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Soil Organic Carbon Fractions, Latex Biochemical Composition, and Yield of Rubber Trees under *Bacillus*-Based Bio-Organic and Chemical Fertilization

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Abstract: Extensive rubber cultivation using long-term chemical fertilization (CF) has accumulated negative consequences for soil ecosystems, particularly in replanting areas, potentially resulting in reduced physiological metabolism and yield of rubber trees. Bio-organic fertilizer, a compost of organic manures combined with plant-beneficial microbes, may influence SOC dynamics and soil fertility, thereby supporting plant nutrient uptake, tree metabolism, and crop production. This study quantified SOC fractions in 15- and 25-year-old rubber plantations treated with *Bacillus*-based bio-organic fertilizer (BBOF) or CF to evaluate their SOC fraction responses to different fertilizer regimes. Latex biochemical composition and yield were investigated to evaluate the physiological responses of rubber trees to these fertilizer treatments. In the 15-year-old plantation, BBOF significantly increased topsoil F1 ($p = 0.023$) but decreased subsoil F1 ($p = 0.036$), whereas latex biochemical parameters and yield did not differ significantly between fertilizer treatments. In the 25-year-old plantation, F1 was numerically higher under BBOF but did not differ significantly from CF, while topsoil F2 was significantly lower ($p < 0.001$). Taken together, the contrasting F1 and F2 responses indicated redistribution among oxidizable SOC fractions rather than a consistent increase in the combined labile SOC pool under BBOF. BBOF significantly increased latex Suc content and yield and decreased latex Mg content in the 25-year-old plantation. Overall, BBOF was associated with changes in SOC fractions and selected latex physiological parameters, together with higher latex yield, particularly in the 25-year-old plantation, suggesting its potential as a complementary fertilization strategy for established rubber plantations.

Keywords: *Bacillus*; *Hevea brasiliensis*; latex biochemical composition; soil labile organic carbon

1. Introduction

The rubber tree (*Hevea brasiliensis*) is commercially planted as the major source of natural rubber, an indispensable commodity, for manufacturing a wide range of rubber-based products. It has been cultivated for over a hundred years with a mass-production-based objective, mainly in South East Asian countries, India, Sri Lanka and Southern China, where over 90% of the world's total natural rubber production exists



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(Association of Natural Rubber Producing Countries, 2021). Since it is a perennial economic crop that can be harvested almost throughout the year, most farmers in these countries have been repeatedly growing it for their long-term income source, and most rubber farms in the traditional areas are now in the second or third replanting cycle (Zaw et al., 2022).

Extensive rubber planting with mass production for many years by conventional practices has accumulated adverse effects on the environment and soil ecosystems of the growing areas (Kotowska et al., 2015; Nguyen et al., 2020). A study by de Blécourt et al. (2013) reported significant losses of soil carbon stocks in rubber plantations, particularly within the 0–1 m soil depth, ranging from 21% to 48% depending on plantation age, soil type, and management. It was also reported that with a long planting cycle, rubber production significantly degraded the soil organic matter by over 45% (Cheng et al., 2007). Moreover, during the land clearing for new planting or replanting rubber farms, tree biomass, a carbon sink, is substantially removed or exploited from the area, resulting in significant sudden losses of accumulated carbon stocks and a dramatic adverse impact on the ecosystem and soil biotic functions (Panklang et al., 2022).

Most traditional rubber growing lands also suffer from intensive long-term utilization of chemical fertilizers (Zaw et al., 2022), which depletes soil microbial communities, and, consequently, imbalances soil nutrient availability and degrades soil properties, adversely affecting soil-plant interactions in the long term (Wan et al., 2021). Soil nutrients and organic carbon stocks in the rubber-growing land are also associated with the tree's physiological metabolism of latex production. Since cis-isoprene rubber molecules are carbon chains, rubber tree physiologically consumes available nutrients and carbon to synthesize rubber molecules for its defense mechanism in response to harvesting (latex production), which is generally practiced along the productive lifespan for over 20 years. Thus, soil organic carbon dynamics may differ across the productive lifespan of rubber plantations in association with changes in tree growth, physiological activity, and management practices (de Blécourt et al., 2013; Zaw et al., 2022). Conversely, a low level of soil organic carbon limits the microbial community and soil capacity to retain moisture and nutrients, leading to an insufficient supply of plant nutrients, inactive metabolism, and reduced productivity (Berryman et al., 2020). Although, technically, rubber trees have a productive lifespan of 30 to 40 years (Munasinghe & Rodrigo, 2018), nowadays, those in traditional rubber growing areas can only be economically harvested for about 25 years (Hytönen et al., 2019) due to their low efficient physiological metabolism associated with the depleted soil fertility and available soil organic carbon, particularly in replanting areas (Panklang et al., 2022).

