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LEAF ACCLIMATION OF ROBUSTA COFFEE TREES TO PHOTOSYNTHETIC EFFICIENCY OF SHADE-GROWN CONDITIONS IN RUBBER-COFFEE INTERCROPPING SYSTEMS

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ABSTRACT

Robusta coffee plant is typically grown under the canopy of shade-providing trees in lowland areas of tropical regions. The study aimed to assess the leaf acclimation and the photosynthetic capacity of Robusta coffee trees under different levels of shade provided by rubber trees in rubber-coffee intercropping systems. The efficiency of photosynthetic traits was studied in 5-year-old Robusta coffee trees intercropped under four different levels of rubber tree shades (SH1 – slight shade, SH2 – moderate shade, SH3 – high shade, and SH4 – heavy shade). The results showed that the SH3 and SH4 (45% and 15% transmission) plantations had lower light transmission than the SH1 and SH2 (80% and 70% transmission) plantations. There was no statistical difference in canopy temperatures among the Robusta coffee trees under the SH2 (30.46 °C), SH3 (30.19 °C), and SH4 (29.85 °C) plantations. The chlorophyll fluorescence (F_v/F_m) ranged from 0.770 to 0.799 showing similar values in SH2, SH3, and SH4 plantations. The greatest total chlorophyll content (Chl_{total}) values were recorded in SH3 and SH4 plantations. Additionally, gas-exchange rates in Robusta coffee trees were higher under the slight shade (SH1) condition, with optimal performance at higher light intensity (1,200 μmol/m^2/s) and higher temperatures at 35°C. The net photosynthetic rate (P_n) response curves exhibited dominance in the SH1 and SH2 plantations, maintaining a similar pattern throughout all light-saturating conditions. The highest values of photosynthetic capacity under biochemical limitations (P_{max}, I_s, $V_{c,max}$, J_{max}, and TPU) were also observed in both SH1 and SH2 plantations, which were 61.37%, 56.29%, 45.44%, 56.84% and 45.84%, respectively higher than those of SH3 and SH4 plantations. According to the results, optimizing light transmission could potentially lead to more efficient Robusta coffee's photosynthetic capacity as an intercropping condition. This study suggests that the Robusta coffee tree could adapt to fluctuating light transmission, more than 50% rubber-shaded conditions could ultimately lead to reduced photosynthetic efficiency, resulting in slower growth and lower yield potential in a rubber-coffee intercropping system.

Keywords: Chlorophyll content, leaf photosynthetic capacity, rubber agroecology, rubber tree age, shade acclimation

INTRODUCTION

Robusta coffee (*Coffea canephora*) plays a significant role in sustainable coffee production in tropical countries because of its adaptation to shaded environments, which contributes to the agroecology of tree-crop agroforestry systems (Kiyingi and Gwali, 2012; Alemu, 2015; Prastowo and Arimarsetiowati, 2019).

Shade-grown Robusta coffee exhibited complex interactions between shade levels and photosynthetic performance. Some studies have indicated that moderate shade could enhance the growth and yield of Robusta coffee, with specific clones, showing increased productivity under shaded conditions (Araújo *et al.*, 2016). The productivity of Robusta coffee is also influenced by the intensity and the species of shade trees. Dense shading, such as that provided by rubber trees, could lead to reduced yield due to excessive etiolation and leaf expansion (Araújo *et al.*, 2016; Chiarawipa *et al.*, 2021b). *Gliricidia sepium* provides higher yields of Robusta coffee than other shade types (Anim-Kwapong *et al.*, 2016). Despite the benefits, excessive shade (>30%) reduced beverage quality and yield of the Robusta coffee (Piato *et al.*, 2022).

Additionally, light intensity has a direct influence on photosynthesis rate and an indirect effect on leaf temperature (Pimentel *et al.*, 2007). Shade trees could improve the microclimate by reducing temperature and increasing humidity. The type of shade tree and the intensity of shade are important factors, contributing to the photosynthetic performance of the coffee tree (Franck and Vaast, 2009). For instance, Robusta coffee under sengon (*Albizia chinensis*) shade showed higher photosynthetic activity than lamtoro (*Leucaena leucocephala*) shade, influenced by stomatal conductivity and light intensity (Pribadi *et al*., 2023). Additionally, the timing of shade could affect gas exchange performance, with morning shade showing better results than afternoon shade (Rodríguez-López *et al*., 2013). The physiological traits of Robusta coffee, such as faster photosynthetic induction in shade leaves, allow it to maximize energy use from sunflecks, contributing to a positive carbon balance under intense shading (Martins *et al*., 2013). Under high light, leaf damage could occur at temperatures over 40 °C, with significant physiological impacts (Yamane *et al*., 2022). Also, variations in growth traits of coffee trees were primarily influenced by light, while photosynthetic traits were more affected by CO_2 (Marçal *et al*., 2021). Significant variations in physiological traits were observed among the genotypes, with water deficit causing reductions in net photosynthetic rate, stomatal conductance, and water use efficiency (Tezara *et al*., 2024).

The carboxylation phase of the Calvin cycle, catalyzed primarily by the enzyme Rubisco, is a critical step in carbon assimilation for coffee leaves. This process involves the fixation of atmospheric CO_2 into organic compounds, ultimately leading to the production of carbohydrates (Bernacchi *et al*., 2003). The activity of Rubisco, influenced by factors such as temperature, light intensity, and CO_2 concentration, directly impacts the rate of carboxylation (Pimentel *et al*., 2007; Ding *et al*., 2016). By understanding the factors affecting carboxylation efficiency and implementing strategies to optimize these factors, it is possible to enhance carbon assimilation in coffee leaves, leading to improved coffee growth, yield, and quality (Marçal *et al*., 2021; Scheffen *et al*., 2021; Piato *et al*., 2022).

