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Research paper

Photosynthetic advantage without growth superiority: Sorghum's paradoxical response to future climate stresses compared to maize

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ABSTRACT

Rising atmospheric CO_2 is projected to reach 700 µmol/mol by 2100, increasing global temperatures by ~3 °C and exacerbating drought frequency. Sorghum (*Sorghum bicolor*), a drought-tolerant alternative to maize (*Zea mays*), may gain relevance under future climates. Here we grew sorghum and maize in growth chambers under ambient (400 µmol/mol CO_2, 24/18 °C day/night) and future (700 µmol/mol CO_2, 27/21 °C day/night) environmental conditions, with and without drought. Drought was imposed by withholding water until soil moisture reached 20 % field capacity, which was then maintained for one week. Under high CO_2 and high temperature, although both maize and sorghum achieved comparable biomass, growth was only promoted in sorghum by 67 %. Its stomatal anatomical traits reduced cumulative transpiration by 18 % while maintaining carbon assimilation, thereby improving water-use efficiency. With combined high CO_2, high temperature and drought, maize and sorghum exhibited comparable biomass, but sorghum maintained gas exchange and quantum yield of photosystem II (PSII). Additionally, sorghum increased root growth, further mitigating future drought impacts on its growth compared to future control conditions. In contrast, maize photosynthesis and the quantum yield of PSII were severely impaired, thereby restricting growth. Thus the photosynthetic and water-use efficiency strategies of sorghum show its considerable potential as a future alternative crop, but further studies should verify whether these strategies persist during the reproductive stage and contribute to enhanced yield stability.

1. Introduction

Anthropogenic CO_2 emissions have increased atmospheric CO_2 from 320 to 420 µmol/mol since 1970, contributing to a nearly 1 °C increase in global temperature (NOAA, 2023; The Core Writing Team IPCC, 2021). This has altered precipitation patterns, causing extreme drought events in regions where they were previously rare (NOAA, 2023). Projections estimate atmospheric CO_2 to reach 700 µmol/mol and temperatures to rise by 3 °C by the end of the century, further intensifying drought risk (The Core Writing Team IPCC, 2021). Thus, climate change is one of the major threats for agricultural production and future food supply, particularly given the expanding population and growing reliance on freshwater resources (NOAA, 2023; The Core Writing Team IPCC, 2021).

Drought and high temperature are among the most detrimental abiotic stresses for crops, as they limit growth and yield. Under drought, plants reduce stomatal conductance (*gs*) and transpiration to minimise water loss, which over time restricts photosynthesis and biomass accumulation (Farooq et al., 2009). Conversely, high temperature initially promotes stomatal opening to enhance transpiration and leaf cooling, but prolonged exposure increases water demand, leading to stomatal closure and damage to the photosynthetic machinery (Mathur et al., 2014). In contrast, high CO_2 promotes stomatal closure and improves water-use efficiency (*WUE*), while also sustaining photosynthesis and stimulating growth (Kimball, 2016). Thus, high CO_2 mitigate the negative impacts of drought and high temperature (Ainsworth and Long, 2021). However, most research on high CO_2 effects has primarily been conducted in C_3 plants, with limited attention given to C_4 plants.

Despite C_4 species representing only 3 % of all plants species (Sage, 2004), maize (*Zea mays*) accounts for 40 % of global cereal production (FAOSTAT, 2025). Sorghum (*Sorghum bicolor*), another C_4 crop, plays a crucial role as a staple in semi-arid regions like Africa (FAOSTAT, 2025; Bozal-Leorri et al., 2021), as its expanded root system, greater stomatal closure and improved *WUE* under water-limited conditions help

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maintain biomass accumulation (de Souza Rodrigues et al., 2023; Goche et al., 2020; Hadebe et al., 2020; Schittenhelm and Schroetter, 2014). Moreover, it has great ability to recover when re-watered (Hnilička et al., 2024; Martínez-Goñi et al., 2023b). Although sorghum has traditionally been considered more drought-resilient than maize and is expected to gain relevance as an alternative in drought-prone regions (Farré and Faci, 2006; The Core Writing Team IPCC, 2021), recent studies have challenged this assumption (Parra et al., 2020; Rotundo et al., 2024). Therefore, further research comparing maize and sorghum growth under drought in future environmental conditions is necessary to verify sorghum's potential as a viable alternative.

Even though high CO_2 enhances growth of both maize and sorghum (Al-Salman et al., 2023; Asadi and Eshghizadeh, 2021; De Souza et al., 2008), sorghum can overcome photosynthetic limitations caused by reduced *gs* and seems more responsive to high CO_2 (Al-Salman et al., 2023; Cano et al., 2019; Ziska and Bunce, 1997). This suggests considerable potential to withstand high temperature or drought stress under future climatic conditions. Although the *WUE* of both maize and sorghum increased under high CO_2 and high temperature, net CO_2 assimilation rate (*A*) and growth were only promoted in sorghum (Prasad et al., 2009; Ruiz-Vera et al., 2015). Under combined high CO_2 and drought, both crops maintain *A*, soil water content and biomass by reducing *gs* and increasing *WUE* (Allen et al., 2011; Ripley et al., 2022; Wei et al., 2022), but sorghum exhibited an increased water-absorbing capacity by allocating more resources towards root growth and decreasing water potential (Ψ_w) (Martínez-Goñi et al., 2023b). Collectively, although high CO_2 alleviates adverse effects of high temperature or drought on both maize and sorghum, the mitigating effect of high CO_2 may benefit sorghum more.

However, the combined impact of high CO_2, high temperature and drought on maize and sorghum is poorly understood. C_4 crops are expected to face a trade-off between reduced *gs* for drought tolerance and increased *gs* required for heat dissipation (Al-Salman et al., 2023). Therefore, stomatal regulation emerges as a crucial mechanism as its influence on *WUE* will determine the resilience of C_4 crops to future environmental conditions. This research characterised the physiological responses of maize and sorghum to the combination of high CO_2, high temperature and drought by measuring gas exchange, stomatal traits, water relations, growth parameters and *WUE*. We hypothesise that the combination of high CO_2 and high temperature will 1) improve stomatal regulation in sorghum but not in maize, causing greater water loss and impacts on photosynthesis and growth of maize. Likewise, we expect the high CO_2, high temperature and drought environment to 2) enhance sorghum's existing drought tolerance mechanisms, improving its *WUE* and mitigating the negative effects on its photosynthetic processes, leading to 3) an improved growth compared to maize.

2. Materials and methods

2.1. Plant material and experimental design

Seeds of *Zea mays* var. Luigi CS (maize, FAO ripening classification 250) were obtained from NEIKER (Basque Institute for Agricultural Research and Development, Basque Country, Spain). Seeds of *Sorghum bicolor* var. Néctar (sorghum, late-ripening) were provided by Semillas Fitó (Catalonia, Spain). The selection of these varieties is based on the fact that maize Luigi CS is used by NEIKER in the Basque Country (Spain), while sorghum Néctar was the most widely produced variety in Spain during the 2017–2024 growing seasons. Seeds were planted in 3 L pots containing a substrate mixture of perlite and vermiculite in a 3:1 (v/v) ratio. The experimental setup was structured to ensure at least a minimum of 6 biological replicates (pots) per species and treatment, with two plants per pot (technical replicates).

