

Gas exchange and water status of cacao under intercropping and monoculture with contrasting irrigation levels

Trocas gasosas e estado hídrico em cacau consorciado e monocultivo sob diferentes lâminas de irrigação

Hugo A. S. Silva¹, Vivian D. da S. Farias², Andressa J. S. Vasconcelos³, Israel A. de Oliveira³, Matheus L. Rua³, Miguel G. M. Santos³, Paulo J. de O. P. de Souza³

¹Instituto Federal de Educação, Ciência e Tecnologia do Pará, Altamira, PA, Brazil. ²Universidade Federal do Pará, Altamira, PA, Brazil. ³Socio-environmental and Water Resources Institute, Universidade Federal Rural da Amazônia, Belém, PA, Brazil.

ABSTRACT - Cacao is of major socioeconomic importance in the Trans-Amazonian region, where irregular rainfall may affect plant physiological performance. This study evaluated gas exchange and water status in seed-propagated cacao grown under intercropping and monoculture systems and subjected to irrigation levels corresponding to 0, 65, 100, and 130% of reference evapotranspiration. The experiment was conducted under field conditions using a split-plot design with five blocks. Leaf water potential (Ψ_f), net CO_2 assimilation rate (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci), leaf transpiration (E), instantaneous water-use efficiency (WUE), and carboxylation efficiency (CE) were evaluated. During the rainy season, no significant differences in Ψ_f were observed among treatments, with values ranging from -0.17 to -0.55 MPa, indicating high water availability. During this period, plants grown in monoculture showed higher A, gs, and E due to greater availability of photosynthetically active radiation, whereas WUE was approximately 38% lower in monoculture cacao than in cacao grown in the intercropping system. During the less rainy season, increasing irrigation levels progressively increased Ψ_f , indicating mitigation of water stress. Mean Ψ_f was higher in the monoculture system (-0.75 MPa) than in the intercropping system (-1.07 MPa). These results indicate that irrigation is essential for maintaining plant water status during the less rainy season, whereas during the rainy season, the two cropping systems exhibit distinct responses associated with radiation availability and water-use efficiency.

RESUMO - O cacau possui elevada relevância socioeconômica na região da Transamazônia, onde a irregularidade das chuvas pode afetar o desempenho fisiológico das plantas. Este estudo avaliou as trocas gasosas e o estado hídrico do cacau seminal cultivado em sistema consorciado e em monocultivo, submetido às lâminas de 0, 65, 100 e 130% da evapotranspiração de referência. O experimento foi conduzido em campo, em delineamento de parcelas subdivididas, com cinco blocos. Foram avaliados o potencial hídrico foliar (Ψ_f), a taxa de assimilação líquida de CO_2 (A), condutância estomática (gs), concentração intercelular de CO_2 (Ci), transpiração da folha (E), eficiência instantânea do uso da água (WUE) e a eficiência de carboxilação (CE). No período chuvoso, não foram observadas diferenças significativas no Ψ_f entre os tratamentos, cujos valores do Ψ_f das plantas variaram de $-0,17$ a $-0,55$ MPa, indicando elevada disponibilidade hídrica. Nesse período, o monocultivo apresentou maiores valores de A, gs e E, em função da maior disponibilidade de radiação fotossinteticamente ativa, enquanto a WUE foi aproximadamente 38% menor no monocultivo do que no sistema consorciado. No período menos chuvoso, o aumento das lâminas de irrigação elevou progressivamente o Ψ_f , indicando mitigação do estresse hídrico. O monocultivo apresentou maior Ψ_f médio ($-0,75$ MPa) que o consórcio ($-1,07$ MPa). Conclui-se que a irrigação é determinante para a manutenção do estado hídrico durante o período menos chuvoso, enquanto, no período chuvoso, os sistemas apresentam respostas distintas, relacionadas à disponibilidade de radiação e à eficiência no uso da água.

Keywords: Irrigation. *Theobroma cacao*. Intercropping.

Palavras-chave: Irrigação. *Theobroma cacao*. Consórcio.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.



This work is licensed under a Creative Commons Attribution-CC-BY <https://creativecommons.org/licenses/by/4.0/>

Received for publication in: December 20, 2025.
Accepted in: September 23, 2026.

Editor in Chief: Aurélio Paes Barros Júnior
Section Editor: João Everthon da Silva Ribeiro

Data Availability: The data that support the findings of this study can be made available, upon reasonable request, from the corresponding author.

***Corresponding author:**
<hugo.amancio@ifpa.edu.br>

INTRODUCTION

Cacao (*Theobroma cacao* L.) plays a major socioeconomic and environmental role in the Trans-Amazonian region of Pará State, Brazil. Historically, a large proportion of cacao plantations in the region were established using seed-propagated planting material, which, despite its high genetic variability, is characterized by a robust root system that enhances the exploitation of soil resources in deeper layers (BRASIL, 2021). However, the physiological performance of these plants remains intrinsically dependent on water availability, which becomes particularly critical during periods of lower rainfall and adverse events that lead to irregular precipitation.

Reduced soil water availability triggers a cascade of adaptive responses, in which stomatal closure acts as the first line of defense against the decline in plant water status. Although essential for water conservation, this mechanism imposes a physical barrier to CO_2 uptake, leading to a progressive reduction in net photosynthesis as internal CO_2 availability declines. This process is coordinated by systemic chemical signaling driven by the synthesis of abscisic acid (ABA) in roots and leaves, which conveys the severity of water deficit and modulates guard-cell turgor (ALMEIDA; VALLE, 2007).

In this context, irrigation represents an essential technology for mitigating the effects of water deficit, helping to maintain leaf water potential and sustain gas exchange at optimal levels. For seed-propagated cacao, the management of different irrigation levels is particularly important because it allows the identification of a balance between water conservation and maximum physiological efficiency of the plants (ALMEIDA et al., 2002).

