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Partial Root-Zone Drying Associated with Kaolin Foliar Application in *Coffea canephora*. I—Leaf Gas Exchange, Photosynthetic Performance Index and Leaf Temperature Responses

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ABSTRACT: Two sustainable strategies were applied simultaneously to *Coffea canephora* grown under tropical conditions: (1) alternate partial root-zone drying (PRD), where half of the root zone is alternatively wetting and drying, and (2) spraying with a calcined kaolin particle film (KF). The aim was to evaluate effects of irrigation [Conv100 replaced 100% reference evapotranspiration (ET_0) at one plant side; PRD100, PRD70 and PRD30 irrigated at 100%, 70% or 30% ET_0 , respectively, alternating the plant sides every week] and kaolin (with and without) on leaf ecophysiological parameters of adult *C. canephora*. Measurements were performed over two diurnal periods (morning and midday), in two seasons (dry winter and hot/wet summer) for two years. Leaf assimilation rate (A) and stomatal conductance (g_s) did not decline, while intrinsic water use efficiency increased under PRD70 and PRD30. Under PRD30, carboxylation efficiency (CE) was similar to other irrigation levels, or even superior (dry winter of second year). Thermal-imaging parameters [leaf temperature (T_{leaf}), relative stomatal conductance index (I_g), and crop water stress index (CWSI)] consistently responded to KF, with limited irrigation effects. KF reduced midday T_{leaf} and CWSI and increased I_g across seasons. The additive and interaction effects in photosynthetic performance index (PI_{ABS}) were expressed during both diurnal periods in both winter seasons, and at midday of the second-year summer. The morning and midday responses had a similar frequency of significance for leaf gas exchange parameters, while the additive and/or interaction effects of PRD and KF were the most frequently observed during the dry and harsh first-year winter. No differences in A , g_s , and CE were registered among irrigations during morning of the second winter, with the highest values shown under PRD30 at midday. Such dynamics suggested a stress-memory-like acclimatization effect on leaf gas exchange responses under PRD30. PI_{ABS} in the second year presented higher values compared to the first year, especially at midday; values of CWSI were lower and of I_g were higher in the second than in the first year, supporting stress-memory-like acclimatization. PI_{ABS} could be interpreted as the most generalist indicator of combined effects of two applied technologies on *C. canephora* in all analyzed diurnal periods and seasons. From a leaf-physiological perspective, PRD70 and PRD30 appear to be promising water-saving irrigation strategies for adult *C. canephora*, with KF providing additional protection at high-temperature. Agronomic validation based on yield, bean quality, and long-term soil-water dynamics is still required before broad field recommendations can be made.

KEYWORDS: Carboxylation efficiency; crop water stress index; deficit irrigation; infrared thermography; iWUE; PI_{ABS} ; PRD; Robusta coffee; stress-memory-like acclimatization; water balance

1 Introduction

Global coffee consumption has increased steadily, leading to growing demands for beans from the two main cultivated coffee species, namely *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* Pierre ex A. Froehner (Robusta coffee), both native to tropical Africa [1]. The increasing demand for coffee has led to the expansion of cultivation into areas with suboptimal climatic conditions [2,3]. Additionally, fluctuations in rainfall patterns, rising temperatures and frost events [4], increasing pest pressure and disease severity [5] constrain coffee growth and production under ongoing climate change.

Although coffee plants can tolerate temperatures ranging from 8 to 37°C, their growth is markedly reduced when average field temperatures fall below 17°C or exceed 31°C [6,7]. At the leaf scale, *C. canephora* begins to exhibit changes in chlorophyll *a* fluorescence at temperatures around 45°C, with water deficit increasing heat tolerance in a genotype-dependent manner [8]. Different coffee genotypes exhibit distinct acclimation responses to environmental stress, depending on the prevailing environmental and microclimatic conditions [9]. Indeed, coffee plants can develop “stress memory”, which is manifested as acclimation to recurrent stress events induced by successive drought cycles [10]. Two genotypes of *C. canephora* showed distinct recovery patterns after the first drought cycle but similar ecophysiological dynamics during the second cycle, in which the coffee plants exhibited evidence of “stress memory”, particularly through changes in the maximum quantum yield of primary photochemical reactions and the photosynthetic performance index (PI_{ABS}) [11]. On the other hand, *C. arabica* grown under rainfed conditions required four drought cycles to exhibit acclimation to soil water stress, as reflected by changes in net assimilation rate (*A*), stomatal conductance (g_s), leaf transpiration (*E*), intrinsic water use efficiency (iWUE), and carboxylation efficiency (*CE*) [12]. Because leaf temperature may exceed air temperature under high radiation and low atmospheric humidity, strategies that reduce canopy heat may be particularly relevant for maintaining photosynthetic performance in coffee plants under field conditions [13].

Irrigation is widely used to mitigate the harmful effects of prolonged drought periods in coffee-growing regions, which may also help reduce biennial bearing [14]. Full irrigation is widely used in crop production worldwide, in which crop water requirements are fully replenished [15,16]. Given the importance of water for agriculture and the increasing competition between agricultural and human water demand, research efforts have focused on developing new irrigation strategies, optimizing the efficiency of existing systems, and improving crop profitability [16,17].

Partial root-zone drying (PRD) is an irrigation strategy that may affect yield but generally increases water use efficiency [18]. This method consists of irrigating only a portion of the root zone during each irrigation event, while the remaining portion is allowed to dry until a crop-specific soil moisture threshold is reached, depending on the crop and its water requirements [19]. When water is consistently applied to the same side of the root system, the method is referred to as fixed PRD. In alternate PRD, the wet and dry sides of the root system are switched between irrigation events, allowing the previously irrigated side to dry while the previously dry side receives irrigation [20]. Fixed PRD may affect root activity due to the heterogeneous distribution of soil moisture [19].

In crops such as pepper [21], apple [22], potato [23], tomato [24,25], cotton [26], grape [27,28], and papaya [29], PRD has proven to be an effective strategy, for saving water, improving fruit quality, increasing

the concentration of total soluble solids, total sugars, and total acids, and enhancing fruit firmness compared with conventional irrigation [30–34]. In Ethiopian Arabica coffee orchards, PRD reduced irrigation water requirements by 50% compared with full irrigation [35]. In China, PRD saved 32.1% of irrigation water and increased water use efficiency by 29.9% and was therefore recommended for the management of young Arabica coffee plants [36]. However, most studies on PRD in coffee have been conducted under pot conditions, whereas field studies evaluating PRD in mature coffee plants remain scarce, particularly regarding the physiological and ecological mechanisms underlying plant responses to different irrigation systems [37]. Therefore, it remains unclear whether severe reductions in irrigation levels under alternate PRD can maintain leaf-level physiological performance in adult field-grown *C. canephora*, especially under contrasting seasonal conditions.

In addition to deficit irrigation techniques, another strategy that may protect plants from excessive solar radiation and high temperatures is the application of processed, calcined, and purified kaolin particle film (KF), which may also improve water use efficiency [38]. Kaolin application reduces leaf temperature (T_{leaf}) [39], water stress [40,41], water consumption [42], and sunscald damage to leaves and fruits [43–45]. Kaolin spraying can reduce heat load by increasing leaf reflectance and dissipating excess radiation, thereby potentially reducing oxidative pressure and allowing a greater proportion of photoassimilates to be allocated to growth and production [46,47]. In warm climates, reduction in leaf temperature may improve ecophysiological traits including A , g_s and water use efficiency [39,48–50]. These responses are strongly influenced by plant species and environmental and biological factors [51,52].

PRD [35] and KF [39,41] have previously been studied separately in *C. canephora* cultivation, with both management showing beneficial effects, but their simultaneous application has not yet been tested. We therefore hypothesized that the additive effects of PRD and KF technologies would: (I) increase leaf gas exchange in *C. canephora*, including A , g_s , the intrinsic water use efficiency (iWUE), as well as the relative stomatal conductance index (I_g), and the PI_{ABS} , while reducing T_{leaf} and the crop water stress index (CWSI); (II) be more pronounced at midday (when diurnal temperatures are the highest) than in the morning, and during winter (dry season) than during summer (wet season); and (III) increase ecophysiological performance in the second year, potentially reflecting a stress-memory-like acclimatization response to repeated exposure to PRD and kaolin treatments. Therefore, this study aimed to evaluate the independent, additive, and interactive effects of PRD and KF technologies on leaf ecophysiological parameters of adult *C. canephora* plants during two diurnal periods (morning and midday), across two contrasting seasons (winter and summer) over two consecutive years.

2 Methods

2.1 Experimental Site and Plant Description

The experiment was conducted in Atilio Vivacqua (20°58'53" S, 41°12'55" W and 650 m a.s.l.), Espírito Santo State, Brazil. The climate is tropical rainy (Cwa) with a dry season in winter, according to Köppen's classification [53].

Four-month-old *Coffea canephora* seedlings (originated from 'Instituto Capixaba de Pesquisa', Vitória ES, Brazil) were transplanted from the nursery to the experimental field in October 2020. Planting arrangement was 2.50 m × 0.60 m, totaling a population of 6667 plants ha⁻¹. Plants were trained with a single orthotropic branch. *C. canephora* is predominantly self-incompatible and therefore requires cross-pollination among genetically distinct individuals for successful reproduction and fruit production. For that reason, five clones were planted in the experimental area, but observations here were focused only on 'A1' clone, which has

shown high productivity when cultivated at 150–850 m altitudes. The ‘A1’ clone is included in both the ‘Andina’ [54] and ‘Tributun’ [55] clonal cultivars.

