


# *Sorghum bicolor* prioritizes the recovery of its photosynthetic activity when re-watered after severe drought stress, while manages to preserve it under elevated CO<sub>2</sub> and drought

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## Funding information

Eusko Jauriaritza, Grant/Award Number: GRUPO Gobierno Vasco-IT022-16

## Abstract

Understanding plant response and resilience to drought under a high CO<sub>2</sub> environment will be crucial to ensure crop production in the future. *Sorghum bicolor* is a C<sub>4</sub> plant that resists drought better than other crops, which could make it a good alternative to be grown under future climatic conditions. Here, we analyse the physiological response of sorghum under 350 ppm CO<sub>2</sub> (aCO<sub>2</sub>) or 700 ppm CO<sub>2</sub> (eCO<sub>2</sub>) with drought (D) or without drought (WW) for 9, 13 and 16 days; as well as its resilience under long (R1: 9D + 7R) or short (R2: 13D + 3R) recovery treatments. Sorghum showed elevated rates of *g<sub>s</sub>* under aCO<sub>2</sub> and WW, which resulted in a significant decrease in  $\Psi_w$ , *g<sub>s</sub>*, *E*,  $\Phi_{PSII}$ ,  $F_v'/F_m'$  when exposed to drought. Consequently, *A* was greatly decreased. When re-watered, both re-watering treatments prioritized *A* recovery by restoring photosynthetic machinery under aCO<sub>2</sub>, whereas under eCO<sub>2</sub> plants required little recovery since plant were hardly affected by drought. However, sorghum growth rate for aboveground organs did not reach control values, indicating a slower long-term recovery. Overall, these results provide information about the resilience of sorghum and its utility as a suitable candidate for the drought episodes of the future.

## KEYWORDS

C<sub>4</sub>, climate change, gas exchange, photochemistry, recovery, resilience

## Key points

- We evaluate the response and resilience of sorghum to drought combined with ambient and elevated CO<sub>2</sub>.
- Under elevated CO<sub>2</sub> sorghum managed to preserve plant water status and carbon assimilation.
- Sorghum is capable of initiating the recovery as soon as water is replenished.
- We foresee sorghum as a suitable crop to ensure future food safety.

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## 1 | INTRODUCTION

Since the 1960s, environmental CO<sub>2</sub> has been increasing exponentially all over the world. Currently, the concentration of the atmospheric CO<sub>2</sub> is of approximately 80% more than 60 years ago (<https://www.co2.earth>). Not only that, but it is also estimated that the CO<sub>2</sub> will continue rising until it reaches a concentration of 700 ppm by the end of the 21st century. This increase in the CO<sub>2</sub> will be accompanied by different environmental alterations, such as changes in the rainfall, causing extreme droughts in various regions of the planet, and increases in global temperatures, which will induce greater evaporation and expansion of arid regions (IPCC 2014). As a consequence, different territories of the planet that are not currently affected by drought are likely to be negatively impacted by water scarcity, limiting the amount of arable land as well as food production.

Drought is a complex abiotic stress that causes great damage to crops and limits their production (Boyer 1982). It has been reported that drought induces a wide variety of physiological changes within the plant, including a decrease in stomatal conductance (*g<sub>s</sub>*) to prevent transpiration and decrease the water loss. However, reduction in *g<sub>s</sub>* decreases the fixation of environmental CO<sub>2</sub>, increasing the production of ROS species and negatively affecting photosynthesis (Ahmad et al. 2018). Generally, these responses induce a substantial decrease in the biomass and yield, with their production being diminished circa 50% when subjected to severe drought stress (Samarah et al. 2009; Prasad et al. 2011; Paeßens et al. 2019). On the contrary, a CO<sub>2</sub>-enriched environment has been shown to alleviate to some extent the negative impact of drought by decreasing evapotranspiration and increasing soil water availability (Kimball 2016; Jin et al. 2017). However, most of the researches have focused on analysing the effect of high CO<sub>2</sub> and drought in C<sub>3</sub> plants, with fewer studies being conducted on C<sub>4</sub> plants (Ainsworth and Long 2020). Therefore, the combined effect of elevated CO<sub>2</sub> and water deficiency in C<sub>4</sub> plants is not widely understood.

Despite C<sub>4</sub> species account for only 3% of all plants, they represent almost a third of world production (Sage and Stata 2015), with maize, sorghum and millet accounting for the 40% of all produced cereals (FAOSTAT 2009–2019). In contrast with C<sub>3</sub> metabolism, C<sub>4</sub> metabolism allows plants to saturate Rubisco enzyme with CO<sub>2</sub> at low environmental CO<sub>2</sub> concentration, limiting the photorespiration process and promoting carbon fixation (Sage 2003). Previous researches showed that an environment enriched in CO<sub>2</sub> alone could increase the rates of CO<sub>2</sub> assimilation and thus increase the biomass of C<sub>4</sub> plants (Ghannoum et al. 2000). Nonetheless, significant increases in yield of C<sub>4</sub> plants induced by high CO<sub>2</sub> are not always observed, as this response could be dependent on several factors such as nutrition, light intensity and water supply (Ghannoum et al. 2000). However, stimulation of biomass and yield in C<sub>4</sub> plants has been observed when high CO<sub>2</sub> is combined with drought stress, since elevated CO<sub>2</sub> could increase soil water disposal by reducing transpiration and improving water use efficiency (Ottman et al. 2001; Tari et al. 2013; Manderscheid et al. 2014; Ainsworth and Long 2020).

In this respect, under future climate change conditions periods of water scarcity are expected to be alternated with rainfalls, pointing out the importance of understanding the resilience of plants to drought conditions. Some researchers reported that rehydration was enough to recover control values of some physiological traits (Dinh et al. 2019), even to enhance the effect to those of control conditions (Gallé et al. 2007). However, other researchers observed that plants were unable to recover after being subjected to drought stress (Xu et al. 2009). The reason for these rather contradictory results, although unclear, might be found in the different factors that influence drought recovery, including plant species, nutrient availability and drought intensity (Gallé et al. 2007). C<sub>4</sub> plants have been reported to have a better response to the soil water stress than C<sub>3</sub> plants, although the majority of the researches including drought recovery in C<sub>4</sub> plants have been focused on grasslands (Hoover et al. 2014; Hamdani et al. 2019), paying little or no attention to food crops such as sorghum. Likewise, few researchers have addressed the question of knowledge regarding the resilience of C<sub>4</sub> crops to drought in a high CO<sub>2</sub> environment, which will be a determining factor for cultivars during the following years.