The carboxylation of ribulose-1,5-bisphosphate (RuBP) by Rubisco and the regeneration of RuBP are critical processes in photosynthesis, influencing the plant's carbon assimilation efficiency (Chiarawipa *et al*., 2021a). In different species, the ratio of RuBP regeneration to carboxylation ($J_{max}/V_{c,max}$) varies with temperature growth, affecting photosynthetic rates. For instance, *Polygonum cuspidatum* showed an increased $J_{max}/V_{c,max}$ ratio at lower temperatures, which alleviates the limitation on RuBP regeneration, whereas *Fagus crenata* did not exhibit such changes, indicating species-specific responses to temperature (Onoda *et al*., 2005a). In summer and autumn leaves, acclimation to elevated CO_2 levels results in a partial adjustment of the balance between carboxylation and RuBP regeneration capacities with a greater reduction in carboxylation capacity compared to being more reduced than RuBP regeneration at higher CO_2 levels (Onoda *et al*., 2005b). All this indicates that the leaves' ability to use CO_2 for photosynthesis varied with the seasonal climatic conditions.

Due to climate variability, including less rainfall and longer, more unpredictable droughts, shaded-coffee systems are becoming a viable nature-based solution for coffee production under climate change (Chemura *et al*., 2021). These systems offer benefits such as improved microclimate conditions and enhanced biodiversity. Additionally, the integration of agroforestry practices is deemed essential for sustainable coffee cultivation in the face of climate challenges (Koutouleas *et al*., 2022). Robusta coffee is often chosen as a companion crop for rubber trees because it is well adapted to shady conditions (Chiarawipa *et al*., 2021b). The differential canopy density associated with age-related variations in rubber intercropping systems might induce photosynthetic adaptations in Robusta coffee plants, thereby affecting their growth and yield potential. Moreover, the design of sustainability in an agro-ecological landscape allows efficient land use within rubber plantations. However, challenges arise when dealing with mature rubber plantations. As rubber trees mature, their canopy becomes denser, leading to reduced sunlight penetration and a more humid microclimate within the plantation.

A comprehensive understanding of the spatial interactions within rubber-coffee intercropping agroecosystems is imperative to optimize the photosynthetic efficiency of Robusta coffee, which is intricately linked to light intensity, shade acclimation, and understory climatic factors. Therefore, this study was conducted to investigate the photosynthetic efficiency of Robusta coffee intercropped with rubber trees at different maturity stages. The objectives were to: (i) assess how coffee leaves acclimate to varying light conditions caused by rubber tree age (5, 10, and 15 years) and the expanded row spacings (3×10 m), and (ii) investigate changes in the photosynthetic capacity of acclimated leaves of Robusta coffee trees along rubber-shaded conditions.

MATERIALS AND METHODS

Plant materials and experimental sites: The experiment was conducted in a rubber plantation located in Sadao district, Songkhla province, southern Thailand (latitude 6°43'07.3"N, longitude 100°16'34.0"E, and altitude 92 m above sea level). The study period was established in rubber-coffee intercropping plots from October 2021 through December 2022. The region experiences a distinct monsoon climate, dominated by the southwest monsoon from June to September, bringing approximately 200 mm of rainfall per month. This is followed by the northeast monsoon from October to December, which is characterized by heavier rainfall, averaging around 400 mm per month. The dry season typically occurs from mid-January to mid-May with a mean temperature of around 28 °C (Thai Meteorological Department, 2019).

Five-year-old Robusta coffee trees (*Coffea canephora* cv. Robusta) were used to investigate leaf photosynthetic acclimation to light conditions. The study focused on the effects of four different light transmission conditions caused by the age of intercropped rubber trees (RRIM 600 clone): SH1 (slight shade between expanded rows (3×10 m) of 5-year-old rubber trees), SH2 (moderate shade between rubber rows (3×7 m) of 5-year-old rubber trees), SH3 (high shade between rubber rows (3×7 m) of 10-year-old rubber trees), and SH4 (heavy shade between rubber rows (3×7 m) of 15-year-old rubber trees).

To assess the impact of rubber tree age on light transmission, rubber trees were categorized into three stages: immature (5 years), first tapping panel (10 years), and second tapping panel (15 years). Five-year-old Robusta coffee trees were intercropped with rubber trees in all plantations. A single row of Robusta coffee was planted between rows of rubber trees, which were spaced conventionally at 3×7 m. An exception was made for the expanded row of 5-year-old rubber plantations, spaced at 3×10 m, which provided a slightly shaded condition for the Robusta coffee trees.

Measurements of physiological responses, chlorophyll content and chlorophyll fluorescence: All physiological parameters of Robusta coffee trees were measured after the dry season and during the period of new rubber leaf flush (May to September). This timing was selected to mitigate the influence of varying LAI on the measured parameters.

Light intensity was measured between 10:00 and 14:00 hours on clear days using a light meter (Sun system, USA). The average light intensity between the rubber rows was recorded under both full sun and shaded conditions. The coffee trees' canopy temperature was measured using a thermal infrared camera (Testo 875-2i, Germany) to determine the average temperature of Robusta coffee leaves. Fully expanded leaves were randomly selected for physiological response measurements. A portable gas-exchange system (LI-6800, Li-Cor Inc., USA) was used to measure leaf gas exchange parameters, including net assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E). In addition, a Handy-PEA chlorophyll fluorometer (Hansatech, UK) was used to measure fluorescence parameters to analyze PSII function, specifically the maximal quantum yield of photochemistry of PSII (F_v/F_m). For a non-destructive assessment of total chlorophyll content (Chl_{total}), ten leaf samples per Robusta tree were measured using a chlorophyll meter (SPAD-502 Plus, Minolta, Tokyo, Japan). The equation of Maxiselly *et al.* (2022) was employed for calibration: Chl_{total}= 0.0080x^2 + 0.5104x + 0.281 (r^2= 0.95), where x represents the leaf greenness value (SPAD reading).

Measurements of photosynthetic capacity: The response of net photosynthetic rate (P_n) of fully expanded leaves was measured from three randomly selected individual Robusta coffee trees in the field trial. P_n response curves were determined using a portable photosynthesis system (LI-6800, Li-Cor Inc., USA) at a saturating photosynthetic photon flux density (*PPFD*) of 1,200 µmol/m^2/s under an ambient LED light source. Ambient CO$_2$ concentration was maintained at a constant level of 400 mmol CO$_2$/mol. The leaf chamber environment was controlled at 30±2 °C and 70±10% relative humidity (*RH*).