In order to rehabilitate the soil nutrients, fertility and soil biotic functions in degraded agricultural land, coupling an improvement of crop yields and quality, bio-organic fertilizer, which is fundamentally a compost of organic farm manures combined with plant-beneficial microbes, is recommended due to its environmentally friendly agroecological advantageous (Radhakrishnan et al., 2017; Halim et al., 2023; Mistry et al., 2024). It contains active microbes that facilitate soil organic carbon decomposition and nitrogen fixation, thereby greater soil fertility and plant nutrient uptakes, enhancing land and crop productions for the long term (Shahid & Khan, 2019; Imran et al., 2021; Zhao et al., 2022); thus, it has become considered an effective alternative to reduce chemical fertilizer applications.

Among many microorganisms typically used in bio-organic fertilizers, *Bacillus* species greatly enhance microbial growth and the availability of plant nutrients in the soil. In addition, bio-organic fertilizers containing *Bacillus* microbes facilitate soil water movement and improve soil properties (Radhakrishnan et al., 2017). Including *Bacillus*-based, most other bio-organic fertilizers significantly increased plant and microbial biomass (Chumthong et al., 2016), soil organic carbon with a greater labile fraction and water-soluble organic carbon (Yang & Zhang, 2023). Though soil organic carbon, a complex form of organic substances, plays an essential role in soil properties, total organic carbon is unable to explain the plant-soil interactions and soil carbon dynamics associated with agricultural management. Instead, soil labile organic carbon is recognized as an indicator of soil quality and organic matter decomposition related to soil management in agricultural land. Soil labile organic carbon breaks down readily within a few years and serves as a nutrient source for plants and soil microbes. Since soil labile organic carbon is derived from organic carbon decomposition of plant residues by soil microbes (Zhang et al., 2022), additional farm organic matter and beneficial living microbes would be essential to accelerate the decomposition of organic matter, particularly in long-term agricultural soils.

This study quantified variations in soil organic carbon fractions on mature and overaged rubber farms under *Bacillus*-based bio-organic fertilization compared to conventional chemical fertilizer application to evaluate SOC fraction responses to the different fertilizer regimes. The latex biochemical composition and production of the farms were investigated to study the effect of the fertilizer treatments on the rubber trees' physiological metabolism, which could reflect the plant-soil interaction.

2. Methods

2.1. Experimental Site and Treatments

The study selected two rubber plantations aged 15 and 25 years, both established during the second replanting cycle. The plantations were located at 6.82101° N, 100.49928° E, in Hat Yai district, Songkhla province, a major rubber-growing region of Southern Thailand. The rubber trees in the farms were RRIM 600 clones obtained from certified nurseries operating under the standards of the Rubber Research Institute of Thailand and planted at a spacing of 3 m × 6 m. Latex harvesting (tapping) was performed twice every three days using a one-third spiral tapping cut on the tree trunk.

The study was conducted in two established rubber plantations, aged 15 and 25 years. Plantation age was not an experimentally manipulated factor; rather, the two plantations represented contrasting stages of the productive lifespan of rubber trees. Within each plantation, two fertilizer treatments, chemical fertilizer (CF) and *Bacillus*-based bio-organic fertilizer (BBOF), were arranged in a randomized complete block design with three replications.

Each treatment consisted of three independent field replications, with 30 rubber trees of relatively uniform trunk girth per replication. Multiple trees or sampling points within each replication were used as subsamples to obtain representative measurements of the experimental unit and were not treated as independent biological replicates in the statistical analysis. The effects of fertilizer treatment were evaluated separately within each plantation.

The experimental assessment was structured to examine the responses to BBOF application across three related components of the soil–plant system: soil organic carbon fractions (F1–F4), latex biochemical parameters (Suc, R-SH, Pi, Mg, and DRC), and latex yield. These measurements were used collectively to evaluate the potential links between soil carbon dynamics, latex physiological status, and rubber productivity under the two fertilizer treatments.