The cropping system may influence the physiological response of cacao to irrigation. In monoculture systems, greater exposure to solar radiation and atmospheric conditions associated with higher evaporative demand may increase leaf temperature and vapor pressure deficit, thereby increasing plant water demand and the need for photoprotective mechanisms (NIETHER et al., 2018). In contrast, intercropping systems provide a milder microclimate through shading, which may reduce evaporative demand and alter gas exchange dynamics and water-use efficiency (SOMARRIBA et al., 2018).

When other constraints, such as plant nutrition and phytosanitary conditions, are adequately controlled, the successful cultivation of perennial crops in the Amazon region is strongly dependent on climatic and water-related factors. In cacao, seasonal fluctuations in yield are substantial because variations in temperature and rainfall affect vegetative growth, flowering, and fruit filling (LAHIVE; HADLEY; DAYMOND, 2019). Moreover, the adoption of more efficient irrigation strategies has been recognized as an alternative for increasing yield and water-use efficiency in cacao production systems.

In this context, the present study was based on the need to improve water management in seed-propagated cacao plantations by identifying management strategies that reconcile physiological efficiency with adaptation to the climatic conditions of the Trans-Amazonian region of Pará

State, Brazil. Accordingly, the study seeks to contribute to the development of cropping arrangements and management practices capable of mitigating the effects of water stress and promoting physiological stability.

MATERIALS AND METHODS

The experiment was conducted in a commercial cacao plantation located in the municipality of Vitória do Xingu, Pará State, northern Brazil (3°09'46.36" S, 52°06'53.87" W). Evaluations were carried out during the 2024/2025 growing season, from June 2024 to August 2025, in an 11,700-m² field containing 1,300 six-year-old cacao plants. The plantation was established in 2019 using hybrid cacao seeds supplied by CEPLAC, at a spacing of 3 × 3 m. In the area considered as the intercropping system, açaí palm was planted between the cacao trees at a spacing of 9 × 9 m, one year after cacao establishment. The açaí cultivar was BRS Pará, and one stem was maintained per clump.

The regional climate is classified as Am according to the Köppen climate classification, characterized as humid tropical, with the rainy season concentrated between December and May and a less rainy period during the remainder of the year (INMET, 2026). During the experiment, the variability of meteorological conditions was monitored throughout the study period, as shown in Figures 1 and 2. Within-canopy precipitation totaled 2,177 mm, while mean air temperature and relative humidity were 26.8°C (± 0.97) and 87.18% (± 7.26), respectively. During the less rainy period, mean air temperature and relative humidity were 27.9°C (± 0.57) and 77.6% (± 4.26), respectively, whereas during the rainy season (December to May), the corresponding means were 26.3°C (± 0.44) and 92.9% (± 1.68).

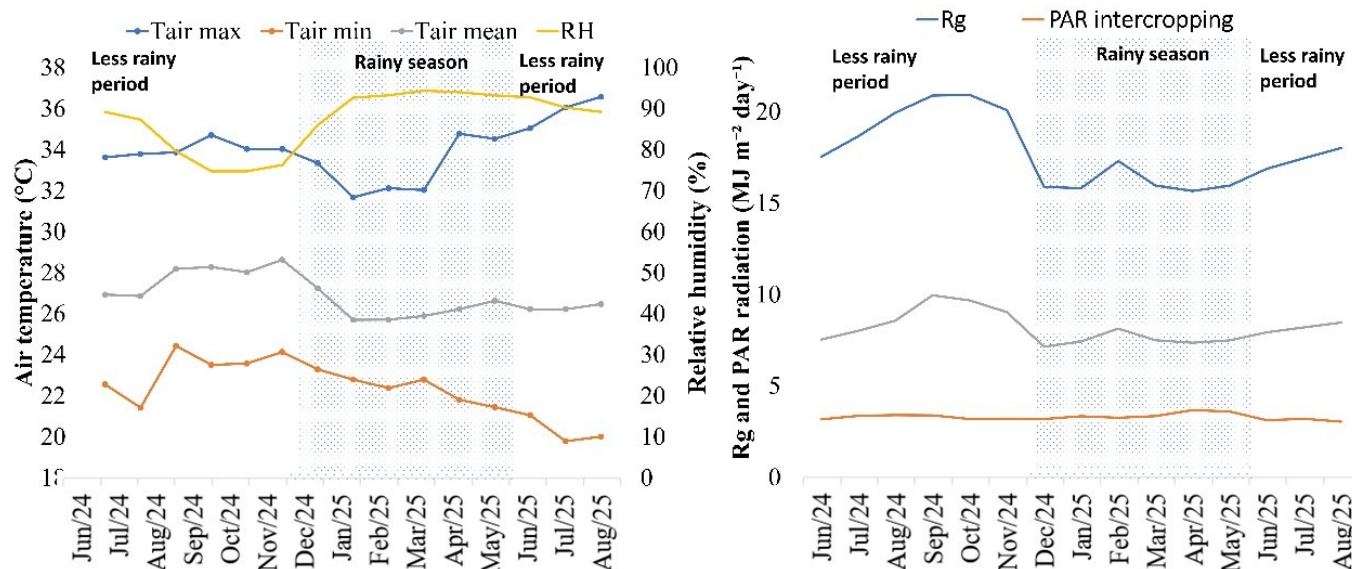


Figure 1. Mean (Tair mean), maximum (Tair max), and minimum (Tair min) air temperature; monthly mean relative humidity (RH); global solar radiation (Rg); net radiation (Rn); and photosynthetically active radiation (PAR).