2.2 Chemical and Physical Soil Conditions

The soil for chemical analyses was collected at a depth of 0 to 20 cm, every year of the experiment, from 2020 to 2024. Such analyses helped to determine nutritional management for the next period. Prior to anthesis, 2000 kg of $\text{CaMg}(\text{CO}_3)_2$ and 1200 kg of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ fertilization were applied. NPK fertilization was carried out at rates of 1800 kg ha^{-1} in 2022 and 2200 kg ha^{-1} in 2023, using a 19:4:19 formulation, performed in split doses during February, September, November, and December. During anthesis, B, Zn, Cu, Fe, Mn, Mo, Co, and Ni micronutrients were applied. Annual changes in soil chemical parameters, including pH, base saturation, Ca, Mg, Al, H, P, K, S, B, Fe, Cu, Mn, and Zn, are shown in Table S1. Soil chemical parameters varied substantially between 2020 and 2024, showing an improvement in fertility and base saturation due to coffee cultivation and mineral supply. The pH (in H_2O) increased from 4.9 in 2020 to 5.5 in 2024, resulting from the application of $\text{CaMg}(\text{CO}_3)_2$, additionally improving base saturation. Base saturation from 10.6% (2020) to 51.7% (2024), indicating a rebalancing of the absorption complex after the coffee planting.

Soil samples for physical property determination were collected in May 2022, 50 cm from the planting lines, at five 20-cm soil layers (0 to 1 m). Soil density (S_d , Mg m^{-3}) was obtained by the volumetric ring method, in 8.0 cm $\times$ 5.0 cm volumetric cylinders, collecting undisturbed soil samples. Soil particle density (S_{pd} , Mg m^{-3}) was determined by the ratio between the soil mass and the soil particle volume. Total soil porosity (T_{sp} , $\text{m}^3 \text{m}^{-3}$) was determined as $1 - S_d/S_{pd}$ [56]. Soil moisture at field capacity (θ_{FC} , $\text{m}^3 \text{m}^{-3}$ estimated at 10 kPa of water tension), soil moisture at permanent wilting point θ_{PWP} , $\text{m}^3 \text{m}^{-3}$, estimated at 1500 kPa of water tension and available water (A_W , $\text{m}^3 \text{m}^{-3} = \theta_{FC} - \theta_{PWP}$) were also evaluated. Soil was classified as Red-Yellow Latosol [57].

S_{pd} showed little variation over the soil profile, evidencing relatively uniform mineralogy along the profile (Table S2). S_d varied across different layers, with the highest values in the layers closest to the surface, and tended to decrease with depth, consistent with a greater porosity in the subsurface. T_{sp} progressively increased from 0–20 to 80–100 cm layers, resulting in a difference of $0.047 \text{ m}^3 \text{m}^{-3}$ between the densest and most porous layers. θ_{FC} generally increased with depth, with a maximum at 40–60 cm ($0.402 \text{ m}^3 \text{m}^{-3}$). θ_{PWP} was also the highest at 40–60 cm ($0.334 \text{ m}^3 \text{m}^{-3}$), indicating an increase in water content strongly retained in the intermediate layers. As a consequence, A_W remained relatively stable along the profile, with available water from 0.076 (0–20 cm) to $0.072 \text{ m}^3 \text{m}^{-3}$ (80–100 cm). Converting this availability to the scale of each 20 cm layer, the available water capacity (AWC, mm) was 15.13 mm in the 0–20 cm layer, 14.20 mm in 20–40 cm, 13.56 mm in 40–60 cm, 14.01 mm in 60–80 cm, and 14.37 mm in 80–100 cm, so that the accumulated AWC in the profile (0–100 cm) totaled 71.32 mm, characterizing a soil with moderate water storage potential and little variation in water availability between the evaluated layers.

2.3 Microclimate Measurements and Water Balance Estimations

Micrometeorological variables were obtained during the years 2022 and 2023 using the WRF-3S agrometeorological station (Ciclus Meteorological Stations, Colombo PR, Brazil). Air minimum, maximum, and mean temperature ($T_{\min}$, $T_{\max}$, T_{mean} , respectively, $^{\circ}\text{C}$), relative air humidity (RH_{air} , %), and photosynthetically photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) during the diurnal period from 7:00 to 17:00 h were recorded every 30 min. Rainfall was measured using a rain gauge made of colorless crystal plastic (thermoplastic), manufactured from polyethylene, with a graduation scale from 0 to 150 mm.

The water balance (sum of the amounts of water that enter and leave a certain portion of the soil in a given time interval) was calculated during the years 2022 and 2023, using the model proposed by Thornthwaite [58] and modified by Mather [59]. This method considered estimates of actual evapotranspiration, water deficit, and water surplus. The estimated rate of water loss through evapotranspiration varies linearly with soil water storage. The water balance can be expressed by Eq. (1):

$$P + I - ET_0 - S_r \pm D_d \pm \Delta_{vwc} = 0 \quad (1)$$

where P is precipitation, I is irrigation, ET_0 is reference evapotranspiration, S_r is surface runoff, D_d is deep drainage, and Δ_{vwc} is the variation in soil water content [60]. The water balance was calculated based on the sum of AWC over the soil profile of 1 m (Table S2).

2.4 Irrigation and Kaolin Applications

2.4.1 Irrigation Installations

From planting (October 2020) until the installation of the PRD (January 2022), the plants were irrigated by micro-sprinkler. Irrigation scheduling was based on ET_0 [61] depending on information about daily T_{min} , T_{max} , T_{mean} and PPFD (Fig. 1), provided from micrometeorological measurements, in order to maintain the soil at field capacity. Throughout the initial 15-month period, there was a rainfall of 2247 mm, and an irrigation depth of 684 mm, the same for all treatments, totaling 2931 mm. In January 2022, conventional irrigation was replaced by a system with self-compensating drippers with a flow rate of 4.0 L h^{-1} , spaced 30 cm from the coffee plant stems, and 60 cm apart. For each row, two 12 mm polyethylene pipes were used, one on each side of the rows. Based on daily calculations of ET_0 , four irrigation treatments were applied: (1) supplying 100% ET_0 on a single fixed side of the plant (Conv100), which operationally corresponds to a fixed PRD100 treatment. It was considered here as the conventional reference treatment, because it represents the irrigation practice most commonly adopted by local coffee growers; (2) alternately supplying 100% ET_0 on two plant sides (PRD100); (3) alternately supplying 70% ET_0 on two plant sides (PRD70); (4) alternately supplying 30% ET_0 on two plant sides (PRD30). Two irrigations were performed per week, while the irrigated side was alternated each week in PRD treatments.

Precipitation volume totaled 1104 mm from autumn 2022 to summer 2023, when 1438 mm of irrigation was applied in Conv100 and PRD100, 1007 mm in PRD70, and 437 mm in PRD30; precipitation volume was 1477 mm in a period from autumn 2023 to summer 2024, when 1476 mm of irrigation was applied in Conv100 and PRD100, 1033 mm in PRD70, and 443 mm in PRD30 (Table 1).

Higher temperatures throughout 2023/24 were associated with greater irrigation requirements, even though it was a year with higher precipitation compared with 2022/23 (Table 1). Throughout the experiment, irrigation was resumed seven days after a rainfall event. In 2023/24, some irrigation events were followed by rainfall the following day. A limitation of this study was that soil water content was not monitored separately on the wet and dry sides of the root zone. Despite the fact that physiological responses were measured on the side being irrigated at the time of measurements, they were interpreted based on the imposed irrigation regime rather than direct measurements of root-zone water heterogeneity.

Table 1: Precipitations (P , mm) and water supply (I , mm) for each irrigation treatment (Conv 100, PRD100, PRD70, PRD30), calculated based on reference evapotranspiration (ET_0), for each observed season and summed for annual periods from autumn 2022 to summer 2023 and from autumn 2023 to summer 2024.

Seasons	ET_0	P	Conv100		PRD100		PRD70		PRD30	
			I	$P + I$	I	$P + I$	I	$P + I$	I	$P + I$
Autumn 2022	302	116	302	418	302	418	211	328	90	207
Winter 2022	297	64	297	361	297	361	208	272	89	153
Spring 2022	397	387	397	784	397	784	278	665	119	506
Summer 2022/23	443	537	443	979	443	979	310	846	133	669
Total 2022/23	1438	1104	1438	2542	1438	2542	1007	2111	431	1535
Autumn 2023	296	418	296	714	296	714	207	626	89	507
Winter 2023	294	129	294	424	294	424	206	335	88	218
Spring 2023	468	291	468	759	468	759	328	619	140	431
Summer 2023/24	417	639	417	1056	417	1056	292	931	125	764
Total 2023/24	1476	1477	1476	2953	1476	2953	1033	2510	443	1920

2.4.2 Fine Calcined Kaolin-Based Particle Film Application

KF was always applied as a 5% solution concentration, where 1.0 kg of KF was dissolved in 20 L of water [39]. Plants not treated with KF were called ‘green leaves’, or GL. KF applications began when seedlings were transplanted from the nursery to the field. Since planting, KF was applied in October, December, February, April, and July. In the first year of orchard development (2020), the plants still had a low leaf area, and 15 kg KF ha⁻¹ year⁻¹ was applied at a spray volume of 60 L ha⁻¹ of solution. In the second year (2021), 30 kg KF ha⁻¹ year⁻¹ was applied at a spray volume of 120 L ha⁻¹. From the third year of orchard development (2022) onward, as plant size increased, 50 kg KF ha⁻¹ year⁻¹ was applied at a spray volume of 200 L ha⁻¹.