P_n response curves were calculated and modeled using the following equation (Eq. 1) based on the methods described by Thornley (1998):

$$Pn = \frac{\Phi.I+Pmax-\sqrt{(\Phi.I+Pmax)^2-4\Theta.\Phi.I.Pmax)}}{2\Theta} - Rd \qquad (1)$$

Where, P_{max} is the maximum net photosynthesis rate, ϕ is the apparent quantum yield, Θ is the light curve convexity, R_d is the dark respiration, the light saturation point (I_s), and the light compensation point (I_c), which were considered by fitting based on a non-rectangular hyperbola (*NRH*) model.

The *A/Ci* response curve was determined under controlled conditions within a leaf chamber. The chamber environment was maintained at 30±2 °C, a *PPFD* of 1,200 µmol/m^2/s, and a *RH* of 70±10%. Ambient CO$_2$ concentration was sequentially adjusted from 400 to 0 mmol CO$_2$/mol and then up to 1,500 mmol CO$_2$/mol.

The maximum rate of carboxylation by Rubisco ($V_{c,max}$), the apparent maximum rate of electron transport for RuBP regeneration (J_{max}), and triose-phosphate utilization (*TPU*) were analyzed by fitting curves to the *A/Ci* response data. The analysis followed the methodology described by Pimentel *et al.* (2007) using equations (2), (3), and (4):

$$W_c = \frac{V_{c,max}.C_i}{C_i + K_c\left(1 + O/K_o\right)} \qquad (2)$$

$$J_{max,c} = \frac{(A_{max} + R_d)(4.5C_i + 10.5\Gamma^*)}{(C_i - \Gamma^*)} \qquad (3)$$

$$W_p = 3.V_{TPU} \qquad (4)$$

Where, W_c is the potential rate of CO$_2$ assimilation due to Rubisco activity, C_i is the intercellular partial pressure of CO$_2$; O is the oxygen concentration, K_c and K_o are the Michaelis-Menten constants of Rubisco activity for carboxylation and oxidation, respectively, A_{max} is the maximum carbon assimilation rate (µmol CO$_2$/m^2/s), and Γ^* is the CO$_2$ compensation point. Finally, the phosphate-limited photosynthetic rate (W_p)

which is limited at high CO_2 concentration levels, was estimated from the velocity of *TPU* (V_{TPU}).

To estimate the values of $V_{c,max}$, J_{max}, and *TPU*, linear regression was applied to the Rubisco-limited and RuBP-limited portions of the A/C_i response curves, following the method described by Pimentel *et al.* (2007). This study additionally employed equation (3) to estimate J_{max}, denoted as $J_{max,c}$. This value, derived from gas exchange measurements, closely correlates with the previously described method (Bernacchi *et al.*, 2003).

Statistical analysis: Statistical analyses were performed using R-Studio (Olivoto and Lúcio, 2020). One-way analysis of variance (ANOVA) was used to assess the significance of data. The treatments means were compared using Duncan's multiple range test (DMRT) at $P \leq 0.05$ where the effect was significant. A simple linear regression analysis was conducted to explore the linear relationship between light-saturating conditions and photosynthetic parameters. Data were collected from four rubber plantations and presented as mean ± standard deviation. Statistical significance was determined ($P \leq 0.05$).

RESULTS

Changes in light intensity and canopy temperature: The average photosynthetically active radiation (*PAR*) in rubber plantations is shown in Figure 1. Under full sun conditions, light intensity was similar across all plantations, ranging from 1,507.00 to 1,616.50 µmol/m^2/s. In shade conditions, light intensity varied among the different ages of rubber trees (Figure 1a). However, similar trends of light transmission were observed in the SH1 (77.38%) and the SH2 (69.94%) plantations. In contrast, shade values decreased with rubber tree ages in the SH3 (45.97%) and reached the lowest level in the SH4 (16.31%) plantation.

The study observed changes in the canopy temperatures of Robusta coffee trees by the different ages of rubber plantations. Although the average shade level between rows decreased the canopy temperature, the highest temperature (34.63±0.82 °C) was recorded in the SH1 plantation. The canopy temperature values in the SH2, SH3, and SH4 plantations were similar, showing 30.46±0.65 °C, 30.19±0.95 °C, and 29.85±0.57 °C, respectively (Figure 1b).

Changes in leaf physiological responses: The F_v/F_m values of the SH2, SH3, and SH4 plantations ranged from 0.770±0.019 to 0.799±0.017, showing similar values. However, there was significantly lower in the SH1 plantation (0.744±0.024) (Figure 2a). Similar to chlorophyll content, leaf greenness values were significantly lower in the SH1 plantation than in both SH3 and SH4 plantations (Figure 2b). Among all the plantations, the SH3 exhibited the greatest value Chl_{total} (46.60±5.41 mg/cm^2). Meanwhile, SH1 and SH2 plantations had Chl_{total} values ranging from 31.64±5.04 to 35.53±3.71 mg/cm^2, with a significant difference.

The assimilation rates (*A*) for both SH1 and SH2 plantations were very similar (10.42±0.21 and 10.11±0.13 µmol CO_2/m^2/s) (Figure 3a). The transpiration rate (*E*) was lowest in the SH3 (1.41±0.18 mmol H_2O/m^2/s) and SH4 (1.42±0.24 mmol H_2O/m^2/s) plantations, while the SH1 plantation had a significantly higher rate (1.93±0.19 mmol H_2O/m^2/s) compared to the others (Figure 3b). Stomatal conductance (g_s) showed a steady decrease in all the plantations, ranging from 9.70±2.01 mmol/m^2/s in the SH1 to 7.44±2.40 mmol/m^2/s in the SH4 plantations (Figure 3c).

Changes in photosynthetic efficiency under light-saturating conditions: In all four light-saturating conditions, the Robusta coffee leaves showed significant acclimation to light (Figure 4). The results showed that P_n increased sharply from 0 to 200 µmol PPFD/m^2/s, followed by a gradual increase from 600 to 2,000 µmol PPFD/m^2/s. The maximum P_n values were achieved in the SH1 (14.27±1.12 µmol/m^2/s) and SH2 (15.55±0.78 µmol/m^2/s) plantations, which maintained a similar pattern under all light-saturating conditions. However, the maximum P_n values in the SH3 (5.36±1.15 µmol/m^2/s) and SH4 (6.17±0.88 µmol/m^2/s) plantations were lower than those in the SH1 and SH2 with significant differences, following similar trends.