The present study has been performed in sorghum (*Sorghum bicolor* L. Moench), a C<sub>4</sub> plant and the fifth most cultivated cereal worldwide, with great importance as a staple food in different regions of developing countries in Africa and Asia (Chadalavada et al. 2021). Sorghum is capable of coping with drought better than other cereals that currently have greater relevance and therefore could be a good alternative to be cultivated under future adverse conditions (Yahaya and Shimelis, 2021). We hypothesize that sorghum will be a resilient crop to drought under elevated CO<sub>2</sub>, making it a good candidate to be sown under high CO<sub>2</sub> and water scarcity. Thus, the objective of our research was to determine whether sorghum is a suitable candidate to be sown in the future, by analysing its physiological response under (1) high CO<sub>2</sub> (700 ppm) versus ambient CO<sub>2</sub> (350 ppm), (2) drought versus well-watered regime, (3) high CO<sub>2</sub> and drought versus ambient CO<sub>2</sub> and drought and (4) the ability of sorghum to recover from drought stress under both, ambient and high CO<sub>2</sub> environments.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material and growth conditions

Sorghum (*Sorghum bicolor* L. Moench) PR8500 seeds (Pioneer Hi-Bred International, Inc.) were sown and grown in 2.5 L pots containing a mixture of perlite: vermiculite (3:1, v/v) in a Conviron E15 (Conviron, Manitoba, Canada) controlled environmental chamber. Plants were grown under a daily regime of 14 h of light and 10 h of darkness, with a temperature of 24/20 °C and a relative humidity of 60/80% for day and night. Photosynthetically active radiation (PAR) was of 400 μmol m<sup>-2</sup> s<sup>-1</sup> under light period, with light supplied by a combination of incandescent bulbs and warm-white, fluorescent lamps (Sylvania F48T12SHO/VHO). Growth chambers were supplied with an environmental concentration of 350 ppm (ambient, aCO<sub>2</sub>) or 700 ppm (elevated, eCO<sub>2</sub>) CO<sub>2</sub> throughout the trial period.

Sorghum pots were watered three times per week with a complete Hoagland solution (Arnon and Hoagland 1940). After 18 days of growth, the drought treatment was imposed to half the plants by withholding water (van Rensburg and Kruger 1993) for 9, 13 and 16 days (mild, 9D; moderate, 13D; and severe, 16D). After 9D and 13D, re-watering treatments started. The well-watered (WW) plants served as control group. The effect of drought was analysed at the end of each drought period, while water recovery was analysed after applying 7 days of re-watering to 9 days droughted plants (R1), and 3 days after re-watering 13 days droughted plants (R2). A graphic representation of the applied treatments could be found in the Figure S1.

## 2.2 | Water parameters

Plant water parameters were analysed as described in Robredo et al. (2007). Briefly explained, relative soil water content (RSWC) was determined as  $RSWC = 100 [(SFW - SDW)/(SFW_i - SDW)]$ , where SFW, SDW and  $SFW_i$  were the soil fresh weight, soil dry weight oven-dried for 72 h at 105°C and the initial soil fresh weight, respectively (Epron 1997). 100% water content for soil at field capacity was assumed.

Leaf water potential ( $\Psi_w$ ) was measured using the Scholander pressure-equilibration technique (Scholander et al. 1965). For determining leaf osmotic potential ( $\Psi_o$ ), freezing point of sap of leaf segments was analysed using an Osmomat 030 osmometer (Gonotec GMBH). Osmotic potential values were calculated as  $\Psi_o = M \times T \times 0.00832$ , where M was the concentration (osmol), and T was the temperature (K) of the sample (Wyn & Gorham, 1983). Pressure potential ( $\Psi_p$ ) was calculated as the difference between  $\Psi_w$  and  $\Psi_o$ .

## 2.3 | Gas exchange parameters

Net photosynthetic  $CO_2$  assimilation (A) and instantaneous transpiration (E) were measured in the first full expanded intact leaf, using a Li-Cor 6400-02B (Li-Cor Inc., Lincoln, NE, USA) programmable, open-flow portable photosystem system as described in Robredo et al. (2007). The temperature of the cuvette was held at 24 °C at a relative humidity of 60%, while the  $CO_2$  concentration of the cuvette was the same as that of the growth conditions. Measurements were made 3 h after dawn under a photosynthetic flux density of  $400 \text{ mmol m}^{-2} \text{ s}^{-1}$ , provided by a red/blue LED light source (model LI 6400-02B, Li-Cor Inc.). Intercellular  $CO_2$  concentration (Ci) and stomatal conductance (gs) were calculated from A and E according to von Caemmerer and Farquhar (1981).

## 2.4 | Fluorescence parameters

Chl-*a* fluorescence was measured as described in González-Moro et al. (2003) using a OS5-FL modulated fluorometer (Opti-Sciences,

Inc.). Photochemical parameters were measured as described in Robredo et al. (2010). Briefly, maximum yield of PSII ( $F_v/F_m$ ) was calculated as  $[F_v/F_m = (F_m - F_o)/F_m]$ , where  $F_o$  was the background fluorescence signal and  $F_m$  the dark-adapted maximum fluorescence, respectively. The quantum yield of PSII ( $\Phi_{PSII}$ ) was calculated as  $(F'_m - F_s)/F'_m$ , where  $F_s$  refers to the variable fluorescence at steady state and  $F'_m$  to the maximum light-adapted fluorescence (Schindler and Lichtenthaler 1996). The photochemical (qP) and non-photochemical (NPQ) quenching parameters were calculated as  $qP = (F'_m - F'_s)/(F'_m - F'_o)$  being  $F'_o$  the minimum light-adapted fluorescence (Schindler and Lichtenthaler 1996) and  $NPQ = (F_m - F'_m)/F'_m$  (Demmig-Adams et al. 1997).

## 2.5 | Growth parameters

Sorghum plants were harvested and separated into leaves, stems and roots at days 9, 13 and 16 for all the treatments (WW, D or D+R). Leaf area (LA) was measured with a Li-Cor 6400-02B (Li-Cor Inc.). After LA determination, dry weight (DW) was determined by drying samples at 80°C for 48 h. Water use efficiency (WUE) was calculated as estimated dry mass accumulation between two harvests divided by transpiration per plant over the same period. Transpiration was calculated by the difference between evapotranspiration in each pot and evaporation in a pot without plants.

