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# An impact of agroforestry-based coffee cultivation and nitrogen fertilization on soil carbon dynamics and microbial metabolic activity: a controlled incubation study

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## Abstract

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Land use changes significantly impact soil carbon dynamics, as the soil microenvironment beneath different canopy structures and the distinct root properties of various plant species influence soil respiration rates and microbial communities. In northern Thailand, substantial forest areas have been converted to agricultural land, necessitating a comprehensive understanding of the resulting effects on soil carbon processes. This study aims to quantify soil CO<sub>2</sub> emissions and dissolved organic carbon (DOC) concentrations across diverse coffee cultivation systems under nitrogen-fertilized and unfertilized conditions. The research was conducted at the Arabica coffee plantation within the Nhong Hoi Highland Agricultural Research Station, Chiang Mai province, Thailand. Four land use types were investigated: (1) Forest, (2) coffee monoculture, (3) coffee grown with forest, (4) coffee grown with horticulture (persimmon). The study examined the effects of nitrogen fertilization on soil microbial respiration and carbon mineralization dynamics. Soil samples were collected from the topsoil layer (0–20 cm) and subjected to laboratory incubation experiments. Destructive sampling was performed at days 1, 4, 7, 14, 21, 35, and 65 post-incubations for DOC and CO<sub>2</sub> emission analyses through microbial respiration. The highest soil carbon emission (0.1261 mg C g<sup>-1</sup> soil<sup>-1</sup>) was observed in unfertilized coffee-forest integration systems, followed by nitrogen-fertilized coffee-forest integration (0.1085 mg C g<sup>-1</sup> soil<sup>-1</sup>). Nitrogen fertilization in natural forest soils significantly increased DOC concentrations ( $p < 0.05$ ), while unfertilized natural forest soils showed no significant difference. For cumulative CO<sub>2</sub> emissions, unfertilized coffee-forest integration systems exhibited the highest cumulative CO<sub>2</sub> emissions (10.3 mg kg<sup>-1</sup>), while unfertilized natural forest soils demonstrated the lowest (4 mg kg<sup>-1</sup>). These findings suggest that coffee cultivation integrated with forest or horticultural species may offer a promising approach for Arabica coffee production, potentially enhancing biodiversity, soil conservation, and carbon sequestration compared to monoculture systems. The study underscores the complex interactions between land use practices, nitrogen fertilization, and soil carbon dynamics in highland agroecosystems. Further research is warranted to elucidate the long-term effects of these integrated cultivation systems on soil health, ecosystem services, and climate change mitigation potential in highland tropical environments.

## 1. Introduction

Over the past decade, global coffee consumption has exhibited a consistent annual growth rate exceeding 2%. Thailand's coffee industry underwent substantial growth from 2016 to 2020. During this period, the country's mean annual coffee bean consumption approached 79,000 metric tons, indicating a noteworthy increase in domestic demand and market expansion

(Chaovanapoonphol et al., 2023). The adoption of *Coffea arabica* L. cultivation by indigenous hill tribe communities in Thailand's northern highlands has become a pivotal economic activity. This agricultural shift is facilitated by the region's suitable edaphoclimatic conditions and influenced by complex socioeconomic dynamics. A significant trend in land-use change involves the transformation of native forest ecosystems into coffee monocultures and the implementation of coffee-centric agroforestry

systems (Soilueang et al., 2023). Thailand's agricultural sector has experienced significant intensification and extensification, characterized by a marked increase in export-oriented crop production. This agricultural transformation has been primarily achieved through two key processes: (i) Land-use conversion: the systematic alteration of natural ecosystems, particularly forests, into cultivated areas, (ii) Agricultural intensification: the implementation of high-input farming practices, including (i) increased use of agrochemicals (synthetic fertilizers and pesticides), (ii) adoption of improved crop varieties, (iii) mechanization of farming operations, (vi) enhanced irrigation systems. These processes have led to higher yields per unit area and expanded cultivated land, but also raise concerns about long-term sustainability and environmental impacts (Arunyawat and Shrestha, 2016).