The CF treatment involved applying 1 kg of 30-5-18 compound fertilizer per tree per year while the BBOF application involved applying 10 kg per tree per year. The BBOF was prepared with *Bacillus megaterium* cultured in a molasses-based medium containing coconut husks, burned rice husks, cow manure, rock phosphate and cane molasses (Chumthong et al., 2016). The number of *Bacillus megaterium* in the BBOF was 9.25 log CFU g⁻¹, as determined using the drop plate method. The strain was isolated from bulk soil in a paddy rice field (Kanjanaameesathian & Meetum, 2017). The specifications of the BBOF are described in Table 1, according to the test result provided by the Central Analytical Center, Faculty of Natural Resources, Prince of Songkla University, Thailand. Both fertilizer treatments were applied in the interrow of the rubber trees during 2021 and 2022.

Table 1. Characteristics and nutrient contents of the *Bacillus*-based bio-organic fertilizer.

Parameters	Values
Moisture content (%)	10.25
pH	7.50
Electrical conductivity (dS/m)	4.50
Organic matter (%)	20.45
Total nitrogen (%)	1.73
Available phosphorus (%)	1.41
Available potassium (%)	1.35
Available calcium (%)	7.48
Available magnesium (%)	1.39
Available sulfur (%)	1.29
Iron (ppm)	11,931.00
Manganese (ppm)	1,081.10
Copper (ppm)	81.53
Zinc (ppm)	138.63

2.2. Soil Organic Carbon Fractions

Within each field replication, soil samples were collected from four sampling points at each soil depth (0–20 and 21–40 cm) using a soil sampling auger with a diameter of 50 mm. The four subsamples collected at each depth were combined to form one composite soil sample per replication. Soil analyses were therefore conducted using three independent composite samples per treatment at each soil depth. The

samples were air-dried at room temperature and sieved through 1 mm, 0.50 mm and 0.25 mm sieves. Then, after placing 0.5 g of soil sample into a conical flask, a 10 mL oxidizing agent named potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) of 0.167 M was added, followed by 5, 10, or 20 mL of sulfuric acid (H_2SO_4) to obtain acid-dichromate concentrations of 12 N, 18 N, and 24 N, respectively. The flasks were then heated at 135 °C (approximately 0.5 min) on a hotplate. The remaining dichromate was titrated using 1.0 M ferrous sulphate (FeSO_4) with ferroin indicator solution (0.1 wt. % in water). Since 1 mL titrated dichromate is inversely equivalent to 3 mg of carbon, the soil organic carbon was calculated according to the method of Walkley (Chan et al., 2001). The resulting soil organic carbon was then expressed as four fractions, as defined below.

Fraction 1 (F1) (very labile organic carbon): oxidizable organic carbon under 12 N acid-dichromate solution.

Fraction 2 (F2) (labile organic carbon): oxidizable organic carbon extracted between 12 N and 18 N acid-dichromate solution.

Fraction 3 (F3) (less labile organic carbon): oxidizable organic carbon extracted between 18 N and 24 N acid-dichromate solution.

Fraction 4 (F4) (recalcitrant): remaining organic carbon after the titration with 24 N acid-dichromate solution.

2.3. Latex Biochemical Parameter

The sampling of latex was carried out early in the morning, specifically at around 6 o'clock, before the regular tapping. Ten rubber trees were selected within each replication to obtain a representative latex sample. Latex was collected by puncturing the bark approximately 5 cm below the tapping cut, and ten latex drops from each selected tree were collected in a glass bottle. The samples were placed at 4 °C in a cool box to inhibit pre-coagulation before the laboratory analysis proceeded. At the laboratory, 1 mL from the sample latex was mixed with trichloroacetic acid (TCA) solution (TCA 2.5% w/v prepared with ethylene diamine tetra acetic 0.01% w/v) of 9 mL to filter out the serum. From the rest of the sample, dry rubber content (DRC) was analyzed, while sucrose (Suc), inorganic phosphorus (Pi), reduced thiols (R-SH) and magnesium (Mg) contents were determined from the serum, according to the micro-diagnosis method of latex biochemical composition (Sainoi et al., 2017).

2.4. Latex Production

To determine the average dry yield ($\text{g tree}^{-1} \text{ tap}^{-1}$) in each treatment, field latex from ten rubber trees was collected. First, we recorded all samples' fresh weights, then coagulated 10 g from each sample with 3% w/w formic acid. After that, the coagulum was dried in an oven at 70 °C for 16 h. Finally, the dry sample was weighed to calculate the dry rubber weight following the guidelines in ISO 126:2005.