The experiment was conducted in a Conviron PGR15 growth chamber (Conviron, North Dakota, USA), which provides approximately 1.5 m^2 of growth area and 1.47 m of growth height. Two environmental setups were established: ambient CO_2 and temperature (400 μmol/mol CO_2 with day/night temperatures of 24/18 °C) and future conditions (700 μmol/mol CO_2 with day/night temperatures of 27/21 °C). CO_2 concentration was continuously monitored and automatically regulated by the internal control system of the chamber, during day and night. A 16-hour photoperiod with 400 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) was provided by a combination of incandescent bulbs (Osram 70 W Halogen Eco, Munich, Germany) and warm-white fluorescent lamps (Philips Master TL-D 70 W 848, Amsterdam, The Netherlands), measured at the top of the canopy and adjusted as plants grew. Day/night humidity levels were maintained at 60/80 % in both environments. Plants were rotated to prevent environmental gradients and watered (well-water treatment) three times a week to field capacity using a Hoagland solution (Hoagland and Arnon, 1938), which was adjusted to pH 6.50 during preparation. Field capacity was determined gravimetrically (Cassel and Nielsen, 1986). First, pots filled with dry substrate were saturated with water, allowed to freely drain and weighed to obtain wet field capacity weight. The dry weight of the substrate was obtained after oven-drying at 80 °C for 72 h. The difference between the wet field capacity and dry weights was used to calculate the amount of water retained at 100 % field capacity. For the experimental pots, field capacity was calculated as the proportion of water retained in each pot relative to the amount of water corresponding to 100 % field capacity. At 14 days after sowing (DAS), drought treatment started by stopping irrigation for half of the plants until the field capacity of the non-irrigated pots decreased to 20 % ± 5 %. Once pots reached 20 % field capacity, that moisture level was maintained for one week (ending of drought treatment). Overall, plants were subjected to four treatments: ambient control (AC), ambient drought (AD), future control (FC) and future drought (FD).

In vivo measurements and the collection of plant material were conducted at the end of the drought treatments. AD lasted on average 43 DAS and 48 DAS in maize and sorghum, respectively, while FD lasted on average 42 DAS and 46 DAS. Both plant types were in the vegetative stage during trait measurements and harvest. To maintain consistency, each drought-treated pot had a corresponding control pot harvested at the same time.

2.2. Leaf gas exchange and photochemistry

Gas exchange parameters were determined by measuring the leaf preceding the flag leaf (one leaf per plant) for each plant type and treatment using a Li-Cor 6400XT instrument (Li-Cor Inc., Lincoln, NE, USA). The cuvette temperature and CO_2 were set to 24 °C and 400 μmol/mol CO_2 for ambient conditions, and 27 °C and 700 μmol/mol CO_2 for future conditions. Relative humidity of the cuvette was maintained at 60 %. A red/blue LED light source (model Li 6400–40, Li-Cor Inc.) was used to ensure a photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1} for each measurement. Gas exchange measurements were taken at 12:00, three hours after dawn. Stomatal conductance (*gs*) and net photosynthetic CO_2 assimilation (*A*) were obtained based on von Caemmerer and Farquhar (1981).

Measurement of the quantum yield of PSII (Φ_{PSII}) was performed using an integrated leaf fluorescence chamber (Li-6400–40, Li-Cor Inc.). It was calculated as:

$$\Phi_{PSII} = \frac{F_m{'} - F_s}{F_m{'}} \tag{1}$$

Where *Fs* is the variable fluorescence at steady state and *Fm'* is the maximum light-adapted fluorescence (Schindler and Lichtenthaler, 1996).

2.3. Stomatal density and size

Leaf imprints of the leaf preceding the flag leaf (one per plant) were prepared for the adaxial (*AD*) and abaxial (*AB*) surfaces following the procedure by Casado-García et al. (2020). A Nikon ECLIPSE 50i fluorescence microscope (Nikon corporation, Japan) and a Leica DFC 420 C camera (Leica Microsystems, Germany) were used to obtain three photos of the adaxial and three photos of the abaxial surfaces for each plant type and treatment. Stomatal density (*SD*) was obtained using the LabelStoma tool (Casado-García et al., 2020). Stomatal size (*S*) was defined by guard cell length (*L*) and width (*W*), and calculated as:

$$S = L \times W \tag{2}$$

2.4. Soil and leaf water relations

Pots were weighed three times per week in order to calculate *CuTr*, as explained by Martínez-Goñi et al. (2024). Shortly, pots were weighed in the morning before watering to determine evapotranspired water. Then, pots were watered to reach field capacity and weighed. The water loss due to substrate evaporation was measured using three pots without plants but filled with substrate. This loss was then subtracted to determine transpiration. The soil fresh weight (*SFW*), soil dry weight (*SDW*) and initial soil fresh weight (*SFWi*) were used to calculate the soil relative water content (*SRWC*) using the following equation:

$$SRWC = 100 \times \frac{SFW - SDW}{SWF_i - SDW} \tag{3}$$

SFWi was determined by weighing a fresh soil portion, while *SDW* was determined by weighing the soil portion after oven-drying it for 72 h.

The Scholander pressure-equilibration technique (Scholander et al., 1965) was used to obtain leaf water potential (Ψ_w) at 15:00, six hours after dawn. The cryoscopic osmometer OSMOMAT 030 (Gonotec GMBH, Berlin, Germany) was used to analyse the freezing point of the sap from leaf segments, which was then used to calculate leaf osmotic potential (Ψ_o). Specifically, Ψ_o was determined using the following equation:

$$\Psi_o = M \times T \times R \tag{4}$$

Where M is the concentration (osmol), T is the sample temperature (298 K) and R is the molar gas constant (0.00832 L MPa K^{-1} mol^{-1}). The difference between Ψ_w and Ψ_o was used to obtain the pressure potential (Ψ_p). Similarly, to calculate the osmotic potential at full turgor (Ψ_o^{100}), leaves from each plant type and treatment were cut and soaked in deionized water at 4 °C in darkness for 24 h.

Leaf fresh weight (*FW*), leaf dry weight (*DW*) and leaf turgid weight (*TW*) were used to determine leaf relative water content (*LRWC*) with the following equation:

$$LRWC = 100 \times \frac{FW - DW}{TW - FW} \tag{5}$$

2.5. Biomass and growth related traits

Leaves, stems and roots of plants per treatment were harvested, separated, washed and oven-dried for 72 h to determine dry-weight (*DW*). A minimum of six plants per treatment and plant type were used. *Total Biomass* was divided by the cumulative transpiration (*CuTr*) to estimate *WUE*. *Shoot/Root* was measured by dividing the sum of the biomass of aerial organs (*Leaf Biomass* and *Stem Biomass*, *Shoot*) by *Root Biomass*. Leaf area (*LA*) was measured as explained in Martínez-Goñi et al. (2023a). Shortly, *LA* was calculated using photos of freshly harvested leaves with the open-source software Fiji (Fiji Is Just ImageJ; Schindelin et al. (2012).