Land use changes, particularly the conversion of natural forests to agricultural areas, have been extensively studied due to their significant environmental impacts. The transformation of forests and grasslands into agricultural landscapes is a global concern, contributing to environmental degradation and climate change (Kooch et al., 2016). These land-use/land-cover changes (LULCC) lead to substantial alterations in biogeochemical cycles, especially carbon (C) and nitrogen (N) dynamics. Research has consistently demonstrated the negative effects of deforestation on terrestrial carbon storage. For instance, Arunyawat and Shrestha (2016) reported a decrease in terrestrial carbon densities over their study period, attributing this decline to the accelerated transition from natural forest to cultivated land due to anthropogenic influences. Their findings revealed a clear gradient in carbon storage capacity across different land use types such as Evergreen forests (highest carbon density (427 Mg ha<sup>-1</sup>), Deciduous forests: (intermediate carbon density (304 Mg ha<sup>-1</sup>), Croplands (lowest carbon storage capacity (19 Mg ha<sup>-1</sup>). These results highlight the substantial difference in carbon sequestration potential between natural forest ecosystems and agricultural landscapes, underscoring the importance of forest conservation and sustainable land management practices in mitigating climate change. Rehman et al. (2022) demonstrated that reductions in land area allocated to crop production correlated with increased CO<sub>2</sub> emissions in both short-term and long-term scenarios. Moreover, multiple studies have established a positive relationship between increased fertilizer application and elevated CO<sub>2</sub> emissions across various temporal scales. In field trials, Liu et al. (2020) observed an inhibitory effect of nitrogen fertilizer on soil organic matter content. Their 25-year longitudinal study revealed a decline in total nitrogen and organic carbon levels, ranging from low to very low, indicative of soil fertility degradation. Prolonged application of inorganic fertilizers resulted in diminished soil nitrogen and organic carbon concentrations, as well as alterations in the composition of beneficial soil microbiota (Salamat et al., 2021).

The terrestrial biosphere functions as both a potential source and sink for CO<sub>2</sub> from the atmosphere, with vegetation and soil contributing to the residual terrestrial carbon uptake (Guo and Gifford, 2002; IPCC, 2000). Research by Dynarski et al. (2020) established that in terrestrial ecosystems, soil carbon pools typically exceed those in living vegetation. Consequently,

it is crucial to understand the intricacies of soil carbon to clarify the carbon balance of terrestrial ecosystems and its impact on the global carbon cycle. Carbon stock serves as a critical indicator of land's potential carbon sequestration capacity, with fluctuations potentially impacting the socioeconomic well-being of local communities and biodiversity (Havemann, 2009).

Despite these findings, there remains a paucity of data regarding the effects of applying fertilizer on changes in land use in highland environments. To address this knowledge gap, the present study aims to: (i) quantify and compare soil CO<sub>2</sub> emissions and dissolved organic carbon (DOC) concentrations across various coffee cropping systems and (ii) assessing the impact of nitrogen fertilizer application on soil microbial respiration rates in various land-use types within highland agroecosystems.

## 2. Materials and methods

### 2.1. Site description

The study was carried out in the Nhong Hoi Highland Agricultural Research Station, situated in Pong Yang Sub-district, Mae Rim District, Chiang Mai Province, Thailand. The exact coordinates of the station are 18°55'19.6"N, 98°48'55.0"E, as shown in Fig. 1. The property is located at an altitude ranging from 850 to 900 meters above mean sea level.

The area is classified under the Köppen-Geiger climate system as Am (tropical monsoon). It exhibits a mean annual precipitation of 1,354 mm, distributed across three distinct hydrometeorological periods: a wet season (May-October), a cool dry season (November-January), and a hot dry season (February-April). The region's climatic profile is further characterized by a mean annual air temperature of 28.5°C and a mean relative humidity of 81.1%.

### 2.2. Soil sampling and preparation

Soil samples were collected from randomly selected points within four land-use types: (1) forest (comparing site) (2) coffee monoculture (3) coffee grown with forest, and (4) coffee grown with horticulture (persimmon) (Fig. 1). Sampling was conducted at a depth of 0–20 cm using a standardized core sampler. The soil samples were dried in the air at room temperature until they reached a consistent mass. Then, they were mechanically broken apart and passed through a sieve with a 2 mm opening to remove any large rocks and organic debris. The soil samples were then used for the nitrogen fertilization experiment. The experimental design employed was a completely randomized factorial design (Factorial in CRD) with two factors including (1) land use type as mentioned earlier and nitrogen fertilizer addition (control: no nitrogen fertilizer addition and nitrogen fertilizer addition at a rate of 100 mg N kg<sup>-1</sup> soil (dry weight basis). Each treatment was replicated in triplicate to ensure statistical robustness. Based on the USDA soil textural triangle, the soil was classified as clay loam. Table 1 presents a comprehensive summary of the key physicochemical properties of soils from the four study sites.