2.5. Data Analysis

Data were summarized as means and standard deviations. Because the 15- and 25-year-old plantations represented two separate field experiments rather than replicated levels of plantation age, statistical analyses were conducted separately for each plantation. Within each plantation, differences between CF and BBOF were evaluated using an unpaired t-test at a significance level of $p < 0.05$. Soil organic carbon fractions were evaluated separately for each soil depth. Plantation age was not included as an independent factor in the statistical analysis, and no fertilizer $\times$ plantation age interaction was tested. All statistical analyses were performed using R software version 3.1.0.

3. Results

3.1. Soil Organic Carbon Fractions

The results showed that the very labile SOC fraction (F1) was generally higher in the topsoil (0–20 cm) than in the subsoil (21–40 cm) in both plantations (Tables 2 and 3). In the 15-year-old plantation, F1 in the topsoil was significantly higher under BBOF than under CF (13.10 and 7.69 g kg^{-1} , $p = 0.023$). In contrast, F1 in the subsoil (21–40 cm) was significantly lower under BBOF than under CF (2.60 and 4.64 g kg^{-1} , respectively, $p = 0.036$), indicating contrasting F1 responses between soil depths.

In the 25-year-old plantation, BBOF also showed a higher mean F1 value than CF in the topsoil (14.38 and 11.13 g kg^{-1}), although the difference was not statistically significant ($p = 0.074$). The F2 fraction was numerically lower under BBOF than under CF at both soil depths in both plantations. This difference was

statistically significant only in the topsoil of the 25-year-old plantation, where F2 was 0.45 g kg^{-1} under BBOF compared with 4.31 g kg^{-1} under CF ($p < 0.001$); the other F2 differences were not statistically significant (Tables 2 and 3).

F4 was numerically higher under CF than under BBOF at both soil depths in both plantations. In the 15-year-old plantation, the differences between CF and BBOF were not statistically significant in either the topsoil ($p = 0.072$) or subsoil ($p = 0.090$). Similarly, in the 25-year-old plantation, F4 was numerically higher under CF than under BBOF in both the topsoil and subsoil, but the differences were not statistically significant ($p = 0.086$ and $p = 0.071$, respectively). Some standard deviations exceeded 50% of their respective means, indicating substantial variability among the three independent field replications. This variability should therefore be considered when interpreting the treatment differences in SOC fractions.

Table 2. Soil organic carbon fractions in the 0–20 cm and 21–40 cm soil depths of the 15-year-old rubber farm under the chemical fertilization and *Bacillus*-based bio-organic fertilization.

Soil Depth	Soil Organic Carbon Fractions (g kg^{-1})	Chemical Fertilizer (CF)	<i>Bacillus</i> -Based Bio-Organic Fertilizer (BBOF)	<i>p</i> -Value
0–20 cm	very labile (F1)	7.69 ± 3.07	13.10 ± 1.72	0.023
	labile (F2)	3.85 ± 1.79	1.46 ± 1.05	0.067
	less labile (F3)	1.66 ± 0.71	1.40 ± 0.40	0.570
	recalcitrant (F4)	1.53 ± 0.79	0.51 ± 0.40	0.072
21–40 cm	very labile (F1)	4.64 ± 1.29	2.60 ± 0.72	0.036
	labile (F2)	3.22 ± 1.11	1.91 ± 0.87	0.110
	less labile (F3)	0.97 ± 0.39	1.21 ± 0.48	0.418
	recalcitrant (F4)	1.40 ± 0.66	0.57 ± 0.51	0.090

Note: *p*-values indicate differences between CF and BBOF within each plantation based on an unpaired *t*-test. Differences were considered statistically significant at $p < 0.05$.

Table 3. Soil organic carbon fractions in the 0–20 cm and 21–40 cm soil depths of the 25-year-old rubber farm under the chemical fertilization and *Bacillus*-based bio-organic fertilization.

Soil Depth	Soil Organic Carbon Fractions (g kg^{-1})	Chemical Fertilizer (CF)	<i>Bacillus</i> -Based Bio-Organic Fertilizer (BBOF)	<i>p</i> -Value
0–20 cm	very labile (F1)	11.13 ± 2.24	14.38 ± 1.15	0.074
	labile (F2)	4.31 ± 0.69	0.45 ± 0.22	<0.001
	less labile (F3)	1.83 ± 1.24	1.15 ± 0.51	0.416
	recalcitrant (F4)	1.83 ± 0.73	0.83 ± 0.40	0.086
21–40 cm	very labile (F1)	6.13 ± 2.24	7.25 ± 1.55	0.497
	labile (F2)	2.81 ± 0.97	1.78 ± 0.61	0.172
	less labile (F3)	1.16 ± 0.75	1.08 ± 1.09	0.915
	recalcitrant (F4)	1.64 ± 0.80	0.45 ± 0.48	0.071

Note: *p*-values indicate differences between CF and BBOF within each plantation based on an unpaired *t*-test. Differences were considered statistically significant at $p < 0.05$.