Growth related parameters were measured at the individual plant level as explained by Boughalleb and Hajlaoui (2011); Poorter (1993). For each treatment, specific sets of plants were destructively harvested at t_1 (14 DAS, start of drought treatment) and t_2 (harvest DAS) to determine: the initial (DW_1) and final (DW_2) total dry weights, the initial (A_1) and final (A_2) leaf area and the initial (LDW_1) and final (LDW_2) leaf weights. relative growth rate (*RGR*), net assimilation rate (*NAR*), leaf area ration (*LAR*), specific leaf area (*SLA*), leaf weight ratio (*LWR*), stem weight ratio (*SWR*) and root weight ratio (*RWR*) were calculated as follows:

$$RGR = \frac{\ln\left(\frac{DW_2}{DW_1}\right)}{t_2 - t_1} \tag{6}$$

$$NAR = \frac{DW_2 - DW_1}{A_2 - A_1} \times \frac{\ln\left(\frac{A_2}{A_1}\right)}{t_2 - t_1} \tag{7}$$

$$LAR = \frac{A_2 - A_1}{DW_2 - DW_1} \times \frac{\ln\left(\frac{DW_2}{DW_1}\right)}{\ln\left(\frac{A_2}{A_1}\right)} \tag{8}$$

$$SLA = \frac{A_2 - A_1}{LDW_2 - LDW_1} \times \frac{\ln\left(\frac{LDW_2}{LDW_1}\right)}{\ln\left(\frac{A_2}{A_1}\right)} \tag{9}$$

$$LWR = \frac{LDW_2 - LDW_1}{DW_2 - DW_1} \times \frac{\ln\left(\frac{DW_2}{DW_1}\right)}{\ln\left(\frac{LDW_2}{LDW_1}\right)} \tag{10}$$

The shoot weight ratio (*SWR*) and root weight ratio (*RWR*) were calculated similarly to *LWR*, but considering stem and root weights, respectively, instead of leaf weights.

Leaves, stems and roots from plants harvested at the start of drought treatment were used to determine leaf biomass (*Leaf Biomass$_0$*), stem biomass (*Stem Biomass$_0$*), root biomass (*Root Biomass$_0$*), total biomass (*Total Biomass$_0$*) and leaf area (LA_0) at day 0 of drought.

2.6. Statistical analysis

GraphPad Prism software version 8.2.1 (GraphPad Software, San Diego, CA USA, www.graphpad.com) was used to create all figures. SPSS software version 28.0.1.1 for Windows (IBM Corp, Armonk, NY USA) was used to perform the statistical analysis. The effect of environmental treatment (ambient and future), water treatment (control and drought), plant type (maize and sorghum) and their interaction was determined using a three-way ANOVA (Table 1). Means were compared using Duncan's multiple range test, and values were considered to be statistically significant when $p < 0.05$. Levene's test was used to assess the homogeneity of variance, while Kolmogorov-Smirnov test was used to evaluate the normality of data distribution. In cases where normality was not met, data transformation was performed prior to ANOVA testing.

3. Results

3.1. Sorghum preserves higher net photosynthetic CO_2 assimilation under the combination of high CO_2 and high temperature, while drought severely downregulates photosynthetic processes in maize

With respect to gas exchange parameters, we found maize to have lower *gs*, *A* and Φ_{PSII} values irrespective of the applied treatment (Fig. 1A-C and Table 1). As a matter of fact, *gs*, *A* and Φ_{PSII} values under AC in sorghum were 34 %, 35 % and 21 % higher compared to maize (Fig. 1A-C).Even though under FC *gs* was decreased by 36 % in maize and by 31 % in sorghum (Fig. 1A), these reductions did not alter *A* and Φ_{PSII} values (Fig. 1B-C). Furthermore, these reductions in *gs* appeared to reduce *CuTr* only in maize, but not in sorghum (Fig. 2A). Moreover,

Table 1
Three-way ANOVA for plant type (P), water treatment (W), environmental treatment (CO_2+T) and their interaction for all the analysed variables. Results are represented as ns (non-significant) and * ($p < 0.05$).

	P	**W**	**CO_2+T**	**P × W**	**P × CO_2+T**	**W × CO_2+T**	**P × W × CO_2+T**
gs	*	*	*	*	*	ns	*
A	*	*	ns	*	ns	*	ns
Φ_{PSII}	*	*	ns	*	ns	*	ns
SD_{AB}	*	*	ns	*	ns	ns	ns
SD_{AD}	*	ns	ns	ns	*	ns	ns
Total SD	*	ns	ns	ns	ns	ns	ns
S_{AB}	*	*	*	ns	ns	ns	ns
S_{AD}	*	*	*	ns	ns	ns	*
R	*	ns	ns	ns	*	ns	ns
CuTr	ns	*	ns	ns	ns	ns	ns
WUE	ns	ns	*	ns	ns	ns	ns
Leaf Biomass	*	*	ns	ns	*	ns	*
Stem Biomass	*	*	*	ns	*	ns	*
Root Biomass	ns	*	ns	*	*	ns	ns
Total Biomass	*	*	ns	ns	*	ns	*
LA	*	*	ns	ns	*	ns	*
Shoot/Root	*	*	*	ns	*	ns	ns
RGR	*	*	ns	ns	ns	ns	ns
NAR	*	ns	*	*	*	ns	ns
LAR	ns	*	ns	*	*	ns	ns
SLA	ns	*	ns	ns	ns	ns	ns
LWR	*	*	ns	*	*	ns	ns
SWR	*	*	*	ns	*	ns	ns
RWR	*	*	*	*	*	*	ns
Leaf Biomass$_0$	*	-	*	-	ns	-	-
Stem Biomass$_0$	*	-	*	-	*	-	-
Root Biomass$_0$	*	-	ns	-	ns	-	-
Total Biomass$_0$	*	-	*	-	ns	-	-
LA_0	*	-	*	-	ns	-	-
Ψ_w	ns	*	ns	ns	*	ns	ns
Ψ_o	ns	ns	ns	ns	ns	ns	ns
Ψ_p	ns	*	ns	ns	ns	ns	ns
LRWC	ns	*	ns	*	ns	ns	ns

contrary to expectations based on the higher *gs* in sorghum, we found that *CuTr over time* was lower in sorghum than in maize, regardless of the treatment (Fig. 2B). This divergence was promoted early in development, as maize exhibited significantly faster initial growth, accumulating five times greater biomass (*Total Biomass*$_0$) and developing a leaf area (LA_0) seven times larger than that of sorghum by 14 DAS (Table 2). This greater LA_0 in maize resulted in substantially higher *CuTr* during the early growth stages, despite its lower *gs* by the end of the experiment. In order to determine whether the differences in *gs* and *CuTr* values among species were also explained by stomatal anatomy, we analysed stomatal traits (Fig. 3A-B). We found *SD* and S to be affected by the species, but not by the water or environmental treatments (Fig. 3A-B and Table 1). Although maize was the species with the lowest *SD* under AC and FC, it also had the highest values for S (Fig. 3A-B). In fact, the average values for maize were 50 mm^{-2} for *SD* and 2.39×10^3 μm^2 for S, whereas these values were 77 mm^{-2} and 1.61×10^3 μm^2 respectively in sorghum (Fig. 3A-B). While *SD* remained unchanged when exposed to FC in both species, S increased by 29 % on both leaf surfaces in maize compared to AC (Fig. 3A-B). In contrast, in sorghum, only S_{AB} was increased by 46 % (Fig. 3A-B).