2.5 Ecophysiological Measurements

Ecophysiological analyses (leaf gas exchange, chlorophyll a fluorescence, and thermography) were performed in four seasons: August 2022 (winter 2022), March 2023 (summer 2022/23), August 2023 (winter 2023), and December 2023 (summer 2023/24). A total of 32 tagged plants were evaluated, with four biological replicates (plants) per treatment. The same plants were followed across all four seasonal evaluations. Measurements were performed on a single day in each season, when the north side of the plants was being irrigated, the same side on which the measured leaves were located. Readings were taken on mature leaves, on the third pair of fully expanded leaves, counting from the axis apex, on a 2nd order plagiotropic axis from the middle third of the canopy, facing north, exposed to sun. The same leaf from each plant was used for leaf gas exchange and PI_{ABS} measurements during the same season. Three additional leaves were selected for infrared thermography measurements, which were always chosen from a neighboring 2nd order plagiotropic branch in relation to other ecophysiological measurements, always on north side. In order to prevent kaolin contamination of the equipment, the KF was always carefully removed from leaves before measurements, using cotton puffs. The winter 2022 and winter 2023 evaluations were performed after fruit harvest and before flowering.

2.5.1 Leaf Gas Exchange Measurements

Leaf gas exchange was measured using a portable open-system infrared gas analyzer, LI6400 (Li-COR, Lincoln NE, USA). During gas-exchange measurements, the reference CO₂ concentration was set to 415 $\mu\text{mol mol}^{-1}$ and PPFD to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Irradiance was provided by a Li-COR 6400-02B LED light source

consisting of 80% red and 20% blue light. Chamber temperature and relative humidity were maintained at 25°C and 60%, respectively. Leaf net CO₂ assimilation (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), leaf stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular CO₂ concentration (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1}$) were measured, while intrinsic water use efficiency (iWUE, $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) and instantaneous carboxylation efficiency (CE , $\mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$) were additionally calculated as A/g_s and A/C_i , respectively. Leaf gas exchange was measured in the morning (8:00–9:00 h) and at midday (12:00–13:00 h).

2.5.2 Photosynthetic Performance Index Measurements

Photosynthetic performance index (PI_{ABS}) was estimated based on fluorescence emitted by chlorophyll *a* [62]. Measurements were performed using an unmodulated light fluorimeter, model Pocket PEA (Plant Efficiency Analyser, Hansatech Ltd., Norfolk, UK). The coffee leaves were dark-adapted for 30 min, using leaf clips, to turn the reaction centers into an “open” (oxidized Q_A) state. After dark adaptation, fluorescence was induced with red light at 650 nm and an intensity of up to 3500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. In each season, the evaluations were carried out in the morning and midday.

2.5.3 Infrared Thermography Measurements

The thermal images were obtained with a Flir i50 mid-wave infrared camera (Flir Systems, Wilsonville OR, USA), with a camera emissivity set to 0.96. To acquire images, the camera was positioned approximately 0.50 m from the plant. Images were acquired using a focal-plane array detector at a resolution of 140 × 140 pixels. Images were acquired from three leaves located on the 2nd order plagiotropic axis of each plant. Petroleum jelly was applied to the abaxial surface of the first leaf 30 min before image acquisition (T_{dry}), in order to achieve maximum leaf temperature due to transpiration blockage [63]. A second leaf was moistened on the adaxial surface (T_{wet}) one minute before the thermographic recording. No intervention was made on the third leaf, which represented the leaf temperature (T_{leaf}). The crop water stress index (CWSI) was calculated as: $\text{CWSI} = (T_{\text{leaf}} - T_{\text{wet}})/(T_{\text{dry}} - T_{\text{wet}})$ [64]. The relative stomatal conductance thermal index (I_g) was calculated as $I_g = (T_{\text{dry}} - T_{\text{leaf}})/(T_{\text{leaf}} - T_{\text{wet}})$ [65]. Thermographic measurements were performed only at midday.

2.6 Statistical Analysis

All analyses were performed in the R programming language [66], with assistance of RStudio software (Posit Software, PBC, Boston MA, USA) as an interface. Our experiment was conducted in a completely randomized factorial design, with two to four factors, depending on the variable. The factors evaluated in *C. canephora* plants were: (i) presence and absence of kaolin in the leaves (KF, with kaolin; GL, without kaolin); (ii) four different irrigation treatments (Conv100, PRD100, PRD70 and PRD30), (iii) four seasonal periods (winter 2022, summer 2022/23, winter 2023, summer 2023/24), and (iv) two diurnal periods (morning and midday). To analyze A , g_s , iWUE, CE and PI_{ABS} responses, two-way ANOVAs were performed comparing effects of kaolin, irrigation and their interaction. For those variables, the additional two-way ANOVAs were performed to analyze effects of seasons, diurnal periods, and their interaction. For T_{leaf} , I_g , and CWSI, two-way ANOVAs were performed to analyze effects of kaolin, irrigation and their interaction at midday, with additional one-way ANOVAs analyzing effects of seasons. Linear mixed-effects models were fitted using the ‘nlme’ package, with irrigation and kaolin (or season and diurnal period, depending on the analysis) as fixed effects and plant identity as a random effect. The Bartlett homogeneity and the Shapiro normality tests were performed for each variable. Statistical significance was assessed at $\alpha = 0.05$ for all analyses. p -values between 0.05 and 0.10 were reported only as marginal trends. When the interaction

effect was not significant in the two-way ANOVA, a reduced additive model was fitted to interpret the main effects. *Post-hoc* pairwise comparisons of the estimated marginal means were performed using Tukey's honestly significant difference test via the 'emmeans' and 'multcomp' packages. The results are presented graphically as estimated means $\pm$ standard errors, including *p*-values. Differences among levels in each treatment are only shown when significance is expressed. Individual effect *p*-values were assigned, but when interaction was significant, only interaction *p*-value was shown.

3 Results

3.1 Microclimate Conditions and Water Balance during the Experiment Period

The variation in minimum, mean, and maximum air temperatures ($T_{\min}$, T_{mean} , and $T_{\max}$) throughout 2022/23 and 2023/24 revealed contrasting seasonal patterns, with a colder winter in 2022 and the occurrence of extreme heat in spring and summer 2023/24 (Fig. 1a,b). The lowest $T_{\min}$, 3.9°C, was recorded in May 2022 (Fig. 1a). Although the winter of 2022 was characterized by low temperatures, $T_{\max}$ during summer 2022/23 remained below 35°C (Fig. 1a). In 2023/24, $T_{\min}$ ranged from 6.3°C in July 2023 to 16.2°C in January 2024 (Fig. 1b). $T_{\max}$ reached 37.0°C in November 2023, exceeding the values recorded in other months and in the previous year (2022), characterizing an extreme heat event at the end of spring and the beginning of summer. In addition, September 2023 already showed a high $T_{\max}$ of 35.1°C, indicating an intensification of warming.

Minimum relative humidity ($RH_{\min}$) showed a wide range in 2022/23, reaching its lowest value, 17%, in May, which indicated strong atmospheric evaporative demand during part of the day and likely intensified midday water stress (Fig. 1c). $RH_{\min}$ ranged from 21% in November 2023 to 41–44% in February–March 2024 (Fig. 1d). Maximum relative humidity ($RH_{\max}$) remained at 100% in all months of both years, except in November 2022 and February–March 2024, when it reached only 93% and 91%, respectively (Fig. 1c).

Maximum photosynthetic photon flux density ($PPFD_{\max}$) measured at midday, reached peaks close to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during most of the year (Fig. 1e,f). In general, PPFD showed a slight increase during autumn, particularly in March and April, followed by a reduction at the end of winter and beginning of spring, especially in September, for both the broader diurnal period and the midday interval.

Air temperature during the five days preceding each ecophysiological evaluation was higher in summer 2023/24 than in summer 2022/23 throughout the day (Fig. S1a). Daytime temperatures in both summer periods were approximately 5–10°C higher than those recorded in winter, while winter 2022 was approximately 5°C cooler than winter 2023 during daylight hours. Relative humidity was lowest during daylight hours in winter 2022 (Fig. S1b). In summer 2023/24, despite high air temperature and PPFD, relative humidity increased around midday but remained comparatively low during the morning. Midday PPFD was lowest in winter 2022 and highest in summer 2023/24 (Fig. S1c). Across all evaluation periods, midday PPFD approached or exceeded the 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD used for gas exchange measurements.

The water balance showed a typical seasonal pattern for the Cwa climate, with water surplus concentrated in summer and water deficits extending from autumn to spring (Fig. 2). While February 2023 recorded the highest water surplus, a water deficit was observed in February 2024, followed by another peak surplus in March 2024. In 2022/23, water surplus occurred over four months, totaling 460 mm (Fig. 2a), whereas in 2023/24, it occurred over six months—particularly in December, January, March, April, May, and June (Fig. 2b)—totaling 612 mm, which is 25% more than recorded in the previous year. During the 2022/23 period, a water deficit persisted for eight months, driven by a continuous dry spell that began in March and extended through autumn and winter. This drought intensified during the transition to spring, culminating in its most severe level in October at –59 mm (Fig. 2a). During the 2023/24 season, the water deficit was

primarily confined to a five-month dry period spanning from July to November, adding an anomalously dry February 2024 (−46 mm) (Fig. 2b).

