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## Short-term exposure to elevated CO<sub>2</sub> stimulates growth and metabolic responses that alleviate early-stage iron deficiency symptoms in soybean

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### ABSTRACT

Elevated CO<sub>2</sub> (eCO<sub>2</sub>) increase plant biomass and might lead to nutritional losses. The results showed that eCO<sub>2</sub> under Fe-deficiency stimulates root dry weight from 0.37–0.80 g plant<sup>-1</sup> and shoot dry weight from 0.82–2.10 g plant<sup>-1</sup>, without compromising root/shoot ratio. Besides, eCO<sub>2</sub> improved the chlorophyll content of Fe-deficient plants. However, downregulation of photosynthesis, reduction in stomatal conductance and transpiration rate, and an increase in water-use efficiency was observed due to eCO<sub>2</sub>. Moreover, under Fe-deficiency, eCO<sub>2</sub> decreased K and Mg in roots, and Mg in leaves, whereas increased P and Zn in roots and leaves. In Fe-sufficient plants, eCO<sub>2</sub> increased K, P, Mn, Zn and Fe in leaves and did not change the concentration in roots. The Fe-deficiency-induced responses in roots, including the increase in FCR activity, and the expression of Fe-uptake genes were stimulated by eCO<sub>2</sub>, but were not sufficient to increase Fe concentration in Fe-deficient plants.

### ARTICLE HISTORY

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### KEYWORDS

Biomass; elevated CO<sub>2</sub>; Fe-deficiency; soybean

### 1. Introduction

The global atmospheric carbon dioxide concentration increased by 48% since the beginning of the industrial era from 280 ppm to the current level of about 420 ppm, (<http://www.esrl.noaa.gov/gmd/ccgg/trends/>) and is predicted to reach 700–1000 ppm by the end of this century (IPCC 2014). This steady increase in atmospheric CO<sub>2</sub> is responsible for global climate change affecting crops worldwide (Bencke-Malato et al. 2019). Therefore, one of the great questions for agriculture in the upcoming future is to improve biomass production and plant product quality, and atmospheric CO<sub>2</sub> concentration and regulation of mineral concentrations are major actors in this scenario (Briat et al. 2015). Soybean is a consistent source of nutrients as consumption of soybean-based products is on the rise due to its high content of proteins, fatty acids, natural antioxidants, vitamins, and minerals (Zheng et al. 2020). Previous findings have demonstrated that elevated CO<sub>2</sub> (eCO<sub>2</sub>) promotes photosynthetic CO<sub>2</sub> assimilation rate in C3 plants, increasing biomass and yield (Haase et al. 2008; Leakey et al. 2009; Bishop et al. 2015a; Kimball 2016; Bencke-Malato et al. 2019). For example, Kimball (2016) showed that biomass and seed yield increased in C3 species under FACE conditions. Yields of C3 grain crops increased by an average of 19%. In another study, eCO<sub>2</sub> stimulated above-ground biomass by 22% and seed yield by 9% among 18 soybean genotypes grown under field conditions (Bishop et al. 2015b). Plants exposed to eCO<sub>2</sub> changed their root size and activity to increase nutrient uptake and translocation for a given nutrient (Pérez-López et al. 2014 Guo et al. 2015). The results from Guo et al. (2015) in rice suggest that eCO<sub>2</sub> might support the translocation of calcium (Ca), magnesium (Mg),