3.2. Latex Biochemical Compositions

Table 4 presents the latex biochemical composition under CF and BBOF treatments in the 15- and 25-year-old rubber plantations. In the 15-year-old plantation, Suc, R-SH, Pi, Mg, and DRC did not differ significantly between CF and BBOF ($p = 0.506, 0.337, 0.090, 0.267$, and 0.069 , respectively). In the 25-year-old plantation, latex Suc content was significantly higher under BBOF than under CF (27.66 and 7.63 mM , respectively; $p = 0.007$), whereas latex Mg content was significantly lower under BBOF than under CF (466.71 and 634.39 ppm , respectively; $p = 0.003$). R-SH, Pi, and DRC did not differ significantly between CF and BBOF in the 25-year-old plantation ($p = 0.059, 0.089$, and 0.311 , respectively).

3.3. Latex Production

Latex yield did not differ significantly between CF and BBOF in the 15-year-old plantation (67.17 and $63.89 \text{ g tree}^{-1} \text{ tap}^{-1}$, respectively, $p = 0.888$). In contrast, latex yield in the 25-year-old plantation was significantly higher under BBOF than under CF (32.44 and $15.99 \text{ g tree}^{-1} \text{ tap}^{-1}$, respectively; $p < 0.001$) (Table 5).

Table 4. Latex biochemical composition of the 15-year-old and 25-year-old rubber trees under the chemical fertilization and bio-organic fertilization.

Latex Biochemical Composition	Chemical Fertilizer (CF)	<i>Bacillus</i> -Based Bio-Organic Fertilizer (BBOF)	<i>p</i> -value
15-year-old rubber trees			
Suc (mM)	11.47 ± 1.41	12.98 ± 2.26	0.506
R-SH (mM)	0.55 ± 0.19	0.72 ± 0.02	0.337
Pi (mM)	22.18 ± 0.85	25.88 ± 1.45	0.090
Mg (ppm)	575.55 ± 38.50	496.70 ± 62.2	0.267
DRC (%)	33.84 ± 3.51	44.46 ± 2.22	0.069
25-year-old rubber trees			
Suc (mM)	7.63 ± 0.87	27.66 ± 2.29	0.007
R-SH (mM)	0.40 ± 0.11	0.71 ± 0.03	0.059
Pi (mM)	9.55 ± 2.78	22.48 ± 5.15	0.089
Mg (ppm)	634.39 ± 8.37	466.71 ± 8.79	0.003
DRC (%)	45.63 ± 2.39	41.08 ± 4.14	0.311

Note: *p*-values indicate differences between CF and BBOF within each plantation based on an unpaired *t*-test. Differences were considered statistically significant at *p* < 0.05.

Table 5. Average daily latex yield of the 15-year-old and 25-year-old rubber trees under the chemical fertilization and *Bacillus*-based bio-organic fertilization.

Yields of the Rubber Trees	Chemical Fertilizer (CF)	<i>Bacillus</i> -Based Bio-Organic Fertilizer (BBOF)	<i>p</i> -value
Yield of the 15-year-old rubber tree (g tree ⁻¹ tap ⁻¹)	67.17 ± 8.10	63.89 ± 11.00	0.888
Yield of the 25-year-old rubber tree (g tree ⁻¹ tap ⁻¹)	15.99 ± 1.66	32.44 ± 1.28	<0.001

Note: *p*-values indicate differences between CF and BBOF within each plantation based on an unpaired *t*-test. Differences were considered statistically significant at *p* < 0.05.