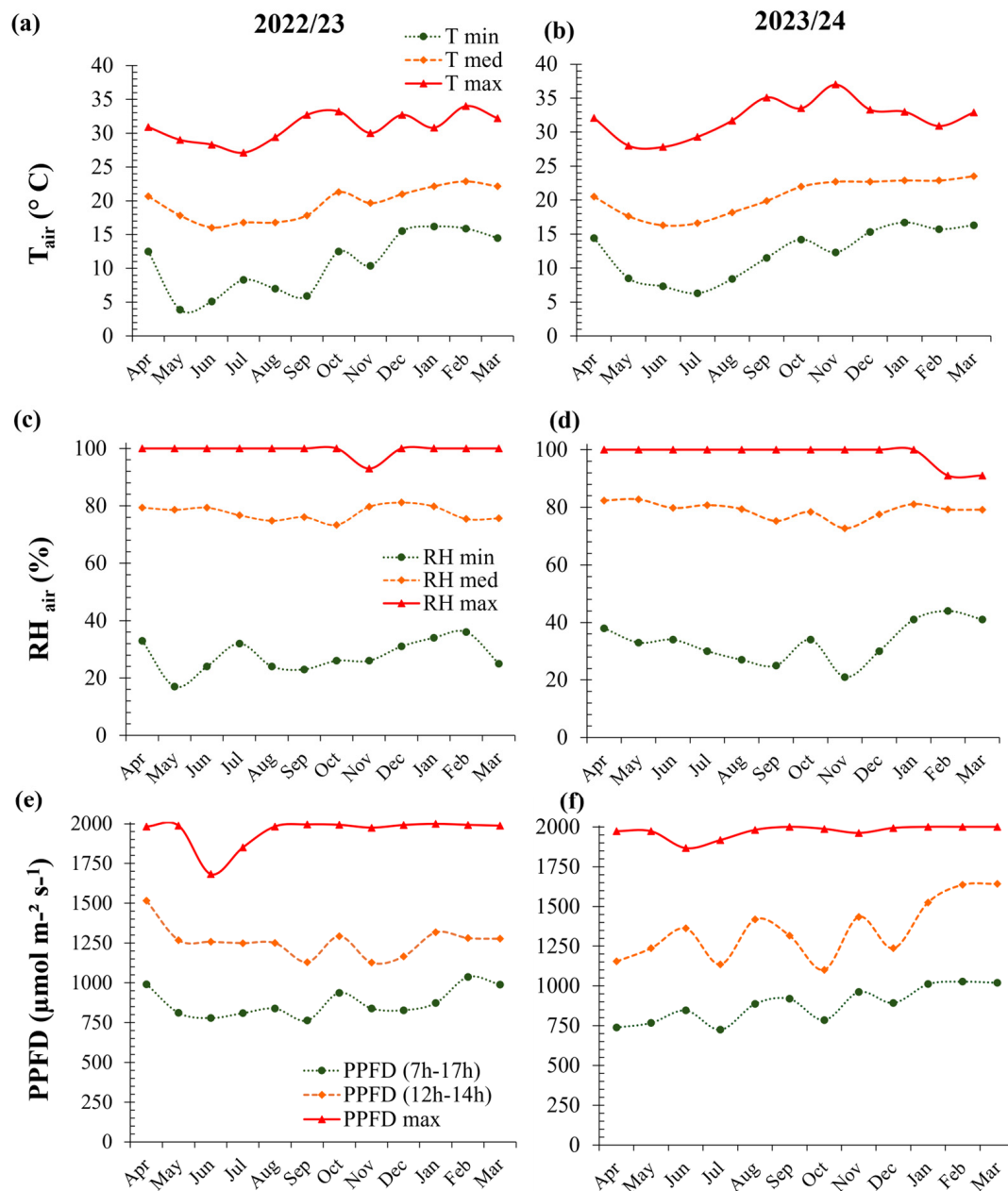


Figure 1: Microclimate: (a,b) monthly absolute minimum air temperature ($T_{\min}$); monthly average air temperature (T_{med}); monthly absolute maximum air temperature ($T_{\max}$); (c,d) monthly absolute minimum relative humidity ($RH_{\min}$); monthly average relative humidity (RH_{med}); monthly absolute maximum relative humidity ($RH_{\max}$); (e,f) monthly average photosynthetically photon flux density (PPFD) over the broadest diurnal period (7:00–17:00 h), or during the interval of the greatest intensity (12:00–14:00 h), and monthly absolute maximum ($PPFD_{\max}$), during 2022/23 and 2023/24, respectively.

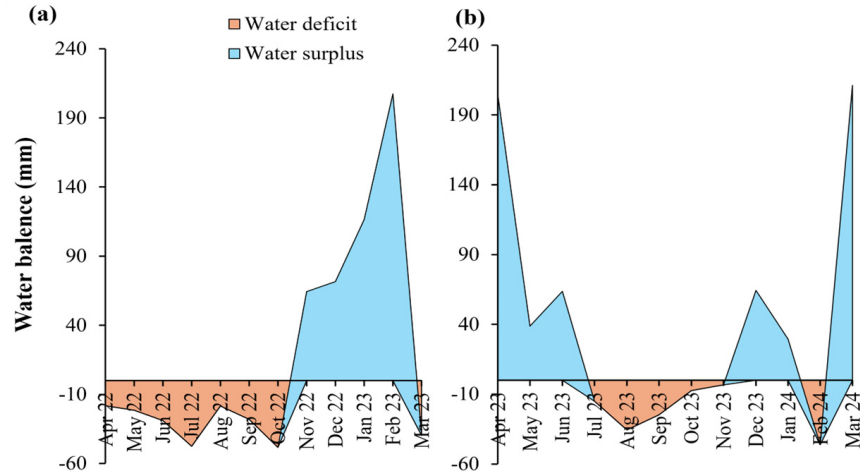


Figure 2: Graphical presentation of water balance, showing the water deficit and surplus (mm) in a soil with a water storage capacity of 71.32 mm (0–100 cm) throughout the months of the experimental period: (a) 2022/23 and (b) 2023/24.

3.2 Leaf Gas Exchange Responses to PRD and Kaolin Treatments

Net photosynthetic rate (A) varied over the observed seasons, indicating general climate effects (Fig. 3). The lowest A values ($2.17\text{--}5.40\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$) were recorded in winter 2022 (in both diurnal periods), whereas the highest morning A values were observed in winter 2023 and summer 2023/24 ($7.17\text{--}11.80\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$, respectively). The low A values observed in the winter 2022 may be associated with the low temperatures prevailing during the measurement period (Fig. S1a), although additional uncontrolled environmental or phenological factors cannot be excluded. The morning evaluations consistently showed A values higher than those observed at midday (Fig. 3).

Irrigation had a significant effect on A during the morning period in winter and summer 2022/23, but this effect was no longer detected in 2023, which may reflect acclimation overtime, although year-to-year environmental differences may also have contributed to this pattern (Fig. 3a). During the morning period in winter 2022, corresponding to the dry season, PRD30 resulted in the highest A values, whereas Conv100 and PRD70 showed the lowest. The opposite pattern was observed during the same diurnal period in summer 2022/23, corresponding to the wet season, when A under PRD30 was lower than under the other three irrigation treatments, regardless of kaolin application. At midday, the effect of irrigation on A was significant only during the dry winter season of 2023, when the highest A was observed under PRD30 (Fig. 3b). These results suggest that the reduced irrigation supply applied under PRD30 maintained A values equal or even higher than those observed under treatments with greater inputs, especially during dry seasons, when crop water demand was the highest. The comparatively high A observed under PRD30 during winter may reflect a protective mechanism similar to that reported under frost conditions, in which lower water availability was associated with reduced damage from low temperatures.

The application of KF increased A compared with the GL treatment during the morning period of both seasons evaluated in 2022, but not in 2023, suggesting a possible acclimation of coffee plants to elevated temperatures in terms of A (Fig. 3a). During midday, kaolin had a significant effect only in winter 2022 (Fig. 3b), under the seasonal influence of lower temperatures (Fig. 1a). These contrasting diurnal responses suggest that additional physiological parameters should be considered to clarify the effects of kaolin management. No interaction irrigation and kaolin application was observed for A over eight seasonal periods.



Figure 3: Leaf CO_2 assimilation (A) in *Coffea canephora* plants subjected to four irrigation treatments (Conv100, PRD100, PRD70 and PRD30), and two kaolin treatments (with-KF or without-GL), in four seasonal periods (winter 2022, summer 2022/23, winter 2023, summer 2023/24), and two diurnal periods (a) morning (8:00–9:00 h), and (b) midday (12:00–13:00 h). Estimated mean $\pm$ standard error and p -values (in bold when significant, $\alpha = 0.05$) are presented. Different black lowercase letters indicate differences among irrigation levels for each kaolin treatment, while different uppercase letters indicate differences between kaolin treatments for each irrigation level ($n = 4$) assigned from Tukey test. A second group of evaluations compared seasonal and diurnal periods, presented by the orange-colored p -values above the graphs ($n = 32$, bold when significant). Different capital letters in orange color (within parentheses) indicate differences in A among seasons for the same diurnal period, while different exponent numbers indicate differences between the two diurnal periods for each season as determined by Tukey test.