## 2.6 | Statistical analysis and figures

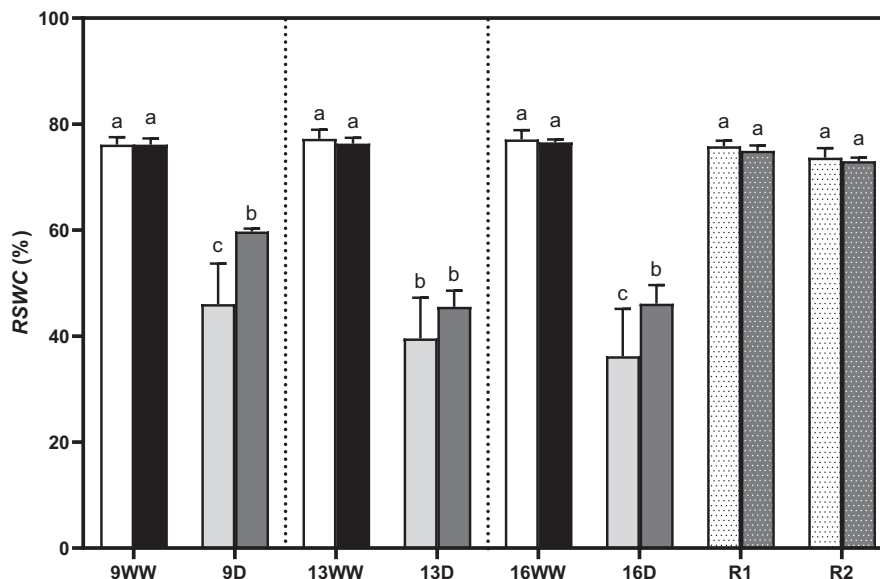
Results are shown as the mean  $\pm$  standard error of three independent experiments with at least three replicates (plants) from different pots measured per experiment. Figures were performed using GraphPad Prism version 8.0.2 for Windows. Data analyses were performed using the SPSS 20.0 software package. Two-way analysis of variance was used to evaluate the effects of drought,  $CO_2$  and their interaction with all dependent variables. The means were compared using Duncan's multiple range tests. *p* values  $\leq 0.05$  were considered statistically significant.

# 3 | RESULTS

## 3.1 | Soil and plant water status

Drought reduced RSWC by 47% and 33% under control and  $eCO_2$  conditions, respectively (Figure 1), and it was stabilized at 46% after 13D in  $eCO_2$ , reaching the same values as the ones of  $aCO_2$  after 9D. When analysing the impact of the  $CO_2$  concentration in the RSWC after recovery, similar values to WW conditions were obtained, irrespective of the drought and subsequent recovery days (Figure 1).

No differences were detected in the  $\Psi_w$  when grown under  $eCO_2$  or  $aCO_2$  and WW conditions, whereas throughout the 16 days of drought,  $\Psi_w$  had a progressive decrease in  $aCO_2$  conditions, reaching



**FIGURE 1** Effect of ambient and elevated CO<sub>2</sub> in combination with well-watered (WW), drought (D) and recovery (R) conditions on relative soil water content (RSWC). Days under drought and recovery are indicated in the x-axis. Sorghum was grown under the following conditions: WW + 350 ppm CO<sub>2</sub> (white bar), WW + 700 ppm CO<sub>2</sub> (black bar), D + 350 ppm CO<sub>2</sub> (light grey bars), D + 700 ppm CO<sub>2</sub> (dark grey bars), R + 350 ppm CO<sub>2</sub> (white bars with black dots) and R + 700 ppm CO<sub>2</sub> (grey bars with white dots). R1 and R2 refer to long (9D + 7R) and short (13D + 3R) recovery treatments, respectively. Each bar represents the mean ± S.E. of 3 replicates. For each measurement day, thus 9, 13 and 16, different letters are used to represent values significantly different at  $p \leq .05$

values of  $-0.85$  MPa after 16D (Figure 2a). However, at eCO<sub>2</sub> this reduction was stabilized approximately at  $-0.46$  MPa after 13D. Regardless of the recovery treatment, re-watering caused an increase in the  $\Psi_w$  values until they reached well-watered values under both, aCO<sub>2</sub> and eCO<sub>2</sub> (Figure 2a).

$\Psi_o$  was significantly affected by the interaction of drought and measurement day (Table 1), while there were no significant differences in the well-watered  $\Psi_o$  under aCO<sub>2</sub> and eCO<sub>2</sub> (Figure 2b). Under eCO<sub>2</sub> and drought,  $\Psi_o$  exhibited no significant variations, while under aCO<sub>2</sub> at 16D it decreased by 22% when compared to WW.

Sorghum with well-watered irrigation regime conserved similar levels of  $\Psi_p$  regardless of the environmental CO<sub>2</sub> concentration (Figure 2c), while mild and severe drought significantly decreased it. After 13D,  $\Psi_p$  dropped by 37.4% and 24.1% at aCO<sub>2</sub> and eCO<sub>2</sub>, respectively, and remained virtually unchanged for 16D. Both re-watering treatments were enough to recover well-watered values of  $\Psi_p$  regardless of the environmental CO<sub>2</sub>.

### 3.2 | Gas exchange parameters

We found  $g_s$  and  $E$  to be 50% and 43% lower under eCO<sub>2</sub> conditions when compared to aCO<sub>2</sub> (Figure 3a,b). When drought was applied under aCO<sub>2</sub>,  $g_s$  and  $E$  were reduced by 32% and 27% in comparison with the corresponding controls. Re-watering treatments increased  $g_s$  by 35% and 19% and  $E$  by 33% and 17%, which was enough to reach 16WW values. On the contrary, under eCO<sub>2</sub>,  $g_s$  and  $E$  values were preserved regardless of the treatment, although R1 managed to significantly increase  $E$  in 91%.

Overall, A under eCO<sub>2</sub> showed similar values than under aCO<sub>2</sub> for control conditions (Figure 3b). However, we observed decreases of 35%, 36% and 41% in A under aCO<sub>2</sub> for 9D, 13D and 16D in comparison to their controls, while under eCO<sub>2</sub> it was not affected. In contrast to 16D, re-watering increased A approximately 35% and 19% under aCO<sub>2</sub> conditions after R1 and R2, respectively. However, these values were 21% and 30% lower than the well-watered values for the same day.