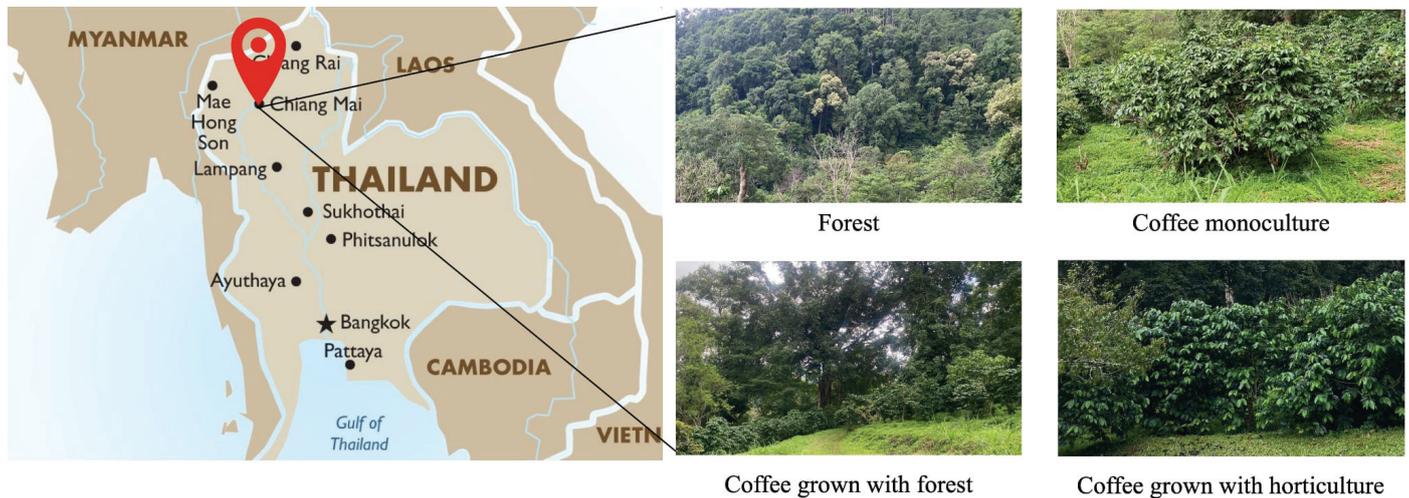


Fig. 1. Nhung Hoi Highland Agricultural Research Station located in Pong Yang Sub-district, Mae Rim District, Chiang Mai Province, Thailand (18°55'19.6"N, 98°48'55.0"E)

Prior to the incubation experiment, soil subsamples were adjusted to 60% water-holding capacity, a moisture level optimal for microbial activity (Linn and Doran, 1984). The samples were thereafter pre-incubated at a temperature of  $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for a duration of 7 days to allow for the stabilization of microbial communities and to minimize the confounding effects of the disturbance caused by sample handling (Franzluebbers, 1999).

### 2.3. Soil carbon concentrations

Total carbon content was quantified using a high-temperature dry combustion method with a CNH elemental analyzer (828 Series Combustion, LECO company, Germany). Oven-dried ( $105^{\circ}\text{C}$  for 24 h) and finely ground ( $<250\ \mu\text{m}$ ) soil subsamples (5–10 mg) were combusted at  $950^{\circ}\text{C}$  in a pure oxygen atmosphere. The resulting  $\text{CO}_2$  was separated chromatographically and quantified using a thermal conductivity detector. Calibration was performed using acetanilide as a standard (C = 71.09%, N = 10.36%, H = 6.71%). All measurements were performed in triplicate, total carbon concentration was calculating according to Wright and Bailey (2001).

### 2.4. Soil microbial respiration and dissolved organic carbon analysis

#### 2.4.1. Incubation experiment for soil microbial respiration analysis

The incubation experiment carried out at the Highland Agrobiodiversity Laboratory, Faculty of Agriculture, Chiang Mai University. Soil microbial respiration rates were quantified using a standardized alkali absorption-titration method (Meena and Rao, 2021). During the experiment, soil moisture levels were consistently maintained at 60% of the water holding capacity (WHC) through precise gravimetric adjustments.

Following a modified protocol based on Kuzyakov and Cheng (2001),  $\text{CO}_2$  evolution was measured by trapping in 0.5 M NaOH solution. Sampling was performed at 1, 4, 7, 14, 21, 35, and 65 days of incubation. At each sampling interval, the NaOH traps were replaced, and soil moisture was readjusted to 60% WHC after weighing. The quantity of  $\text{CO}_2$  sequestered was determined via titration. Specifically, a portion of the NaOH solution was combined with 0.5 M  $\text{BaCl}_2$  to precipitate carbonates. The remaining NaOH was then measured by titration with 0.1 M