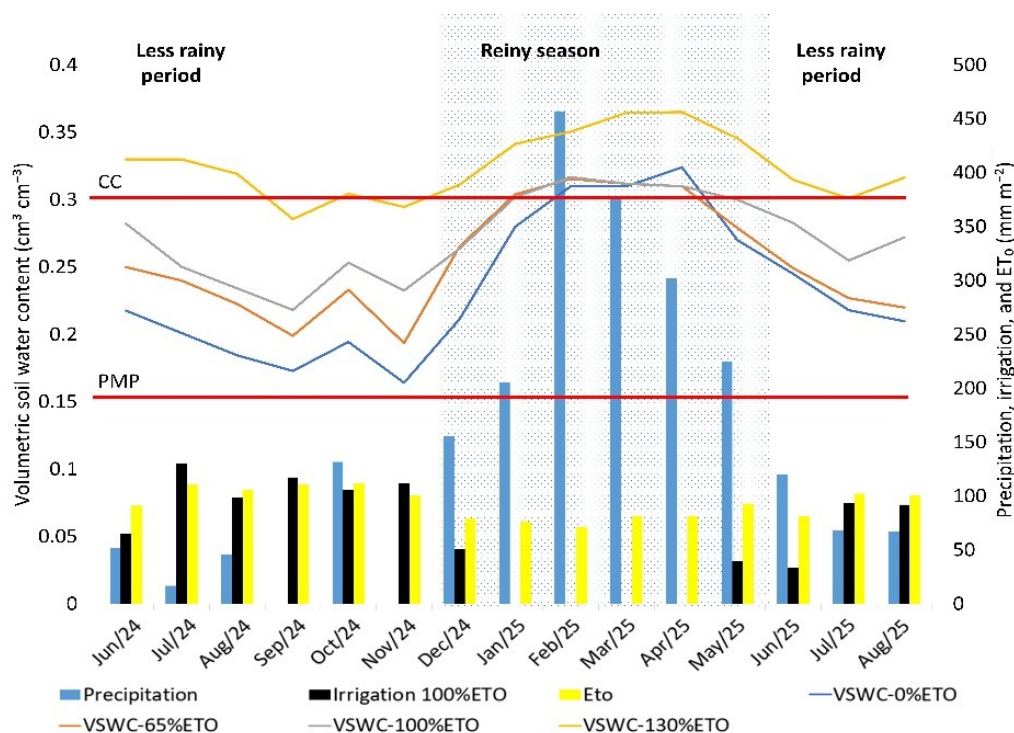


Figure 2. Precipitation, irrigation corresponding to 100% of reference evapotranspiration (ET_0), reference evapotranspiration (ET_0), and volumetric soil water content. 0% ET_0 : non-irrigated treatment; 65% ET_0 : irrigation at 65% of reference evapotranspiration; 100% ET_0 : irrigation at 100% of reference evapotranspiration; 130% ET_0 : irrigation at 130% of reference evapotranspiration.

During the experiment, incident global solar radiation above the canopy averaged $17.8 \text{ MJ m}^{-2} \text{ day}^{-1}$ (± 1.9), while photosynthetically active radiation (PAR) averaged $8.2 \text{ MJ m}^{-2} \text{ day}^{-1}$ (± 0.8) in monoculture cacao (PARm) and $3.3 \text{ MJ m}^{-2} \text{ day}^{-1}$ (± 0.2) in cacao intercropped with açai palm (PARc). Over the experimental period, mean reference evapotranspiration was 3.1 mm day^{-1} (± 0.5), whereas mean crop evapotranspiration was 2.7 mm day^{-1} (± 0.3), totaling 980 mm year^{-1} , based on the Kc reported by Rua et al. (2026).

The soil in the experimental area was classified as Latossolo Amarelo distrófico, with a clay loam texture, a pH of 5.1, and base saturation of 43% (SANTOS et al., 2018). Regarding soil hydraulic properties, field capacity and permanent wilting point were estimated using the empirical equations proposed by Saxton and Rawls (2006), based on soil textural composition. The estimated field capacity and permanent wilting point were 0.30 and $0.15 \text{ cm}^3 \text{ cm}^{-3}$, respectively. During the experiment, crop management practices followed the technical recommendations adopted for cacao cultivation in the region (BRASIL, 2021). Fertilization was carried out during the rainy season and split into three applications throughout the growing season. Sanitary pruning was performed every two months to remove dry and diseased branches and excessive shoots, whereas maintenance pruning was performed once during the less rainy period to maintain vegetative balance and improve canopy aeration and light penetration. Pest and disease monitoring and phytosanitary control were conducted according to the management practices routinely adopted by the farm owner.

Below-canopy precipitation was monitored daily by measuring throughfall and stemflow. To descriptively characterize the temporal dynamics of volumetric soil water content as a function of irrigation levels, the soil moisture

profile in the 0–40 cm layer was monitored using four time-domain reflectometry (TDR) probes (CS616, Campbell Scientific, Inc.), installed vertically in the plots of the intercropping system, with one probe assigned to each irrigation level. In addition, a set of tensiometers was installed at depths of 0–20 and 20–40 cm in each treatment combination for continuous monitoring of soil matric potential and to support irrigation management, totaling 16 instruments. Soil water content and matric potential data were used strictly for point-based descriptive purposes to illustrate soil water status throughout the experimental period and were not subjected to analysis of variance because of the lack of instrumental replication among blocks.

The experiment was arranged in a split-plot design with five blocks, comprising two whole-plot treatments (cropping systems: cacao monoculture and intercropped cacao) and four subplot treatments (irrigation levels corresponding to 0, 65, 100, and 130% of reference evapotranspiration, ET_0), with five replications. Each block covered an area of approximately $2,600 \text{ m}^2$ and was subdivided into two whole plots. Because interference between the cropping systems had to be avoided, particularly to prevent shading and root expansion from the açai palms from compromising the full-sun conditions required in the monoculture plots, the whole-plot treatments were assigned to fixed positions within each block, resulting in restricted randomization. This strict physical separation was necessary to ensure effective independence between the two cropping systems, although it imposed a limitation on complete spatial randomization within the blocks. Within each whole plot, the subplot treatments were randomly assigned, ensuring randomization of the irrigation levels while controlling environmental variability within blocks. Each experimental

unit consisted of nine cacao plants arranged in three rows of three plants, covering a total area of 81 m². Only the central plants of each plot were used for evaluations, whereas the outer rows served as border plants and received the same management practices and irrigation levels. Overall, the experiment comprised 40 experimental units.