The  $C_i$  remained constant throughout 9WW-16WW and aCO<sub>2</sub>, with an average value of 157 ppm CO<sub>2</sub> (Figure 3c), while under eCO<sub>2</sub>  $C_i$  values had an average value of 274 ppm CO<sub>2</sub> for the same period. The detected values under drought and recovery treatments were the same as those under well-watered treatment for both aCO<sub>2</sub> and eCO<sub>2</sub>.

### 3.3 | Photochemistry

We found no significant effect of environmental CO<sub>2</sub> nor water treatments on  $F_v/F_m$  and  $qP$  (Figure 4a and Figure 4c).

$\Phi_{PSII}$  showed similar values after 9WW-16WW treatments for aCO<sub>2</sub> and eCO<sub>2</sub> (Figure 4b). However, after 16D  $\Phi_{PSII}$  was reduced in 24%, while under eCO<sub>2</sub> it remained constant. Nevertheless, both re-watering treatments increased  $\Phi_{PSII}$  values and reached 16WW values, which were 26% and 31% higher than after 16D.

eCO<sub>2</sub> showed similar  $F_v'/F_m'$  values to aCO<sub>2</sub> for 9WW-16WW treatments, and they were preserved under drought (Figure 4d). On the contrary, after 16D,  $F_v'/F_m'$  was significantly reduced in aCO<sub>2</sub> in comparison to 16WW values. After applying the R1 and R2 recovery

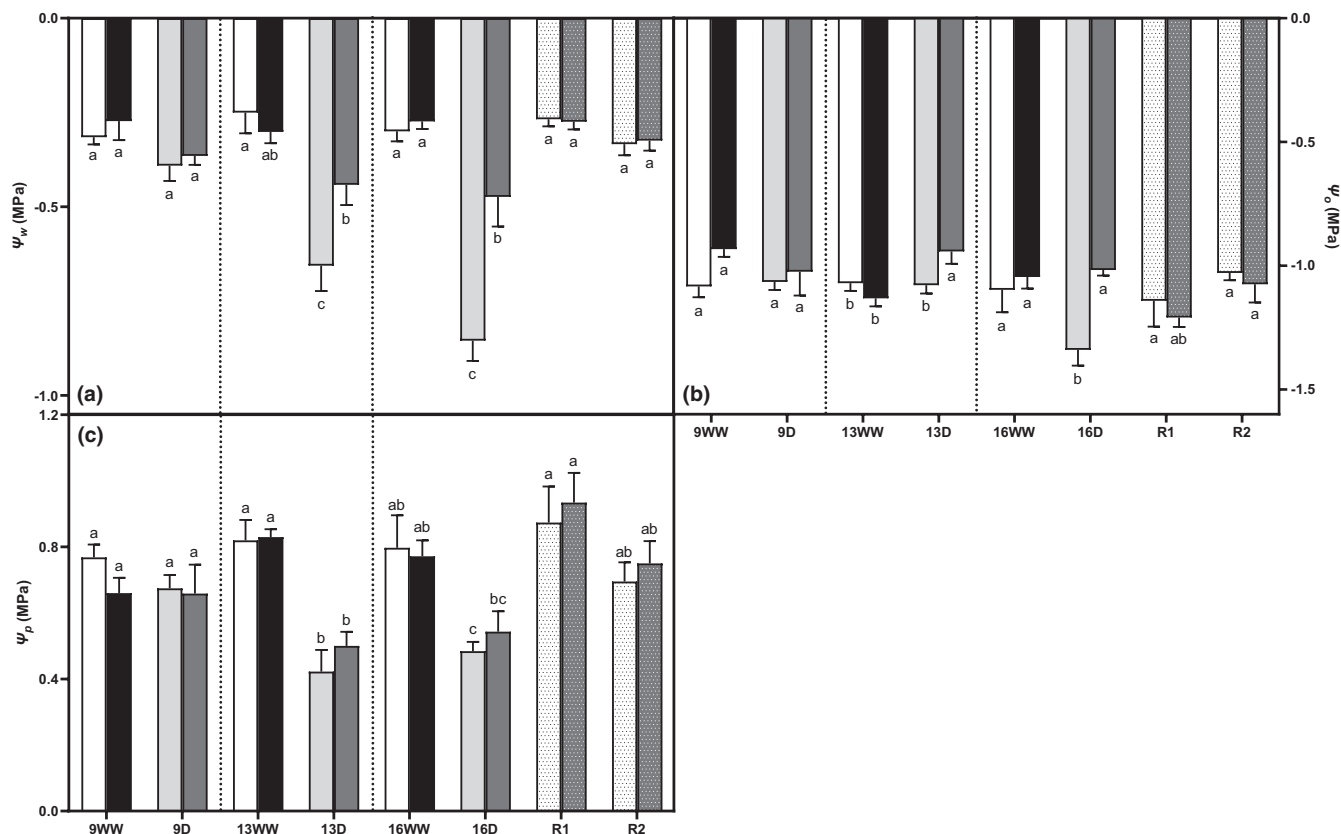


FIGURE 2 Effect of ambient and elevated CO<sub>2</sub> in combination with WW, D and R conditions on (a) water potential ( $\Psi_w$ ), (b) osmotic potential ( $\Psi_o$ ) and (c) pressure potential ( $\Psi_p$ ). Graphic labels, growth conditions and statistical analysis are explained in Figure 1

treatments,  $F_v/F_m'$  reached values 10% and 14% higher than the values at 16D under aCO<sub>2</sub> conditions and recovered control values.

Under aCO<sub>2</sub> the NPQ was increased progressively from 0.344 and 0.426 for 9WW and 9D to 0.518 and 0.641 for 16WW and 16D, respectively (Figure 4e). Nevertheless, under eCO<sub>2</sub>, NPQ remained almost unchanged and was approximately 25%, 31% and 50% lower than under aCO<sub>2</sub> for 9WW-16WW. Likewise, under drought, NPQ was 30%, 36% and 48% lower in the presence of eCO<sub>2</sub> than under aCO<sub>2</sub>. When compared to 16D values, R1 and R2 recovery treatments reduced NPQ in approximately 30% and 28% under aCO<sub>2</sub>, reaching similar values to 16WW.