iron (Fe), zinc (Zn), and manganese (Mn) from soil to stem and panicle. A great deal of attention has been dedicated to the effects of eCO<sub>2</sub> on the nutrient quality of crop plants and expanding this knowledge to Fe status is of particular interest. Fe is a crucial element of biomass production and plant quality due to its essential role in the structure and function of the photosynthetic electron transfer chain and chlorophyll synthesis (Briat et al. 2015). Besides, Fe intake for a significant proportion of the worldwide population is delivered by plant consumption (Smith et al. 2017). Even though the Fe concentration in soils regularly surpasses plant requirements, its bioavailability is often limited, predominantly in calcareous soils representing 30% of cultivated soils (Jin et al. 2009). Furthermore, Fe is also the most commonly deficient micronutrient in the human diet, impacting roughly an estimated 2 billion people (Briat et al. 2015; Smith et al. 2017). Recent studies revealed that C3 plants, legumes, and maize have lower Fe concentrations ranging from 4–10% when grown at eCO<sub>2</sub> (550 ppm) under field conditions (Myers et al. 2014). Similar findings were obtained by Loladze (2014) in C3 plants, reflecting foliar and edible tissues, FACE, and non-FACE studies. Consequently, Fe metabolism in higher plants is likely to be affected by the atmospheric CO<sub>2</sub> concentration, and it is crucial to address the impact of eCO<sub>2</sub> on plant Fe nutrition. Jin et al. (2009) reported a relative increase in tomato biomass and the root/shoot ratio at eCO<sub>2</sub> under Fe-limited and Fe-sufficient conditions. Under eCO<sub>2</sub>, the Fe-deficiency-induced responses in roots, including ferric chelate reductase (FCR) activity, proton secretion, subapical root hair development, and the expression of FER, FRO1, and IRT genes, were higher than in plants grown at aCO<sub>2</sub>. Also, eCO<sub>2</sub> has a positive effect on biomass when Fe is limited in barley (Haase

et al. 2008). Thus, understanding the Fe-uptake mechanisms and Fe-metabolism under eCO<sub>2</sub> conditions is crucial for selecting nutrient-rich and more tolerant genotypes to Fe-deficiency (Morrissey and Guerinet 2009). Moreover, little is known about the interaction of eCO<sub>2</sub> and Fe-deficiency in soybean plants, and the underlying mechanisms responsible for such changes. In plants, the Fe-uptake mechanism is divided into two different approaches: strategy I occur in nongraminaceous plants, and Fe<sup>3+</sup> is reduced via a membrane-bound reductase to make it accessible for uptake by a Fe<sup>2+</sup> transporter, and strategy II occurs in grasses which secrete phytosiderophores that readily bind Fe<sup>3+</sup>, and these complexes are then transported back into the roots (Jin et al. 2009 Morrissey and Guerinet 2009). In the present study, soybean plants were grown in a hydroponic culture system to report the effects of the interaction of eCO<sub>2</sub> and Fe-deficiency in the morphological, physiological, and molecular responses under controlled conditions. It was previously demonstrated in tomato (Jin et al. 2009) and barley (Haase et al. 2008) that eCO<sub>2</sub> has a positive impact on plant biomass when Fe is limited. Here, we aimed to understand if eCO<sub>2</sub> may have a positive effect on soybean growth under Fe-deficiency without impairing mineral accumulation, since Fe homeostasis and eCO<sub>2</sub> could be linked to the interplay occurring among nutrient uptake and translocation.

## 2. Material and methods

### 2.1. Plant culture

Soybean (*Glycine max* cv 'Winsconsin Black') seeds were surface sterilized with 75% (v/v) ethanol, rinsed four times with ultra-pure water, and germinated at 25 °C in the dark for 5 days. Then, plants were transplanted to black plastic pots (5 L) filled with an aerated, full-strength nutrient solution with the following composition: 1.2 mM KNO<sub>3</sub>, 0.8 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.3 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 25 μM CaCl<sub>2</sub>, 25 μM H<sub>3</sub>BO<sub>3</sub>, 0.5 μM MnSO<sub>4</sub>, 2 μM ZnSO<sub>4</sub>·H<sub>2</sub>O, 0.5 μM CuSO<sub>4</sub>·H<sub>2</sub>O, 0.5 μM MoO<sub>3</sub>, 0.1 μM NiSO<sub>4</sub>, and 20 μM Fe(III)-EDDHA. The solution was buffered with the addition of 1mM MES (pH 5.5) and changed every 3 days. Plants were grown in the controlled environment at 75% humidity with a daily cycle of 16 h at 25 °C (day) and 8 h at 20 °C (night). The daytime light intensity was 325 μmol s<sup>-1</sup> m<sup>-2</sup> of the photosynthetic photon flux density at the plant level. After 7 days of pre-treatment in the full-strength nutrient solution, plants were transferred to a nutrient solution with Fe(III)-EDDHA at 0.5 μM (Fe-deficiency) or 20 μM (Fe-sufficiency) for 12 days. The plants were grown at 400 ± 10 ppm (aCO<sub>2</sub>) or 800 ± 10 ppm (eCO<sub>2</sub>). The CO<sub>2</sub> concentration was continuously monitored and maintained by an automated CO<sub>2</sub> control system, which measured and adjusted the CO<sub>2</sub> concentration from soybean planting to the end of the experiment.