P_{max} and I_s in the SH1 and SH2 plantations showed higher values, while the lowest values were observed in both the SH3 and SH4 plantations (Figure 5a and 5b). Conversely, I_c decreased significantly in both SH1 and SH2 plantations, exhibiting a similar pattern to R_d across all light-saturating conditions for all Robusta coffee leaves (Figure 5c and 5d).

Changes in photosynthetic capacity under biochemical limitations: $V_{c,max}$, J_{max}, and *TPU* values were significantly higher in the SH1 and SH2 than in the SH3 and SH4 plantations (Figure 6). In all the plantations, the $V_{c,max}$ value in the SH2 (20.57±7.61 µmol/m^2/s) was the highest, followed by that in the SH1 (15.80±2.27 µmol/m^2/s) (Figure 6a). The SH1 plantation exhibited the highest J_{max} (79.13±12.83 µmol/m^2/s) and *TPU* (5.74±1.76 µmol/m^2/s). However, $V_{c,max}$, J_{max}, and *TPU* were markedly lower in the SH3 (10.55±2.20, 33.53±4.70, and 2.11±0.29 µmol/m^2/s, respectively) and SH4 (8.70±4.99, 30.53±15.01, and 3.21±0.35 µmol/m^2/s, respectively) compared to those in the SH1 and SH2 plantations (Figure 6b and 6c).

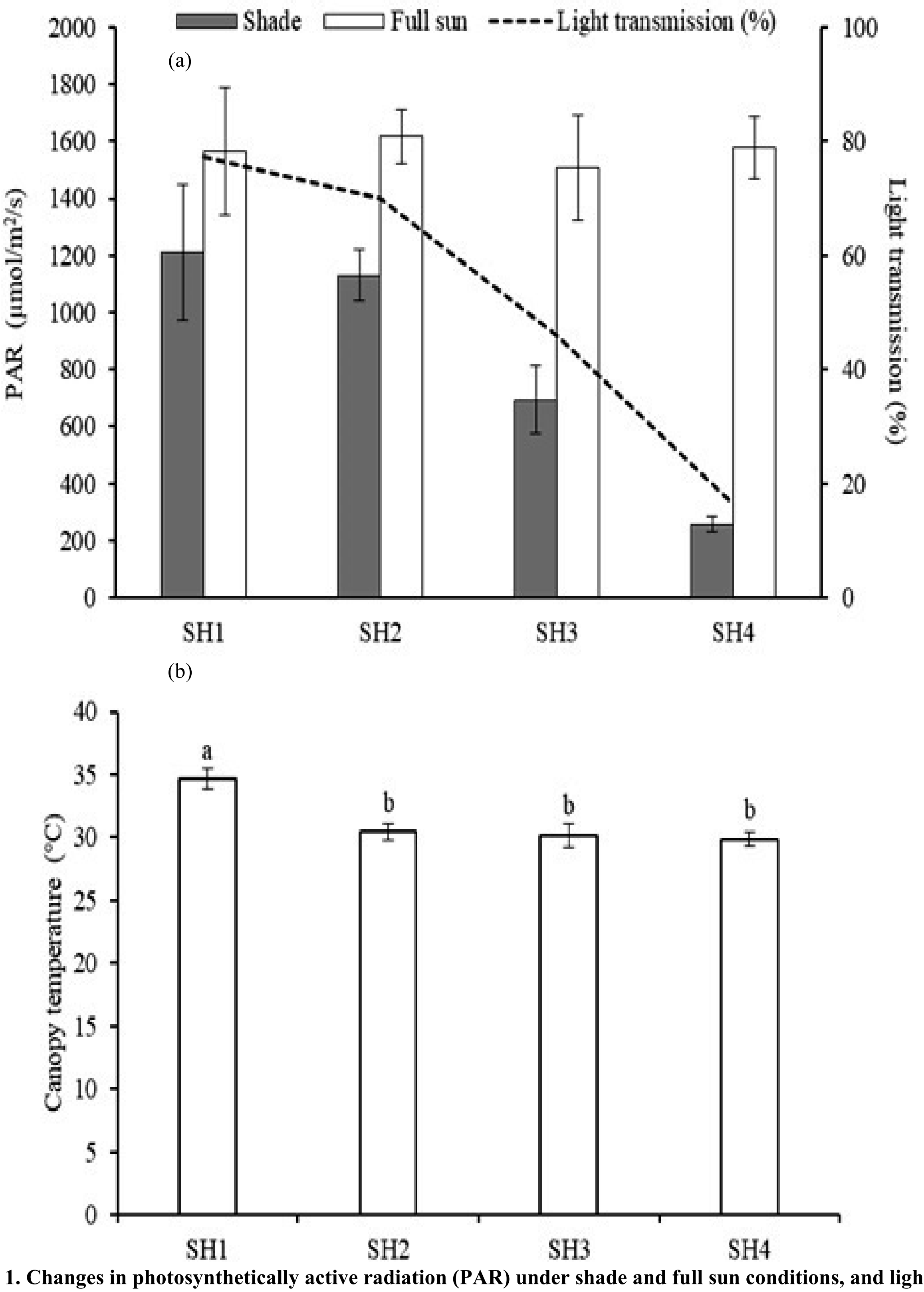

Figure 1. Changes in photosynthetically active radiation (PAR) under shade and full sun conditions, and light transmission (a), and canopy temperature (b) of Robusta coffee trees in the SH1, SH2, SH3, and SH4 plantations