### 3.4 | Growth parameters

Regardless of the water treatment, the DW of well-watered leaves, stems and roots was slightly higher under eCO<sub>2</sub>, although this difference was not statistically significant (Table 2). In comparison to WW, we observed a drop in the DW of leaves of 55%, 57% and 68% for the days 9D, 13D and 16D under aCO<sub>2</sub>, whereas in stems this decrease was of 44%, 52% and 60%. Similarly, under eCO<sub>2</sub>, drought caused a decrease of 41%, 51% and 60% in leaves and 31%, 42% and 56% in stems for the same period. The aboveground biomass decrease under drought was also related to a decrease in LA, which was on average 66% and 54% lower under aCO<sub>2</sub> and eCO<sub>2</sub>,

respectively (Table 2). On the contrary, the growth of roots was larger under drought conditions, irrespective of the environmental CO<sub>2</sub>. In comparison with the well-watered regime, the DW of roots was on average 50% and 43% higher under control and eCO<sub>2</sub>, respectively (Table 2). After 16D, an increase in DW of 21% was observed under eCO<sub>2</sub> compared with aCO<sub>2</sub> conditions. Likewise, the observed trend for each organ individually was reflected in total DW, with eCO<sub>2</sub> showing a slightly but non-significant increases throughout the growth period (Table 2). Compared with well-watered conditions, the average growth under drought was 27% and 23% lower under aCO<sub>2</sub> and eCO<sub>2</sub>, respectively.

Under aCO<sub>2</sub>, R1 treatment increased DW of leaves by 69% in comparison with 16D, although this value was still 47% lower than under 16WW (Table 2). Stems were not significantly influenced by recovery treatments and showed similar values to 16D regardless of environmental CO<sub>2</sub> (Table 2). On the contrary, under eCO<sub>2</sub>, both recovery treatments had a significant impact in the DW of roots (Table 2): R2 decreased root DW by 23%, reaching 16WW values; while R1 treatment decreased root growth by 44%, with values significantly lower than after 16WW. In the case of total DW, none of the recovery treatments had a significant impact (Table 2). Regarding LA, none of the recovery treatments caused a significant increase, except for the observed 70% increase after R1 under aCO<sub>2</sub> (Table 2). However, it was not sufficient to recover the well-watered values.

TABLE 1 Summary of the analysis of variance (ANOVA)

	Analysed variables																			
	RSWC	$\Psi_w$	$\Psi_o$	$\Psi_p$	Gs	A	Ci	E	WUE	$F_v/F_m$	$\Phi_{psII}$	qP	$F_v/F_m'$	NPQ	Leaf DW	Stem DW	Root DW	Total DW	LA	
CO <sub>2</sub>	*	***	**	-	***	***	***	***	***	***	***	*	***	***	**	**	*	***	***	-
Drought	***	***	-	***	***	-	***	***	***	**	**	-	**	**	***	***	***	***	***	***
Age	-	***	**	-	**	***	***	*	*	***	***	-	***	**	***	***	***	***	***	***
CO <sub>2</sub> +Drought	*	***	-	-	**	***	-	-	*	-	-	-	-	-	-	-	-	-	-	-
CO <sub>2</sub> +Age	-	-	***	-	*	***	***	-	-	***	*	-	***	**	-	-	-	-	-	-
Drought + Age	-	***	-	***	-	-	-	-	***	*	*	-	***	-	***	***	*	**	***	***

Note:  $p \leq .05$ ,  $p \leq .01$  and  $p \leq .001$  are represented with \*, \*\* and \*\*\*, respectively.

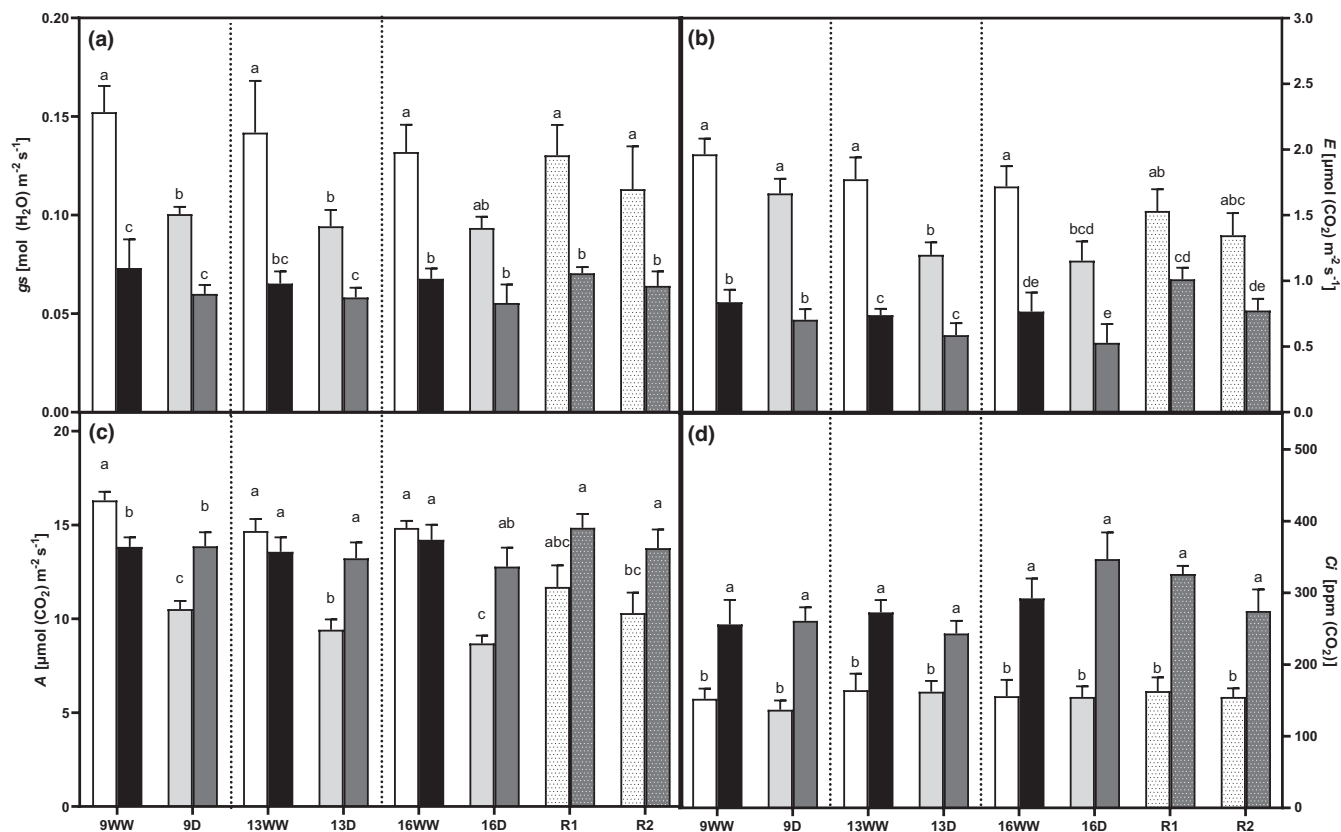
Total WUE was on average 85% higher in the presence of eCO<sub>2</sub> in contrast to the WUE under aCO<sub>2</sub> for the period 9WW-16WW (Table 2). Drought caused WUE to increase by 68%, 151% and 170% under aCO<sub>2</sub> and by 39%, 108% and 152% under eCO<sub>2</sub> for days 9D, 13D and 16D when compared to control values (Table 2). Among stressed plants, WUE was approximately 61%, 52% and 74% higher under eCO<sub>2</sub> than under aCO<sub>2</sub>. R1 treatment decreased WUE in 36% under aCO<sub>2</sub> when compared to 16D, while R2 had no significant effect (Table 2). Under eCO<sub>2</sub>, WUE was reduced in 50% and 20% after R1 and R2, respectively, even though only R1 was capable of reaching control values.

