculture (CA), coffee intercropped with forest trees (CF), and coffee intercropped with persimmon trees (CP). Soil samples were randomly collected in triplicate from each sampling site across three seasons, which were the rainy season (September), the cold season (December), and the summer season (April) of the 2022–2023 period. At each sampling site, soil profile pits were dug, and soils were collected at depths of 0–20 cm, 20–40 cm, and 40–60 cm, using both disturbed and undisturbed methods (soil core method). The disturbed soil samples were carefully processed by removing stones and roots, air drying, and sieving through a 2 mm mesh to eliminate unwanted particles. The processed soils were then stored in zip-lock bags for further analysis. A subset of samples was kept at 4 °C for subsequent analysis of soil microbial activity. Weather data, including temperature and humidity, were recorded daily from 9:00 a.m. to 4:00 p.m. using TinyTag Plus 2 data loggers (Gemini Data Loggers, Chichester, UK) for a period of 223 days, spanning from 9 October 2022 to 20 April 2023 (Figure 2).

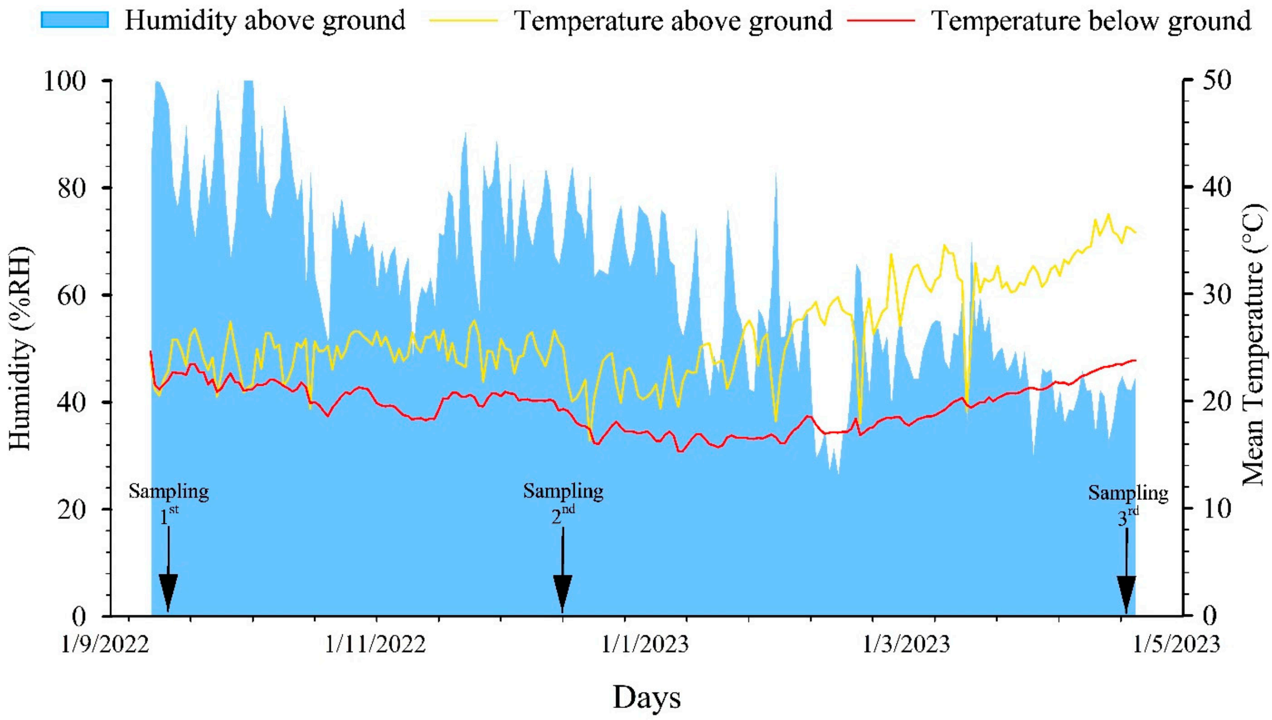

Figure 2. Mean temperature (aboveground and belowground) and relative humidity during the experimental period (September 2022 to April 2023).

2.3. Soil Physicochemical and Microbial Analysis

Undisturbed soil samples were collected for three replicates of each soil depth to determine bulk density (BD) using the core method [18]. The soil water content (SWC) was measured gravimetrically by drying in an oven at 105 °C until a consistent weight was obtained [19]. The air-dried and sieved soil samples were analyzed for various physico-chemical properties. Soil particle size distribution was determined using the hydrometer method [20] to assess soil texture. Soil pH and electrical conductivity (EC) were measured in deionized water (1:5 soil/water ratio) using a pH meter (Mettler Toledo AG, Schwerzenbach, Switzerland) [21] and an EC meter (Thermo Fisher Scientific, Waltham, MA, USA) [22], respectively. Total carbon and nitrogen concentrations were determined by dry combustion using an elemental analyzer (CHN-O-RAPID, Heraeus, Hanau, Germany). Soil available phosphorus (P) was extracted using the Bray II method and quantified colorimetrically [23]. Soil exchangeable potassium (K), exchangeable calcium (Ca), and magnesium (Mg) were extracted with ammonium acetate and measured by Atomic Absorption Spectrometer (PerkinElmer Inc, Shelton, CT, USA) [24]. Soil carbon stock (C stock) and soil nitrogen stock (N stock) for each soil depth were calculated using the following Formulas (1) and (2), respectively:

$$\text{C stock} = \text{C}\ (\%) \times \text{BD} \times \text{d} \tag{1}$$

$$\text{N stock} = \text{N}\ (\%) \times \text{BD} \times \text{d} \tag{2}$$

where C stock indicates soil C stock (t C ha^{-1}); N stock indicates the soil total nitrogen (t N ha^{-1}); C is the percentage of total C content; N is the percentage of total nitrogen; BD is the bulk density (g cm^{-3}); and d is the soil thickness (cm) [25].

To assess soil organic matter mineralization, soil microbial activity, including the microbial respiration rate (MR), microbial biomass carbon (MBC), and dissolved organic carbon (DOC), freshly collected surface soil samples (0–20 cm depth) were stored at 4 °C prior to analysis. MR was determined by quantifying the CO_2 evolved during a 50-day incubation period at 25 °C, using a modified alkaline adsorption method [26,27]. Fifty grams of soil was placed in a 1 L jar with 1 bottle of 0.1 M NaOH 20 mL solution, then the jar was sealed. NaOH was then titrated with 0.1 M HCl using a 0.5 M BaCl solution and 1% phenolphthalein on days 3, 7, 14, 21, 35, and 50 after incubation and changing the NaOH solution every time [28]. MBC and DOC were measured using the chloroform fumigation–extraction method [29]. A soil sample (10 g) was extracted with 50 mL of 0.5 M K_2SO_4 for 45 min on a shaker (240 RPM). The extractable carbon was released into a K_2SO_4 extracted solution from non-fumigated soil [16], and the soil was fumigated with chloroform for 48 h at 25 °C. The extracted solution was analyzed for carbon content by the wet oxidation method with 0.07 M $K_2Cr_2O_7$, then titrated with 0.01 M $(NH_4)_2$ $[Fe(SO_4)]_2 \cdot 6H_2O$. The DOC and MBC were calculated using Equations (3) and (4), respectively.

$$\text{DOC } (\mu\text{g kg}^{-1} \text{ soil}) = \text{Cfum} \quad (3)$$

$$\text{MBC } (\mu\text{g kg}^{-1} \text{ soil}) = (\text{Cfum} - \text{Cnon-fum}) \quad (4)$$

where

DOC = dissolved organic carbon;
MBC = microbial biomass carbon;
Cfum = carbon in fumigated sample;
Cnon-fum = carbon in non-fumigated sample.