Table 1

Soil properties at four investigated sites

Experimental sites	BD ( $\text{g cm}^{-3}$ )	pH	EC ( $\mu\text{S cm}^{-1}$ )	C (%)	N (%)	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Sand (%)	Silt (%)	Clay (%)
Forest (control)	1.11	5.75	44.30	2.48	0.19	3.16	556	751	35.85	44.67	27.33	28.00
Coffee monoculture	1.32	5.86	58.90	1.83	0.16	45.6	214	1,119	12.40	40.00	28.67	31.33
Coffee grown with forest	1.14	6.01	85.83	2.34	0.23	7.5	46	1,722	48.38	41.33	20.67	38.00
Coffee grown with horticulture	1.22	5.68	45.13	1.76	0.16	9.9	272	1,357	13.68	50.00	28.00	22.00

Note: BD = Bulk Density, EC = Electrical Conductivity

HCl, with phenolphthalein serving as an indicator. Three blank NaOH samples were included as controls for each titration set. The CO<sub>2</sub> evolution rate was calculated based on the difference in titration volumes between samples and blanks (Hussain et al., 2023; Bottomley et al., 2020; Zibilske, 1994).

2.4.2. Dissolved organic carbon

Dissolved organic carbon (DOC) quantification was performed using a modified protocol based on Vance et al. (1987). Soil samples were subjected to extraction using 0.5 N K<sub>2</sub>SO<sub>4</sub> solution at a soil-to-solution ratio of 1:5 (w/v). The mixture was agitated on a reciprocal shaker at 250 rpm for 45 minutes and then filtered through a 0.45 μm. The concentration of DOC in the filtrate was measured using wet oxidation with potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). Briefly, an aliquot of the filtrate was mixed with 0.07 M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and concentrated H<sub>2</sub>SO<sub>4</sub> (98%) at a volumetric ratio of 1:1:2. After cooling, the surplus dichromate was subjected to titration using a 0.007 M solution of ferrous ammonium sulfate, with ferroin serving as the indicator. A calibration curve was prepared using glucose as a standard. DOC concentration was calculated based on the difference in titration volumes between samples and blanks and expressed as mg C kg<sup>-1</sup> dry soil.

2.5. Statistical analyses

Statistical analyses were conducted utilizing Statistix 10. Analyses of variance (ANOVA) were performed to evaluate the impact of land use type on the assessed variables. Post-hoc comparisons were conducted with Fisher's least significant difference (LSD) test, applying Bonferroni correction to account for multiple comparisons. The threshold for statistical significance was established at *p* = 0.05 and 0.01 for all tests.

Principal Component Analysis (PCA) was used to identify and visually represent the main elements that affect soil carbon emissions. The effect sizes for ANOVA results were estimated using partial eta-squared (η<sup>2</sup>). The findings of all studies are reported as the mean value plus or minus the standard error (SE) unless otherwise stated. The study examined the relationships between variables by calculating Pearson's correlation coefficients. To account for the possibility of making multiple comparisons, the significance levels were modified using the Holm-Bonferroni method, which helps control for the overall chance of making a false positive.

3. Results

3.1. Soil microbial respiration as influenced by nitrogen fertilizer application

Soil microbial respiration rates were measured across all land use types over a 65-day incubation period (Fig. 2a). The respiration dynamics exhibited an initial decrease over the course of the first 4 days, which was then followed by a period of stability throughout the rest of the incubation. Significant differences in CO<sub>2</sub>-C emission were observed among the land use types (*p* < 0.001). The coffee-forest agroforestry system exhibited the highest mean microbial respiration rate (0.1085 ± 0.0052 mg C g<sup>-1</sup> soil day<sup>-1</sup>), which was significantly higher than all other land use types (*p* < 0.05). This was followed by coffee monoculture (0.0782 ± 0.0041 mg C g<sup>-1</sup> soil day<sup>-1</sup>) and coffee-horticulture (persimmon) agroforestry (0.0645 ± 0.0038 mg C g<sup>-1</sup> soil day<sup>-1</sup>), which were not significantly different from each other (*p* = 0.089). Contrary to our hypothesis, the native forest (control) exhibited the lowest microbial respiration rates (0.0412 ± 0.0029 mg C g<sup>-1</sup> soil day<sup>-1</sup>), significantly lower than all other land use types (*p* < 0.01).