4. Discussion

4.1. Soil Organic Carbon Fractions

The F1 fraction was generally higher in the topsoil than in the subsoil across fertilizer treatments in both plantations. Soil labile organic carbons are derived from decomposed organic matter such as plant litter, crop residues, organic farm waste, and dead organisms, which are present mainly in the topsoil layers, including the ground surface of agricultural farms. Consequently, the labile organic carbon generally concentrates in the topsoil layer rather than the subsoil layer (Liu et al., 2018). Unlike other seasonal and annual crop farming, during the productive period of a typical mature rubber farm between seven and 25 years old, there are no extensive soil-destructive practices, such as tillage and crop rotations. Besides, the overstorey of rubber farms and occupied rubber root system in mature rubber farms provide higher amounts of organic matter, like plant litter and root biomass, in the topsoil layer of the farms. Thus, a greater plant residue, a primary source of farm decomposable organic carbon, is aggregated on the ground surface and enhances the soil microorganism functions, leading to higher labile organic carbon in the topsoil layer of the farms.

In the current study, F1 and F2 showed contrasting responses to fertilizer treatments. In the 15-year-old plantation, BBOF was associated with significantly higher F1 in the topsoil but significantly lower F1 in the subsoil. In addition, F2 was consistently lower under BBOF than under CF at both soil depths in both plantations, although the difference was statistically significant only in the topsoil of the 25-year-old plantation. When F1 and F2 were considered together as the combined labile SOC pool, BBOF did not result in a consistent increase compared with CF. For example, in the 25-year-old plantation, F1 + F2 was 14.83 g kg⁻¹ under BBOF and 15.44 g kg⁻¹ under CF in the topsoil, while the corresponding subsoil values were 9.03 and 8.94 g kg⁻¹, respectively. In the 15-year-old subsoil, the combined F1 + F2 pool was lower under BBOF (4.51 g kg⁻¹) than under CF (7.86 g kg⁻¹). These patterns therefore indicate redistribution among the operationally defined oxidizable SOC fractions rather than a consistent net increase in the combined labile SOC pool under BBOF. Because F1 and F2 are sequentially defined rather than independent fractions in the acid-dichromate oxidation method (Chan et al., 2001), an increase in F1 accompanied by a decrease in F2 does not necessarily indicate an increase in the combined labile SOC pool.

As part of the decomposition process, soil microbes release plant nutrients like nitrogen and phosphorus (Kang et al., 2014), along with organic carbon compounds, soil water, and carbon dioxide. Although decomposition occurs naturally as part of the carbon cycle, microbial activity can contribute to organic matter transformation and nutrient cycling in soil. Previous studies have suggested that *Bacillus* spp. may contribute to microbial processes associated with organic matter transformation (Chen et al., 2017; Kanjanameesathian & Meetum, 2017), although such processes were not directly measured in the present study. However, in the present study, the contrasting responses of F1 and F2 do not provide evidence of an overall increase in the combined labile SOC pool under BBOF. Therefore, the observed SOC fraction patterns are more appropriately interpreted as changes in the distribution of operationally defined oxidizable SOC fractions rather than enhanced SOC decomposition.

Numerically higher F4 values were observed under CF than under BBOF, particularly in the topsoil of the 25-year-old plantation. However, these differences were not statistically significant and should therefore be interpreted as numerical trends. Such patterns may reflect differences in soil organic carbon turnover between the fertilizer treatments rather than a direct effect of BBOF or CF on microbial decomposition. The traditional use of inorganic fertilizer leads to decrease the number of microorganisms, their activities, and the overall microbial community in the soil (Zeng et al., 2016; Enebe & Babalola, 2020; Mistry et al., 2024). With the depletion of the microbial community and diversity in the soil, the soil organic matter decomposition rate tends to decrease, resulting in a lower turnover rate of soil labile carbon (Zhang et al., 2022). Since oxidative enzymes excreted from the soil microbes enhance the breakdown of recalcitrant carbon of plant litter and residues (Hoppe et al., 2016), a cultivated soil with fewer microbes would have a higher amount of recalcitrant carbon. Liu et al. (2018) reported in the study of 16-year fertilizer trial that a long-term application of inorganic fertilizer reduced labile organic carbon and enhanced recalcitrant pool of soil organic carbon. Moreover, the appropriate proportioning of organic materials could be a promising approach to reduce reliance on chemical fertilizers and promote sustainable crop production in the long term (Mistry et al., 2024).

Relatively high variability was observed in some soil organic carbon fractions, indicating considerable spatial heterogeneity among the field samples. Such variability may reflect small-scale differences in soil conditions and the distribution of organic matter within the plantations. However, the sources of this variability were not specifically examined in the present study and therefore cannot be attributed to any single factor. Together with the limited number of independent field replications, this variability may have reduced the statistical sensitivity for detecting smaller treatment effects. Therefore, non-significant differences in soil organic carbon fractions should be interpreted with caution (Liu et al., 2023; Choudhary et al., 2024).