2.4. Statistical Analyses

Statistical analyses were conducted using Statistix version 10 software. Analysis of variance (ANOVA) coupled with the least significant difference (LSD) test ($p \leq 0.05$) was used to assess the main and interactive effects of land use and soil depth on the measured soil properties. The *t*-test was employed to assess alterations in carbon stock comparing natural forest areas and coffee cultivation areas, specifically the changes from NF to CA, CF, and CP, to better understand and assess changes in carbon stocks at different depths (0–20, 20–40, and 0–60 cm). Linear regression analysis was performed to examine the relationships between microbial activity parameters (MR, MBC, and DOC) and C stock. Pearson's correlation coefficients were calculated to quantify the strengths of associations among the measured soil parameters.

3. Result

3.1. Soil Physical and Chemical Characteristics Before Experiment

The statistical analysis of soil characteristics between different land uses and soil depths is presented in Table 1. The significant interactions between the land use type and soil depth were observed for key soil properties, including bulk density, clay content, total C and N, and available P. These interactive effects indicate that the influence of land use on these soil attributes varied across different soil depth intervals or, conversely, the depth-wise patterns of these properties differed among the various land use systems.

Table 1. Result of ANOVA for soil characteristics in different land use and soil depths before experiment.

Land Use × Depth (cm)	Physical Characteristics				Chemical Characteristics								
										Available	Exchangeable		
	BD	Sand	Silt	Clay	pH	EC	C	N	C/N	P	K	Ca	Mg
	g cm^{-3}	%	%	%		µS cm^{-1}	%	%		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
NF 0–20	1.11 e	44.67 cd	27.33	28.00 ef	5.76	49.82	2.29 b	0.19 b	12.68	1.92 d	461	760	26.61
NF 20–40	1.15 e	50.67 a	24.67	24.67 fg	5.73	34.31	1.39 d	0.13 c	10.79	1.22 d	327	616	20.67
NF 40–60	1.23 cd	36.00 g	16.67	47.33 a	5.39	27.55	0.91 fg	0.09 d	11.26	0.49 d	301	520	17.05
CA 0–20	1.30 ab	40.00 efg	28.67	31.33 cde	5.95	64.73	1.76 c	0.15 c	12.15	43.14 a	251	1116	10.59
CA 20–40	1.33 a	50.00 ab	26.00	24.00 fg	5.95	44.63	0.99 fg	0.09 d	10.87	20.73 b	274	801	7.03
CA 40–60	1.31 a	46.00 bc	22.67	31.33 cde	6.07	45.18	0.91 fg	0.09 d	11.63	4.67 cd	255	779	7.13
CF 0–20	1.12 e	41.33 def	20.67	38.00 b	6.12	76.38	2.68 a	0.25 a	11.36	5.97 cd	380	1889	37.91
CF 20–40	1.24 bcd	44.00 cde	26.00	30.00 de	6.22	52.62	1.36 de	0.13 c	10.29	1.55 d	341	1293	28.56
CF 40–60	1.27 abcd	44.00 cde	21.33	34.67 bcd	6.21	41.82	0.97 fg	0.09 d	10.52	1.00 d	385	1128	25.85
CP 0–20	1.22 d	50.00 ab	28.00	22.00 g	5.92	55.95	1.81 c	0.16 bc	11.68	8.93 c	417	1430	15.29
CP 20–40	1.28 abc	38.67 fg	26.67	34.67 bcd	5.92	43.99	1.12 fg	0.10 d	12.05	4.45 cd	338	1151	8.83
CP 40–60	1.32 a	43.33 def	28.67	35.33 bc	5.84	41.23	0.76 g	0.08 d	10.25	0.88 d	319	1031	6.63
p values	0.013	0	0.112	0	0.67	0.530	0	0	0.890	0	0.358	0.094	0.403
F-test$_{0.05}$	*	*	ns	*	ns	ns	*	*	ns	*	ns	ns	ns
CV	5.21	6.24	16.14	9.14	7.66	30.37	19.43	23.88	27.72	82.71	37.75	26.07	31.24

Note: BD: bulk density; EC: electrical conductivity; C (%): carbon; N (%): nitrogen; C/N: C:N ratio; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium. *: significant level at $p < 0.05$. ns: non-significant level at $p > 0.05$. CV: coefficient of variation. Different letters indicate the statistical significance at $p < 0.05$.

3.2. Changes in Soil Characteristics Across Different Land Uses and Seasons

The average values of soil characteristics across the different land uses in various seasons is shown in Figure 3a–l. As a BD result analysis (Figure 3a), the NF system exhibited the lowest BD among three seasons, indicating a less compacted soil structure within the study area. In contrast, the CA system consistently demonstrated the highest BD values, suggesting increased soil compaction likely due to intensive management practices. During the summer season, the CP system exhibited the highest BD values. Soil pH varied significantly among land uses and seasons (Figure 3b). During the rainy season, the coffee–forest (CF) and CA systems exhibited higher pH values compared to NF and CP. In the cold season, the pH order was CF > CP > CA > NF. However, no significant differences in pH were observed among the land uses during the summer season. Soil EC patterns also differed across land uses and seasons (Figure 3c). In the rainy season, EC followed the order CF > CA > CP > NF, while during the cold season, the order was CF > CP > CA > NF. Notably, no significant differences in EC were detected among the land uses during the summer season. However, total C and N concentrations, the C/N ratio, and C and N stocks did not exhibit significant variations across the three seasons, regardless of land use (Figure 3d–h). The CA system consistently exhibited the highest levels of P across all three seasons, potentially due to fertilization practices associated with monoculture coffee cultivation (Figure 3i). Exchangeable K levels varied among land uses and seasons (Figure 3j). During the rainy season, CF and NF had higher exchangeable K levels than CA and CP, while in the cold season, CF and CP had higher exchangeable K than CA and NF. However, no significant differences in exchangeable K were observed during the summer season. The CF system exhibited the highest levels of exchangeable Ca, while NF had the lowest (Figure 3k). Furthermore, exchangeable Mg levels were significantly higher in CF and NF compared to CA and CP (Figure 3l).