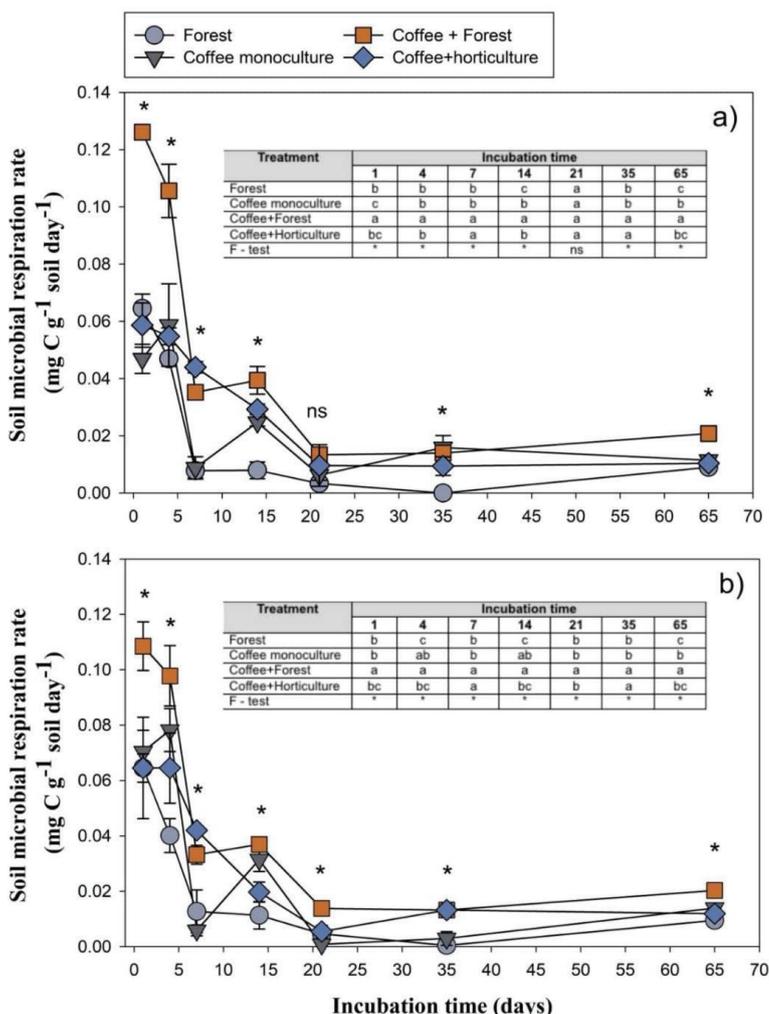


Fig. 2. Changes of soil microbial respiration rate across land-use change (a) without fertilizer and (b) with fertilizer on different land use types

**Note:** The error bars indicate the standard error of the mean, with a sample size of 3. The presence of distinct lowercase letters within each panel indicates notable variations in land-use types within each soil layer. The disparities were identified utilizing the least significant difference (LSD) test with a significance level of *p* ≤ 0.05.

### 3.2. Soil microbial respiration as influenced by non-nitrogen fertilizer application

Land use change significantly influenced soil microbial respiration rates compared to the natural forest control (Fig. 2b). The forest (control) exhibited the lowest mean microbial respiration rate ( $0.0412 \pm 0.0029 \text{ mg C g}^{-1} \text{ soil day}^{-1}$ ), which was significantly lower than all other land use types ( $p < 0.001$  for all comparisons). The coffee grown with forest demonstrated the highest mean respiration rate ( $0.0806 \pm 0.0041 \text{ mg C g}^{-1} \text{ soil day}^{-1}$ ), representing a 95.6% increase over the forest. This rate was also significantly higher than both the coffee grown with horticulture and coffee monoculture ( $p < 0.05$  for both comparisons). The coffee grown with horticulture and coffee monoculture exhibited identical mean respiration rates ( $0.0587 \pm 0.0035 \text{ mg C g}^{-1} \text{ soil day}^{-1}$ ), which were 42.5% higher than the forest. However, these two land use types did not differ significantly from each other. The LSD value at  $p = 0.05$  was calculated as  $0.0138 \text{ mg C g}^{-1} \text{ soil day}^{-1}$ . This indicates that any two means differing by more than this value are considered significantly different at the 5% level.

### 3.3. Cumulative CO<sub>2</sub> emission from different coffee cropping systems

The analysis of cumulative CO<sub>2</sub> emission from Fig. 3a non-nitrogen fertilizer indicate the coffee grown with forest had the highest of cumulative CO<sub>2</sub> emission ( $10.4247 \text{ mg kg}^{-1}$ ), Following by coffee grown with horticulture, coffee monoculture and natural forest, reaching values of  $6.3790 \text{ mg kg}^{-1}$ ,  $5.0571 \text{ mg kg}^{-1}$ ,  $4.0912 \text{ mg kg}^{-1}$ , respectively. Similar to the results from Fig. 3b nitrogen fertilizer applied under coffee grown with forest was significantly higher compared to different land use types.