Overall, stomatal conductance (g_s) values ranged from 0.029 to 0.320 $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ in the morning (Fig. 4a) and from 0.030 to 0.134 $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ at midday across seasons (Fig. 4b), indicating that seasonal conditions also influenced stomatal opening. The g_s values were consistently higher in the morning than at midday, except in winter 2022, when both diurnal periods showed similar g_s values. During that season, the g_s reached its lowest values.

Irrigation level had significant effects on g_s during only two seasonal periods: (i) the summer 2022/23 (morning), when the lowest values were observed under PRD30 (Fig. 4a), and (ii) winter 2023 (midday) when the lowest g_s was recorded under Conv100 treatment. These results suggest that the reduced irrigation supply under PRD30 generally maintained g_s values similar to those observed under treatments with greater

water inputs. Throughout the experimental period, KF application had a significant effect on g_s , and no interaction between irrigation levels and kaolin applications was observed for this variable across the eight season $\times$ diurnal-period combinations.

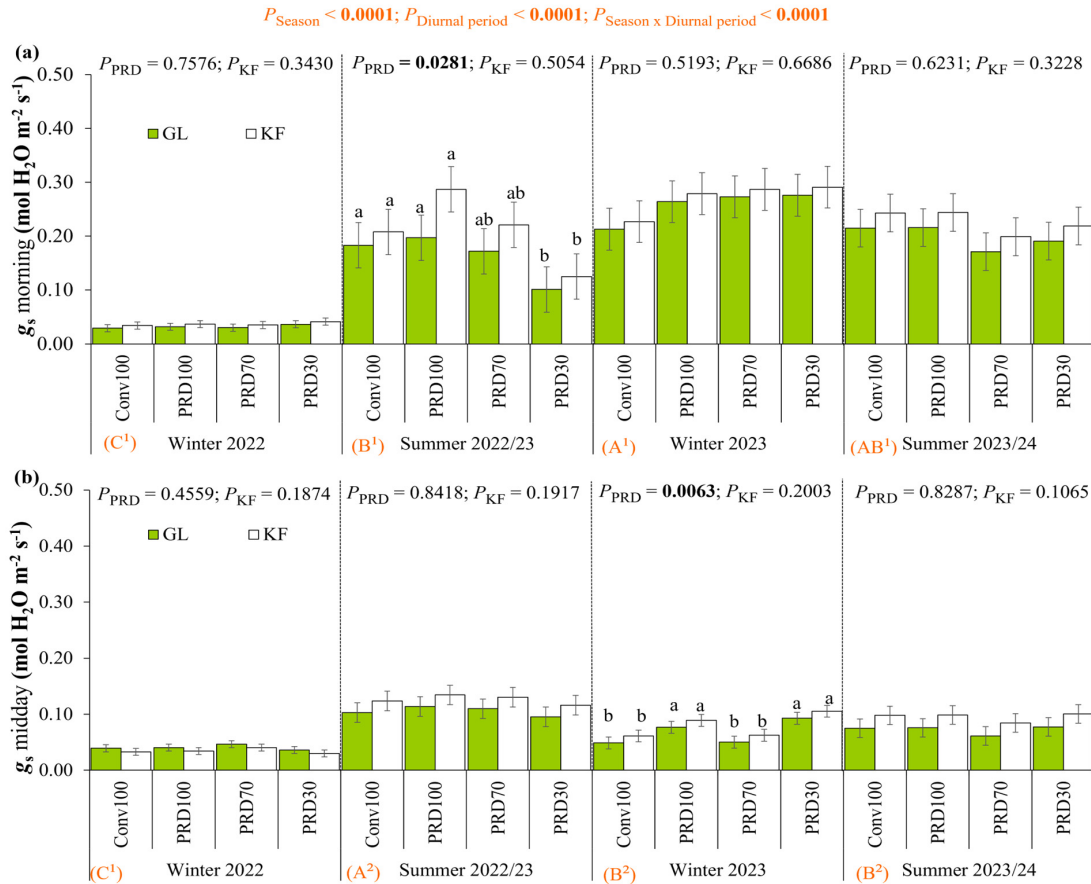


Figure 4: Stomatal conductance (g_s) in *Coffea canephora* plants subjected to four irrigation treatments (Conv100, PRD100, PRD70 and PRD30), and two kaolin treatments (with–KF or without–GL), in four seasonal periods (winter 2022, summer 2022/23, winter 2023, summer 2023/24), and two diurnal periods (a) morning (8:00–9:00 h), and (b) midday (12:00–13:00 h). Estimated mean $\pm$ standard error and p -values (in bold when significant, $\alpha = 0.05$) are presented. Different black lowercase letters indicate differences among irrigation levels for each kaolin treatment ($n = 4$), assigned from Tukey test. A second group of evaluations compared seasonal and diurnal periods, presented by the orange-colored p -values above the graphs ($n = 32$, bold when significant). Different capital letters in orange color (within parentheses) indicate differences in g_s among seasons for the same diurnal period, while different exponent numbers indicate differences between the two diurnal periods for each season as determined by Tukey test.

A progressive increase in carboxylation efficiency (CE) was observed throughout the experiment during the morning period, with the lowest values recorded in winter 2022 ($0.279 \mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$) and the highest in summer 2023/24 ($0.408 \mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$) (Fig. 5a). At midday, CE reached the highest values in summer 2022/23 ($0.321 \mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$), whereas the lowest values were recorded in winter 2022 ($0.163 \mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$) (Fig. 5b). When comparing diurnal periods within the same season, CE was consistently higher in the morning than at midday, except in summer 2022/23, when no difference between diurnal periods was observed (Fig. 5).



Figure 5: Carboxylation efficiency (CE) in *Coffea canephora* plants subjected to four irrigation treatments (Conv100, PRD100, PRD70 and PRD30), and two kaolin treatments (with-KF or without-GL), in four seasonal periods (winter 2022, summer 2022/23, winter 2023, summer 2023/24), and two diurnal periods (a) morning (8:00–9:00 h), and (b) midday (12:00–13:00 h). Estimated mean $\pm$ standard error and p -values (in bold when significant, $\alpha = 0.05$ including one marginal trend of p -value < 0.10) are presented. Different black lowercase letters indicate differences among irrigation levels for each kaolin treatment, while different uppercase letters indicate differences between kaolin treatments for each irrigation level ($n = 4$) assigned from Tukey test. A second group of evaluations compared seasonal and diurnal periods, presented by the orange-colored p -values above the graphs ($n = 32$, bold when significant). Different capital letters in orange color (within parentheses) indicate differences in CE among seasons for the same diurnal period, while different exponent numbers indicate differences between the two diurnal periods for each season as determined by Tukey test.

During the morning period, irrigation treatments had a significant effect on CE only in summer 2022/23, when PRD30 showed the lowest CE values (Fig. 5a). At midday, irrigation significantly affected CE during the dry winter seasons (Fig. 5b). In winter 2022, CE was higher for coffee plants under the Conv100 than under PRD70 treatment, whereas in winter 2023, CE was higher under PRD30 than under Conv100 at midday. Our results suggest that the reduced irrigation supply applied under PRD30 maintained CE values similar to those observed under treatments with higher water inputs and even showed higher values during the dry winter season in 2023. The KF application led to increases in CE only in winter 2022, during both the morning (Fig. 5a) and midday (Fig. 5b) periods, indicating that this season may be particularly important for the effectiveness of this management strategy. No interaction was observed between irrigation levels and kaolin applications for CE across the eight seasonal and diurnal period combinations, suggesting independent or additive effects of these management practices.

Intrinsic water use efficiency (iWUE) varied throughout the experimental period and was strongly dependent on season and diurnal period, with mean values ranging from 42 to 148 $\mu\text{mol mol}^{-1}$ (Fig. 6). During the morning period, the highest iWUE was recorded in winter 2022 (Fig. 6a), whereas at midday, iWUE was generally higher in winter than summer seasons (Fig. 6b). When comparing diurnal periods within each season, iWUE was higher in the morning than at midday in winter 2022 and summer 2023/24, while the opposite pattern was observed in summer 2022/23 and winter 2023 (Fig. 6).



Figure 6: Intrinsic water use efficiency (iWUE) in *Coffea canephora* plants subjected to four irrigation treatments (Conv100, PRD100, PRD70 and PRD30), and two kaolin treatments (with-KF or without-GL), in four seasonal periods (winter 2022, summer 2022/23, winter 2023, summer 2023/24), and two diurnal periods (a) morning (8:00–9:00 h), and (b) midday (12:00–13:00 h). Estimated mean $\pm$ standard error and p -values (in bold when significant, $\alpha = 0.05$ including three marginal trends of p -values < 0.10) are presented. Different black lowercase letters indicate differences among irrigation levels for each kaolin treatment, while different uppercase letters indicate differences between kaolin treatments for each irrigation level ($n = 4$) assigned from Tukey test. A second group of evaluations compared seasonal and diurnal periods, presented by the orange-colored p -values above the graphs ($n = 32$, bold when significant). Different capital letters in orange color (within parentheses) indicate differences in iWUE among seasons for the same diurnal period, while different exponent numbers indicate differences between the two diurnal periods for each season as determined by Tukey test.