### 2.2. Physiological parameters

At the end of the experiment, the chlorophyll content was evaluated using the first expanded trifoliate leaf with a chlorophyll meter (SPAD-502; Minolta). Then, plants were measured for length and separated into shoots and roots,

and the material was dried at 70 °C until constant weight and stored for ICP-OES analysis. Each analysis was performed from 5 plants and mean values calculated.

### 2.3. Determination of FCR activity

Root FCR activity was measured as described by Vasconcelos et al. (2006). The assays were performed by the spectrophotometric determination of Fe<sup>2+</sup> chelated to BPDS (bathophenanthroline disulfonic acid). Plant roots were submerged in the assay solution containing: 1.5 mM KNO<sub>3</sub>, 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 3.75 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 0.25 mM MgSO<sub>4</sub>, 25 μM CaCl<sub>2</sub>, 25 μM H<sub>3</sub>BO<sub>3</sub>, 2 μM MnSO<sub>4</sub>, 2 μM ZnSO<sub>4</sub>, 0.5 μM CuSO<sub>4</sub>, 0.5 μM H<sub>2</sub>MoO<sub>4</sub>, 0.1 μM NiSO<sub>4</sub>, 100 μM Fe(III)-EDTA, 100 μM BPDS, and buffered with 1 mM MES at pH5.5. The assays were conducted under dark conditions at room temperature for 60 min. Absorbance values were obtained spectrophotometrically at 535 nm, and the solution with no roots was used as blank. Rates of reduction were determined using the molar extinction coefficient of 22.14 mM<sup>-1</sup>cm<sup>-1</sup>. The assays were performed from 5 samples and mean values calculated.

### 2.4. Mineral analysis by ICP-OES

About 200 mg of the roots and leaves dried samples were mixed with 5 mL of 65% HNO<sub>3</sub> (v/v) and 1 mL of H<sub>2</sub>O<sub>2</sub> 30% (v/v) in a Teflon reaction vessel and heated in a Speedwave TMMWS- 3+ (Berghof, Germany) microwave system. The digestion procedure was described in detail by Santos et al. (2015), and the resulting solutions were diluted to 50 ml with ultra-pure water for further analysis. Determination of mineral concentrations was performed using the ICP-OES Optima7000 DV (Perkin Elmer, USA) with a radial configuration from five independent digestions of each plant organ from all the treatments. The assays were performed from 5 samples and mean values calculated.

### 2.5. Leaf gas exchange parameters

Gas exchange parameters were performed in the last fully expanded leaves, ten days after the Fe-treatment, using a portable photosynthesis system (LI-6400XT; LICOR, Inc.). The CO<sub>2</sub> in the leaf chamber was set to match the CO<sub>2</sub> treatment with a PPFD of 500 μmol photon m<sup>-2</sup> s<sup>-1</sup> at 25 °C. A/Ci curves were also measured, and data were analyzed using the PS-FIT software (<http://www.life.illinois.edu/bernacchi/links.html>). This software uses the leaf model of photosynthesis (Farquhar et al. 1980) to calculate the maximum rates of electron transport ( $J_{max}$ ) and maximum velocity of carboxylation by Rubisco ( $V_{c,max}$ ). Moreover, the transpiration rate ( $T_r$ ), stomatal conductance ( $g_s$ ), and water use efficiency ( $WUE = A/T_r$ ) were determined. The assays were performed from 3 plants and mean values calculated.