Upon exposure to FD, we found no variations in *gs*, A and Φ_{PSII} in maize compared to AD (Fig. 1A-C). On the contrary, stomatal closure was significantly increased in sorghum, with a *gs* value 46 % lower under FD compared to AD (Fig. 1A). Sorghum preserved similar A and Φ_{PSII} values to those observed under AD (Fig. 1B-C). Under AD and FD conditions, while the decreases in A and Φ_{PSII} in sorghum were 23 % and 15 % compared to AC, in maize these decreases were 63 % and 58 %, respectively (Fig. 1B-C). Thus, photosynthetic traits in maize were more affected by drought than those in sorghum. Drought was the determining factor causing variations in A and Φ_{PSII}, while the combination of the environmental CO_2 and temperature did not seem to have an influence (Fig. 1B-C and Table 1). Under AD and FD, *CuTr* in maize followed a similar trend to *gs*, but this did not occur in sorghum (Fig. 2A). FD led to greater transpiration in maize compared to AD, as indicated by the higher *CuTr over time* values under FD (Fig. 2B). Nevertheless, this did not appear to be influenced by stomatal traits, as none of them varied in maize, while in sorghum both S_{AD} and S_{AB} were significantly increased under FD by 51 % and 11 %, respectively (Fig. 3A-B).

3.2. *Sorghum exhibits enhanced growth under the combination of high CO_2 and high temperature due to improved water-use efficiency*

Both species showed statistically similar values for *WUE* regardless of the environmental and water treatment, even though the environment influenced *WUE* (Fig. 4 and Table 1). In fact, upon exposure to FC, there was a significant increase of approximately 46 % in *WUE* in maize and sorghum in comparison to AC (Fig. 4), likely attributed to the decreased *gs* and lower *CuTr* under FC (Fig. 1A and Fig. 2A-B). Despite maize exhibiting significantly greater early growth (14 DAS), with nearly five times higher *Total Biomass*$_0$ and seven times larger LA_0 compared to sorghum (Table 2), FC ultimately inhibited the growth of maize by 24 % (Fig. 5A-D) while increasing the biomass of aboveground organs in sorghum by 81 % (Fig. 5A-B and Fig. 5E). This particularly noticeable by the 122 % increase in *Stem Biomass*. Therefore, FC caused *Total Biomass* to significantly decrease from 16.05 g to 12.29 g in maize and promoted its increase from 7.91 g to 13.23 g in sorghum (Fig. 5D). The *Shoot/Root* ratio for maize remained relatively constant in both AC and FC conditions (Fig. 5F), whereas the *Shoot/Root* value in sorghum followed a similar trend to *Total Biomass* (Fig. 5D and Fig. 5F). We found no variations in *RGR* in maize nor sorghum when compared to AC (Fig. 6A). Nevertheless, our results revealed disparities in the response of maize and sorghum for *NAR* and *LAR* (Fig. 6B-C): on the one hand, these parameters remained unchanged in maize under FC compared to AC; on

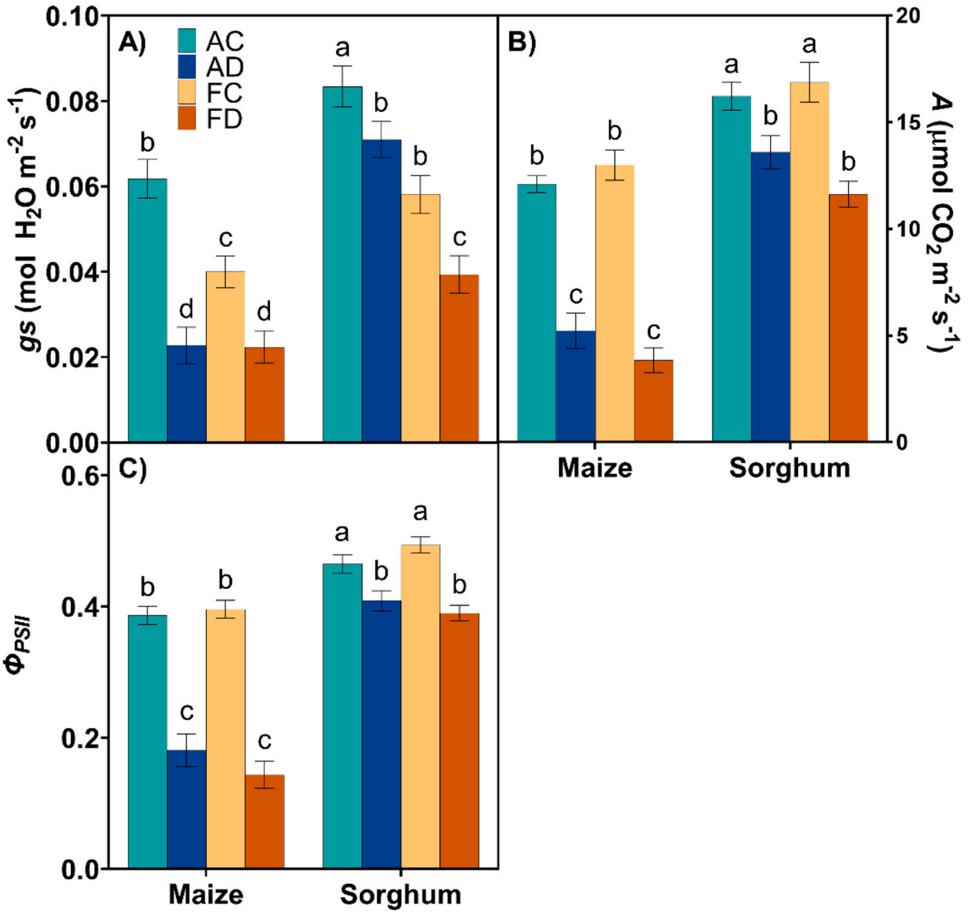

Fig. 1. Impact of ambient control (AC), ambient drought (AD), future control (FC) and future drought (FD) treatments on maize and sorghum at the end of the experiment (after drought treatments) in **A)** stomatal conductance (*gs*), **B)** net photosynthetic CO_2 assimilation (*A*) and **C)** quantum yield of PSII (Φ_{PSII}). The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. The treatments are represented as AC (light blue bars), AD (dark blue bars), FC (light orange bars) and FD (dark orange bars). Significantly different values ($p < 0.05$) are indicated by different letters.

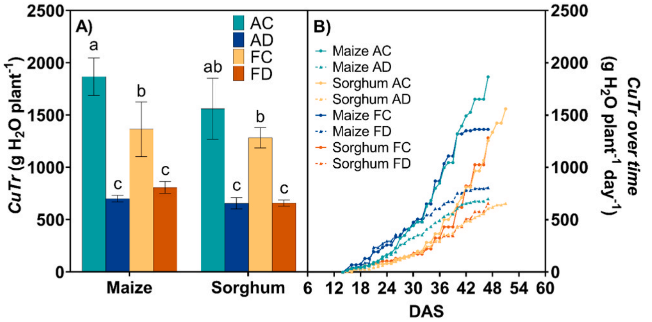

Fig. 2. Impact of AC, AD, FC and FD treatments on maize and sorghum at the end of the experiment (after drought treatments) in **A)** cumulative transpiration (*CuTr*) and **B)** cumulative transpiration per day (*CuTr over time*). The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1.

the other hand, *NAR* in sorghum was increased by 23 %, while *LAR* was decreased by 18 %.