Irrigation levels were defined based on daily reference evapotranspiration (ET_0) because information on maximum crop evapotranspiration for cacao under the regional conditions is not available. Daily ET_0 was estimated using the Penman–Monteith method (SZILAGYI; HOGAN, 2020), based on data from the automatic weather station of the Brazilian National Institute of Meteorology (INMET), located 12.2 km from the experimental area.

The daily gross irrigation depth was applied using a localized micro-sprinkler irrigation system operated at a pressure head of 10 m of water column. Water distribution efficiency was assessed using the Distribution Uniformity Coefficient (DUC), which yielded a value of 85%. Based on the daily ET_0 values, the irrigation depths for each treatment were determined according to Equation 1:

$$GI = \frac{(ET_0 * \%Treat) - P_{in}}{ef} \quad (1)$$

where: GI = gross irrigation depth (mm); ET_0 = daily reference evapotranspiration (mm); $\%Treat$ = percentage of ET_0 replacement corresponding to each treatment (0.00, 0.65, 1.00, or 1.30); P_{in} = throughfall (mm day⁻¹); and ef = irrigation system efficiency (%).

When throughfall (P_{in}) exceeded the water requirement of a given treatment ($ET_0 \times \%Treat$), irrigation was suspended for the corresponding plot. The irrigation duration for each treatment was estimated according to Equation 2:

$$IT = \frac{GI}{WA} \quad (2)$$

where: IT = irrigation time (h); GI = gross irrigation depth (mm); and WA = water application rate (mm h⁻¹).

The primary factor consisted of the cropping system, either intercropped or monoculture. In the intercropped plots, açai palms were spaced at 9 × 9 m.

Leaf water potential (Ψ_f) was measured during the less rainy period (September 2024) and the rainy period (April 2025) using a portable Scholander pressure chamber (model SEC-3115, P40G4V; Soilmoisture Equipment Corp.). Measurements were performed before sunrise, with one leaf evaluated per experimental unit. Leaves selected for measurement were fully expanded, mature, and located in the middle third of the plants. Leaf water potential was determined immediately after each leaf was detached from the plant.

Gas exchange parameters were monitored in February 2025, during the regional rainy season, on the first fully

expanded mature leaf from the terminal shoot of fruit-bearing branches located in the middle third of the plant, following procedures adopted in previous studies (LAHIVE et al., 2021). Net CO₂ assimilation rate (A ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance to water vapor (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intercellular CO₂ concentration (C_i ; $\mu\text{mol CO}_2 \text{ mol}^{-1}$), leaf transpiration rate (E ; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), instantaneous water-use efficiency (WUE ; $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$), and carboxylation efficiency (CE ; $\text{mol m}^{-2} \text{ s}^{-1}$) were measured between 9:00 and 11:00 a.m. using a portable infrared gas analyzer (LI-6400XT; LI-COR Biosciences Inc., Nebraska, USA) at an external CO₂ concentration of 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ and a photosynthetic photon flux density (PPFD) of 1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (OLIVEIRA et al., 2018). Instantaneous water-use efficiency (WUE) and carboxylation efficiency (CE) were calculated as A/E and A/C_i , respectively. Owing to technical problems with the equipment, gas exchange measurements could not be performed during the less rainy period.

Prior to analysis of variance, the data were subjected to the Shapiro–Wilk and Levene tests to assess residual normality and homogeneity of variances, respectively, at the 5% significance level. Once these assumptions were met, the data were subjected to analysis of variance (ANOVA) for a split-plot design. The effects of the whole-plot factor (cropping systems) were tested using residual error (a), corresponding to the block × cropping system interaction, whereas the effects of the subplot factor (irrigation levels) and the cropping system × irrigation level interaction were tested using residual error (b). For the cropping system factor, means were compared using the Tukey test ($p < 0.05$). The effect of irrigation levels was evaluated by polynomial regression analysis, and the best-fitting model was selected based on the significance of the regression coefficients according to the F-test ($p < 0.05$) and the highest coefficient of determination (R^2). All statistical analyses and data modeling were performed in R.

RESULTS AND DISCUSSION

Leaf Water Potential and Soil Matric Potential

Plant water status, assessed by predawn leaf water potential (Ψ_f), differed between evaluation periods. During the rainy season, no significant differences were observed among treatments ($p > 0.05$), with Ψ_f values ranging from –0.17 to –0.55 MPa. During the same period, soil matric potential ranged from –0.0022 to –0.0158 MPa, indicating high soil water availability.

Analysis of cacao leaf water potential (Ψ_f) during the less rainy period revealed distinct responses to irrigation treatments and cropping systems (Table 1). A clear improvement in plant water status was observed with increasing irrigation level, regardless of the cropping system, with Ψ_f values becoming progressively less negative from the non-irrigated treatment (0% ET_0) to the 130% ET_0 treatment (Figure 3).

Table 1. Summary of the analysis of variance for predawn leaf water potential (Ψ_f) of cacao under different cropping systems (M) and irrigation levels (L).

SV	df	Calculated F	
		Rainy season	Less rainy season
Cropping system	1	5.5172 ns	33.5984**
Block	4	0.5616 ns	1.5505 ns
Error (a)	4		
Irrigation level	3	0.4109 ns	31.3032**
Cropping system $\times$ irrigation level	3	0.4520 ns	2.473 ns
Error (b)	24		
Total	39		
C.V.1(%)		17.58%	17.34%
C.V.2(%)		28.08%	25.37%

ns: not significant; * $p < 0.05$; ** $p < 0.01$. df: degrees of freedom; CV: coefficient of variation.