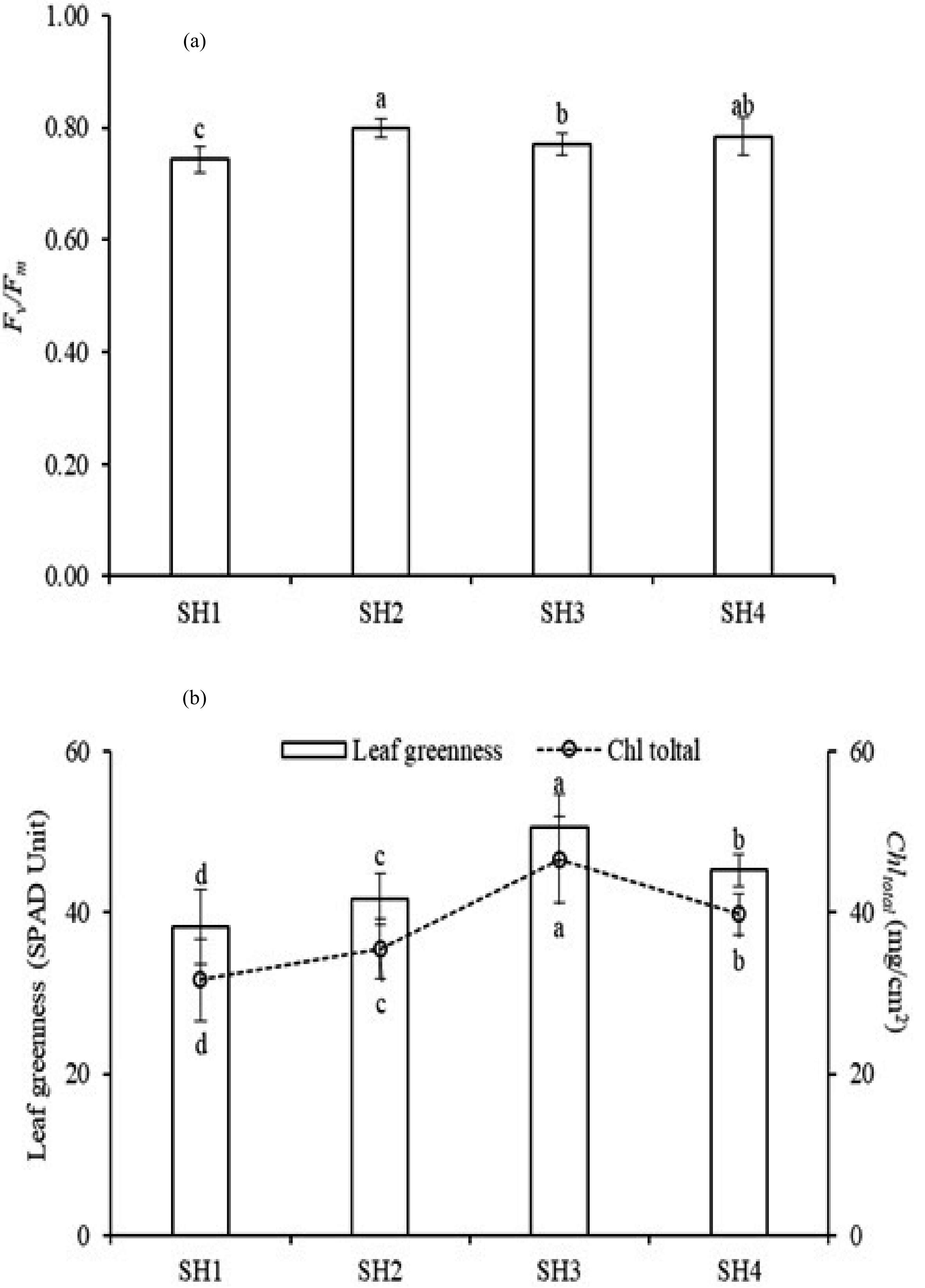

Figure 2. Chlorophyll fluorescence (F_v/F_m) (a) and leaf greenness, and total chlorophyll content (Chl_{total}) (b) in Robusta coffee leaves in the SH1, SH2, SH3, and SH4 plantations