3.3. Sorghum exhibits more efficient water management under future conditions as a result of an increased root system and higher water conservation

Upon examining the water consumption of both species under the drought treatments, we found that *SRWC* was reduced faster in maize, which exhibited a similar decrease regardless of the environmental treatment (Fig. 7). Overall, it took approximately 36 and 35 days in maize to reach 20 % under AD and FD, while in sorghum it took 41 and 39 for the same treatments (Fig. 7). In order to identify the differences in water consumption, we examined parameters related to water uptake in both species. On the one hand, maize reduced Ψ_w by 50 % when exposed to FD compared to AD and AC (Fig. 8A), while showing no variations in the resource allocation toward roots (Table 3). On the other hand, under FD sorghum exhibited similar Ψ_w values compared to AD (Fig. 8A). Sorghum allocated resources towards stems rather than roots under FD compared to AD (Table 3). In fact, in comparison to AD, when exposed to FD *SWR* was increased by 178 %, and *RWR* was decreased by 20 % (Table 3). Nevertheless, root growth under AD and FD was similar, and *RWR* remained 18 % higher compared to AC (Fig. 5C and Table 3). Ψ_p showed statistically similar values in maize between FD and AD (Fig. 8C). However, the reduction in Ψ_w and lack of effect on Ψ_o resulted in Ψ_p having 28 % and 42 % lower values under AD and FD, respectively, than under AC. In contrast, Ψ_p in sorghum remained unaffected regardless of the treatment (Fig. 8C and Table 1). As a result, while sorghum was capable of preserving adequate *LRWC* under both AD and FD compared to AC, maize exhibited significant reductions (Fig. 8D).

3.4. The combination of high CO_2 and temperature alleviates the negative impact of drought in the growth of sorghum

When comparing the growth under FD and AD, we found that maize showed no differences in the growth of any organ (Fig. 5A-D), whereas

Table 2
Effect of ambient control (AC), ambient drought (AD), future control (FC) and future drought (FD) treatments in leaf dry-weight (*Leaf Biomass$_0$*), stem dry-weight (*Stem Biomass$_0$*), root dry-weight (*Root Biomass$_0$*), total dry-weight (*Total Biomass$_0$*) and leaf area (*LA$_0$*) at days 0 of drought treatment. The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1.

Plant type	Treatment	*Leaf Biomass$_0$* (g DW)	*Stem Biomass$_0$* (g DW)	*Root Biomass$_0$* (g DW)	*Total Biomass$_0$* (g DW)	*LA$_0$* (cm^2)
Maize	AC	0.106 ± 0.020^{b}	0.057 ± 0.009^{b}	0.098 ± 0.014^{a}	0.260 ± 0.043^{b}	30.78 ± 4.64^{b}
	FC	0.181 ± 0.031^{a}	0.119 ± 0.022^{a}	0.123 ± 0.032^{a}	0.423 ± 0.073^{a}	53.96 ± 9.33^{a}
Sorghum	AC	0.020 ± 0.003^{c}	0.011 ± 0.002^{c}	0.017 ± 0.003^{b}	0.047 ± 0.006^{c}	4.86 ± 0.67^{c}
	FC	0.032 ± 0.003^{c}	0.021 ± 0.002^{c}	0.034 ± 0.004^{b}	0.087 ± 0.008^{c}	7.64 ± 0.67^{c}

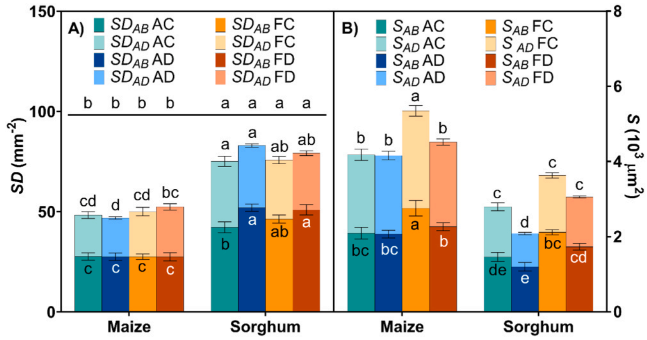

Fig. 3. Impact of AC, AD, FC and FD treatments on maize and sorghum at the end of the experiment (after drought treatments) in **A)** stomatal density (*SD*) and **B)** stomatal size (*S*). Ambient conditions are represented by blue colour palettes, while future conditions use orange palettes. Within each palette, lighter shades denote adaxial stomata for control treatments (AC, FC) and slightly darker shades indicate abaxial stomata for these controls. For drought treatments (AD, FD), vivid shades represent adaxial stomata and the darkest shades correspond to abaxial stomata. The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1.

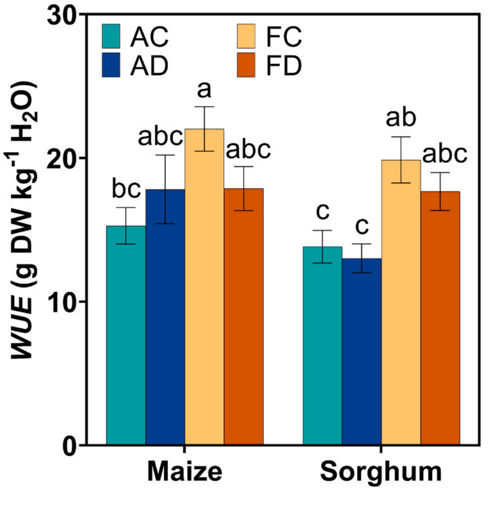

Fig. 4. Impact of AC, AD, FC and FD treatments on maize and sorghum at the end of the experiment (after drought treatments) in water-use efficiency (*WUE*). The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1.

sorghum significantly promoted *Stem Biomass* by 114 % under FD (Fig. 5B). When exposed to FD, the growth of sorghum was slightly enhanced, as *Total Biomass* reached values statistically intermediate between AC and AD (Fig. 5D). However, as occurred with *WUE*, when analysing the data of each species individually, we found the growth in sorghum under FD to be statistically similar to that under AC, but not under AD. *RGR* showed no variations between AD and FD in either maize or sorghum, and was decreased by 23 % and 14 % in maize and sorghum, respectively, compared to AC values (Fig. 6A). Although *NAR* and *LAR* in maize under AD and FD showed no variation, in sorghum *NAR* was slightly (but not significantly) increased, and *LAR* was significantly decreased by 12 % due to the decrease in *SLA* (Fig. 6B-D).

4. Discussion

4.1. High CO$_2$ and high temperature enhance sorghum transpiration efficiency and growth while limiting maize growth

Despite higher stomatal conductance, sorghum's smaller stomata optimised gas exchange and minimised water loss, allowing greater stem biomass accumulation under future conditions. Enhanced early growth of maize was offset by worse water management, respiratory costs and thermal stress, leading to comparable final biomass among maize and sorghum but with divergent physiological responses.

High CO$_2$ and high temperature increase stomatal closure and decrease transpiration in C$_4$ species (Al-Salman et al., 2023; Leakey, 2009; Martínez-Goñi et al., 2023b; Wand et al., 1999). Decreased *gs* under these conditions decreased *CuTr* in maize, but not in sorghum (Figs. 1, 2). Despite higher steady-state *gs*, sorghum had lower *CuTr* throughout its early growth, matching maize only by the end of the experiment. Greater leaf area of maize compared to sorghum during early stages under FC (Table 2) explained its higher *CuTr* despite lower *gs* at the end of the experiment. As leaf area of both species became more comparable (Fig. 5E), *CuTr* values converged as the plants grew, emphasising that leaf morphology determines *CuTr*. High densities of smaller stomata as in sorghum (Fig. 3) can optimise gas exchange by providing a shorter diffusion path, thereby improving transpiration efficiency and reducing water loss while preserving high CO$_2$ uptake (De Boer et al., 2012; Drake et al., 2013; Franks and Farquhar, 2007). These results partially confirm our first hypothesis, as the high CO$_2$ and high temperature improved stomatal regulation in sorghum but not in maize, leading to improved water preservation in sorghum.