## 4 | DISCUSSION

Climate change is one of the main issues that will limit crop production during the following years, among others, due to the increases in drought episodes. However, drought episodes will be alternated with periods of rainfalls, and thus, understanding plant recovery to drought is one of the greatest challenges to ensure crop survival. *Sorghum bicolor* is considered to be a drought-tolerant crop, and thus has the potential to be widely cultivated under the future climate change conditions. Therefore, in this research we have analysed the resilience of sorghum to drought stress by applying two re-watering treatments (long, R1; short, R2) under different environmental CO<sub>2</sub> levels.

Preserving water when its availability is reduced is a determining factor to ensure plant survival under drought stress. In our study, we found  $\Psi_w$  to be significantly reduced after moderate and severe drought stresses, especially under aCO<sub>2</sub>.  $\Psi_w$  is indicative of the whole plant water status and its decrease is associated with reductions in leaf RWC (Levitt, 1980). However, in our case, leaf RWC was maintained regardless of the treatment (data not shown). This is possibly explained due to the higher concentration of water in the leaf as a consequence of the reported reduction in its biomass and surface (Table 2). Likewise, we observed a decrease in  $\Psi_o$  after severe drought under aCO<sub>2</sub>, but not under eCO<sub>2</sub>. Reductions in  $\Psi_o$  under drought could be explained as a consequence of accumulating solutes by dehydration and/or by inducing osmotic adjustment to maintain water uptake and metabolic activities (Ashraf and Ahmad 1998; Nieves-Cordones et al. 2019). Maintaining high  $\Psi_w$  is considered to be related to dehydration avoidance, and thus, its rapid recovery after the short re-watering treatment could be an indicative improved drought tolerance in sorghum. This would also explain the recovery of turgor in cells and the increases in  $\Psi_p$  as water flows inside cells.

High CO<sub>2</sub> has been shown to alleviate to some extent drought damage in plants by affecting different physiological parameters such as gas exchange and photochemical parameters (Wall et al. 2001). We found that gs and E showed significantly lower values under eCO<sub>2</sub> and remained constant regardless of the water treatment. Low gs and E values have been related to an improved the use of water resources, resulting in less water loss under drought and