Irrigation treatments significantly affected iWUE during the morning period, except in winter 2023, when no significant effect was observed and the lowest values were recorded (*ca.* 45 $\mu\text{mol mol}^{-1}$) (Fig. 6a). In other seasons, iWUE showed higher values under PRD30 (summer 2022/23) or values comparable to those observed under the highest water supply treatments (winter 2022 and summer 2023/2024). A similar pattern was observed for PRD70 during the morning period across most seasons, except in summer 2022/23, when this irrigation level resulted in the lowest iWUE. At midday, no significant differences among four irrigation treatments were observed in summer 2022/23 and winter 2023, suggesting that, in these seasons, even reduced irrigation level were sufficient to sustain photosynthetic activity (Fig. 6b). However, during this diurnal period, the lowest iWUE was recorded under PRD70 in winter 2022 and under PRD30 in summer 2023/2024, indicating that the choice of reduced irrigation level should be adjusted according to seasonal water demand. KF application significantly affected iWUE only in winter 2022 (Fig. 6). During this season, KF had a positive impact only under PRD70 during the morning period (Fig. 6a), whereas at midday, its positive effect was observed across all irrigation treatments (Fig. 6b).

3.3 Responses of Photosynthetic Performance Index (PI_{ABS}) to PRD and Kaolin Managements

PI_{ABS} reached the highest values in summer 2023/24, followed by summer 2022/23, whereas lower photosynthetic performance was recorded during the winter seasons, particularly in winter 2022 (Fig. 7). This pattern was observed across both diurnal periods, however, PI_{ABS} was generally higher in the morning than at midday, except in winter 2023, when PI_{ABS} did not differ between diurnal periods.

During the morning period in both summer seasons, irrigation levels had no significant effect on PI_{ABS} , indicating that PRD30 could replace Conv100, without reducing photosynthetic efficiency (Fig. 7a). A similar pattern was observed at midday in summer 2022/23, whereas in summer 2023/24, the highest PI_{ABS} was recorded under PRD30 when KF was applied (Fig. 7b). Under these conditions, reduced irrigation with alternating sides, combined with KF application, appeared to be more effective than the conventional irrigation system with KF. During the two dry winter seasons, characterized by water deficit conditions (Fig. 2), PRD30 and PRD70 tended to show PI_{ABS} values equal to or higher than those observed under Conv100 in both diurnal periods (Fig. 7). The positive responses of PI_{ABS} under PRD30 and PRD70 were enhanced by KF applications during the dry winter seasons in both diurnal periods (Fig. 7), except at midday in winter 2023, when no KF effect was observed (Fig. 7b). In addition, KF application also had a positive impact on PI_{ABS} during the two summer seasons in both diurnal periods, except during the morning period in summer 2023/24, when neither irrigation level nor KF application had a significant effect (Fig. 7a). However, during midday in summer 2023/2024, KF was required to maximize PI_{ABS} under PRD30 (Fig. 7b), further indicating the importance of KF applications under reduced water supply. Great responsiveness of PI_{ABS} suggests that this parameter integrated photochemical changes not fully captured by instantaneous gas exchange measurements.

3.4 Responses of Infrared Thermography Parameters to PRD and Kaolin Managements

Mean midday leaf temperature (T_{leaf}) values ranged from 26.9 to 39.3°C, with the lowest values observed in winter 2023 and the highest in summer 2023/2024 (Fig. 8a). Higher T_{leaf} values were observed during both summer seasons, whereas winter seasons showed milder temperatures, particularly in winter 2023, when midday T_{leaf} ranged from 26.9 to 34.5°C, including the lowest values recorded throughout the experiment. The different irrigation treatments did not affect T_{leaf} throughout the experimental period. In contrast, T_{leaf} was strongly affected by KF application, which reduced at midday throughout the experimental period. The mean reduction in KF-protected plants was 2.5°C in winter 2022, 2.4°C in summer 2022/23, 4.8°C in

winter 2023, and 4.3°C in summer 2023/24. The greater efficacy of kaolin in winter 2023 may be associated with specific environmental conditions, possibly because, during that season, PPFD between 12:00–14:00 h reached monthly average values exceeding 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with peaks approaching 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figs. 1f and S1c).

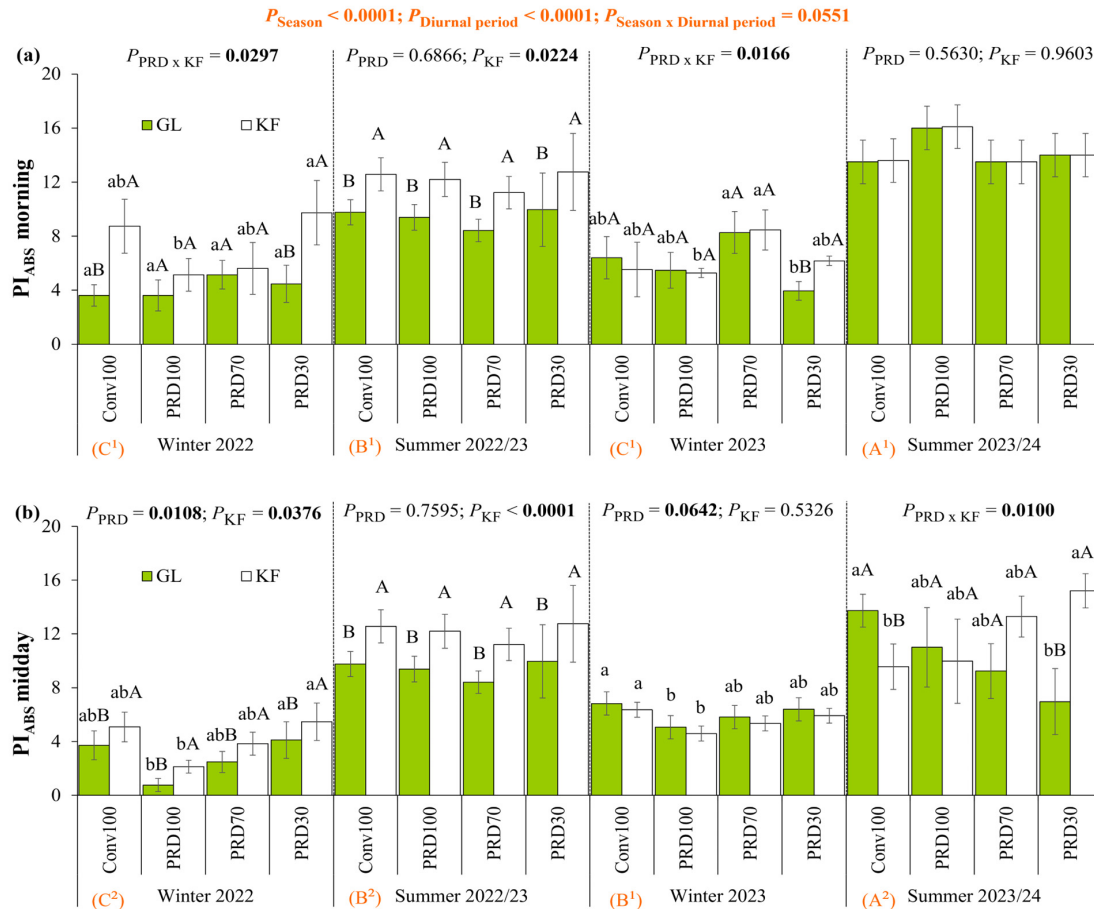


Figure 7: Photosynthetic performance index (PI_{ABS}) in *Coffea canephora* plants subjected to four irrigation treatments (Conv100, PRD100, PRD70 and PRD30), and two kaolin treatments (with–KF or without–GL), in four seasonal periods (winter 2022, summer 2022/23, winter 2023, summer 2023/24), and two diurnal periods (a) 8:00–9:00 h, and (b) midday (12:00–13:00 h). Estimated mean $\pm$ standard error and *p*-values (in bold when significant, $\alpha = 0.05$ including one marginal trend of *p*-value < 0.10) are presented. Different black lowercase letters indicate differences among irrigation levels for each kaolin treatment, while different uppercase letters indicate differences between kaolin treatments for each irrigation level ($n = 4$) assigned from Tukey test. A second group of evaluations compared seasonal and diurnal periods, presented by the orange-colored *p*-values above the graphs ($n = 32$, bold when significant). Different capital letters in orange color (within parentheses) indicate differences in PI_{ABS} among seasons for the same diurnal period, while different exponent numbers indicate differences between the two diurnal periods for each season as determined by Tukey test.

Midday Ig values were the lowest in winter 2022 and highest in summer 2023/24 (Fig. 8b). The intermediate midday Ig values observed in winter 2023 were characterized by marked differences between KF-treated (from 1.646 to 1.792) and GL plants (from 0.456 to 0.602). Ig values remained relatively stable across different irrigation levels, with significant differences observed only in summer 2022/23, when

Conv100 and PRD30 showed the highest values, while PRD100 showed the lowest values, regardless of KF application. Interestingly, I_g did not differ among irrigation levels in other seasons, suggesting that the lowest water supply under alternate PRD (PRD30) was sufficient to maintain midday leaf temperature within a range compatible with stomatal opening and leaf carbon and water fluxes. KF-treated plants exhibited significantly higher I_g values than GL plants across all irrigation levels and seasonal periods (Fig. 8b), indicating a consistent and robust effect of KF on this variable. Across seasons, I_g was two to three-fold higher in KF-treated than in GL plants, with the strongest effect during the dry winter period, indicating that KF substantially reduced midday water stress.