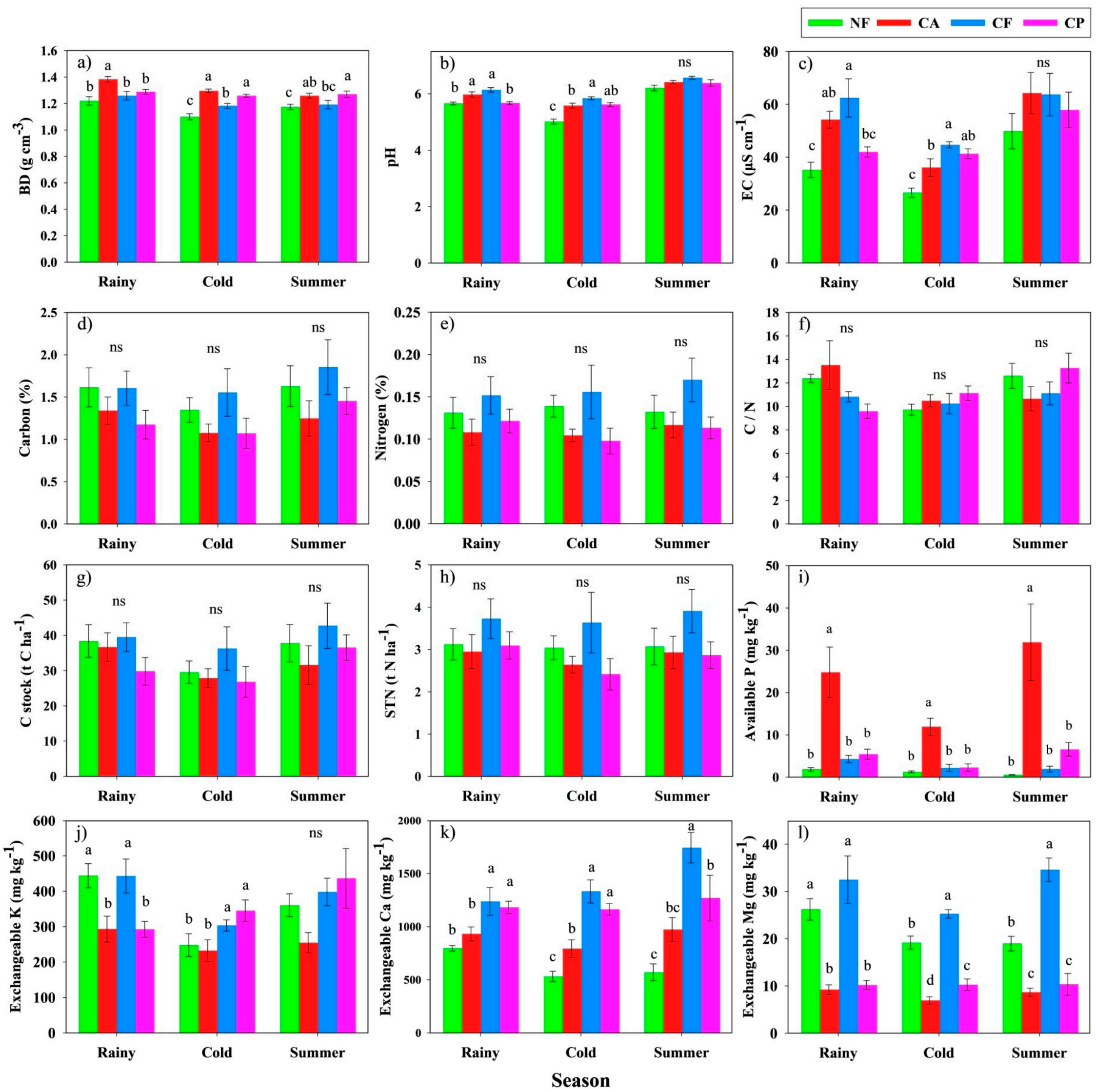

Figure 3. Soil characteristics across different land uses in three seasons, namely (**a**) BD, (**b**) pH, (**c**) EC, (**d**) C (%), (**e**) N (%), (**f**) C/N, (**g**) C stock, (**h**) STN, (**i**) available P, (**j**) exchangeable K, (**k**) exchangeable Ca, and (**l**) exchangeable Mg. Note: NF: natural forest; CA: coffee monoculture; CF; coffee combined with forest; CP: Arabica coffee combined with persimmon; BD: bulk density; EC: electrical conductivity; C (%): carbon; N (%): nitrogen; C/N: C:N ratio; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium. Different letters indicate the significance of different land uses. ns: non-significant level at $p > 0.05$.

3.3. Impacts of Coffee Agroforestry Systems on Soil Carbon Stock

The impact of soil depth on C stock was evident. Each land use type experienced distinct seasonal changes in C stock (Figure 4a–c). Changes in C stock were observed in the 0–20 cm soil layer (Figure 4a). Under rainy conditions, the C stock changes followed the pattern CF = NF > CA > CP. In contrast, under cold conditions, the pattern was CF > NR = CP > CA. And there was a change in the pattern again in the summer, namely CF > NR > CA > CP. During the summer, the C stock in the 20–40 cm soil layer decreased in the CF compared to the CA (Figure 4b). The CA exhibited the lowest C stock, with a decrease in the 40–60 cm soil layer (Figure 4b). However, no significant difference in C stock was observed due to land utilization. When comparing the 0–60 cm depth across seasons, no significant differences in C stock were found among the land use types (Figure 5).

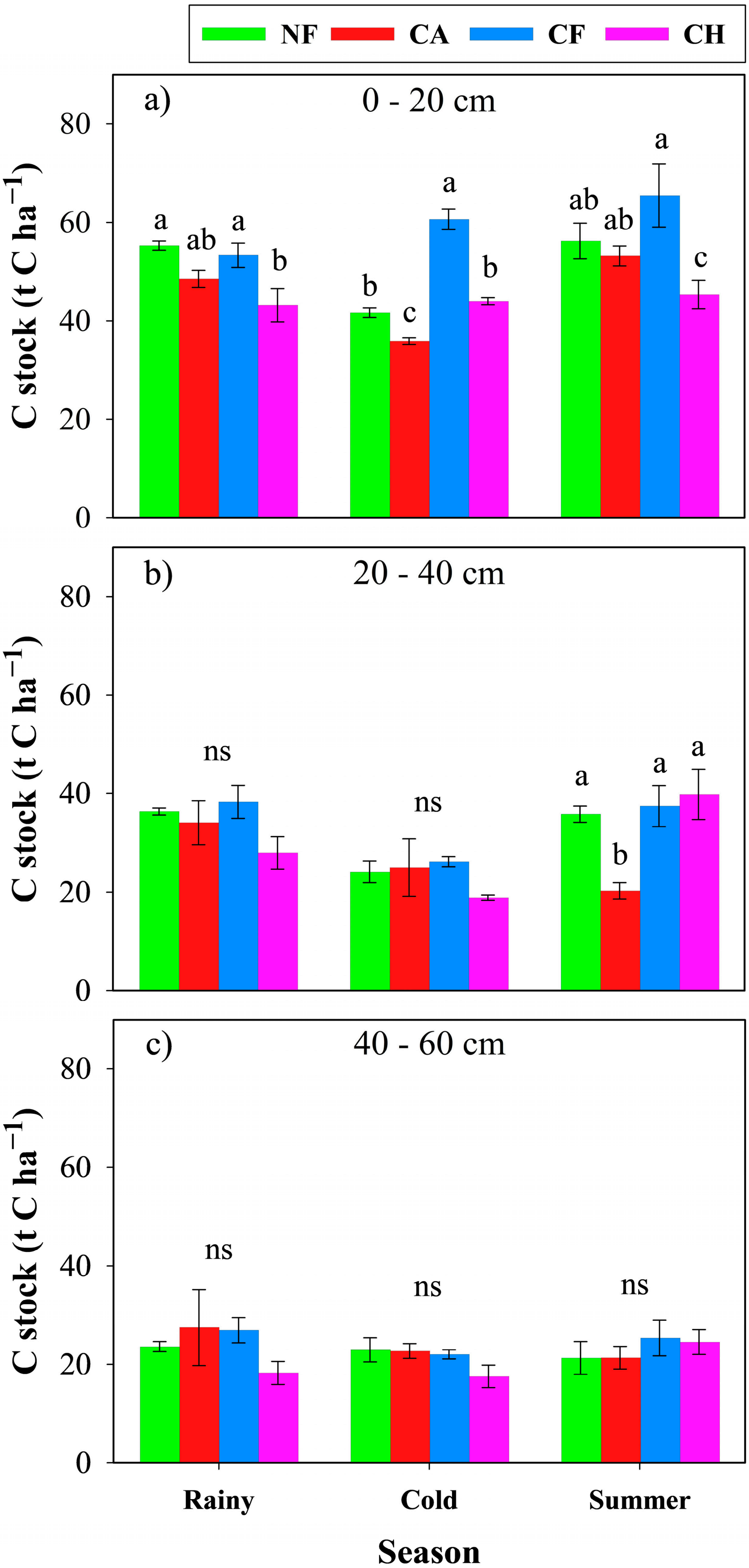

Figure 4. Soil C stock across the different land uses in three seasons and three depths, with depths of (**a**) 0–20 cm., (**b**) 20–40 cm., and (**c**) 40–60 cm. NF: natural forest; CA: coffee monoculture; CF; coffee combined with forest; CP: Arabica coffee combined with persimmon. Different letters indicate the statistical significance at $p < 0.05$. ns: non-significant level at $p > 0.05$.