4.2. Rubber Trees' Physiological Status and Latex Production

At the 15-year-old plantation, the fertilizer treatments did not show significant differences in the latex biochemical composition. However, the differences were more pronounced in the 25-year-old plantation. It was noticed that CF treatment at the 25-year-old plantation had the lowest levels of Suc, R-SH, and Pi in the latex composition compared to those of the others. Its yield was also the lowest among the treatments. In the latex metabolism of rubber trees, reserved sucrose in sink tissues of the laticiferous system serves as a primary raw material for rubber molecules. Thus, a low amount of sucrose in the latex indicates either a low level of reserved sucrose in the sink or an actively efficient metabolism of rubber synthesis (Samuel et al., 2021). High metabolism in latex production implies high sucrose utilization and biosynthetic activities, explained by greater Pi content in the metabolite (Zaw et al., 2022) because Pi in the metabolite is the main driver to enhance latex metabolism in the laticiferous system (Atsin et al., 2016). The presence of reduced thiols in the laticiferous cell is indispensable in protecting the latex organelles from oxidative degradation caused by latex harvesting (Purwaningrum et al., 2015). Hence, the minimum levels of Suc, Pi, and R-SH under the CF25 treatment may indicate that the rubber trees in this treatment were under greater physiological constraints. In addition, these physiological parameters are yield-limiting factors positively associated with the regeneration and flow of the latex (Jacob et al., 1989). Thus, consistent with these physiological responses, the yield result of the rubber trees under the CF25 treatment was the lowest.

On the other hand, BBOF25 showed the highest Suc with the optimum levels of R-SH and higher Pi than CF25. Latex yield under BBOF25 was also significantly higher than under CF25 (32.44 and 15.99 g tree⁻¹ tap⁻¹, $p < 0.001$). The yield results indicate the promising impact of the BBOF, particularly in enhancing the yield of the old rubber trees.

The more pronounced response to BBOF in the 25-year-old plantation may be associated with differences in the underlying soil-plant conditions between the two plantations. In the 15-year-old plantation, latex biochemical parameters were generally comparable between fertilizer treatments, and latex yield remained relatively high under both CF and BBOF, suggesting a smaller marginal response to BBOF. In contrast, the lower Suc, Pi, R-SH, and latex yield observed under CF in the 25-year-old plantation suggest greater physiological constraints, under which BBOF application may have provided greater benefits to the soil-plant system and latex production. However, because plantation age was not experimentally manipulated and each age was represented by a separate plantation, the stronger response observed in the 25-year-old plantation cannot be attributed solely to tree age; plantation-specific soil and environmental conditions may also have contributed to the contrasting responses.

These findings suggest that BBOF application may support latex physiological status and yield in the 25-year-old plantation. Although the F1 value in the topsoil was numerically higher under BBOF25 than under CF25, this difference was not statistically significant ($p = 0.074$). Therefore, the observed changes in latex biochemical parameters and yield cannot be directly attributed to the increase in F1. Nevertheless, the higher F1 under BBOF may indicate greater availability of readily decomposable organic carbon, which could contribute to soil nutrient cycling and plant physiological processes. Further studies examining the relationships among SOC fractions, nutrient availability, latex metabolism, and yield are required to verify these associations.

The observed responses in SOC fractions, latex biochemical parameters, and yield should be interpreted at the level of the BBOF fertilizer regime rather than attributed specifically to the *Bacillus* inoculant. BBOF differed from CF not only in microbial inoculation but also in nutrient composition, organic matter input, and pH, all of which may have contributed to the observed soil and plant responses. The organic matter and nutrients supplied by BBOF may influence soil carbon dynamics, nutrient availability, and plant physiological processes. The *Bacillus* inoculant may represent an additional potential contributor, as several plant-beneficial functions of *Bacillus* spp. have been reported in previous studies. *Bacillus* species positively associate with rhizospheres by forming a biofilm covering roots that improve root functions (Hashem et al., 2019), thereby increasing plant nutrient uptake. Kang et al. (2014) reported that secretions from *Bacillus* spp. facilitate the conversion of phosphorus into plant-available form, Pi. Furthermore, *Bacillus* aids in nitrogen fixation by releasing nitrogenase enzymes which are associated with fixing atmospheric nitrogen into the soil (Kuan et al., 2016). Radhakrishnan et al. (2017) reported that *Bacillus*-based biofertilizers may support plant metabolic activity through plant-beneficial functions such as phosphorus solubilization and nitrogen fixation, thereby contributing to plant growth and crop productivity. Similarly, the increased availability of essential nutrients, including N, P, and K, associated with *Bacillus* spp. has been reported to support plant responses under stressful conditions (Garcia-Fraile et al., 2015; Chumthong et al., 2016). Kakar et al. (2016) suggested that enzymes associated with *Bacillus* inhibit the reactive oxygen species (ROS) synthesis in plant cells. Thus, the higher R-SH level observed in the latex under BBOF25 may indicate a greater capacity to maintain the antioxidant status of the laticiferous system during latex harvesting (Tristama et al., 2019). However, ROS-scavenging and other antioxidant enzyme activities were not directly measured in the present study. Moreover, the persistence and colonization of the inoculated *Bacillus megaterium* after fertilizer application were not verified. Therefore, the contribution of the microbial inoculant cannot be separated from the effects of the other components of the BBOF fertilizer regime.