### 2.6. Gene expression analysis

For gene expression analysis, root samples were frozen in liquid nitrogen and stored at -80 °C. About 100 mg of tissue was grounded in liquid nitrogen, and total RNA was extracted using a Qiagen RNeasy Plant Mini Kit (Qiagen, USA). Therefore, single-stranded cDNA was synthesized

with the first-strand cDNA synthesis kit (Fermentas, USA) in a Thermal cycler (VWR, Doppio, Belgium), according to the manufacturer's instructions. The mRNA levels were detected by the SYBR Green Supermix (Bio-Rad) with the following pairs of gene-specific primers: *FER* fw, 5'-GAACAAACGTGGTGGAAAA G-3'; rev, 5'-AACTGCACGTCACCATTCTT-3'; *FRO2* fw, 5'-TGCTTGGACTCACACCA GAG-3'; rev, 5'-AGAG GTAGAAACCGGGGAGA-3'; *Ferritin* fw, 5'-CCCCTTATGCCTCTTTCCTC -3'; rev, 5'-GCTTTTCAGCGTGCTCTCTT-3'; *IRT1* fw, 5'-GATTGC ACCTGTGACA CAAA-3'; rev, 5'-CAGCAAAGGCCT-TAACCAT-3'; *DTM1* fw, 5'-GCCGCAAGAAACAGCT-TATG-3'; rev, 5'-AGCTTCTTCCACGAGA ATCG-3'. The respective housekeeping genes were used as reference genes: *Actin* fw, 5'-ATCTTGACTGAGCGTGGT-TATTCC-3'; rev, 5'-GCTGGTCTGGCTGTCTCC-3'; and *ELF1B* fw, 5'-GTTGAAAAGCCAGGGGACA-3'; rev, 5'-TCTTACCCCTTGAGCGTGG-3'. *FER* and *DTM1* gene sequences were searched in the NCBI database, and primers were designed using the Primer-Blast tool from NCBI with an expected PCR product of 100–200 bp. *FRO2*, *ferritin*, and *IRT1* primer pairs were described by Santos et al. (2015), and *Actin* and *ELF1B* by Wan et al. (2017). qPCR reactions were performed on a Chromo4 thermocycler (Bio-Rad, Hercules, CA, USA) with the following reaction conditions: 10 min at 95 °C and 40 cycles with 15 s at 95 °C, 15 s at 58 °C, and 15 s at 68 °C. The  $2^{-\Delta\Delta C_T}$  method (Livak and Schmittgen 2001) was used for the relative quantification of gene expression analysis. All the assays were performed with three biological and two technical replicates.

## 2.7. Organic acids and sugar analysis

The extraction protocol was described in detail by López-Millán et al. (2009). About 100 mg of plant material was grounded using liquid nitrogen and suspended in 5 mM H<sub>2</sub>SO<sub>4</sub>, vortexed for 30 s, and then boiled for 30 min. The samples were centrifuged at 2320 x g for 10 min, the supernatant filtered through a 0.45 mm PTFE filter, and the volume adjusted to 2 ml and stored at -80 °C until further analysis. The HPLC system consisted of an ion exchange aminex HPX-87H Column (300x7.8 mm) (Bio-Rad, USA) maintained at an oven temperature of 40 °C and two detectors in

series (Refractive Index and UV 210 nm; K-2301 and K-2501, Knauer, Germany). The mobile phase was 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.6 mL/min. The assays were performed from 5 samples, and mean values were calculated.

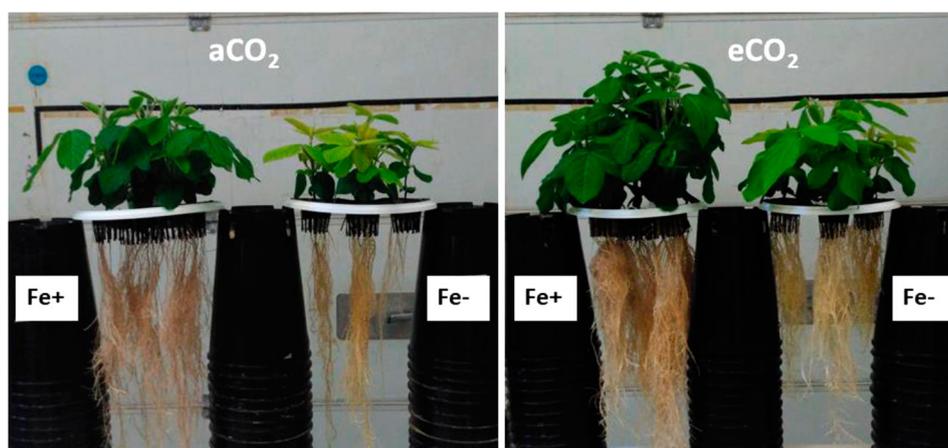
## 2.8. Statistical analysis

The data for each dependent variable was subjected to the two-way ANOVA at  $P < 0.05$  in all cases. All statistical analyses were performed with SPSS software (SPSS version 26.0).