### 3.4. Dissolved organic carbon from different coffee cropping systems

The transformation of natural forest to coffee grown with forest led to a rise in the concentration of dissolved organic carbon (DOC). Nevertheless, there were no statistically significant disparities in dissolved organic carbon (DOC) detected among the natural forest, coffee monoculture, coffee grown with forest, and coffee grown with horticulture systems ( $p > 0.05$ ) (Fig. 4a). In the absence of nitrogen fertilization, DOC contents in coffee monoculture, coffee-forest, and coffee-horticulture systems did not differ significantly from each other ( $p > 0.05$ ). However, these agricultural systems exhibited significantly lower DOC contents compared to the natural forest (control treatment) ( $p < 0.05$ ) (Fig. 4b).

### 3.5. Relationship among parameters in each coffee cropping systems as illustrated by Principal components analysis (PCA)

Our study found that soil carbon stocks are strongly influenced by how the land is used. Principal Component Analysis showed that as dissolved organic carbon (DOC) in soil increased, CO<sub>2</sub> emissions decreased (Fig. 5). However, soil carbon

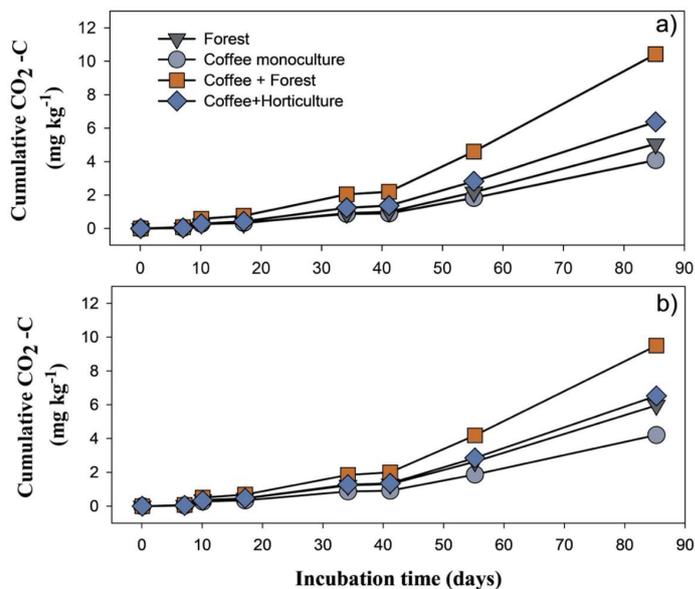


Fig. 3. The cumulative CO<sub>2</sub> (a) with fertilizer and (b) non-fertilizer on different land use types on different land use types

**Note:** The error bars indicate the standard error of the mean, with a sample size of 3. The presence of distinct lowercase letters within each panel indicates notable variations in land-use types within each soil layer. The disparities were identified utilizing the least significant difference (LSD) test with a significance level of  $p \leq 0.05$ .

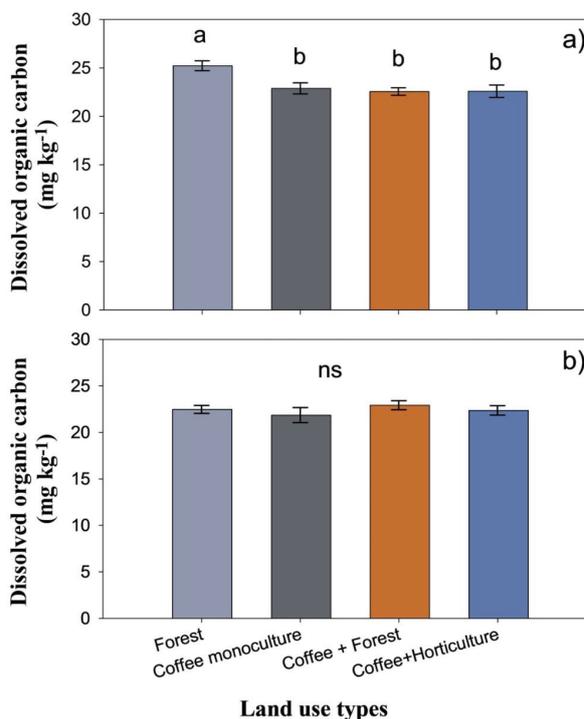
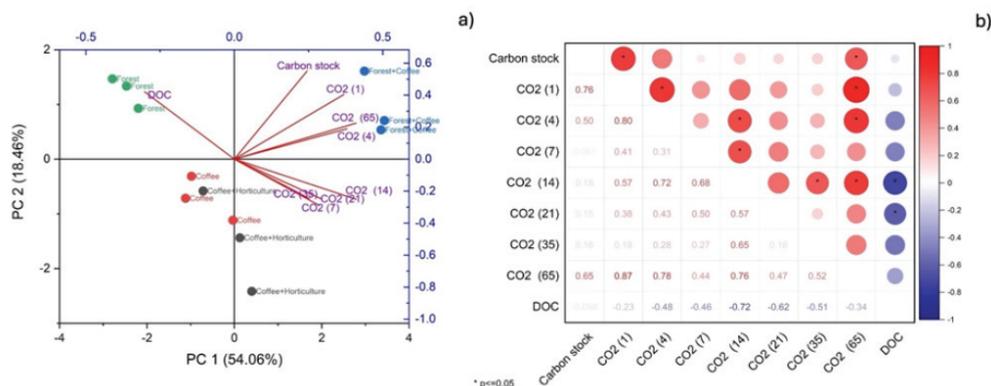