However, no significant effect on *A* was observed in either maize or sorghum compared to ambient conditions (Fig. 1), partially refuting our first hypothesis. These results agree with Kim et al. (2007) and Ruiz-Vera et al. (2015) for maize, but not with Liu et al. (2022) for maize and Prasad et al. (2006), (2009) for sorghum, who reported increases in *A*. Different maize and sorghum varieties, and variations in growth stages, growth conditions and temperatures may explain these disparate results. Interestingly, the *gs* values observed in our study were lower than previously reported for maize and sorghum (Liu et al., 2022; Prasad et al., 2009, 2006), likely attributed to the lower light intensity of our growth chamber (400 µmol m^{-2} s^{-1}) compared to the higher light intensities in those studies (Han et al., 2023). Our *gs* values are consistent with those reported at similar PAR values (Correia et al., 2021; Vuralhan-Eckert et al., 2018; Wei et al., 2022). The lack of effect on *A* between environments may be linked to the diminishing positive effects of high temperature when maintained for long periods (between 40 and 50 days in our experiment). This could result from the C$_3$-like characteristics of young C$_4$ leaves, thermal acclimation of photosynthesis and/or the CO$_2$ saturation characteristics of C$_4$ photosynthesis, where high CO$_2$ mainly affects transpiration rather than assimilation (Ghannoum et al., 2000; Prasad et al., 2009). This is consistent with the higher intercellular CO$_2$

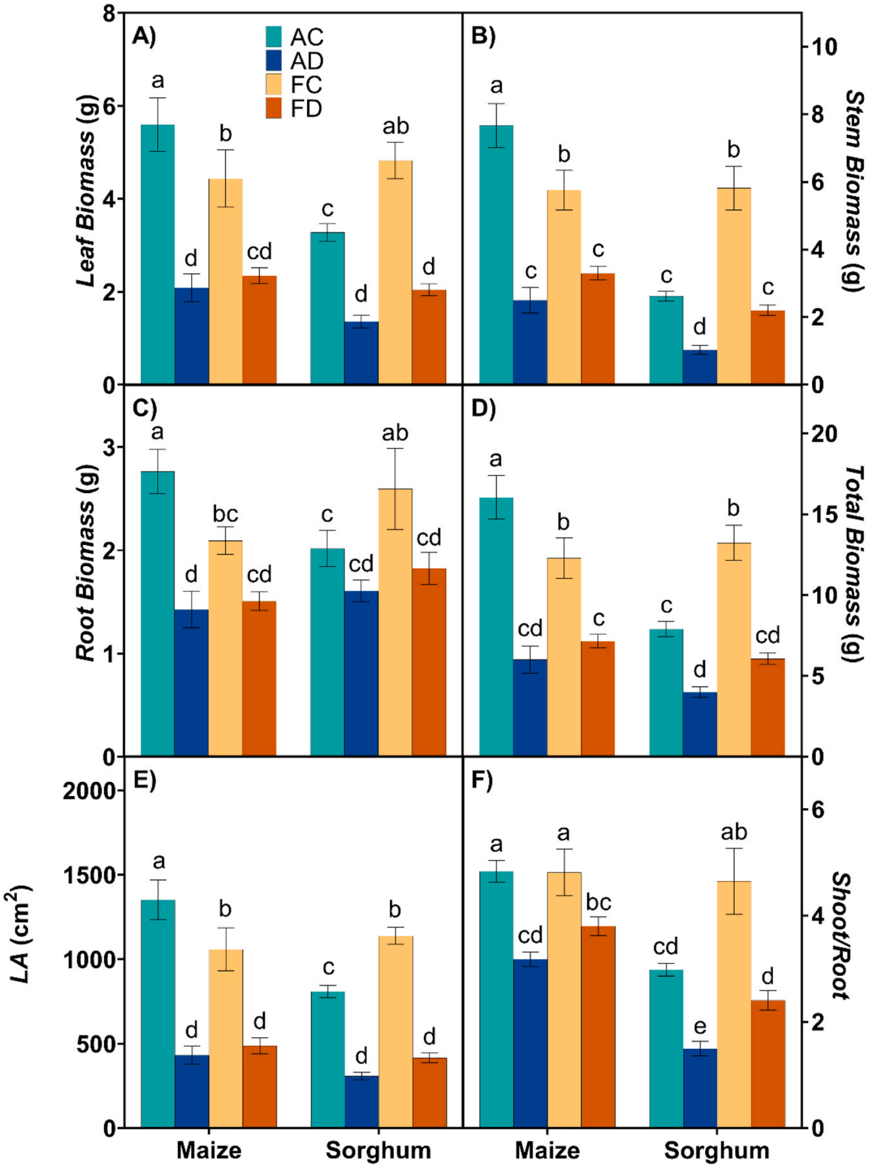

Fig. 5. Impact of AC, AD, FC and FD treatments on maize and sorghum at the end of the experiment (after drought treatments) in **A)** leaf dry-weight (*Leaf Biomass*) **B)** stem dry-weight (*Stem Biomass*), **C)** root dry-weight (*Root Biomass*), **D)** total dry-weight (*Total Biomass*), **E)** leaf area (*LA*) and **F)** *Shoot/Root*. The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1.

in both plants (Sup Fig. 1). Nevertheless, the variability in *gs* and *A* responses across studies makes it challenging to arrive at definitive conclusions regarding the response of C_4 photosynthesis. Therefore, further research on C_4 species is required to understand the interactive effects of high CO_2 and prolonged high temperature, with particular attention to plant developmental stage and leaf age.

The reductions in *gs* but lack of effect on *A* under high CO_2 and high temperature promoted water-use efficiency at the whole-plant level (*WUE*) in both maize and sorghum (Fig. 4). Nonetheless, only sorghum growth increased, whereas maize growth greatly decreased (Fig. 5). High temperature can limit maize growth, both alone and in combination with high CO_2, as high VPD restricts maize leaf expansion (Salah and Tardieu, 1997, 1995), while high temperatures increases respiration rates (Liu et al., 2022). In contrast, the higher optimal temperature for sorghum vegetative growth intensified the growth-enhancing effect of high CO_2 (Al-Salman et al., 2023; Martínez-Goñi et al., 2023b; Prasad et al., 2009). Specifically, sorghum increased the production of new leaf dry matter (*NAR*), enhancing resource allocation towards aboveground organs, particularly the stem, thereby accumulating more biomass (Fig. 6 and Table 2). Higher *NAR* under high CO_2 and high temperature but lack of effect on *A*, suggests a decrease in respiration (Atkin et al., 2006), potentially explaining the reported biomass gain in sorghum (Pérez-López et al., 2013). Adjustments in respiration rates rather than changes in photosynthesis likely account for growth inhibition in maize and growth promotion in sorghum. These findings partially confirm our first hypothesis, as maize growth was indeed inhibited, but also partially refute it.