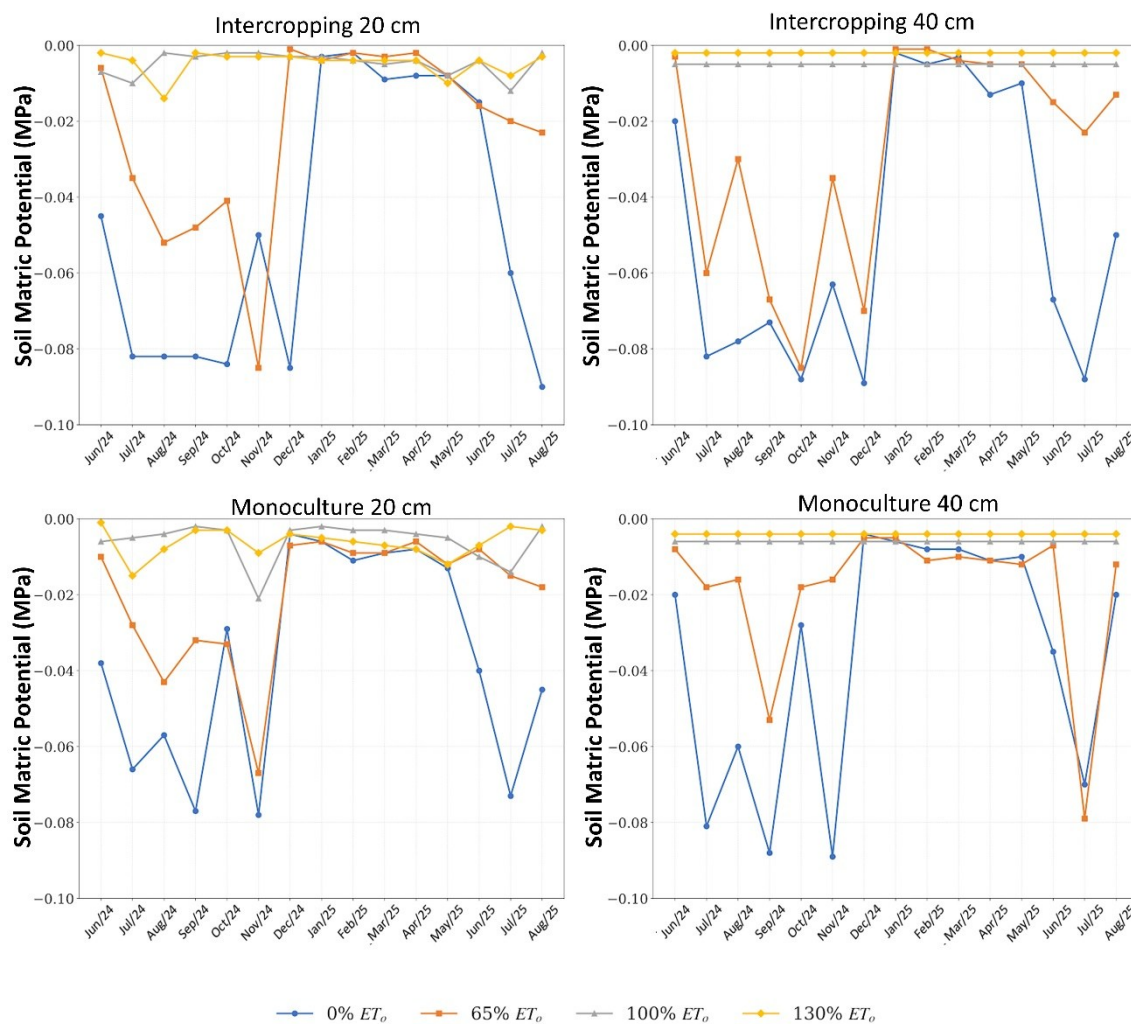


Figure 3. Soil matric potential was measured with tensiometers at depths of 20 and 40 cm.

For leaf water potential (Ψ_f) during the less rainy period, analysis of variance revealed no significant interaction between cropping system and irrigation level ($p > 0.05$), indicating that these factors affected cacao water status independently.

When the main effect of the cropping system was evaluated separately, plants grown in monoculture (M) maintained a significantly higher water status, as indicated by less negative Ψ_f values, with an overall mean of -0.75 MPa. In contrast, plants grown in the intercropping system (C) had an overall mean of -1.07 MPa. These results suggest that, under the conditions evaluated, monoculture imposed less water limitation on cacao than the intercropping system, indicating that potential competition for water within the intercrop may have outweighed the microclimatic benefits of shading during the less rainy period.

When the main effect of irrigation level was evaluated separately, Tukey's test at the 5% significance level

distinguished clear levels of plant water status recovery. The absence of irrigation ($0\% ET_0$) resulted in the most critical plant water status, with an overall mean Ψ_f of -1.48 MPa. Increasing water replacement progressively increased Ψ_f , with mean values of -1.00 MPa at $65\% ET_0$ and -0.70 MPa at $100\% ET_0$. The most favorable water status was observed at $130\% ET_0$, with a mean Ψ_f of -0.47 MPa, confirming the effectiveness of irrigation in mitigating water stress in cacao during the period of lower rainfall (Figure 4).

Although these data were not intended for statistical comparison because of limitations in instrumental replication, point-based monitoring of soil matric potential indicated a tendency toward greater soil water depletion in the intercropping system. Values of -0.077 MPa in monoculture and -0.085 MPa in the intercropping system were observed at a depth of 20 cm, whereas values of -0.088 MPa in monoculture (M) and -0.091 MPa in the intercropping system (C) were recorded at a depth of 40 cm (Figure 3).