Fig. 4. The dissolved organic carbon (a) without fertilizer and (b) with fertilizer application on different land use types

**Note:** The error bars indicate the standard error of the mean, with a sample size of 3. The presence of distinct lowercase letters within each panel indicates notable variations in land-use types within each soil layer. The disparities were identified utilizing the least significant difference (LSD) test with a significance level of  $p \leq 0.05$ .



**Fig. 5.** Principal components analysis (PCA) and Pearson's correlation coefficients to identify the relationships among the soil carbon stock, dissolved organic carbon and Carbon stock each day after incubation

concentration grew significantly as soil CO<sub>2</sub> emissions increased. Coffee plants grown alongside forest trees showed high levels of both DOC and CO<sub>2</sub> emissions, likely due to increased microbial activity. This suggests that the type of land use affects the presence and activity of soil microorganisms. In coffee grown with forest systems, several factors may contribute to these observations by more diverse plant life providing varied organic matter and improved soil structure allowing better gas exchange. While high CO<sub>2</sub> emissions suggest active decomposition by microbes, it does not necessarily mean the system is losing carbon overall. The balance between carbon inputs and outputs determines long-term carbon storage.

#### 4. Discussion

Our first hypothesis posited that different coffee cropping systems would differentially impact soil respiration through microbial activity. Our results strongly support this hypothesis, demonstrating significant variations in CO<sub>2</sub>-C emissions across different cultivation methods. Coffee grown in a forest system exhibited the highest CO<sub>2</sub>-C release, followed by coffee intercropped with fruit trees. In contrast, natural forest and coffee monoculture systems showed significantly lower CO<sub>2</sub>-C emissions. These findings suggest that coffee agroforestry systems in tropical highland conditions contribute to higher CO<sub>2</sub>-C release compared to monoculture or natural forest systems. Several factors may involve in these observations i.e. organic matter input, microclimate modification, root system interaction and nutrient cycling.

One of the key drivers controlling nutrient cycling in agroecosystem is a soil microbe (Tully and Ryals, 2017). Soil microbial respiration serves as an indicator of the catabolic processes carried out by soil microbial communities under aerobic conditions. Our results demonstrated that soil microbial respiration rates were highest in soils amended with nitrogen fertilizer in the form of urea. This finding aligns with Goyal et al. (1999) and López-López et al. (2012), which found that the simultaneous use of chemical fertilizers and organic amendments increases soil microbial activities, resulting in the release of CO<sub>2</sub>-C. The rise in the soil respiration rate could be attributed to the flow of nutrients in the liquid portion of the soil, stimulated by the addition of nitrogen fertilizer. These enhancements are crucial for main-

taining the long-term fertility of soil in tropical environments, where soil organic matter is quickly exhausted as a result of high rates of decomposition. The carbon-to-nitrogen (C:N) ratio provides insight into the soil's capacity to store and cycle energy and nutrients. Cui et al. (2022) observed a decrease in soil C:N ratio following fertilizer application, suggesting an accumulation of nitrogen in the soil pool and increased microbial activity. This change in C:N ratio can have significant implications for nutrient cycling and soil fertility. It is important to note that while increased microbial respiration can indicate higher microbial activity, it may also lead to faster depletion of soil organic matter. Our research showed that soil from coffee grown with forests had the highest microbial respiration rates. Hojjati et al. (2023) have demonstrated that land use changes can significantly affect this microbial activity. The intricate nature of these mixed ecosystems likely fosters a diverse microbial population, resulting in increased activity, as noted by Schlesinger and Andrews (2000). Research by Hararuk et al. (2015), Zhang et al. (2016), and Ren et al. (2018) suggest that land use changes primarily influence soil respiration by altering soil microbial activity. These microbial changes can have wide-ranging impacts on soil health, nutrient cycling, and overall ecosystem function. The higher respiration in coffee-forest systems might be due to varied organic inputs, better soil conditions, and stronger plant-microbe relationships. While increased microbial activity often indicates better nutrient cycling, it may also mean faster soil organic matter breakdown, highlighting the need for careful management of these agroforestry systems.