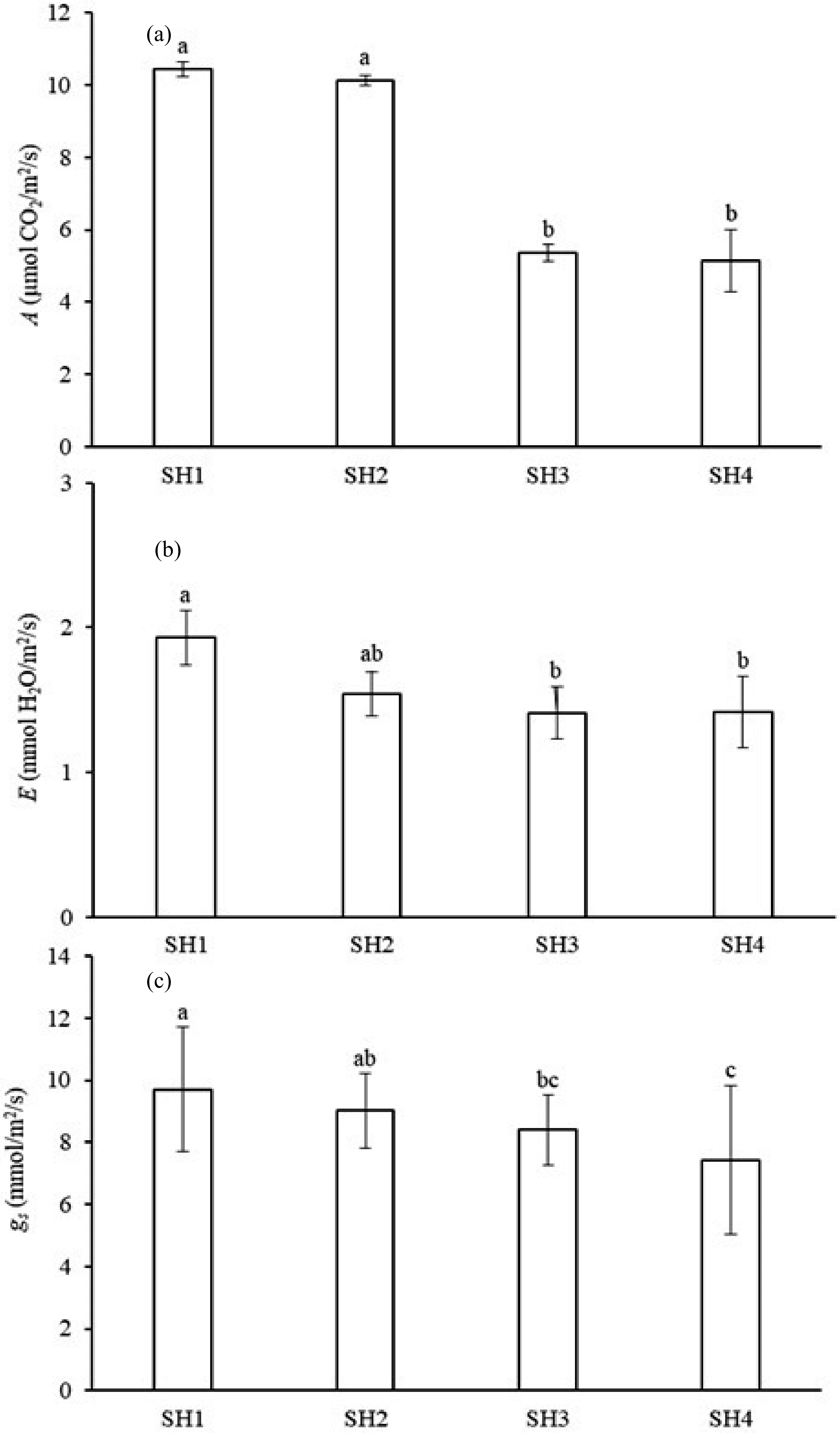

Figure 3. Leaf gas exchange parameters in Robusta coffee leaves of net assimilation rate (*A*) (a), transpiration rate (*E*) (b), and stomatal conductance (g_s) (c) in the SH1, SH2, SH3, and SH4 plantations

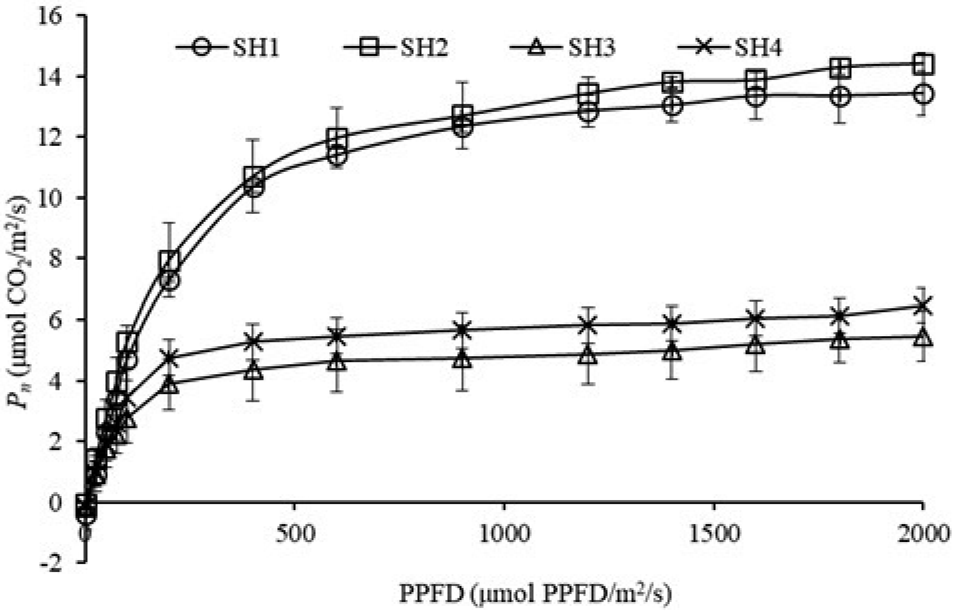

Figure 4. Changes in response of net photosynthetic rate (P_n) under light-saturating conditions in Robusta coffee leaves in the SH1, SH2, SH3, and SH4 plantations