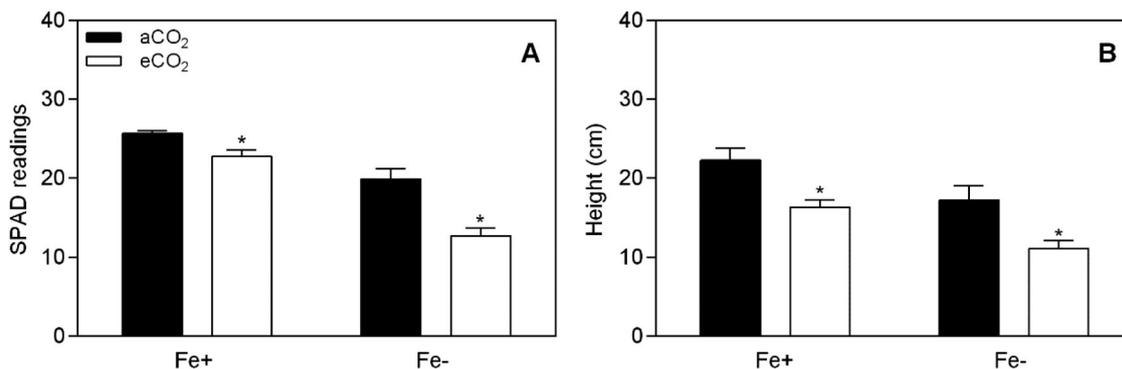
## 3. Results

### 3.1. Effects of eCO<sub>2</sub> and Fe-deficiency on chlorophyll synthesis, plant growth, and Fe-uptake

After 12 days of plant growth at aCO<sub>2</sub> and under Fe-deficiency (0.5 μM Fe-EDTA), the soybean leaves were chlorotic (Figure 1) with an average SPAD reading of 12.7 (Figure 2a). However, leaves of plants grown in the same growth conditions but at eCO<sub>2</sub> had SPAD readings of 19.9 (Figure 2a), indicating that eCO<sub>2</sub> conditions ( $P < 0.05$ ; Table 1) significantly improved the chlorophyll synthesis of plants grown in Fe-deficient conditions. In Fe-sufficient plants (20 μM Fe-EDTA) the chlorophyll content was also higher under eCO<sub>2</sub> in comparison to aCO<sub>2</sub> conditions ( $P < 0.05$ ; Figure 2a). The effects of CO<sub>2</sub> and Fe-supply were statistically significant on SPAD readings ( $P < 0.001$ ), with a significant CO<sub>2</sub> x Fe interaction ( $P < 0.05$ , Table 1). Moreover, in Fe-sufficient plants, the height was higher compared to Fe-deficient plants, and this effect was exacerbated in response to eCO<sub>2</sub> (Figure 2b). In hydroponic growth, root dry weight was increased at eCO<sub>2</sub> by 66.4% in Fe-sufficient ( $P < 0.01$ ) and by 119.7% in Fe-deficient ( $P < 0.001$ ) plants, as demonstrated in Figure 3a. Shoot dry weight (Figure 3b) was also increased under eCO<sub>2</sub> by 40.1% in Fe-sufficient ( $P < 0.001$ ) and by 157.6% in Fe-deficient plants ( $P < 0.001$ ). Furthermore, the root/shoot ratio (Figure 3c) was not affected in Fe-deficient plants ( $P > 0.05$ ) but increased in Fe-sufficient plants ( $P < 0.05$ ) by 20.1% under eCO<sub>2</sub> conditions. The effects of CO<sub>2</sub> levels and Fe-supply were statistically significant on FCR activity ( $P < 0.001$ ), with a significant CO<sub>2</sub> x Fe interaction ( $P < 0.001$ , Table 1). Therefore, FCR activity was induced in plants grown in Fe-deficiency, and this induction was higher when combined



**Figure 1.** Soybean plants grown in nutrient solution at aCO<sub>2</sub> (400 ppm) and eCO<sub>2</sub> (800 ppm). After 7 days of pre-treatment in the complete nutrient solution, plants were transferred to Fe-sufficient (20 μM Fe-EDDHA) and Fe-deficient (0.5 μM Fe-EDDHA) conditions for 12 days.