Figure 8: Infrared thermography responses of *Coffea canephora* plants subjected to four irrigation treatments (Conv100, PRD100, PRD70 and PRD30), and two applications of kaolin (with–KF or without–GL), in four seasonal periods (winter 2022, summer 2022/23, winter 2023, summer 2023/24): (a) leaf/canopy temperature (T_{leaf}), (b) relative

stomatal conductance index (Ig), and (c) crop water stress index (CWSI). Estimated mean $\pm$ standard error and p -values (in bold when significant, $\alpha = 0.05$ including one marginal trend of p -value < 0.10) are presented. Different black lowercase letters indicate differences among irrigation levels for each kaolin treatment, while different uppercase letters indicate differences between kaolin treatments for each irrigation level ($n = 4$) assigned from Tukey test. A second group of evaluations compared seasonal periods, presented by the orange-colored p -values above the graphs ($n = 32$, bold when significant), while the different capital letters in orange color (within parentheses) indicate differences in T_{leaf} , Ig and CWSI among seasons.

Midday CWSI ranged from 0.363 to 0.837 throughout the experimental period, and the intensity of midday water stress estimated by this index decreased from winter 2022 to summer 2023/24 (Fig. 8c), indicating a possible stress-memory-like acclimatization. Across the investigated seasons, CWSI was affected by irrigation level only in summer 2022/23, when CWSI was lower under Conv100 (0.57) than under PRD100 (0.737), and PRD70 (0.743) treatments, while PRD30 showed values similar to Conv100. A consistent reduction in CWSI was observed in KF-treated plants across all evaluated seasons. The greatest benefits of KF application were observed during the winter periods, with a reduction of 30% and 41% in winter 2022 and 2023 respectively, when plants naturally experienced greater water deficit (Fig. 2).

4 Discussion

This study on field-grown *Coffea canephora* plants combined two sustainable agricultural management strategies, kaolin particle film (KF) and partial root-zone drying (PRD) treatments. To the best of our knowledge, the combined application of PRD and kaolin particle film has rarely been investigated under field conditions, particularly in adult *C. canephora* plants. The novelty of this work lies in the finding that reductions of 30% (PRD70) and 70% (PRD30) in water supply did not lead to declines in gas exchange, PI_{ABS} , or infrared thermography parameters compared with the Conv100 or PRD100 treatments, indicating that alternating irrigation between root-zone sides may reduce water inputs by 30–70% in *C. canephora* cultivation, without impairing plant physiological performance. Under these conditions, kaolin applications showed their strongest effects in regulating T_{leaf} , Ig and CWSI, allowing stomatal functioning to be maintained even at midday, although these effects were not reflected in g_s responses.

4.1 Ecophysiological Responses of *Coffea canephora* to PRD

Drying a portion of the root system under PRD management can induce partial stomatal closure, thereby increasing water use efficiency, while still allowing stomatal functioning to be maintained. This response is species-dependent and may also vary according to plant functional group [67]. For example, plants of riparian *Melaleuca* spp., which naturally experience repeated cycles of drying and re-wetting in different portions of their root systems, are able to maintain stomatal opening until chemical signals are transmitted from the drying roots. In this case, root drying triggers rapid changes in the abundance of aquaporins, i.e., specialized membrane proteins that facilitate water transport, allowing roots to dynamically adjust their hydraulic permeability. In addition, increased abscisic acid (ABA) concentration in drying roots and shoot xylem sap, together with increased xylem sap pH, have been identified as key signaling mechanisms involved in stomatal responses to PRD, rather than responses driven solely by plant water deficit [68,69].

In soybean, PRD does not increase foliar ABA content but reduces g_s , a response attributed to decreases in leaf water potential and/or increases in the concentrations of ABA, jasmonic acid, or 1-aminocyclopropane-1-carboxylic acid, the precursor of ethylene, in the xylem sap [70]. From a morphophysiological perspective, PRD may also modulate root growth and metabolism, reprogram the rhizosphere microbiome, and enhance low temperature tolerance in barley by increasing glutathione concentrations [71]. Coffee plants under

the PRD30 exhibited slightly higher A than those under the other irrigation treatments possibly due to (i) increasing glutathione accumulation or (ii) mitigation of cold-stress responses, during winter. The mild water deficit conditions trigger osmotic adjustments that can lower the freezing point of cells, reducing the incidence of frost damage in coffee [72]. Additionally, plants grown under PRD may allocate more resources to root system maintenance, resulting in a higher root-to-shoot ratio than that observed in plants under conventional full irrigation [71]. Such allocation may affect coffee beans production, which should be investigated in future studies.

In the present study, the maintenance of A and g_s under PRD70 and PRD30, compared with conventional full irrigation, regardless of diurnal period or season, suggests that reduced irrigation applied by alternating the irrigated side of the root system did not impair carbon assimilation or stomatal conductance (Figs. 3 and 4). It is possible that since coffee plants grown under PRD70 and PRD30 experienced summer stress during 2022, they may have invested in deeper root growth and in root-system optimization, as observed in barley [71]. Such a strategy would ensure better water uptake—even from deeper soil layers—as observed in 2023 when a shorter dry period than in 2022 (Fig. 2) allowed plants to avoid water stress, potentially aided by stress-memory-like acclimatization. Although g_s was not reduced, iWUE increased in *C. canephora* cultivated under PRD70 and PRD30, depending on the season (Fig. 6). In grapefruit trees, PRD50 increases whole-plant water use efficiency and leaf ABA concentration, while markedly decreasing g_s compared to full irrigation [73]. In olive trees PRD50 increases concentration of leaf ABA, proline and soluble sugars, and decreases A , g_s and WUE when compared to full irrigation [74]. Coffee plant responses partially resemble those observed in grapefruit and olive, but as *C. canephora* evolved as a riparian species, often growing naturally in the understory of dense, humid forests along riverbanks and streams [75], its responses to PRD resemble those observed in *Melaleuca* spp. [67]. This suggests that the mechanisms underlying PRD responses in *C. canephora* may be primarily associated with changes in aquaporins abundance in drying roots and/or in ABA leaf concentration.

In the present study, rainfed plants were not used as an absolute control because reduction in leaf gas exchange would be expected in *C. canephora* [76] and *C. arabica* [12], after prolonged soil water depletion. Complete withholding of irrigation in coffee cultivation can cause biochemical damage [77], reduce RuBisCO activity [72], decrease leaf water potential [78], and lead to serious yield losses [79]. Although some grasses can increase stomatal density to maintain g_s relative to fully irrigated plants [80], stomatal density does not change under severe water stress in *C. canephora* [11]. Declines in A and g_s under PRD treatments have been reported when water supply is reduced by 35% relative to full irrigation in *Carica papaya* during autumn and spring [29]. In contrast, our results indicate that *C. canephora* is a tropical species that tolerates a 70% reduction in irrigation water supply under PRD30, when irrigation alternates between root-zone sides, probably due to higher hydraulic efficiency in Robusta coffee [81] than in papaya [82] plants.

Near-complete recovery of gas exchange parameters after soil rehydration has been reported after a single drought cycle in *Coffea* spp. [83], whereas full recovery of A , g_s , and iWUE has been observed only after two consecutive drought cycles in *C. canephora* [11]. In young *C. canephora* plants, some non-stomatal parameters, such as PI_{ABS} , maximum quantum yield of primary photochemical reactions, and density of reaction centers capable of Q_A reduction, showed an acclimation response to water stress during the second drought event [11]. These findings indicate that photochemical and anatomic adjustments may play a more decisive role than stomatal adjustments in the acclimation process to repeated water stress events [8]. In the present study, adult *C. canephora* plants showed higher A , g_s , and CE values in winter 2023 than in winter 2022 during both diurnal periods (Figs. 3–5).

No differences among irrigation treatments were observed for A , g_s , and CE during the morning in the second winter period, but differences became evident at midday, with PRD30 showing the highest values. This response suggests a possible stress-memory-like acclimatization response in leaf functioning when only one side of the root system was rewetted to 30% of full field capacity and subsequently allowed to dry again.

4.2 Ecophysiological Responses of *Coffea canephora* to KF

KF had no significant impact on g_s in any season or diurnal period in coffee plants (Fig. 4), whereas positive effects were observed for A , CE , and $iWUE$ only in winter 2022 during both diurnal periods (Figs. 3, 5 and 6, respectively). The highest CE and $iWUE$ values were observed under PRD30 + KF, suggesting that, during winter, KF application may enhance the physiological benefits of severe irrigation reduction under PRD management.

PI_{ABS} index, representing the second group of measurements, increased with KF applications across all observed seasons, and when an interaction with PRD was observed, KF had a positive effect under PRD30 and/or PRD70 treatments (Fig. 7). In agreement with the findings that photochemical adjustments are more important after two repeated water stress events than stomatal adjustments [11], PI_{ABS} showed that the second-year seasons presented higher values than the first year, especially at midday, indicating a possible stress-memory-like acclimatization. The improved photochemical performance may also be related to the plants' nutritional status, given the improvement in soil fertility over a time (Table S1). PI_{ABS} is a comprehensive, multi-parametric photosynthetic biomarker derived from the OJIP chlorophyll a fluorescence transient, calculated on absorbance basis [84]. As it represents the efficiency of electron flow in photosynthesis, it provides a highly sensitive measure of plant vitality and photosystem II functionality and may be considered a robust predictor of biomass production under stress conditions [85]. KF mitigated the effects of excessive midday radiation during the summer of 2023 under PRD70 and PRD30 treatments, ensuring an appropriate balance between electron absorption, capture, and transport—reflected by PI_{ABS} —thereby maintaining A values similar to those under full irrigation. Thus, although leaf gas exchange did not show high sensitivity to KF in *C. canephora*, PI_{ABS} may be used in precision agriculture as a predictor of both PRD and KF responses at the leaf scale.