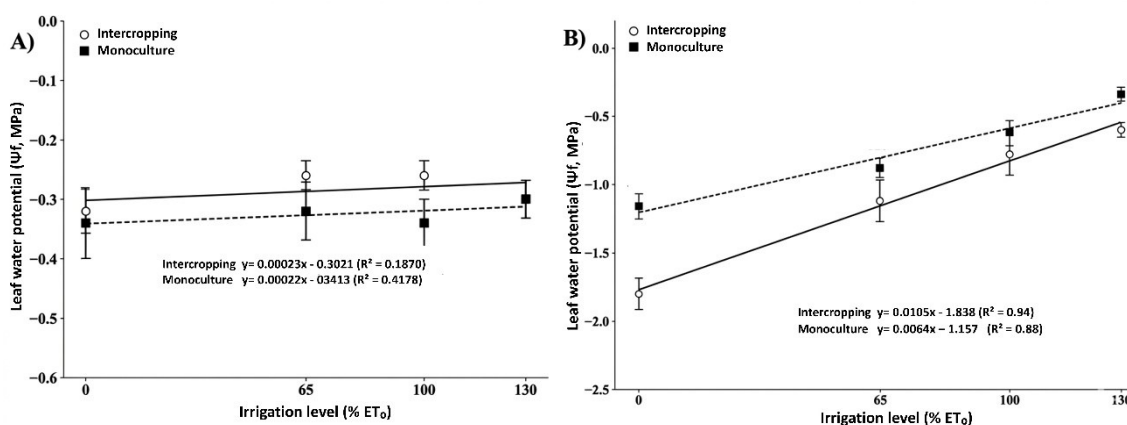


Figure 4. Predawn leaf water potential (Ψ_f) during the rainy season (A) and the less rainy period (B) under the main effects of cropping system and irrigation level.

Leaf Gas Exchange

Based on the analysis of variance, neither irrigation level (L) nor the cropping system $\times$ irrigation level interaction ($M \times L$) had a significant effect ($p > 0.05$). This result is likely related to the timing of gas exchange measurements, which

coincided with intense rainfall (Figure 2), thereby reducing the effect of irrigation level on the crop. When the main effect of the cropping system (M) was evaluated, significant differences were observed for net CO_2 assimilation rate (A), stomatal conductance (gs), transpiration rate (E), and water-use efficiency (WUE) ($p \leq 0.01$) (Table 2).

Table 2. Summary of the analysis of variance for net CO_2 assimilation rate (A), stomatal conductance (gs), transpiration rate (E), intercellular CO_2 concentration (Ci), water-use efficiency (WUE), and carboxylation efficiency (CE), considering the effects of cropping system (M), irrigation level (L), and the $M \times L$ interaction.

Parameters	F value			Coefficient of variation	
	M	L	M x L	CV 1	CV 2
A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	15.0515**	0.2899 ns	0.953 ns	20.91%	19.63%
gs ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	32.147**	0.638 ns	0.938 ns	29.75%	24.77%
E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	54.378**	0.128 ns	0.08 ns	26.64%	27.15%
Ci ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	0.3052 ns	0.2619 ns	2.2655 ns	15.35%	12.35%
WUE [$(\mu\text{mol CO}_2) (\text{mmol H}_2\text{O})^{-1}$]	53.696**	0.071 ns	0.023 ns	20.14%	29.11%
CE ($\text{mol m}^{-2} \text{ s}^{-1}$)	1.4545 ns	0.3150 ns	1.1339 ns	18.40%	25.52%

ns: not significant; * $p < 0.05$; ** $p < 0.01$.

Mean values for the gas exchange variables are presented in Table 3. Stomatal conductance (g_s), transpiration rate (E), and net CO_2 assimilation rate (A) were higher in cacao plants grown under monoculture. Net CO_2 assimilation rate (A), which reflects the net exchange of CO_2 between the

leaf and the atmosphere, was approximately 20% higher in monoculture cacao than in cacao grown in the intercropping system. Mean A was $9.65 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in monoculture cacao and $8.04 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in cacao intercropped with açai palm.

Table 3. Net CO_2 assimilation rate (A), stomatal conductance (g_s) ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration rate (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intercellular CO_2 concentration (C_i) ($\mu\text{mol CO}_2 \text{ mol}^{-1}$), instantaneous water-use efficiency (WUE) [$(\mu\text{mol CO}_2) (\text{mmol H}_2\text{O})^{-1}$], and carboxylation efficiency (CE) as affected by cropping system.

Parameters	Intercropping	Monoculture	p-value (Cropping system)
A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	8.04 b	9.65 a	< 0.01
g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.030 b	0.051 a	< 0.01
E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.566 b	1.077 a	< 0.01
C_i ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	343.10 a	334.00 a	ns
WUE [$(\mu\text{mol CO}_2) (\text{mmol H}_2\text{O})^{-1}$]	14.95 a	9.28 b	< 0.01
CE ($\text{mol m}^{-2} \text{ s}^{-1}$)	0.028 a	0.029 a	ns

Similar to the response observed for A , the cropping system significantly affected stomatal conductance and transpiration. In cacao intercropped with açai palm, mean values were $0.03 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for g_s and $0.566 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for E . In contrast, cacao grown in monoculture showed higher mean values of $0.051 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for g_s and $1.077 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for E , corresponding to increases of 70% and 90%, respectively (Table 3).