**Figure 2.** SPAD readings (a) and height (b) of soybean plants grown in the nutrient solution, depending on Fe-supply (0.5 and 20  $\mu\text{M}$  Fe-EDDHA) and atmospheric  $\text{CO}_2$  concentration (400 and 800 ppm). Data are mean  $\pm$  SEM ( $n = 5$ ). \*, Significant differences ( $P < 0.05$ ) between  $\text{aCO}_2$  and  $\text{eCO}_2$  treatments.

**Table 1.** Two-factorial ANOVA table for the effect of  $\text{eCO}_2$  and Fe-supply on leaf chlorophyll content (SPAD values), plant height (cm), plant biomass (root and shoot dry weight,  $\text{g plant}^{-1}$ ), root/shoot ratio, FCR activity ( $\mu\text{mol Fe g}^{-1} \text{FW h}^{-1}$ ),  $A$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $g_s$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ),  $T_r$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ), WUE ( $\mu\text{mol mol}^{-1}$ ),  $V_{c,\text{max}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and  $J_{\text{max}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

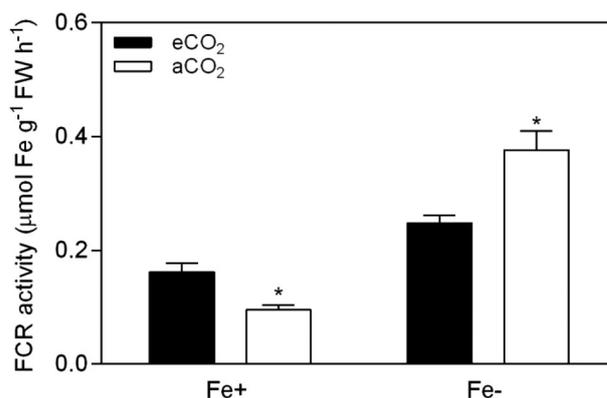
Parameters	d.f.	$\text{CO}_2$	Fe	$\text{CO}_2 \times \text{Fe}$
SPAD	1	<b>31.434;&lt;0.001</b>	<b>77.547;&lt;0.001</b>	<b>5.573;0.025</b>
Height	1	<b>19.286;&lt;0.001</b>	<b>14.147;0.002</b>	0.009;0.924
Root dry weight	1	<b>64.970;&lt;0.001</b>	<b>13.163;0.002</b>	0.280;0.604
Shoot dry weight	1	<b>89.608;&lt;0.001</b>	<b>16.628;&lt;0.001</b>	<b>10.753;0.005</b>
Root/shoot ratio	1	0.031;0.863	0.276;0.606	<b>9.405;0.007</b>
FCR activity	1	<b>2.439;&lt;0.001</b>	<b>82.105;&lt;0.001</b>	<b>23.406;&lt;0.001</b>
$A$	1	0.006;0.939	<b>15.180;&lt;0.001</b>	0.208;0.654
$g_s$	1	<b>84.401;&lt;0.001</b>	4.021;0.059	1.477;0.239
$T_r$	1	<b>68.916;&lt;0.001</b>	<b>5.217;0.035</b>	2.765;0.114
WUE	1	<b>120.798;&lt;0.001</b>	1.296;0.270	0.597;0.450
$V_{c,\text{max}}$	1	<b>41.212;&lt;0.001</b>	4.656;0.063	<b>9.83;0.014</b>
$J_{\text{max}}$	1	<b>20.418;0.002</b>	0.606;0.459	<b>6.943;0.030</b>

$A$ , Photosynthetic carbon assimilation rate;  $g_s$ , stomatal conductance;  $T_r$ , transpiration rate; WUE, water-use efficiency;  $V_{c,\text{max}}$ , maximum velocity of carboxylation by Rubisco;  $J_{\text{max}}$ , maximum rate of electron transport. Results from the analysis of variance with degrees of freedom (d.f.), F ratios and probabilities ( $P$ ) for some parameters. Significant effects are shown in boldface.

with  $\text{aCO}_2$  (Figure 4). Contrastingly, FCR activity was higher under  $\text{eCO}_2$  conditions in Fe-sufficient conditions.