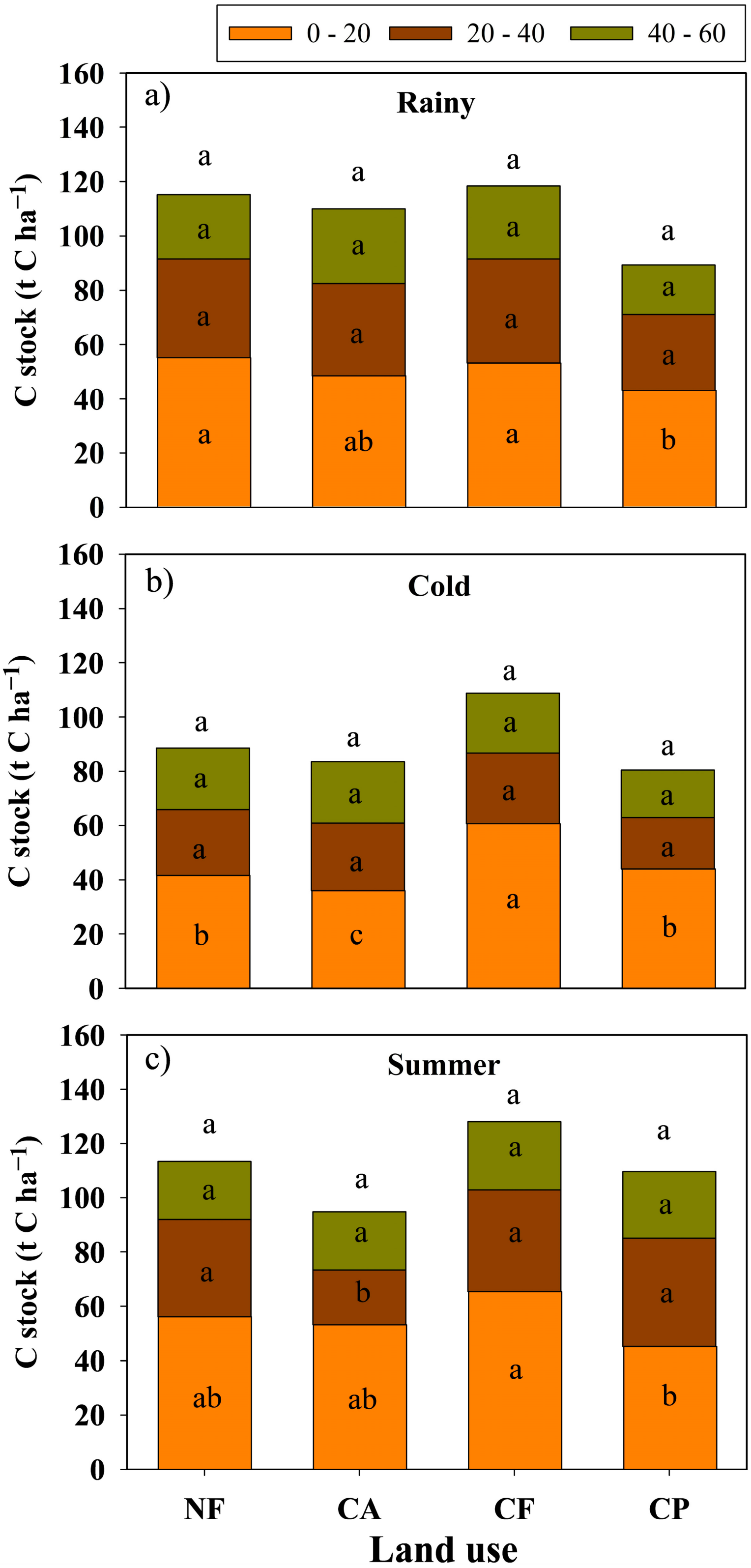

Figure 5. Soil stock C stack bar varies according to various land uses in three seasons and three depths, with the (**a**) rainy season, (**b**) cold season, and (**c**) summer season. NF: natural forest; CA: coffee monoculture; CF: coffee combined with forest; CP: Arabica coffee combined with persimmon. Different letters indicate the statistical significance at $p < 0.05$.

Table 2 displays the results of the alterations in C stock due to the conversion of forest to various coffee growing systems. The soil layer at 0–60 cm has shown an increase in carbon stock accumulation due to conservation farming, with a significance value of $p = 0.0037$. However, C stock in the treatment of CA and CH decreased at the soil depth of 0–60 cm, with values of $p = 0.0729$ and $p = 0.0388$, respectively.

Table 2. Comparison of changes in soil carbon stocks among natural forest and coffee-growing systems.

Depth (cm)	Carbon Stock (t C ha^{-1})	Carbon Stock (t C ha^{-1})	Δ Carbon Stock (t C ha^{-1})	Change Loss	% Change	*p*-Value
	NF	CA				
0–20	51.03 ± 2.61	45.83 ± 2.70	−5.20 ± 1.14	−	10.18	0.002
20–40	32.06 ± 2.15	26.44 ± 2.97	−5.62 ± 3.60	−	17.53	0.157
40–60	22.59 ± 1.27	23.83 ± 2.55	1.24 ± 3.04	+	5.48	0.694
0–60	105.68 ± 4.49	96.10 ± 5.56	−9.58 ± 4.64	−	9.06	0.073
	NF	CF				
0–20	51.03 ± 2.61	59.78 ± 2.72	8.75 ± 4.10	+	17.15	0.065
20–40	32.06 ± 2.15	33.93 ± 2.50	1.87 ± 1.37	+	5.84	0.208
40–60	22.59 ± 1.27	24.76 ± 1.50	2.16 ± 1.37	+	9.57	0.153
0–60	105.68 ± 4.49	118.47 ± 3.73	12.79 ± 3.15	+	12.10	0.004
	NF	CP				
0–20	51.03 ± 2.61	44.13 ± 1.33	−6.90 ± 2.68	−	13.51	0.033
20–40	32.06 ± 2.15	28.88 ± 3.56	−3.18 ± 2.89	−	9.92	0.304
40–60	22.59 ± 1.27	20.11 ± 1.62	−2.49 ± 2.26	−	11.02	0.304
0–60	105.68 ± 4.49	93.12 ± 5.25	−12.56 ± 5.09	−	11.89	0.039

Note: loss (−), gain (+). NF: natural forest; CA: coffee monoculture; CF: coffee combined with forest; CP: Arabica coffee combined with persimmon.

3.4. Impacts of Coffee Agroforestry Systems on Microbial Activity

The results of coffee agroforestry systems on microbial activity, including MBC and DOC, are shown in Figures 6a and 6b, respectively. During the rainy season, the MBC was found to be highest in CF and NF treatments and lowest in the CA treatment (Figure 6a). When entering the cold season, MBC was found to have similar results in NF, CF, and CP treatments, while CA remained the lowest value. However, no significant difference was found in MBC across the land use types in the summer season. The CA treatment had the highest DOC concentration during the rainy season, whereas the NR treatment had the highest DOC value throughout the summer (Figure 6b).

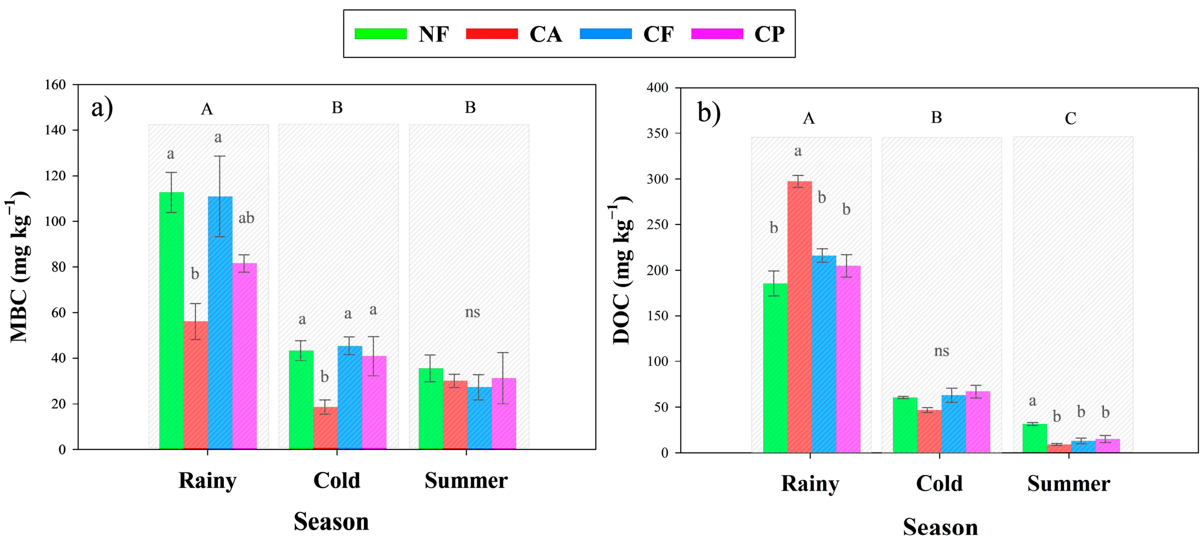

Figure 6. The results of MBC and DOC for different land uses in three seasons by MBC (**a**) and DOC (**b**). NF: natural forest; CA: coffee monoculture; CF: coffee combined with forest; CP: Arabica coffee combined with persimmon; MBC: microbial biomass carbon; DOC: dissolved organic carbon. A, B, and C indicate the statistically significant differences in season at $p < 0.05$. a and b indicate the statistically significant differences in land use at $p < 0.05$. ns indicates non-significant differences within group at $p < 0.05$.