Analysis of intercellular CO_2 concentration (C_i) showed no statistically significant differences ($p > 0.05$) among cropping systems, irrigation levels, or their interaction. The contrasting responses of A , g_s , and E between cropping systems were reflected in differences in water-use efficiency (WUE), whereas carboxylation efficiency (CE) did not differ significantly among treatments. The ratio between net CO_2 assimilation rate and transpiration rate (A/E), used as an indicator of instantaneous water-use efficiency, differed significantly between cropping systems (Tables 2 and 3) and was higher in intercropped cacao. The A/E ratio was approximately 38% lower in monoculture cacao than in cacao intercropped with açai palm, indicating greater water-use efficiency in the intercropping system. No statistically significant differences were detected for CE among treatments, as indicated by the gas exchange results presented in Table 2.

Microclimatic characterization during the experimental period revealed thermal and humidity conditions (26.8°C and $87.18\% \text{ RH}$) that were highly favorable for cacao growth in the Trans-Amazonian region. The maintenance of these variables within suitable ranges suggests that the physiological responses observed were unlikely to result from thermal limitations, but rather from other management-related factors and resource availability. According to Gómez-Yarce et al. (2020), temperatures between 20 and 30°C are within a favorable range for cacao physiological activity, under which stomatal conductance (g_s) is less constrained by high vapor pressure deficit (VPD), thereby supporting the maintenance of photosynthetic processes.

Regarding water dynamics, the results demonstrate that plant water status and gas exchange in cacao are modulated by the interaction between regional seasonality and cropping system. During the rainy season, regular precipitation and the resulting high soil water availability maintained relatively

high and uniform leaf water potential (Ψ_f) across all treatments (-0.17 to -0.55 MPa). This high level of plant hydration indicates conditions of reduced water limitation, under which plants maintain less negative water potential values (ALMEIDA; TEZARA; HERRERA, 2016).

Under these conditions of abundant water supply, supplemental irrigation proved technically redundant because natural soil water availability fully met plant transpirational demand. This response is consistent with the observations of Brito et al. (2020) regarding the pronounced recharge of Amazonian soils during periods of higher rainfall. In addition, the stability of Ψ_f during this phase indicates that, when soil water is not limiting, intercropping with açai palm does not result in detrimental competition, allowing cacao plants to maintain water homeostasis regardless of the planting arrangement.

With water limitation effectively minimized, photosynthetically active radiation (PAR) emerged as the main ecophysiological constraint, as incident radiation in the intercropping system was approximately 60% lower than in monoculture. This contrast in radiation availability helps explain the higher net CO_2 assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E) observed under full-sun conditions, highlighting the physiological plasticity of cacao in responding positively to increased radiation when water supply is adequate (BALIGAR et al., 2008).

Under the higher irradiance conditions of the monoculture system, the greater rate of carbon fixation required a sustained supply of CO_2 to the mesophyll, supported by significantly greater stomatal opening. Although this coordination between gas diffusion and metabolic activity enhances photosynthetic carbon assimilation, it also results in greater water loss through transpiration. Under the high soil water availability prevailing during the rainy season, plants grown in full sun were able to sustain this physiological cost without inducing the stomatal closure that would otherwise be expected under high evaporative demand combined with limited soil water availability (SILVA et al., 2020).

In contrast, an important finding was that water-use efficiency (WUE) was approximately 38% lower in monoculture cacao than in cacao grown in the intercropping system. This response suggests that shading provided by the açai palms improved plant water balance by reducing leaf

temperature and the leaf-to-air vapor pressure deficit (VPD), allowing cacao plants to assimilate carbon with substantially lower water loss (ARAUJO et al., 2017).

The stability of intercellular CO₂ concentration (C_i) observed among treatments during the rainy season, despite pronounced differences in A and g_s, provides an important physiological indicator for understanding cacao responses under the conditions of the region. Because C_i reflects the dynamic balance between CO₂ supply through stomatal conductance and its metabolic consumption in the Calvin–Benson cycle, as reflected by A, the maintenance of similar C_i values suggests that a proportional increase in net CO₂ assimilation capacity accompanied the increase in g_s under monoculture. According to Larcher (2006), when C_i remains relatively stable despite differences in stomatal opening, variation in photosynthesis is generally governed by the availability of radiant energy to the chloroplasts.

This pattern indicates that the lower photosynthetic rate observed in intercropped plants was not primarily associated with physical limitations to gas diffusion or with impairment of the biochemical machinery, but rather with a limitation associated primarily with the lower radiation availability beneath the açaí palm canopy. This response is consistent with the findings of Baligar et al. (2008), who highlighted the sensitivity of the cacao photosynthetic apparatus to photon flux density, even though cacao is considered an understory species. The absence of significant differences in intercellular CO₂ concentration (C_i) and carboxylation efficiency (CE) between cropping systems further supports the interpretation that the lower photosynthetic rate under intercropping was primarily associated with reduced light availability rather than with restricted gas diffusion or impairment of Calvin–Benson cycle biochemistry.

This pattern changed markedly during the less rainy period, when system vulnerability became particularly evident in the non-irrigated plots (0% ET₀). Drying of the soil profile caused a pronounced decline in leaf water potential (Ψ_f), which reached –1.16 MPa in the monoculture system and a more critical value of –1.80 MPa in the intercropping system. The significantly more negative Ψ_f observed in the intercrop under severe water deficit suggests that belowground competition for water between cacao and açaí palm may outweigh the microclimatic benefits of shading during severe dry periods. This physiological response was consistent with the descriptive pattern observed in tensiometer monitoring, which indicated slightly more negative soil matric potential values in the intercropped plots. Studies such as those of Mensah et al. (2023) have demonstrated a close relationship between soil drying and declining leaf water potential in cacao, underscoring the importance of water replenishment for maintaining favorable plant water status.

Considering that the literature indicates the onset of conservative stomatal closure responses in cacao at leaf water potentials approaching –0.8 MPa (BALIGAR et al., 2008), the values recorded during the less rainy period fell below this physiological threshold in the non-irrigated plots. Although gas exchange was not measured during this period because of the operational limitations described above, the pronounced decline in Ψ_f suggests, based on physiological responses reported in the literature, a likely reduction in stomatal conductance and net CO₂ assimilation.