### 3.2. Effects of $\text{eCO}_2$ and Fe-deficiency on the photosynthetic parameters

Leaf photosynthetic assimilation rate ( $A$ ) decreased in Fe-deficient conditions ( $P < 0.05$ ; Table 2) when compared to Fe-sufficient conditions. However, in Fe-sufficient and Fe-deficient plants the response of  $A$  was not affected by exposure to  $\text{eCO}_2$  ( $P > 0.05$ ), with no significant  $\text{CO}_2 \times \text{Fe}$  interaction ( $P > 0.05$ , Table 1). Also,  $\text{eCO}_2$  reduced stomatal conductance ( $g_s$ ) from 0.37–0.13  $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$  in Fe-sufficient ( $P < 0.05$ ) and from 0.30–0.12  $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$  in Fe-deficient ( $P < 0.05$ ) plants. The transpiration rate ( $T_r$ )

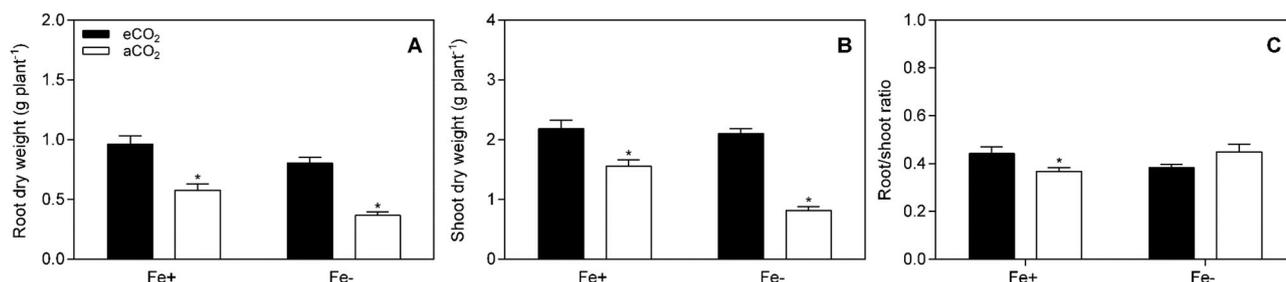


**Figure 4.** Effect of  $\text{eCO}_2$  on FCR activity in soybean plants grown in Fe-sufficient (20  $\mu\text{M}$  Fe-EDDHA) and Fe-deficient (0.5  $\mu\text{M}$  Fe-EDDHA) conditions. Data are mean  $\pm$  SEM ( $n = 5$ ). \*, Significant differences ( $P < 0.05$ ) between  $\text{aCO}_2$  and  $\text{eCO}_2$  treatments.

also decreased by  $\text{eCO}_2$ , in both Fe-supplies. The water-use efficiency (WUE) increased significantly from 2.23–4.76  $\text{mmol mol}^{-1}$  ( $P < 0.01$ ) in Fe-sufficient plants, and from 2.17–3.97  $\text{mmol mol}^{-1}$  ( $P < 0.01$ ) in Fe-limited plants due to exposure to  $\text{eCO}_2$ . The  $A/C_i$  response curves showed a reduction in the  $V_{c,\text{max}}$  and in  $J_{\text{max}}$  under  $\text{eCO}_2$  in Fe-sufficient and Fe-deficient plants ( $P < 0.05$ , Table 2). However, the response of  $V_{c,\text{max}}$  and  $J_{\text{max}}$  were not affected by Fe-supply ( $P > 0.05$ ) but a significant  $\text{CO}_2 \times \text{Fe}$  interaction was found ( $P < 0.05$ , Table 1).