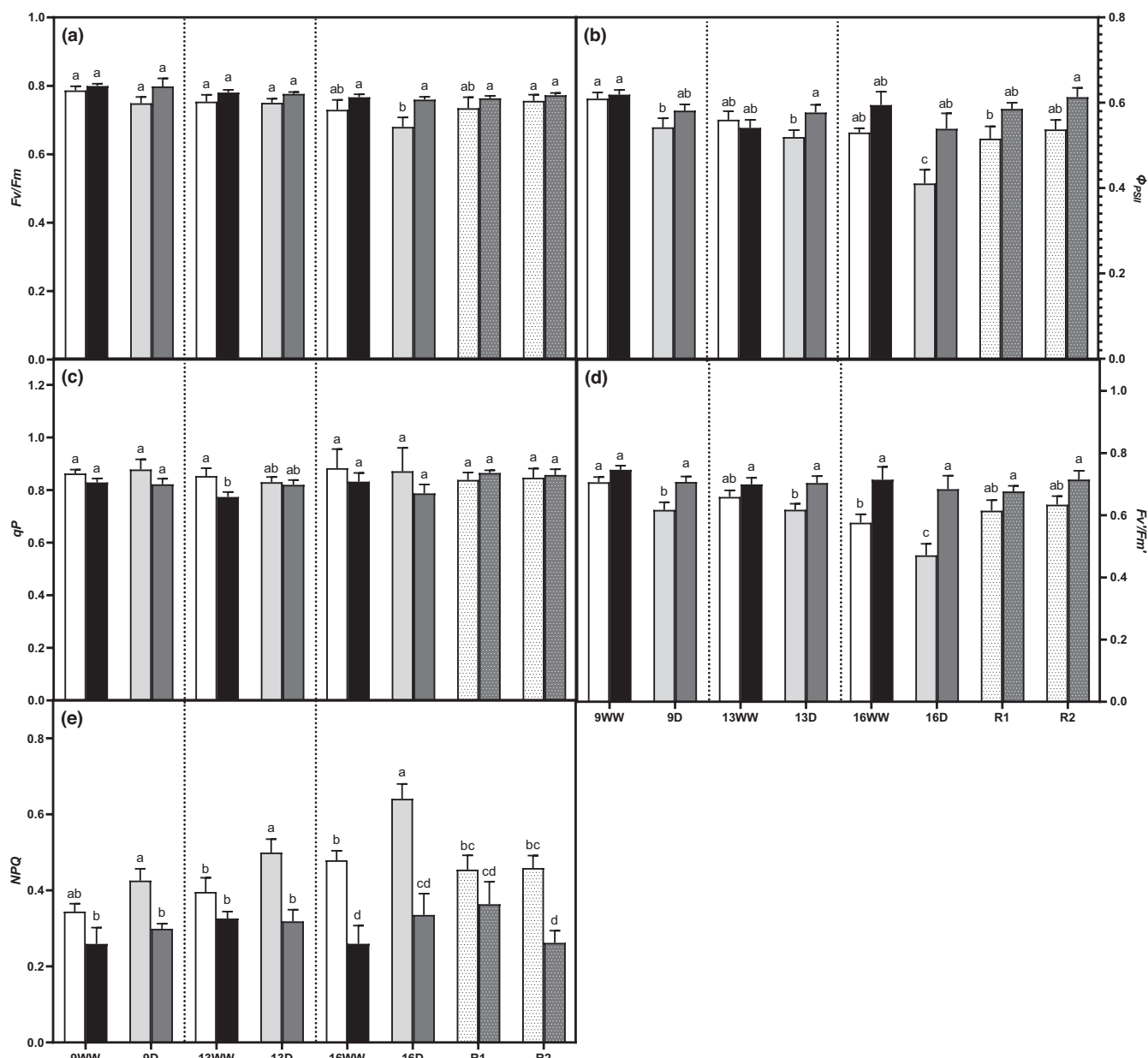


**FIGURE 3** Effect of ambient and elevated CO<sub>2</sub> in combination with WW, D and R conditions on (a) stomatal conductance (*gs*), (b) transpiration rate (*E*) (c) net photosynthetic CO<sub>2</sub> assimilation (*A*) and (d) intercellular CO<sub>2</sub> concentration (*ci*). Graphic labels, growth conditions and statistical analysis are explained in Figure 1

thus, making them desirable traits for crops (Medrano et al. 2002). This is supported by the observed higher values of *WUE* throughout the growth period under eCO<sub>2</sub>. On the contrary, under aCO<sub>2</sub> the observed higher ratios of *gs* and *E* were significantly reduced as the severity of the drought increased. As a consequence, sorghum plants could not preserve *A* under aCO<sub>2</sub>, and it was negatively affected. Decreases in photosynthesis under drought stress can occur by the restricted flux of CO<sub>2</sub> into the leaf caused by reductions in stomatal conductance or the inhibition of metabolic potential for photosynthesis (Lawlor and Tezara 2009). *Ci* remained unchanged through the whole experiment, indicating that the observed variations in *A* is mainly explained by stomatal limitations. When re-watered, sorghum induced a rapid stomatal opening and transpiration increasing under aCO<sub>2</sub>, as a way to recover control values of *A*. Reductions in *A* are related to decreases in plant growth. Therefore, its fast recovery after drought periods is essential for the full recovery of plant development and growth. Nevertheless, neither short nor long re-watering treatments recovered control values of *A*, indicating that the complete recovery of sorghum after the drought requires more than 7 days of re-watering. On the other hand, re-watering treatments had no overall impact in *gs*, *E* or *A* under eCO<sub>2</sub>. This is not particularly surprising given the fact that eCO<sub>2</sub> was capable of avoiding the adverse effects of drought as a result of improved use of water

resources. Thus, these results show that sorghum prioritizes the recovery of *A* when re-watered after a drought period, as well as its potential to be cultivated in environments that will be affected by drought in the future.

In relation to the photochemical parameters, we found  $\Phi_{PSII}$  to have higher values under eCO<sub>2</sub>, and it remained unchanged even in the presence of severe drought stress. This finding is in accordance with results obtained by Long and Drake (1991) and Habash et al. (1995) who observed increases in the  $\Phi_{PSII}$  under enriched CO<sub>2</sub>. In our case, increased  $\Phi_{PSII}$  is explained by a higher  $F_v'/F_m'$ , since  $qP$  remained constant regardless of the water treatments. Therefore, absorbed quanta were transported to the PSII reaction centres with improved efficiency by the greater proportion of light that reaches the electron transport, not by an increase in the use of light in photochemical processes. Additionally, we found drought stress to cause  $\Phi_{PSII}$  reduction under aCO<sub>2</sub>, suggesting a possible downregulation or structural alteration of the photosynthetic apparatus (Foyer et al. 2017). The possible need for an adequately high light intensity to induce PSII inactivation before observing differences in  $F_v'/F_m'$  between treatments could explain the reported lack of differences between water treatments in  $F_v'/F_m'$  (Murchie and Lawson 2013). *NPQ* values remained stable under eCO<sub>2</sub>, which was also observed in barley plants (Robredo et al. 2010) and were



**FIGURE 4** Effect of ambient and elevated CO<sub>2</sub> in combination with WW, D and R conditions on (a) maximum yield of PSII photochemistry ( $F_v/F_m$ ), (b) quantum yield of PSII ( $\Phi_{PSII}$ ), (c) photochemical quenching ( $qP$ ), (d) the efficiency of absorbed quanta to be transferred to the PSII reaction centres ( $F_v'/F_m'$ ) and (e) non-photochemical quenching ( $NPQ$ ). Graphic labels, growth conditions and statistical analysis are explained in [Figure 1](#)

significantly lower than aCO<sub>2</sub> for all the treatments. Thus, the photoprotection induced by NPQ under eCO<sub>2</sub> appeared to be sufficient to prevent PSII from suffering downregulation from excess light energy under severe drought stress, but not under aCO<sub>2</sub>. This different photochemical sensitivity between both CO<sub>2</sub> treatments under water stress could explain the better behaviour of A under elevated CO<sub>2</sub>. When re-watered, even the short re-watering treatment was capable of recovering control values of  $\Phi_{PSII}$ ,  $F_v'/F_m'$  and NPQ, indicating that once water is available, sorghum promotes a rapid repair of the aforementioned downregulation or damage in the PSII and thus, a rapid recovery of A. Therefore, our results show the tendency of sorghum to use water resources to recover normal

photosynthetic activity after a period of drought, recovering A via the repair of the photosynthetic machinery.