The result of the MR analysis under coffee agroforestry systems during rainy, cold, and summer seasons is shown in Figures 7a, 7b and 7c, respectively. The MR rate peaked on day 3 for every treatment. It was found that the order of MR rates was significant, with the order following CF > CA > CP > NF, and MR values gradually decreased in all three studied seasons (Figure 7a–c). CF had the highest MR value and NF had the lowest. The results found that the cumulative MR was highest in CF and lowest in NR during rainy conditions (Figure 7a). Regularly, MR exhibited similar changes across the three seasons, and in the cold season after 10 days, no differences in MR were found between treatments (Figure 7b). As the weather turned to summer, there was a slight reversal of the cumulative MR position, namely CF > CP > NR = CA (Figure 8).

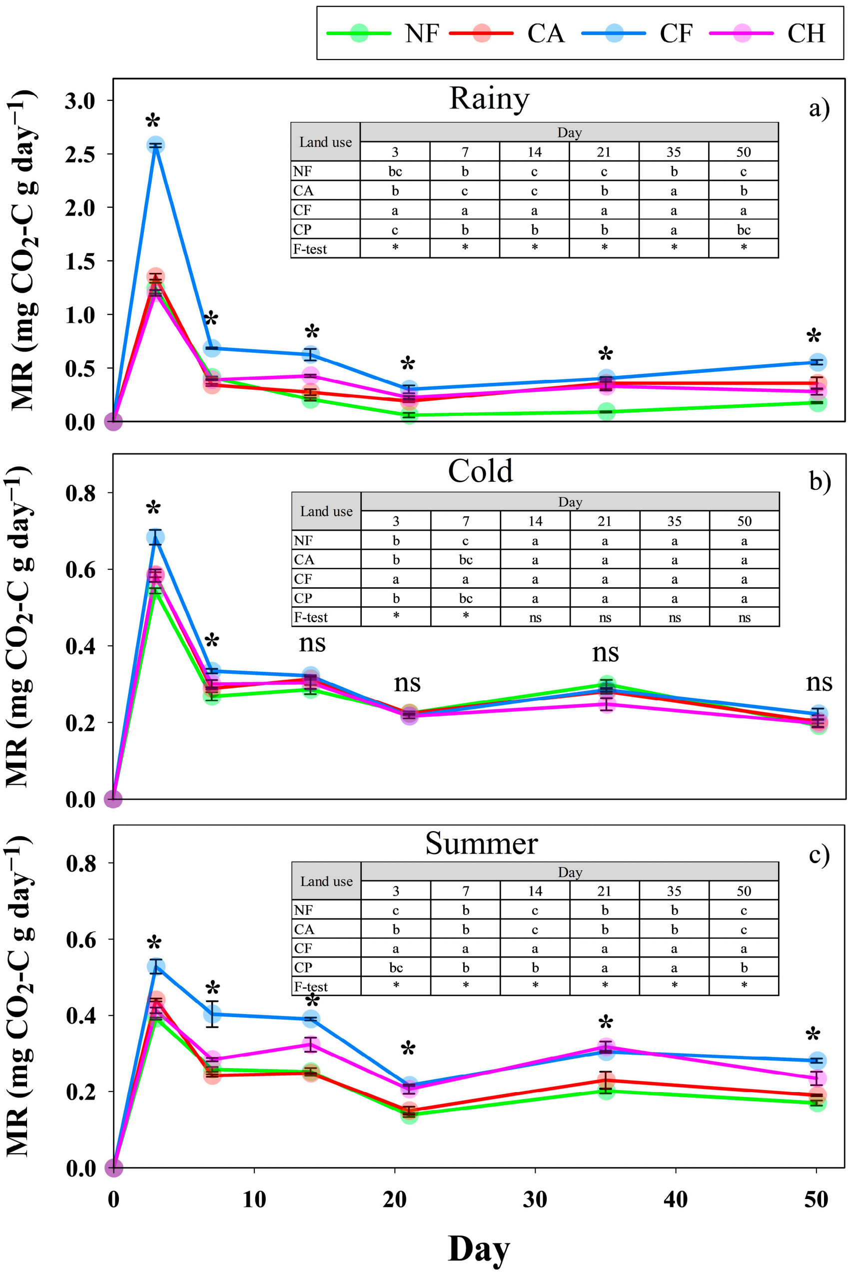

Figure 7. Result of MR across the different land uses in three seasons, namely the (**a**) rainy season, (**b**) cold season, and (**c**) summer season. NF: natural forest; CA: coffee monoculture; CF: coffee combined with forest; CP: Arabica coffee combined with persimmon; MR: microbial respiration; *: significant; ns: non-significant. Different letters in the table indicate the significance of different land uses. Error bars indicate standard error.