Similarly, the severity of water stress observed in non-

irrigated plants under the intercropping system (Ψ_f = –1.80 MPa) raises the hypothesis, based on previous studies (ARAQUE et al., 2012), that non-stomatal or biochemical limitations, such as reduced enzymatic activity, could also occur under such severe stress. However, these potential metabolic responses should be regarded strictly as literature-based inferences because the conclusions of the present study for the less rainy period are limited to the leaf water potential and soil matric potential variables that were directly measured.

CONCLUSION

During the less rainy period, irrigation was a determining factor in mitigating water stress, with increasing irrigation levels improving plant water status in both cropping systems. In contrast, during the rainy season, abundant rainfall ensured high soil water availability, making supplemental irrigation unnecessary regardless of cropping system or irrigation level. During this period, cacao grown under full-sun conditions (monoculture) exhibited higher absolute rates of net CO₂ assimilation, stomatal conductance, and transpiration in response to greater availability of photosynthetically active radiation. Conversely, the cacao–açaí intercropping system enhanced instantaneous water-use efficiency (WUE). These findings demonstrate that the two cropping systems promote distinct ecophysiological strategies, with monoculture favoring greater carbon gain and intercropping favoring water conservation through shading, highlighting the importance of considering light–water interactions in cacao management.

REFERENCES

- ALMEIDA, A.-A. F. et al. Water relations' aspects of *Theobroma cacao* L. clones. **Agrotrópica**, 14: 35-44, 2002.
- ALMEIDA, J.; TEZARA, W.; HERRERA, A. Physiological responses to drought and experimental waterlogging of four cacao clones (*Theobroma cacao* L.) selected for cultivation in Venezuela. **Agricultural Water Management**, 171: 80-88, 2016.
- ALMEIDA, A.-A. F.; VALLE, R. R. Ecophysiology of the cacao tree. **Brazilian Journal of Plant Physiology**, 19: 425-448, 2007.
- ARAQUE, O. et al. Fotossíntese comparativa, relações hídricas, crescimento e taxas de sobrevivência em cultivares jovens de cacau Criollo (*Theobroma cacao*) durante as estações seca e chuvosa. **Agricultura Experimental**, 48: 513-522, 2012.
- ARAUJO, A. V. et al. Trocas gasosas e eficiência no uso da água em variedades de cacau cultivadas em sistema de pleno sol. **Revista Brasileira de Ciências Agrárias**, 12: 488-494, 2017.
- BALIGAR, V. C. et al. Efeitos da densidade de fluxo de fótons fotossintéticos, da concentração de dióxido de carbono e do déficit de pressão de vapor na fotossíntese em mudas de

cacau. **Photosynthetica**, 46: 216-221, 2008.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Manual de boas práticas agrícolas para a cultura do cacau**. Brasília, DF: MAPA/CEPLAC, 2021. 64 p.

BRITO, A. P. et al. Relação entre precipitação e recarga de águas subterrâneas na Amazônia Central. **Águas Subterrâneas**, 34: 39-49, 2020.

GÓMEZ-YARCE, J. P. et al. Eficiência das trocas gasosas no sistema agroflorestal cacau-olmo espanhol no noroeste de Antioquia, Colômbia. **Revista Facultad Nacional de Agronomía Medellín**, 3: 9283-9291, 2020.

INMET - Instituto Nacional de Meteorologia. **Banco de Dados Meteorológicos para Ensino e Pesquisa (BDMEP)**. Brasília, DF: INMET, 2026. Available at: <https://bdmep.inmet.gov.br/>. Access on: May 1, 2026.

LAHIVE, F. et al. Climate change impacts on cacao: genotypic variation in responses of mature cacao to elevated CO₂ and water deficit. **Agronomy**, 11: 818, 2021.

LAHIVE, F.; HADLEY, P.; DAYMOND, A. J. The physiological responses of cacao to the environment and the implications for climate change resilience. A review. **Agronomy for Sustainable Development**, 39: 5, 2019.

LARCHER, W. **Ecofisiologia vegetal**. 3. ed. São Carlos, SP: Rima, 2006. 550 p.

MENSAH, E. O. et al. Efeitos combinados da sombra e da seca na fisiologia, crescimento e produção de cacaueiros adultos. **Science of the Total Environment**, 899: 165657, 2023.

NIETHER, W. et al. Shade trees and tree pruning alter throughfall and microclimate in cocoa (*Theobroma cacao* L.) production systems. **Annals of forest science**, 75: 38, 2018.

OLIVEIRA, M. G. et al. Physiological responses of photosynthesis in black pepper plants under different shade levels promoted by intercropping with rubber trees. **Ciência e Agrotecnologia**, 42: 513-526, 2018.

RUA, M. L. et al. Evapotranspiration and Crop Coefficient of Economically Important Fruit Trees in the Eastern Amazon. **Hydrology**, 13: 108, 2026.

SANTOS, H. G. et al. **Sistema brasileiro de classificação de solos**. 5. ed. rev. ampl. Brasília, DF: Embrapa; 2018. 356 p.

SAXTON, K. E.; RAWLS, W. J. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. **Soil Science Society of America Journal**, 70: 1569-1578, 2006.

SILVA, G. S. et al. Calibration of a weighing lysimeter for measuring cocoa evapotranspiration. **Revista Caatinga**, 33: 803-814, 2020.

SOMARRIBA, E. et al. Carbon stocks and cocoa yields in

agroforestry systems of Central America. **Agriculture, Ecosystems & Environment**, 253: 110-119, 2018.

SZILAGYI, J.; HOGAN, J. P. Computation of daily Penman–Monteith reference evapotranspiration from temperature and incoming solar radiation. **Atmospheric Measurement Techniques**, 13: 251-260, 2020.