Interestingly, although both maize and sorghum achieved similar biomass by the end of the experiment, during early stages under future conditions maize had 5-fold more biomass (Table 2). However, both species had similar *RGR* under these conditions (Fig. 6), likely because the combined effect of high CO_2 and high temperature typically promotes early growth, but its impact diminishes at later stages (Morison and Lawlor, 1999; Zhang et al., 2013). However, this fast-growth strategy in maize caused high water consumption, contributing to growth inhibition at later stages. In contrast, the more conservative developmental strategy of sorghum prioritised root development and water preservation over early aboveground growth. The delayed but

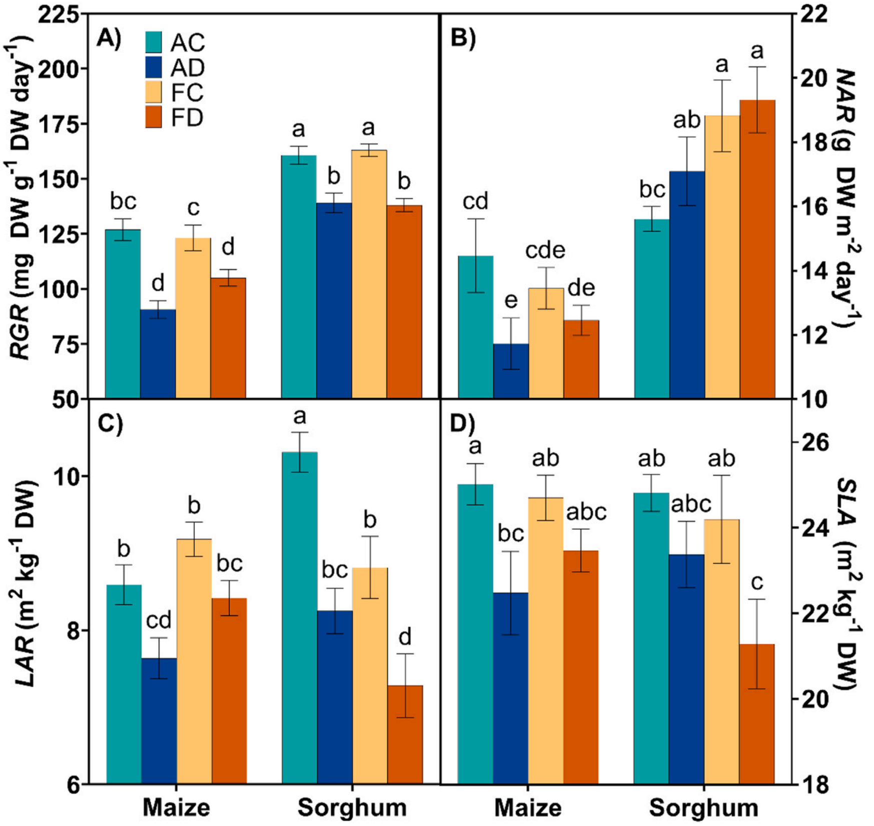

Fig. 6. Impact of AC, AD, FC and FD treatments on maize and sorghum at the end of the experiment (after drought treatments) in **A)** relative growth rate (*RGR*), **B)** net assimilation rate (*NAR*), **C)** leaf area ratio (*LAR*) and **D)** specific leaf area (*SLA*). The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1.

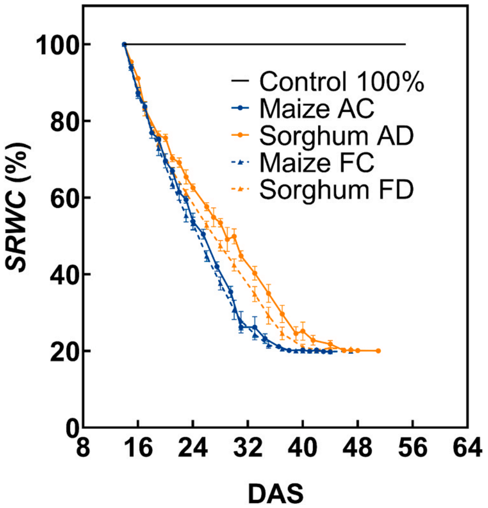

Fig. 7. Impact of AD and FD treatments in soil relative water content (*SRWC*) over time in maize and sorghum. Solid lines represent AD treatments, while dashed lines represent FD treatments. Red circles represent results for maize, while and orange triangles represents results for sorghum. Black line is used to represent control treatment (AC and FC). The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1. DAS means days after sowing.

sustained biomass accumulation in sorghum, supported by its improved transpiration efficiency, stomatal traits and root development, may provide a physiological advantage under prolonged stress conditions.

4.2. Greater effects of high CO$_2$, high temperature and drought on maize photosynthesis than sorghum

Although C_4 species can concentrate CO$_2$, stomatal closure of droughted plants decreased CO$_2$ diffusion to the mesophyll cells and limited photosynthesis, thereby inhibiting *NAR* in maize (Fig. 6). In contrast, drought did not decrease sorghum *NAR*, indicating that *A* was not as affected as in maize (Fig. 1). Even though these conditions decreased sorghum *gs*, *A* was similar to ambient drought, with less impact compared to well-watered conditions and to maize. The higher density of smaller stomata in sorghum (Fig. 2) allows more rapid and efficient regulation of stomatal aperture in water-demanding environments, reducing exposure to transient leaf water deficits (De Boer et al., 2012; Drake et al., 2013; Franks and Farquhar, 2007), and maximising CO$_2$ diffusion into the leaves despite increased stomatal closure. On the contrary, maize had lower densities of larger stomata, increasing *CuTr* and decreasing *A* compared to sorghum despite having lower *gs*. Additionally, Φ_{PSII} in sorghum was consistent across treatments, suggesting an absence of photochemical limitations under drought, as opposed to maize. Thus effective photoprotective mechanisms in sorghum (such as non-photochemical quenching or antioxidant enzymes) could dissipate excess light energy and protect the photosynthetic apparatus from damage.

Reducing Ψ_w during drought allows plant water uptake to maintain turgor (Miranda-Apodaca et al., 2018). Maize Ψ_w and Ψ_o did not vary under ambient drought, but Ψ_w decreased under future drought (Fig. 8). Drought decreased maize *CuTr*, thereby mitigating the impact of drought (Blum, 2005) but with no increase in *WUE* (Fig. 4). While the enhanced drought tolerance mechanisms in sorghum mitigated the impact on photosynthetic processes compared to maize, the absence of improved *WUE* under future drought only partially confirms our second