One of the main concerns regarding the impact of drought on crops is related to growth, since problems in plant development are related to reductions in crop yields. Previous studies have observed a significant increase in the growth of sorghum when exposed to a CO<sub>2</sub>-enriched environment (Prior et al. 2003; Wu et al. 2009), as well as a slight but non-significant increase (Ottman et al. 2001). Similarly, elevated CO<sub>2</sub> has previously been reported to alleviate the effect of drought in sorghum growth (Allen et al. 2011; De Souza et al. 2015). However, in our study, we observed that drought caused an overall growth reduction in



**TABLE 2** Effect of environmental CO<sub>2</sub> in combination with different irrigation regimes on growth parameters Effect of environmental CO<sub>2</sub> in combination with different irrigation regimes on growth parameters

		Leaf DW (g)	Stem DW (g)	Root DW (g)	Total DW (g)	LA (cm <sup>2</sup> )	WUE (g DW g <sup>-1</sup> H <sub>2</sub> O)
aCO <sub>2</sub>	9WW	0.348 ± 0.03a	0.211 ± 0.01ab	0.257 ± 0.01b	0.817 ± 0.06b	172.11 ± 12.49a	7.498 ± 0.401c
	9D	0.156 ± 0.01c	0.117 ± 0.00c	0.345 ± 0.02a	0.619 ± 0.03c	56.92 ± 4.76c	12.585 ± 0.740b
eCO <sub>2</sub>	9WW	0.402 ± 0.03a	0.241 ± 0.02a	0.315 ± 0.01ab	0.959 ± 0.07a	166.31 ± 11.74a	14.533 ± 0.852b
	9D	0.236 ± 0.02b	0.165 ± 0.01b	0.368 ± 0.02a	0.769 ± 0.06b	88.54 ± 7.15b	20.260 ± 1.528a
aCO <sub>2</sub>	13WW	0.500 ± 0.04a	0.317 ± 0.02a	0.353 ± 0.01b	1.171 ± 0.08ab	244.79 ± 17.03a	6.258 ± 0.299d
	13D	0.216 ± 0.01b	0.151 ± 0.01b	0.560 ± 0.02b	0.928 ± 0.05c	82.64 ± 5.28b	15.707 ± 0.593c
eCO <sub>2</sub>	13WW	0.567 ± 0.05a	0.357 ± 0.04a	0.373 ± 0.02a	1.299 ± 0.12a	230.02 ± 15.23a	11.465 ± 0.789b
	13D	0.278 ± 0.03b	0.207 ± 0.02b	0.557 ± 0.01a	1.044 ± 0.07bc	105.44 ± 9.52b	23.843 ± 0.730a
aCO <sub>2</sub>	16WW	0.723 ± 0.06a	0.463 ± 0.03b	0.451 ± 0.02de	1.638 ± 0.11ab	271.48 ± 19.57a	6.367 ± 0.298e
	16D	0.228 ± 0.01c	0.183 ± 0.01d	0.683 ± 0.03b	1.095 ± 0.06cd	92.56 ± 5.01d	17.175 ± 0.951d
	R1	0.386 ± 0.03b	0.258 ± 0.02cd	0.384 ± 0.01de	1.029 ± 0.76d	190.36 ± 15.88b	10.918 ± 0.605de
	R2	0.308 ± 0.02bc	0.212 ± 0.01cd	0.525 ± 0.02cd	1.046 ± 0.07d	131.74 ± 10.01bcd	14.129 ± 0.744cd
eCO <sub>2</sub>	16WW	0.801 ± 0.06a	0.553 ± 0.03a	0.536 ± 0.03cd	1.891 ± 0.13a	272.22 ± 20.26a	11.891 ± 0.693c
	16D	0.320 ± 0.03bc	0.242 ± 0.02cd	0.828 ± 0.07a	1.390 ± 0.13bc	108.91 ± 7.64cd	29.910 ± 2.665a
	R1	0.434 ± 0.05b	0.272 ± 0.03c	0.373 ± 0.01e	1.080 ± 0.09d	132.39 ± 10.29bc	14.983 ± 0.582cd
	R2	0.411 ± 0.04b	0.269 ± 0.02cd	0.637 ± 0.05bc	1.318 ± 0.12cd	150.86 ± 12.70bc	23.798 ± 1.994b

Note: Mean values of ambient and elevated CO<sub>2</sub> in combination with well-watered (WW), water stress (D) and recovery (R) conditions on leaf DW, stem DW, root DW, Total DW, LA and WUE. Growth conditions and statistical analysis are explained in Figure 1. For each measurement day, thus 9, 13 and 16, different letters are used to represent values significantly different at  $p \leq .05$ .

all aboveground organs regardless of the drought period and CO<sub>2</sub> treatments. Root biomass, on the other hand, was significantly increased under both, aCO<sub>2</sub> and eCO<sub>2</sub>. It is likely that the greater root accumulation under eCO<sub>2</sub> concentration is a consequence of the improved water relations, as well as increased Ci and net assimilation of CO<sub>2</sub> as discussed above. Both re-watering treatments suppressed the root growth enhancement under drought and induced a slight growth of aboveground organs. Nevertheless, these increases in biomass in leaves and stems remained significantly far from the growth in control conditions, even after the long re-watering treatment. These results along with the previously discussed ones suggest that the restoration of the growth of sorghum is a slower long-term process, in which the photosynthesis is the first parameter to be recovered.

Briefly, our results show that sorghum plants have a rapid recovery response to water scarcity under aCO<sub>2</sub>, were the recovery of the structural and functional photosynthetic machinery is prioritized. Also, since the sorghum plants grown under eCO<sub>2</sub> were less damaged when subjected to drought, they would require less or no recovery. Overall, these results provide significant information about the resilience of C<sub>4</sub> plants, specifically sorghum, to drought events. Also, maintenance of high rates of  $\Psi_w$ , chlorophyll content (data not shown) and  $F_v/F_m$  under high CO<sub>2</sub> and drought stress suggest great adaptability to drought (Chen et al. 2016), indicating that sorghum could be a suitable candidate to be sown during the adverse drought events of the future.

## AUTHOR CONTRIBUTIONS

AMP, AMR and AR conceived and designed research. AR conducted experiments. XSMG analysed data, made the figures and tables and wrote the manuscript. UPL analysed statistical data and supervised the initial version of the manuscript. AMP, AMR and UPL corrected the manuscript. All authors read and approved the manuscript.

## ACKNOWLEDGEMENTS

This research was financially supported by grant GRUPO Gobierno Vasco-IT022-16. X. S. Martínez-Goñi is the recipient of a grant from Departamento de Universidades e Investigación del Gobierno Vasco (Spain). A. Robredo was the recipient of a grant from Departamento de Educación, Universidades e Investigación del Gobierno Vasco (Spain).

## DATA AVAILABILITY STATEMENT

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

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**How to cite this article:** Martínez-Goñi, X. S., Robredo, A., Pérez-López, U., Muñoz-Rueda, A., & Mena-Petite, A.(2023). *Sorghum bicolor* prioritizes the recovery of its photosynthetic activity when re-watered after severe drought stress, while manages to preserve it under elevated CO<sub>2</sub> and drought. *Journal of Agronomy and Crop Science*, 209, 217–227. <https://doi.org/10.1111/jac.12618>