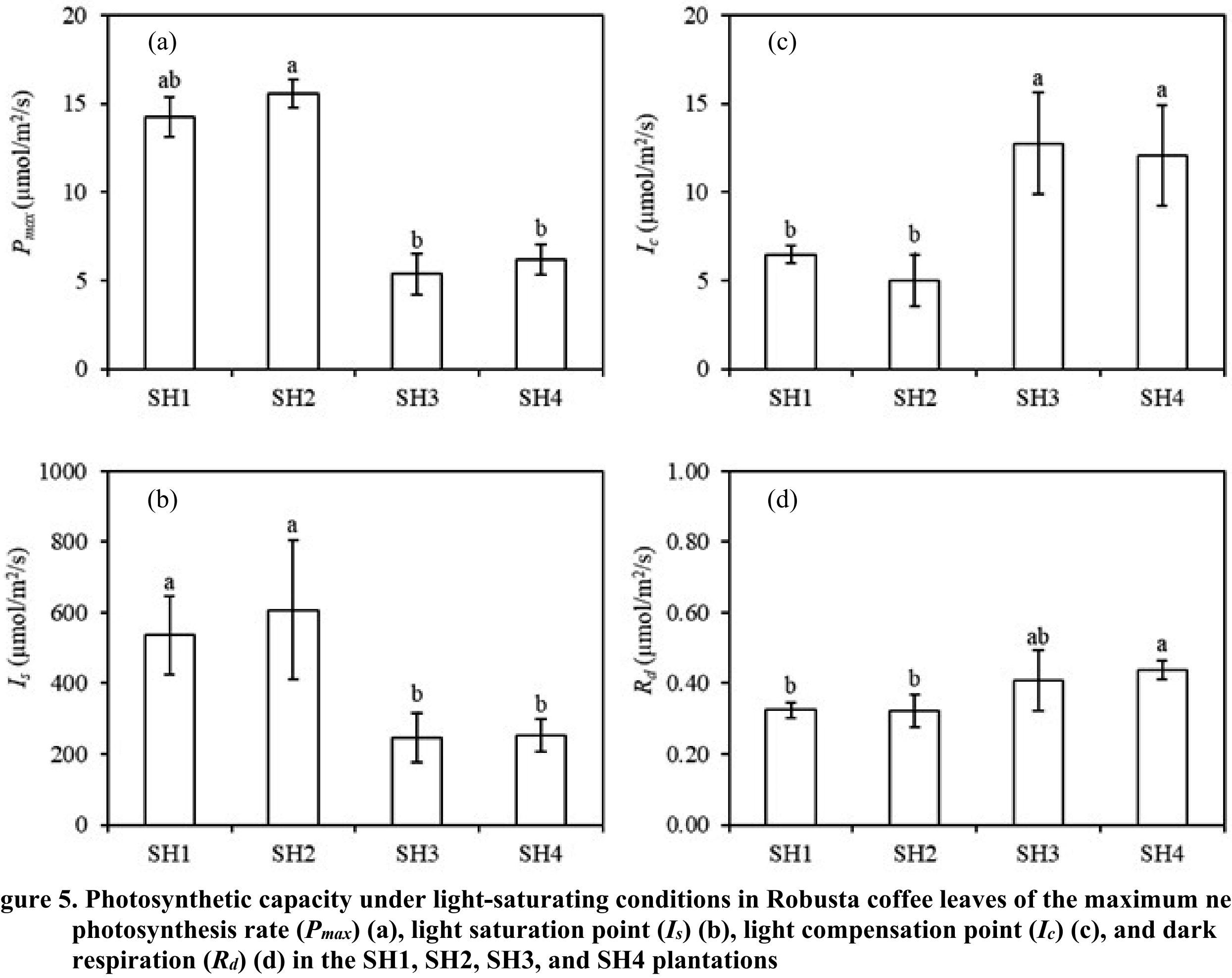

Figure 5. Photosynthetic capacity under light-saturating conditions in Robusta coffee leaves of the maximum net photosynthesis rate (P_{max}) (a), light saturation point (I_s) (b), light compensation point (I_c) (c), and dark respiration (R_d) (d) in the SH1, SH2, SH3, and SH4 plantations

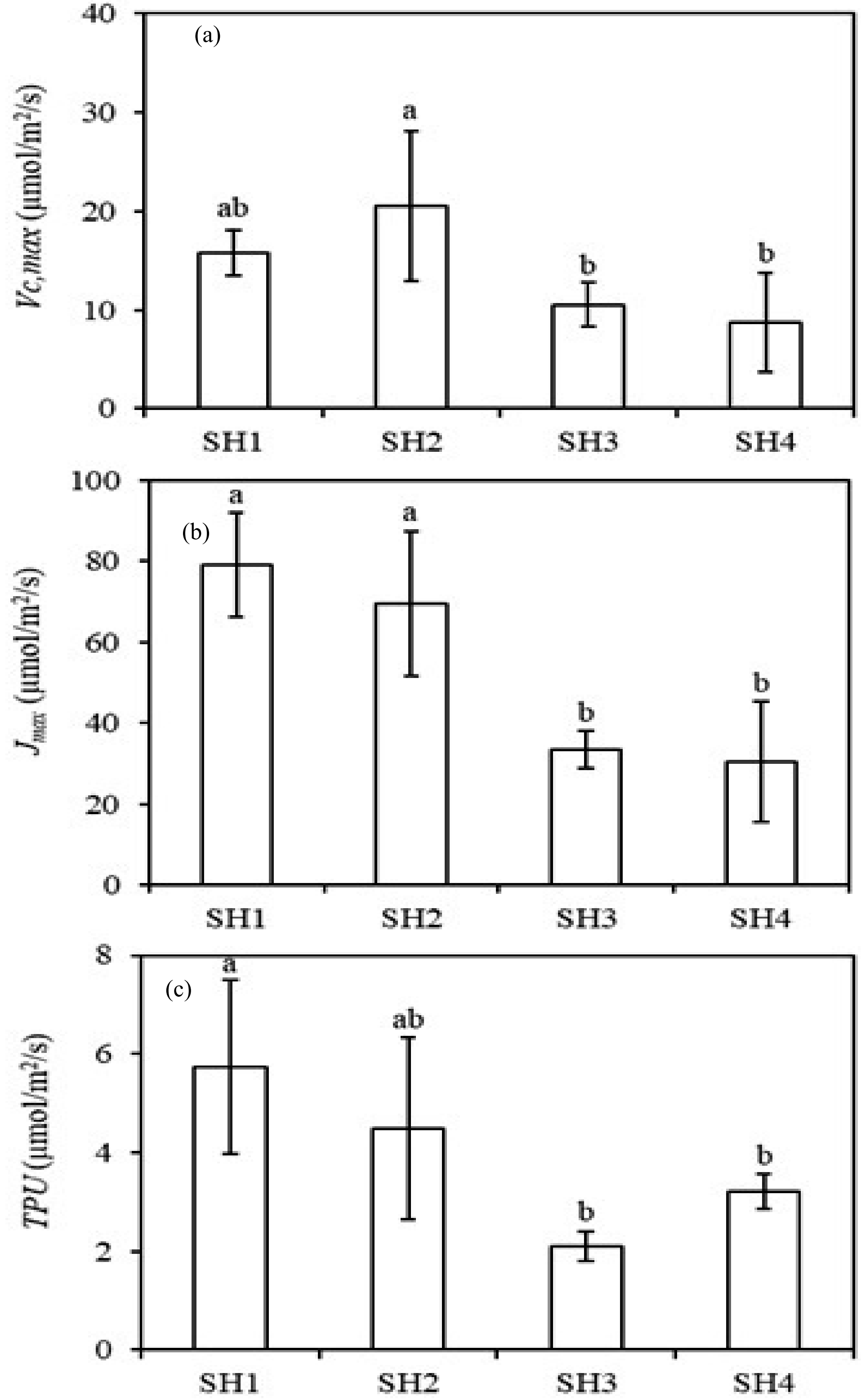

Figure 6. Photosynthetic capacity under biochemical limitations in Robusta coffee leaves of the maximum rate of carboxylation by Rubisco ($V_{c,max}$) (a), the apparent maximum rate of electron transport for RuBP regeneration (J_{max}) (b), and triose-phosphate utilization (*TPU*) (c) in the SH1, SH2, SH3, and SH4 plantations.

DISCUSSION

Leaf shade acclimation and chlorophyll content and fluorescence: Robusta coffee grown in shaded conditions tends to have higher chlorophyll levels than unshaded plants, allowing shaded coffee leaves to photosynthesize more efficiently. This adaptation is likely due to light intensity. In lower-light environments, Robusta coffee plants need more chlorophyll to capture available sunlight effectively. This acclimation translates to increased leaf area in shaded plants to maximize light capture (Nattawit *et al*., 2019; Vasheka *et al*., 2019). Furthermore, shaded Robusta coffee exhibits a higher rate of stomatal closure, decreasing gas exchange for photosynthesis, affecting the coffee tree's ability to absorb CO_2.