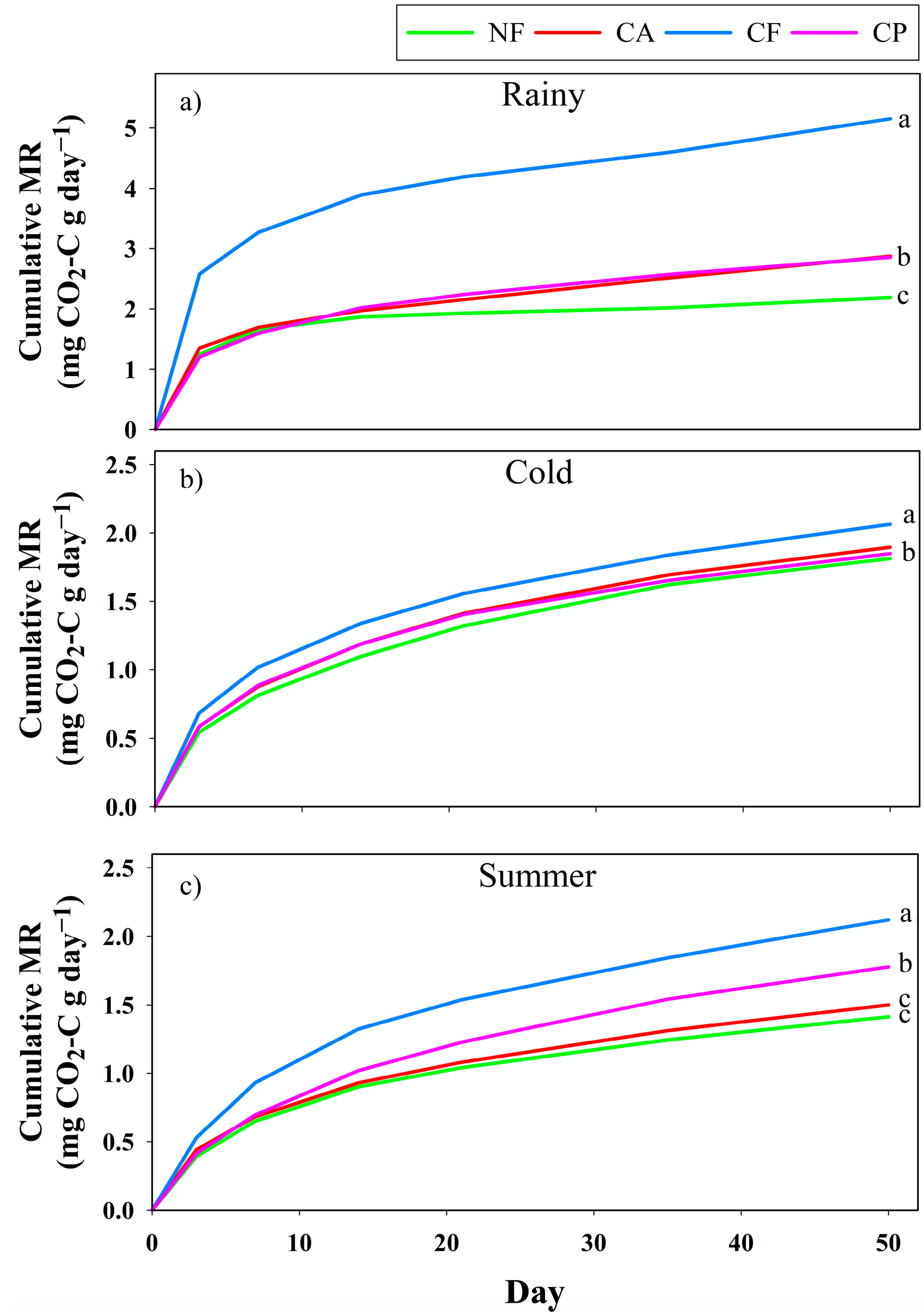

Figure 8. Mean commutative values of MR across the different land uses in three seasons, namely the (**a**) rainy season, (**b**) cold season, and (**c**) summer season. NF: natural forest; CA: coffee monoculture; CF: coffee combined with forest; CP: Arabica coffee combined with persimmon; MR: microbial respiration; Different letters in the table indicate the significance of different land uses.

3.5. Relationships of Soil Parameters Through Linear Regression Correlation and Principal Component Analysis

Figure 9 shows the linear regression correlation between soil characteristics and the influence of C stock factors and soil depth at 0–20 cm, 20–40 cm, and 40–60 cm. For the relationship between soil characteristics and the influence of C stock factors and soil surface area (0–20 cm), positive parameters ($p < 0.001$) were C, N, and STN; ($p < 0.01$) were clay, pH, EC, and Mg; and ($p < 0.05$) were CA, but the negative correlation in the soil surface was silt ($p < 0.001$) and BD ($p < 0.05$) (Figure 9a). Next, for the soil stratum of a 20–40 cm depth

(Figure 9b), only C, N, and STN have a positive connection ($p < 0.001$). The parameters C/N, K, and Mg have p values less than 0.01, while pH has a p value less than 0.05. Lastly, the soil layer measures 40–60 cm in soil depth, and positive correlations were seen with C and C/N ($p < 0.001$) (Figure 9c).

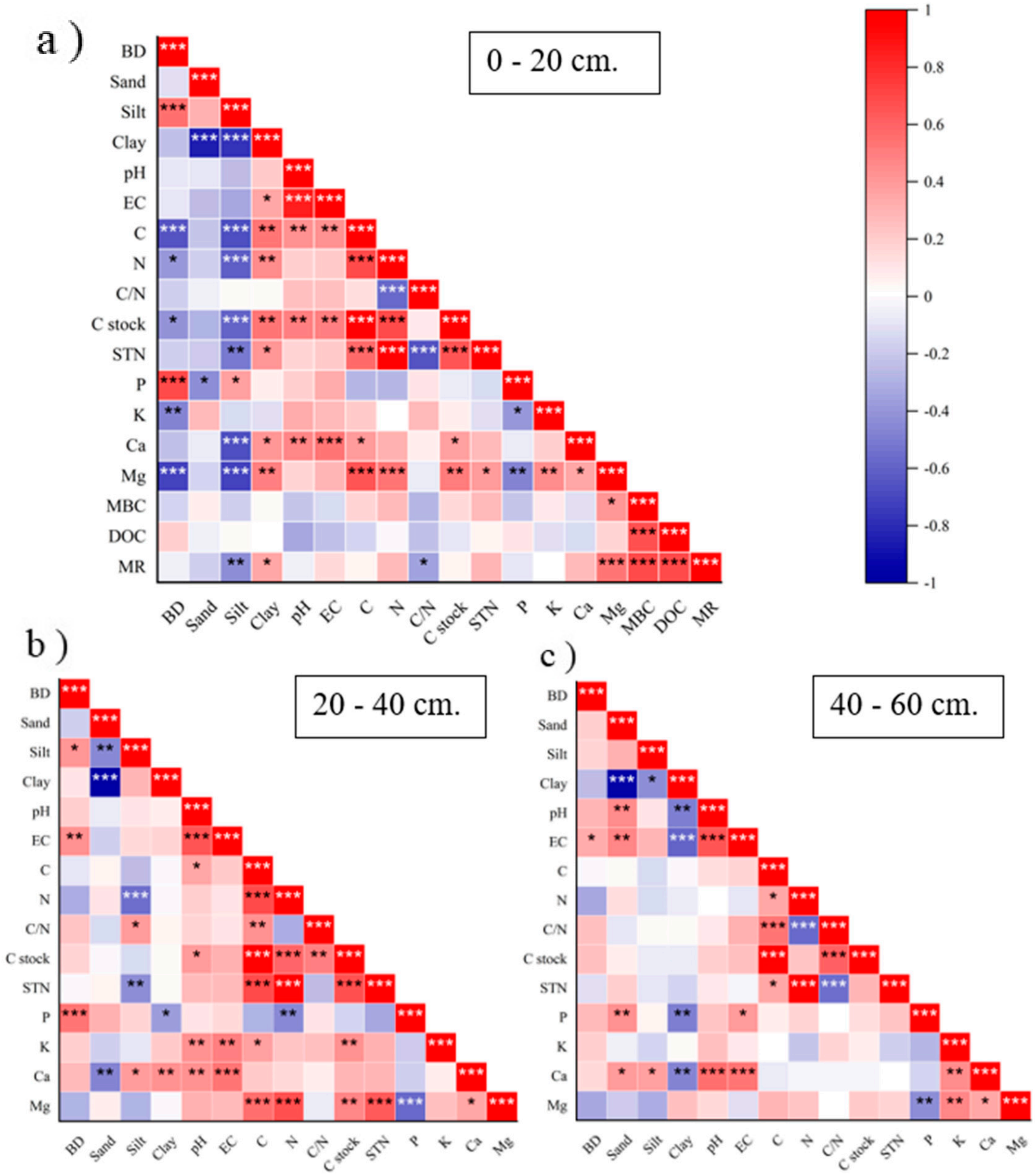

Figure 9. Relationship between soil characteristics with different soil depths of (**a**) 0–20 cm, (**b**) 20–40 cm, and (**c**) 40–60 cm. Note: *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$. BD: bulk density; C: carbon; N: nitrogen; C/N: C:N ratio; C stock: soil carbon stock; STN: soil total nitrogen; P: available phosphorus; K: exchangeable potassium; Ca: exchangeable calcium; Mg: exchangeable magnesium; MR: microbial respiration; MBC: microbial biomass carbon; DOC: dissolved organic carbon.

Figure 10 displays the result of linear regression among soil microbial activity, comprising MBC, DOC, MR, and C stock with different seasons. MBC during the rainy season (R^2 = 0.4567) and winter (R^2 = 0.3531) exhibited a relationship with C stock ($p < 0.05$) (Figure 10a). A similar observation was found in the treatment of MR. During the rainy season (R^2 = 0.5164), MR has a relationship with C stock ($p < 0.05$) (Figure 10b). In terms of DOC, it was determined that all three seasons exhibited a value of ($p > 0.05$) (Figure 10c).