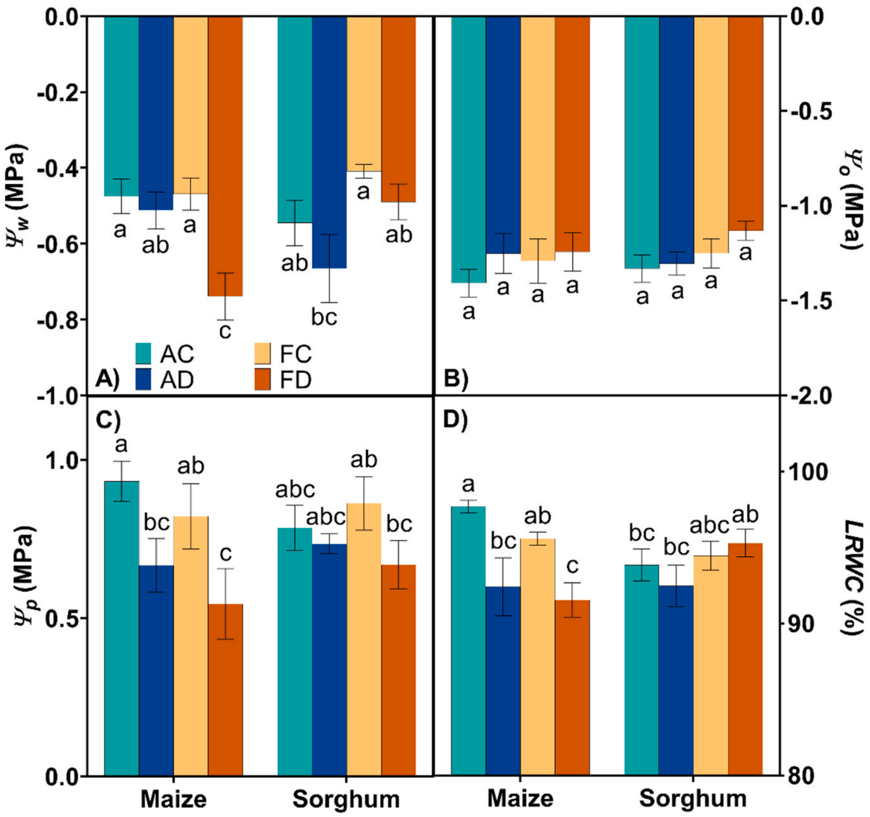

Fig. 8. Impact of AC, AD, FC and FD treatments on maize and sorghum at the end of the experiment (after drought treatments) in **A)** water potential (Ψ_w), **B)** osmotic potential (Ψ_o), **C)** pressure potential (Ψ_p) and **D)** leaf relative water content (*LRWC*). The mean ± S.E. is presented for each plant type and treatment, with a minimum of 6 replicates. Treatments and statistical analysis are described in Fig. 1.

Table 3
Effect of ambient control (AC), ambient drought (AD), future control (FC) and future drought (FD) treatments in leaf weight ratio (*LWR*), stem weight ratio (*SWR*) and root weight ratio (*RWR*). Growth conditions and statistical analysis are explained in Fig. 1. The mean ± S.E. is presented for each species and treatment, with a minimum of 6 replicates. Significant differences ($p < 0.05$) are indicated by different letters.

Plant type	Treatment	*LWR* (g DW g^{-1} DW)	*SWR* (g DW g^{-1} DW)	*RWR* (g DW g^{-1} DW)
Maize	AC	0.349 ± 0.010^{bc}	0.381 ± 0.007^{b}	0.199 ± 0.005^{f}
	AD	0.341 ± 0.008^{c}	0.330 ± 0.005^{c}	0.258 ± 0.006^{cd}
	FC	0.373 ± 0.008^{bc}	0.410 ± 0.006^{b}	0.206 ± 0.008^{ef}
	FD	0.353 ± 0.007^{bc}	0.402 ± 0.005^{b}	0.233 ± 0.006^{de}
Sorghum	AC	0.415 ± 0.005^{a}	0.310 ± 0.007^{c}	0.270 ± 0.006^{c}
	AD	0.348 ± 0.009^{bc}	0.248 ± 0.010^{d}	0.395 ± 0.016^{a}
	FC	0.375 ± 0.015^{b}	0.720 ± 0.042^{a}	0.221 ± 0.018^{ef}
	FD	0.343 ± 0.010^{c}	0.689 ± 0.021^{a}	0.317 ± 0.012^{b}

hypothesis. Despite the limited drought duration (7 days at 20 % field capacity), plants already experienced water scarcity as the soil dried from 100 % field capacity to 20 % field capacity (Fig. 7). As *WUE* may be higher earlier in the experiment, future studies should address the temporal dynamics of *WUE* across growth and leaf developmental stages. Nevertheless, maize was unable to maintain leaf water status, as *LRWC* decreased. Plant water content influences *LAR* which relies on the cell expansion capacity of various organs (Koch et al., 2019). Lower soil water availability decreased maize pressure potential (Ψ_p), which contributed to the inhibition of the leaf expansion rate and consequent reduction in *LAR* compared to well-watered conditions (Salah and Tardieu, 1997, 1995).

In contrast, decreased *LAR* in sorghum was explained by increased resource allocation to root development, with *RWR* increasing (Table 3). This resource allocation strategy allowed sorghum to expand its water-absorbing surface area and enhance soil penetration, increasing root water uptake compared to maize (Gargallo-Garriga et al., 2014). Overall, drought under ambient CO_2 and temperature decreased biomass production, especially in maize (Fig. 5 and Table 3), causing a more substantial decrease in *RGR* (Fig. 6). In contrast, drought effects under high CO_2 and high temperature were attenuated in sorghum. Response of C_4 species are limited by the trade-off between high stomatal conductance required for enhanced heat tolerance under high CO_2 and high temperature, and low stomatal conductance for a higher *WUE* for drought tolerance (Al-Salman et al., 2023). Drought was the predominant abiotic stress influencing growth in maize and sorghum when high CO_2, high temperature and drought were combined (Fig. 5), suggesting that drought tolerance might be a more fundamental factor shaping behaviour in C_4 species. Nonetheless, further validation using a broader panel of C_4 species and varieties is required to verify these trends.

Although our results confirm our third hypothesis, note that maize had greater growth than sorghum under ambient control conditions, which may have contributed to its more pronounced growth limitation when exposed to future drought stress. Furthermore, despite exhibiting similar growth, sorghum's physiological mechanisms coped with future drought conditions by maintaining improved photosynthetic performance and transpiration efficiency compared to maize. Analysing maize and sorghum data separately demonstrates greater reduction in *gs* under future drought significantly increased *WUE* and growth in sorghum compared to ambient drought, without these improvements occurring in maize. Although our research focused on the vegetative growth stage, high temperature and drought stress during this period can significantly decrease long-term growth and grain yield (Li et al., 2022; Prasad et al., 2021). We hypothesise that, the greater photosynthetic and water-use efficiency of sorghum compared to maize may persist into later developmental stages, potentially reducing stress impacts and improving performance during reproductive stage. Future studies should therefore explicitly test whether these vegetative stage advantages promote reproductive resilience and yield under the combination of high CO_2, high temperature and drought.

5. Conclusions

Sorghum outperformed maize by maintaining photosynthetic performance, improving transpiration efficiency and promoting growth under high CO_2 and high temperature, likely because the higher densities of smaller stomata in sorghum optimised CO_2 diffusion. Although both maize and sorghum increased *WUE* and achieved comparable biomass, growth was only promoted in sorghum. While sorghum increased *NAR* and potentially reduced respiration rates, higher maize leaf area during early growth increased transpiration and restricted leaf expansion and potentially increased respiration in maize at later stages, thereby limiting growth. Under high CO_2, high temperature and drought, the improved CO_2 uptake and the reduced exposure to transient leaf water deficits mitigated photosynthetic damage and prevented photochemical limitations in sorghum, unlike in maize. Similarly, while maize failed to maintain *LRWC*, sorghum maintained it by increasing root growth. Thus growth was limited more in maize than in sorghum. Our results also suggest that sorghum may respond more effectively to future environmental conditions than maize, as its improved photosynthetic performance and *WUE* could potentially persist and contribute to more stable yields. However, future research should confirm whether these advantages are preserved and help mitigate stress impacts during the reproductive stage ensuring stable yields.

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CRediT authorship contribution statement

Usue Pérez-López: Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization. **Jon Miranda-Apodaca:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Xabier Simón Martínez de Goñi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (developed by OpenAI) in order to improve readability and text flow. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2025.106210.

Data availability

The raw data supporting this study will be made available upon reasonable request.

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