Leaf age and position significantly impact the rate of photosynthesis in plants. Young leaves typically exhibit a gradual increase in photosynthesis as their foliage expands and internal structures mature, developing essential components for efficient light capture and carbon fixation (Vasheka *et al*., 2019; Chiarawipa *et al*., 2021a). However, once leaves reach full expansion, the photosynthetic rate often begins to decline, partly due to the reallocation of nutrients towards other growing parts of the plant, called sink organs (Araujo *et al*., 2008). The conditions in intercropping Robusta coffee with rubber trees could lead to restrictions for nutrients, water, and sunlight, limiting the growth and yield of Robusta coffee trees (Chiarawipa *et al*., 2021b).

Robusta coffee's chlorophyll content and fluorescence are critical indicators of its photosynthetic efficiency, health, and yield potential. Various factors, including altitude, influence the chlorophyll a and b levels of Robusta coffee leaves (Araújo *et al*., 2016; Prastowo and Arimarsetiowati, 2019). The non-destructive method for estimating chlorophyll content and fluorescence has proven effective as a rapid and accurate assessment of photosynthetic pigments and nitrogen levels in Robusta coffee (França and de Carvalho, 2016) and hazelnut (Catoni *et al*., 2019) leaves. Leaf greenness data is also valuable for predicting coffee leaf chlorophyll content, with finer spatial resolutions yielding (Zanella *et al*., 2020). Higher chlorophyll levels were observed during the vegetative stage in the certain varieties since the chemical composition of leaves, including chlorophyll and nutrient concentrations, varies with the plant's developmental stages (Ji *et al*., 2021; Zaw *et al*., 2023; Javed *et al*., 2024). In addition, soil and leaf nutrient densities, particularly microelements like zinc (Zn), and boron (B), also could play a significant role in determining chlorophyll content and overall coffee quality (Inthasan *et al*., 2021). Based on the result of this study, shade acclimation to chlorophyll fluorescence (F_v/F_m), which represents the maximum efficiency of photosystem II (*PSII*) photochemistry, also depends on the chlorophyll content in shaded leaves of Robusta coffee trees. Then, limitations to *PSII* in Robusta coffee could also be limited by factors as a result of nutrition and water availability, as shown in studies by DaMatta *et al*. (2002).

Leaf shade acclimation and photosynthetic efficiency: Robusta coffee varies photosynthetic efficiency depending on light intensity and shading conditions. In this study, gas exchange rates (A, E, and g_s) in Robusta coffee trees were high under slight shade (SH1) conditions. The optimal photosynthetic performance was recorded at higher light intensity (1,200 $\mu mol/m^2/s$) and temperatures at 35 °C. However, heavy shade in the understory leads to a decrease in gas exchange rates within Robusta coffee leaves. This effect is attributed to the reduced absorption of radiation by the leaf surface (Franck and Vaast, 2009). Additionally, high-temperature leaves in the slight shade condition (SH1) exhibited an acclimation as higher temperatures could reduce mesophyll conductance in photosynthetic responses (Araujo *et al*., 2008). Meanwhile, temperatures exceeding 35 °C could significantly restrict both the activity of ribulose-1,5-bisphosphate (RuBP) and its regeneration (Pimentel *et al*., 2007). In *Coffea arabica*, a temperature tolerance threshold was between 44.0 and 46.3°C. Significant physiological damage was observed after 96 hours at 45°C. Chlorophyll content in leaves declined significantly at higher temperatures, especially above 40°C (Yamane *et al*., 2022).

Despite generally having lower photosynthetic rates under low light conditions, shade leaves of coffee plants maintain strong photosynthetic machinery and are capable of high CO_2 fixation rates when exposed to high light intensities (Onoda *et al*., 2005b; Franck and Vaast, 2009). Furthermore, shade conditions limited photosynthesis in Robusta coffee leaves, as evidenced by the decrease in P_{max} and I_s in light intensity response curves. Interestingly, carboxylation efficiency also suggests that CO_2 concentration could be a limiting factor for photosynthesis. This study suggests that photosynthetic capacity, as indicated by the lower values of $V_{c,max}$, J_{max}, and *TPU* in heavily shaded Robusta coffee leaves, is a sensitive indicator of light acclimation. This is further supported by the observed limitations in Rubisco activity and RuBP regeneration.

The results demonstrate a potential link between light conditions (slight shade vs. heavy shade) and carboxylation efficiency in Robusta coffee trees. However, temporal fluctuations in light, such as morning or afternoon shades, also might affect gas exchange. Morning-shaded plants have better photosynthetic efficiency than afternoon-shaded and full-sun plants (Rodríguez-López *et al*., 2013). Therefore, the photosynthetic potential of the Robusta coffee agroforestry system was notable, with variations observed across different agroecosystems (Martins *et al*., 2013). This highlights the system's contribution to the productivity and physiological responses of the coffee trees (Venancio *et al*., 2019).

This study implies that Robusta coffee trees were adapted to the slight and moderate shaded environments of the young rubber trees and could maintain high photosynthetic performance. The specific conditions of shade, including light transmission, light intensity, and temperature, significantly influenced the overall productivity and photosynthetic capacity of the Robusta coffee trees. The higher photosynthetic capacity is a positive sign of the sustainability and productivity of Robusta coffee trees in rubber-coffee intercropping system, thereby supporting both economic and agroecological goals.

Therefore, the ecophysiological acclimation of Robusta coffee leaves are particularly beneficial in rubber-based agroforestry systems, where intercropping with optimum-shaded rubber trees can reduce plant abiotic stress. This approach can contribute to more sustainable and resilient agricultural practices, economic sustainability, and mitigating the negative impacts of climate change. By understanding and harnessing these crop adaptations, farmers can optimize Robusta coffee production within agroforestry systems, leading to both agroecological and economic benefits, especially in tropical regions where intercropping systems are prevalent.

Conclusions: Although the Robusta coffee tree could adapt to rubber-shaded conditions, excessive shade in mature rubber plantations could ultimately lead to reduced photosynthetic efficiency, resulting in slower growth and lower yield. Therefore, the canopy density of the rubber tree could contribute to the Robusta coffee leaf acclimation with shaded photosynthetic efficiency. The resilience of photosynthetic traits to fluctuating light transmission could be considered a critical indicator of Robusta coffee's vigorousness and yield potential in a rubber-coffee intercropping system.

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Conflict of interest: The authors declare no conflict of interest.

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