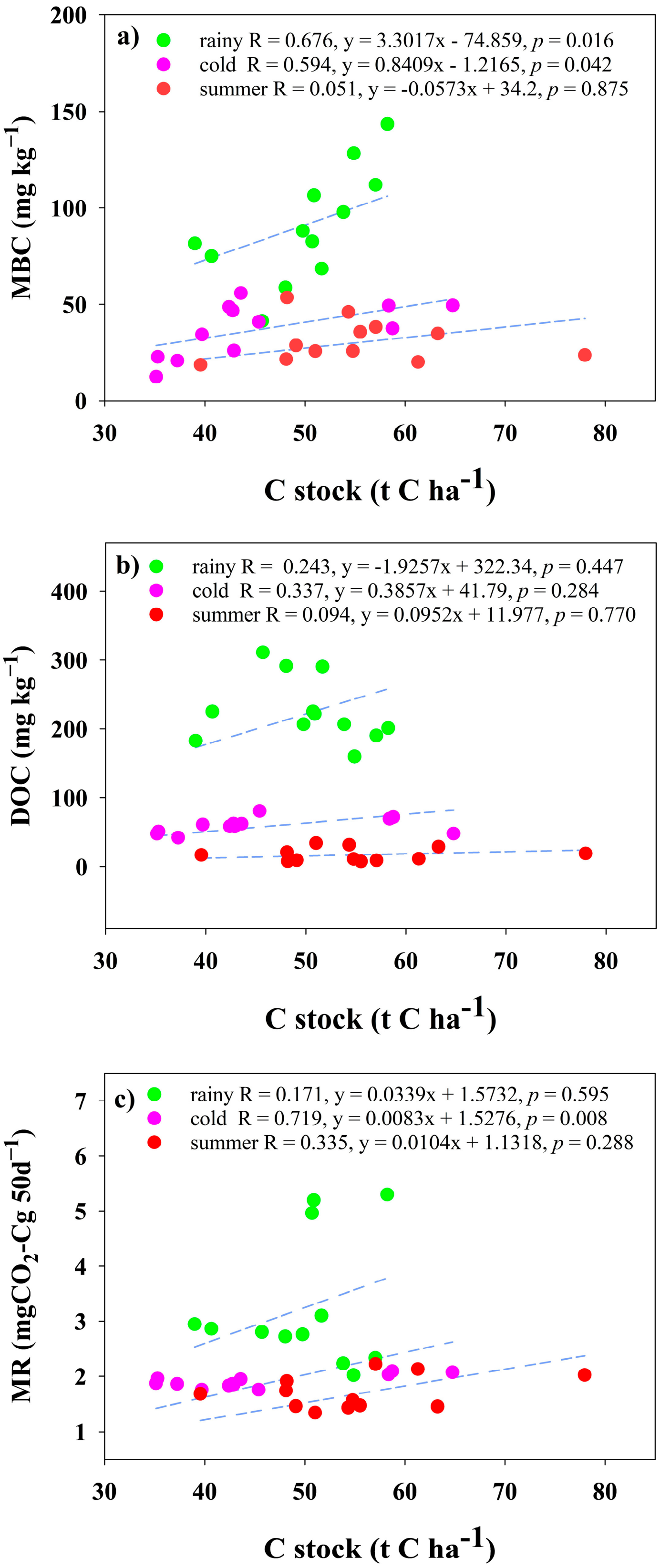

Figure 10. Relationships between C stock and microbial activity for (**a**) MBC, (**b**) DOC, and (**c**) MR. MR: microbial respiration; MBC: microbial biomass carbon; DOC: dissolved organic carbon.

4. Discussion

4.1. Soil Physical and Chemical Characteristics

According to the obtained results from the soil properties analysis under different land use types, the highlights of this study are discussed. Soil BD exhibited a statistically significant variation among land use types ($p < 0.001$). The CA of each area has a unique characteristic, depending on the surface cover [11,12]. Increasing plant cover can help reduce BD due to increased organic matter [30], and reduced plowing can also reduce BD [31]. In general, as the depth increased, the bulk density increased [32,33]. Jagadesh et al. [19] reported that the forest ecosystem had the lowest bulk density value, which was 1.31 g cm^{-3} compared to the tea plantation ecosystem at 1.40 g cm^{-3} and agricultural land at 1.35 g cm^{-3}.

With respect to soil texture, higher sand content typically results in lower C content. Our findings in natural forest (NF) and coffee with forest (CF) areas align with Stevenson et al. [34], who found that forest soils generally have higher clay content. While Castro et al. [7] reported a positive correlation between clay and carbon content, our study found no significant differences in soil texture (sand, silt, and clay contents) across different land uses. These soils exhibit a characteristic clay distribution pattern resulting from pedogenetic processes, specifically clay migration. This process creates an argillic horizon in the subsoil with higher clay content compared to the topsoil. According to Parras-Alcántara and Lozano-García [35], a key characteristic of these soils is their low organic matter content. The soil texture analysis revealed exclusively clay content, which showed a significant increase with depth ($p < 0.01$).

Although no significant difference was found in C content ($p > 0.05$), NF and CF areas showed higher values compared to CA and CP. This can be attributed to their natural forest characteristics and coffee–forest tree intercropping systems. Forest ecosystems generally store more soil carbon than agricultural areas, with fine roots and small soil fragments playing crucial roles [12] alongside traditional plant diversity [36]. Agroforestry systems demonstrate higher soil carbon sequestration compared to conventional row crop systems [37–39].

Nitrogen (N) patterns closely followed carbon trends, as N accumulation typically results from organic matter decomposition in plant residues [30,35,40] and microbial activity [28,41]. High carbon levels typically correlate with elevated nitrogen concentrations [30,42], and agroforestry systems can help maintain nitrogen in its preferred ammonium form [43].

Essential plant nutrients (N, P, K, Ca, and Mg) showed decreasing concentrations with soil depth [33]. Soil available phosphorus (P) was notably high in CA due to P fertilizer applications in this monoculture system. Potassium (K) was lowest in CA, possibly due to reduced plant diversity compared to other systems [44], while NF and CF maintained higher magnesium (Mg) retention. Soil pH significantly influences nutrient availability [12,33,45], and calcium (Ca) enhances organic matter–mineral interactions and microbial activity [46]. Microbial biomass carbon (MBC) in soils without Ca treatment ranged from 11 to 59 μg g^{-1}, which is considered low compared to reported values in similar soils [16].

4.2. Soil Carbon Stock Across Different Land Use Types

Carbon stock is calculated from the total carbon percentage, which comprises both organic and inorganic carbon. In this study, soil samples collected from forest and agricultural areas showed equivalent amounts of soil organic carbon (SOC) to total C. Therefore, this discussion incorporates SOC findings from other research studies [47].

Carbon stocks vary with land use [13,30] and land cover [48,49] through complex processes involving multiple factors. The carbon balance is influenced by additions and losses [13], temperature, humidity, and climate [50], as well as microbial activities [51]. Converting forest land to agricultural use typically reduces soil carbon stocks [11,12]. For instance, Jagadesh et al. [19] found that forest ecosystems contained an average carbon stock of 77.94 t C ha^{-1} at a 0–45 cm depth, significantly higher than agricultural ecosystems at 32.30 t C ha^{-1}. However, agricultural lands can experience rapid carbon increases when supplemented with organic matter [52].

Carbon stock values generally decrease with soil depth [30]. Surface layers are more susceptible to carbon fluctuations due to organic matter decomposition instability [13]. The highest carbon stock concentrations typically occur in the shallow 0–20 cm soil layer, decreasing in deeper layers [53]. Consistent with these findings, our research showed carbon stocks beginning to stabilize at 20–40 cm and 40–60 cm depths.

Land cover patterns primarily influence carbon dynamics, while microbial activity plays a secondary role through the breakdown of plant biomolecules and their constituents, which in turn shapes microbial selection [13]. As mentioned earlier, agroforestry agriculture can promote carbon stock [34,37,54], particularly by increasing soil organic carbon content [55]. Agroforestry areas maintain soil carbon stocks comparable to natural forests and significantly higher than conventional monoculture systems [12,56]. Growing many crops together with agricultural crops increases biodiversity, causing more organic matter to be produced and more material to fall to the ground, facilitating the nutrient cycling process [54,56,57]. Notably, root systems from diverse plant species contribute more stable, long-term carbon compared to leaf litter [12,14]. Carbon assessment is an indicator of soil health and ecosystem preservation [42]. However, agroforestry does not always increase carbon stock, depending on the plants grown in the system. Selecting trees in agroforestry requires local and scientific knowledge, which has many criteria to consider [40]. Chen et al. [9] reported that agroforestry systems (except for growing rubber along with coffee) can increase organic carbon better than growing rubber alone. Our study showed that when coffee was grown with persimmon trees, the amount of carbon stock in the soil layer 0–60 cm decreased from natural forests by 11.89% ($p < 0.05$), which was more than when coffee was grown by itself, which decreased by 9.06% ($p > 0.05$). However, when coffee was grown with forest trees, the amount of carbon stock can increase by 12.10% ($p < 0.01$). Noponen et al. [38] reported that during the first 9 years of the coffee system, soil carbon stocks at depths of 0–40 cm decreased by an average of 12.4% in Costa Rica and 0.13% in Nicaragua. This underscores the need for the effective management of coffee systems and the significance of boosting soil carbon levels.