Infrared thermography represented the third group of measurements in our experiment, (Fig. 8). This method was highly effective in detecting positive KF effects, while showing limited ability to detect variation in PRD responses, although thermography responses were consistent with A and g_s variations across PRD treatments (Figs. 3 and 4, respectively). To some degree, I_g , which estimates gas exchange based on canopy temperature responses [86], was related to midday g_s responses to PRD, but was highly sensitive to KF, consistently showing positive effects of this management strategy across all seasons (Fig. 8b), while such variation was not observed in g_s . Meanwhile, CWSI also did not show variation due to PRD treatments, although it has been shown to be useful for irrigation scheduling in some crops, such as pumpkin [87]. Herein, CWSI was consistently reduced by KF applications, indicating that KF should be applied, as in our experiment, throughout the coffee growing cycle to reduce water stress related to elevated midday temperatures, which was associated with reduced midday T_{leaf} (Fig. 8a). Infrared thermography at crop scale has been shown to be a robust high-throughput phenotyping tool for both biotic and abiotic stresses [88]. In the case of *C. canephora*, this precision agriculture tool allowed us to detect that CWSI was lower in the second than in the first year, while I_g was higher in the second than in the first year, indicating a possible stress-memory-like acclimatization [89]. These responses further suggest that the use of kaolin under moderate stress conditions—whether due to a shorter period of drought exposure in 2023 or the plants' morphophysiological acclimation—sustained carbon gain in the face of water deficit.

4.3 Independent, Additive and Interaction Effects of PRD and KF on *Coffea canephora* Ecophysiological Parameters

Positive effects of PRD on iWUE and A may result from the uncoupling of stomatal and mesophyll conductance, as observed in cotton leaves under simulated water stress conditions [90]. Similarly, positive effects of PRD50 and PRD25 have been associated with improved orange quality, without yield reduction, even under increased soil water deficit [91]. In turn, KF application has been shown to be highly effective in mitigating the deleterious effects of supra-optimal temperature stress at the leaf scale and in increasing bean size and productivity in *C. canephora* [41]. Although both technologies were simultaneously applied in our experiment, the measured ecophysiological parameters most often exhibited independent responses. The first group, leaf gas exchange parameters (A , g_s , CE and iWUE), showed significant responses to either technology only occasionally (Figs. 3–6). When significant effects were detected, PRD affected these variables more frequently than KF, with significant responses observed in 13 out of 32 season $\times$ diurnal period combinations for PRD, compared with 6 out of 32 cases for KF. The frequency of significant responses did not differ between morning and midday evaluations. Additive and/or interaction effects were observed in only 4 out of 32 diurnal/seasonal cases and were the most frequently detected in winter 2022, which represented the most stressful environmental conditions. This suggests that the combined effect of PRD and KF was particularly associated with harsher environmental conditions for *C. canephora*.

The second group of parameters, chlorophyll a fluorescence, represented here by PI_{ABS} , was the most sensitive to the integrated effects of PRD and KF among all parameters investigated (Fig. 7). Additive and/or interaction effects on PI_{ABS} were observed during both diurnal periods in both winter seasons, as well as at midday in summer 2023/24. The third group of parameters, those related to crop temperature regulation and water stress, including T_{leaf} , I_g , and CWSI, consistently responded positively to KF application, whereas additive effects of the combined PRD + KF treatments on I_g and CWSI were detected only in summer 2022/23. Overall, this integrated assessment indicates that PI_{ABS} was the most broadly responsive parameter for detecting the combined effects of the two applied technologies, and that the photosynthetic performance index may serve as a useful indicator of the benefits of PRD and KF across all analyzed seasons.

Our field experiment has several limitations that should be considered when interpreting the results. First, soil water content was not monitored independently on the wet and dry sides of the root zone, preventing direct quantification of the spatial soil-water gradients generated by PRD. Second, environmental conditions differed substantially between years, so temporal changes in plant performance cannot be attributed exclusively to repeated PRD exposure. Third, the study focused on a single *C. canephora* clone and on leaf-level physiological responses; therefore, extrapolation to other genotypes and to agronomic performance requires further validation. Finally, yield and bean quality responses were not evaluated here and should be integrated with physiological responses before recommending reduced irrigation regimes for commercial production.

5 Conclusions

Overall, PRD70 and PRD30 maintained A and g_s at levels comparable to those under the reference irrigation treatment across most seasons and diurnal periods, although season-specific reductions occurred. Thus, reducing irrigation supply while alternating the irrigated side of the root zone did not consistently impair leaf carbon assimilation or stomatal conductance. Although g_s was generally maintained, iWUE increased under PRD70 and PRD30 in specific seasonal periods. CE under PRD30 was generally comparable to that observed under higher irrigation levels and was even higher in winter 2023. These findings support our first hypothesis.

The parameters related to crop temperature regulations and water stress (T_{leaf} , I_g and CWSI measured only at midday) consistently responded positively to KF, whereas additive effects of PRD + KF on I_g and CWSI were observed only in summer 2022/23. Additive and/or interaction effects on PI_{ABS} were observed during both diurnal periods in both winter seasons, and at midday of summer 2023/24. In general, morning and midday responses showed a similar frequency of significant effects for leaf gas exchange parameters, while the additive and/or interaction effects of PRD and KF were most frequently observed in winter 2022, which was the driest and harshest season. These findings partially supported our second hypothesis.

No differences among irrigation treatments in A , g_s , and CE were observed during the morning in the second winter period, but interestingly, at midday in the second winter, PRD30 showed the highest values. These temporal patterns are consistent with, but do not demonstrate, a stress-memory-like acclimatization response under PRD30. Thus, our third hypothesis was only partially supported. Additionally, PI_{ABS} values were higher in the second year than in the first year, especially at midday; CWSI was lower in the second than in the first year, whereas I_g was higher in the second year than in the first. The improved performance of PRD30 during the second winter suggests a possible acclimation response to repeated alternate wetting and drying. However, further studies incorporating direct soil moisture monitoring and mechanistic indicators are needed to confirm whether this pattern represents stress-memory-like acclimatization. Finally, among the evaluated parameters, PI_{ABS} showed the broadest responsiveness to the combined effects of PRD and KF across seasons and diurnal periods.

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Conflicts of Interest: Given their role as guest editors of this journal, Miroslava Rakocevic and Weverton Pereira Rodrigues had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. The authors declare no other conflicts of interest.

Supplementary Materials: The supplementary material is available online at <https://www.techscience.com/doi/10.32604/phyton.2026.088692/s1>. Table S1: Soil chemical parameters: pH (in H₂O); base saturation (V, %); element content in cmol_c dm⁻³ of calcium (Ca), magnesium (Mg), aluminum (Al), hydrogen (H); element content in mg dm⁻³ of phosphorus (P), potassium (K), sulfur (S), boron (B), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn). Table S2: Physical characteristics of the soil over five layers: soil particle density (S_{pd}, Mg m⁻³), soil density (S_d, Mg m⁻³), total soil porosity (T_{sp}, m³ m⁻³), soil moisture at field capacity (θ_{FC}, m³ m⁻³, 10 kPa water tension), soil moisture at permanent wilting point (θ_{PWP}, m³ m⁻³, 1500 kPa water tension), available water (A_w, m³ m⁻³), and available water capacity (AWC, mm). Figure S1: Daytime (a) air temperature (T_{air}, °C), (b) relative air humidity (RH_{air}, %), and (c) photosynthetic photon flux density (PPFD, μmol m⁻² s⁻¹). Mean ± SE (n = 5) for the five days preceding each seasonal group of ecophysiological measurements is shown.

Abbreviations

The following abbreviations are used in this manuscript:

A	Leaf CO ₂ assimilation rate
ABA	Abscissic acid
A _w	Available water in the soil
AWC	Available water capacity
CE	Carboxylation efficiency
C _i	CO ₂ concentration in the leaf mesophyll
Conv100	100% ET ₀ irrigation supplied on a single side
CWSI	Crop water stress index
E	Leaf transpiration rate
ET ₀	Reference evapotranspiration
GL	Plants not treated with kaolin, green leaves
g _s	Stomatal conductance
Ig	Relative stomatal conductance index
iWUE	Intrinsic water use efficiency
KF	Plants treated with kaolin
PI _{ABS}	Photosynthetic performance index
PPFD _{max}	Maximum monthly photosynthetic photon flux density
PRD	Partial root drying
PRD100	100% ET ₀ irrigation alternately supplied on two plant sides
PRD30	30% ET ₀ irrigation alternately supplied on two plant sides
PRD70	70% ET ₀ irrigation alternately supplied on two plant sides
RH _{min}	Absolute minimum monthly relative humidity
S _d	Soil density
S _{pd}	Soil particle density
T _{leaf}	Leaf/canopy temperature
T _{max}	Absolute maximum monthly temperature
T _{mean}	Mean monthly temperature
T _{min}	Absolute minimum monthly temperature
T _{sp}	Total soil porosity
WUE	Instantaneous water use efficiency
θ _{FC}	Soil moisture at field capacity
θ _{PWP}	Soil moisture at permanent wilting point

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