Microbial decomposition releases soil organic carbon as CO<sub>2</sub>, but it can also move in runoff water as particles or dissolved organic carbon (DOC). Converting natural areas to agriculture land can boost DOC in runoff, potentially affecting water quality. The amount of DOC released varies based on soil type and agriculture practice, as Manninen et al. (2018) noted. While farmland soil data is scarce, some studies show higher DOC levels in rivers surrounded by agricultural land (Mattsson et al., 2005; Manninen et al., 2018). Our research supports this, finding natural forests had the most DOC, matching their high carbon concentration. When forests became farmland, DOC dropped significantly, suggesting lower carbon stocks and less DOC formation, likely due to higher carbon emissions. Manninen et al. (2018) found that cultivated fields had higher DOC concentrations in discharge water than Manninen et al. (2018); Graeber

et al. (2012) reported. They also noticed permanent grasslands released more DOC than cultivated soils with mineral fertilizers and manure. This was probably because the grassland's surface soil had more carbon. These DOC differences across land uses might be due to variations in soil organic matter, microbial activity, soil properties, water movement, and farming practices. More research is needed to fully understand how land use affects DOC dynamics and to develop better land management strategies.

We found the natural forests had the lowest cumulative CO<sub>2</sub> emission, the variations in land-use types appear to have an influence on the potential carbon dioxide emissions from the soils. Not only Iqbal et al. (2010) findings corroborate the observation that agricultural soils frequently exhibit greater CO<sub>2</sub> emissions compared to soils covered by native vegetation, but also Iqbal et al. (2008) found that the transformation of forested areas into agricultural land resulted in a considerable rise in soil CO<sub>2</sub> emissions. According to our study, combined coffee and forest cultivation can be an effective and sustainable approach to restore microbial activity following forest conversion. However, not only the different species of coffee can be integrated with the forest, but also the development of their root systems at depth, allowing us to better understand the dynamics of the carbon cycle. Additionally, the method of laboratory incubation is commonly used to evaluate the total amount of CO<sub>2</sub> released from soil. This suggests that the outcomes obtained by laboratory incubation may not precisely reflect the intricacies of field conditions, where multiple biotic and abiotic variables simultaneously influence soil processes. However, there has been a scarcity of research that has carried out on-site assessments of soil CO<sub>2</sub> emission and dissolved organic carbon (DOC). Therefore, additional investigation is required to comprehend the soil CO<sub>2</sub> emission and dissolved organic carbon in soils under field conditions, and it is crucial to conduct laboratory incubations to fill this gap in knowledge. Furthermore, our research was carried out exclusively on coffee plantations that were 30 years old after the conversion from forest. Consequently, our discovery may vary from the findings of the initial era. Hence, additional investigation is necessary to examine the dynamics of soil cumulative CO<sub>2</sub> emissions following the conversion of forests into coffee plants at various stages of growth.

## 5. Conclusions

This study indicated that conversion of forest to coffee monoculture, coffee grown with forest and coffee grown with horticulture, affects soil microbial respiration. The fertilizer application not only increased microbial respiration, but also resulted changes of DOC composition. Thus, combined coffee and forest cultivation offer a promising, sustainable solution for Arabica coffee production in Northern Thailand. This method enhances biodiversity, improves soil health, mitigates climate change, and boosts biodiversity more than coffee monoculture. By adopting these integrated practices, Northern Thailand can achieve long-term agricultural productivity and environmental sustainability. The approach not only supports high-quality coffee production

but also promotes ecological balance and resilience, making it an ideal option for sustainable Arabica coffee cultivation in the region. Therefore, coffee cultivation in association with forests or horticulture can be an option for sustainable Arabica coffee cultivation in Northern Thailand.

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## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Author contribution statement

**Sasiprapa Kullachonphuri:** Writing – review & editing, Formal analysis, Data curation. **Phonlawat Soilueang:** Data curation, Conceptualization. **Piyaphad Ninlaphong:** Methodology, Formal analysis. **Yupa Chromkaew:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization. **Kesinee Iamsaard:** Writing – review & editing, Project administration, Investigation, Data curation. **Nuttapon Khongdee:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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