A research study on agroforestry coffee cultivation found that in the 0–40 cm soil layer, agroforestry coffee cultivation had a carbon stock of 111.29 Mg C ha^{-1}, while monoculture coffee cultivation had a value of 110.11 Mg C ha^{-1}. This indicates that carbon accumulation in the soil is similar for both systems. However, when focusing on the topsoil layer (0–10 cm), agroforestry coffee cultivation showed a higher value than coffee monoculture, with an average of 32.08 Mg C ha^{-1} compared to 29.67 Mg C ha^{-1} [25]. Moreover, Tumwebaze and Byakagaba [55] reported that carbon sequestration under agroforestry systems of Arabica coffee cultivation and monoculture Arabica coffee cultivation in Uganda had the following values: coffee intercropped with fruit trees: 54.010 t C ha^{-1}; coffee intercropped with timber and fruit trees: 54.543 t C ha^{-1}; coffee intercropped with timber trees: 51.170 t C ha^{-1}; and monoculture coffee system: 50.987 t C ha^{-1}.

During the research period, we collected soil samples at three intervals to represent the seasons in Thailand. Rainfall has a direct relationship with organic carbon because it plays a crucial role in supporting plant growth. Plants carry out photosynthesis and produce organic carbon, which is transferred to the soil through roots and dead plant debris. Moreover, rainfall helps dissolve nutrients and promotes microbial activity in the soil [58,59]. The soil carbon stock exhibited minimal variations over the three seasons. It was noted that during summer, values were only marginally elevated compared to other seasons. This occurs because of the seasonal shedding of deciduous leaves resulting from elevated temperatures and reduced moisture from precipitation [60]. The modest rise in soil carbon stock may result from a gradual enhancement in decomposition attributed to the constraints faced by soil microorganisms [51].

4.3. Soil Microbial Respiration and Microbial Biomass Carbon

Microbial respiration (MR) is an important process of organic matter decomposition that releases energy for soil microorganisms and directly affects the release of carbon dioxide (CO_2) into the atmosphere. This process is an indicator of microbial activity in the soil, as well as the soil's ability to cycle nutrients. Assessing the health of the soil and its associated ecosystems involves measuring soil basal respiration rates. MBC has a positive correlation with MR, meaning that as the amount of MBC increases, microbial respiration also increases. This is because microorganisms play a crucial role in decomposing organic matter and releasing CO_2. Studies have found that changes in land use, such as agriculture or afforestation, significantly affect microbial activity and MR [15,51]. Additionally, MBC has a positive correlation with the amount of SOC, which means that an increase in MBC is often associated with an increase in SOC in the soil. A high microbial biomass aids in organic matter decomposition and increases soil carbon content [28,49,51]. Land use changes impact MBC, which can be used to assess the effects of managed land in various forms. MBC and C–CO_2 are important indicators for evaluating land use changes and their effects on carbon cycling in soil ecosystems [15,41,61]. Research has found that C–CO_2 and MBC in soil covered by trees, such as forests, have higher carbon retention compared to soil used for agricultural activities [16]. Singh et al. [50] reported that MBC varies according to the type of area and land use, with several factors affecting the quantity and quality of MBC in different areas. Agroforestry systems have MBC levels that are 6–34% higher compared to agricultural systems. Agroforestry systems are one of the agricultural practices that promote the conservation of biodiversity and soil sustainability [41]. Findings show that coffee agroforestry systems can keep MBC levels the same as natural forests. This is because agroforestry systems are better at supporting soil microbial biomass than monoculture systems. Coffee agroforestry systems have the potential to enhance MBC, which is a crucial indicator of soil health. There is a study on MBC in various systems, finding that the native forest had the highest value at 783.05 $\mu g\ g^{-1}$, followed by coffee with a Grevillea robusta agroforestry system at 259.1 $\mu g\ g^{-1}$, coffee with cedar agroforestry system at 468.43 $\mu g\ g^{-1}$, and coffee monoculture had the lowest value at 187.60 $\mu g\ g^{-1}$. This is similar to our research, where CA had the lowest MBC, while CF had a value close to that of the natural forest (NF). However, a high MBC does not always mean that MR will also be high [28]. When comparing MBC and MR in our study, we found that MR in the NF is relatively low, similar to that of CA, even though NF has the highest MBC. Additionally, we found an inverse relationship between the MR of CF and MBC, which could potentially depend on other factors like soil management and environmental conditions that influence microbial activity and nutrient cycling in the soil system [28]. This is also linked to the DOC in soil. The DOC consists of small organic carbon compounds that are soluble in water, typically resulting from the decomposition of plants and animals, as well as the activity of microorganisms in the soil. DOC is important for ecosystems and soil management because it can affect water quality, nutrient mobility, and carbon storage in the soil. Additionally, erosion and water runoff can lead to the loss of DOC, potentially affecting the soil's total organic carbon content [61], which is most commonly found in monoculture coffee systems.

Seasons affect the decomposition process and SOC. Seasonal changes, as shown in our study, demonstrate the relationship between microbial activity in the soil and the changing environmental conditions throughout the seasons. Higher temperatures can accelerate the decomposition of organic matter, resulting in increased CO_2 emissions and a reduction in SOC levels. Adequate moisture supports microbial activity and carbon storage in the soil, but excessive moisture can lead to oxygen depletion in the soil, thereby reducing SOC retention. Good aeration promotes microbial activity, while poor aeration can decrease microbial respiration and lead to lower carbon storage [50]. Rainfall, an important factor in controlling temperature and soil moisture, helps dissolve nutrients and promotes the decomposition and accumulation of SOC [23]. MBC significantly decreases under conditions of drought and heat stress [62]. Microorganisms in the soil collected from agroforestry systems showed better recovery capabilities after changes in soil moisture [63].

The role of nitrogen in the soil is as follows: a high nitrogen content in the soil can support the growth of microorganisms and lead to an increase in MBC. This is because nitrogen is an essential nutrient for the synthesis of proteins and other biomolecules necessary for microbial growth. A high nitrogen content in the soil can enhance microbial growth and increase MBC as well [15,28,35].

5. Conclusions

The research results describe the impact of land use changes, particularly the alteration of vegetation cover, on soil physicochemical and biological properties and soil carbon stock. The coffee agroforestry system was able to enhance the carbon stock compared to coffee monoculture. Contrarily, compared to the initial levels, the carbon stock decreased in the coffee monoculture and the CP. It is well understood that agricultural activities typically lead to disturbances caused by human actions, resulting in a reduction in carbon stock. However, the finding of CF is an exception to this rule. Carbon stock has a positive correlation with soil total nitrogen, which results in the treatment of CF having a higher total nitrogen content. The study on MBC found the highest levels in natural forests and coffee in agroforestry systems, namely coffee intercropped with forest trees and coffee intercropped with persimmon trees. It was also discovered that there was a significant positive correlation between MR, MBC, and DOC. Despite high MBC levels, low MR levels showed in natural forests, suggesting that high MBC content does not necessarily translate into high microbial activity, and DOC is also present more in coffee monoculture. Seasonal changes also influence the amount of carbon stock and microbial activity, which fluctuate throughout the year in response to various environmental factors. We can conclude that coffee agroforestry can mitigate the effects of deforestation and help maintain soil fertility for long-term sustainable agriculture. Additionally, selecting appropriate species for cultivation has potential benefits for carbon sequestration and soil microbial activity.

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