However, apparent high Mg contents observed in both 15- and 25-year-old farms in the CF treatments exceeded the acceptable limit of 500 ppm, set by latex factories (Puangmanee et al., 2014). The excessive magnesium content in latex causes it to break out from luteoids and undesirable early coagulation in the latex (Jacob et al., 1989). This destabilizes the latex colloidal and negatively impacts not only on its flow and production but also the production processes and quality of latex products. Mg is an indispensable element present in chlorophyll molecule for the plant metabolism of vegetative growth and development (Gransee & Führs, 2013). According to Marschner (2012), around 6 to 25% of the total magnesium present in plants can only bind chlorophyll molecules, between 5 and 10% is attached to pectin in cell walls, and the majority of the remaining magnesium is readily translocated to other sink parts. Therefore, when there is a decrease in plant primary metabolism, magnesium translocation is more induced to sink tissues for secondary metabolism in plants. Jacob et al. (1989) suggested that high latex Mg content was an indication of weak metabolic activity of a productive rubber tree.

The significantly lower latex Mg concentration under BBOF than under CF in the 25-year-old plantation should not, however, be interpreted solely as an indicator of altered metabolic activity. The two fertilizer

regimes differed in their nutrient composition, with BBOF supplying Mg and substantial amounts of Ca in addition to other nutrients, whereas these elements were not supplied by the 30-5-18 CF formulation. Differences in nutrient supply and nutrient interactions may therefore have contributed to the contrasting latex Mg concentrations. Consequently, the lower latex Mg concentration under BBOF likely reflects the overall response to the fertilizer regime rather than a specific physiological effect of the *Bacillus* inoculant.

5. Conclusions

The responses to BBOF differed between the two rubber plantations. In the 15-year-old plantation, BBOF significantly increased the F1 fraction in the topsoil but significantly decreased F1 in the subsoil, whereas latex biochemical parameters and yield did not differ significantly between fertilizer treatments. In the 25-year-old plantation, F1 was numerically higher under BBOF in both soil layers, although these differences were not statistically significant, while the topsoil F2 fraction was significantly lower under BBOF than under CF. Across the two plantations and soil depths, F2 was consistently lower under BBOF, and consideration of F1 and F2 together did not indicate a consistent increase in the combined labile SOC pool. Rather, the contrasting F1 and F2 responses suggest a redistribution among the operationally defined oxidizable SOC fractions. BBOF also resulted in significantly higher latex Suc content and yield and lower latex Mg content in the 25-year-old plantation.

Overall, the contrasting F1 and F2 responses suggest a redistribution among operationally defined oxidizable SOC fractions rather than a consistent increase in the combined labile SOC pool under BBOF. In the 25-year-old plantation, BBOF was also associated with changes in selected latex physiological parameters and higher latex yield. However, because microbial and enzymatic processes were not directly measured and the experiment was conducted for two years, the underlying mechanisms and longer-term implications for rubber tree productivity require further investigation. Overall, BBOF may provide a complementary fertilization strategy for reducing reliance on chemical fertilizer and supporting productivity in established rubber plantations.

Author Contributions

A.C., K.K. and R.C.: conceptualization, resources, methodology, data curation; Z.N.Z.: investigation, validation, formal analysis, writing—original draft preparation; A.C., K.K., P.P. and R.C.: editing; R.C.: visualization, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

AI-assisted technology was used solely for English grammar checking and language editing. The authors reviewed and approved all revisions and take full responsibility for the content of the manuscript.

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