### 3.3. Effects of $\text{eCO}_2$ and Fe-deficiency on the sugar and organic acid concentrations

In hydroponic culture, elevation of atmospheric  $\text{CO}_2$  did not affect sucrose concentration ( $P > 0.05$ ) in roots and leaves of Fe-sufficient and Fe-deficient plants (Table 3). Moreover,  $\text{eCO}_2$  increased glucose concentration in roots of Fe-deficient



**Figure 3.** Root (a), shoot biomass (b) and root/shoot ratio (c) of soybean plants grown in the nutrient solution, depending on Fe-supply (0.5 and 20  $\mu\text{M}$  Fe-EDDHA) and atmospheric  $\text{CO}_2$  concentration (400 and 800 ppm). Data are mean  $\pm$  SEM ( $n = 5$ ). \*, Significant differences ( $P < 0.05$ ) between  $\text{aCO}_2$  and  $\text{eCO}_2$  treatments.

**Table 2.** Effect of eCO<sub>2</sub> on photosynthetic parameters of soybean plants grown in Fe-sufficient (20 µM Fe-EDDHA) and Fe-deficient (0.5 µM Fe-EDDHA) conditions.

Fe treatment	CO <sub>2</sub>	A	g <sub>s</sub>	T <sub>r</sub>	WUE	V <sub>c,max</sub>	J <sub>max</sub>
Fe-sufficient	Ambient	11.43 ± 0.55	0.37 ± 0.03	5.22 ± 0.36 a	2,23 ± 0,39	57.63 ± 2.00	125.95 ± 6.26
	Elevated	11.21 ± 0.43	0.13 ± 0.02 *	2.46 ± 0.19 b	4,76 ± 0,95 *	29.56 ± 3.79 *	70.22 ± 11.46 *
Fe-deficient	Ambient	8.90 ± 0.80	0.30 ± 0.02	4.12 ± 0.29 c	2,17 ± 0,33	42.08 ± 2.71	99.35 ± 4.95
	Elevated	9.21 ± 0.40	0.12 ± 0.01 *	2.28 ± 0.16 b	3,97 ± 0,31 *	32.43 ± 2.95 *	84.68 ± 1.96 *

A, Photosynthetic carbon assimilation rate (µmol m<sup>-2</sup> s<sup>-1</sup>); g<sub>s</sub>, stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>); T<sub>r</sub>, transpiration rate (mol m<sup>-2</sup> s<sup>-1</sup>); WUE, water-use efficiency (µmol mol<sup>-1</sup>); V<sub>c,max</sub>, maximum velocity of carboxylation by Rubisco (µmol m<sup>-2</sup> s<sup>-1</sup>); J<sub>max</sub>, maximum rate of electron transport (µmol m<sup>-2</sup> s<sup>-1</sup>). Data are mean ± SEM (n = 3). \*, Significant differences (P < 0.05) between aCO<sub>2</sub> and eCO<sub>2</sub> treatments.

**Table 3.** Effect of eCO<sub>2</sub> on sugars and organics acids content (µmol gFW<sup>-1</sup>) of soybean plants grown in Fe-sufficient (20 µM Fe-EDDHA) and Fe-deficient (0.5 µM Fe-EDDHA) conditions.

	Roots				Leaves			
	Fe-sufficient		Fe-deficient		Fe-sufficient		Fe-deficient	
	eCO <sub>2</sub>	aCO <sub>2</sub>	eCO <sub>2</sub>	aCO <sub>2</sub>	eCO <sub>2</sub>	aCO <sub>2</sub>	eCO <sub>2</sub>	aCO <sub>2</sub>
Suc	4,41 ± 0,53	3,31 ± 0,21	3,08 ± 0,74	3,96 ± 0,42	16,36 ± 1,40	13,38 ± 0,86	15,29 ± 1,42	14,81 ± 1,54
Gluc	4,91 ± 0,87	3,78 ± 0,68	5,02 ± 0,90	2,67 ± 0,28 *	10,87 ± 1,08	7,31 ± 0,69 *	9,05 ± 1,12	8,71 ± 0,99
Frut	16,85 ± 2,47	5,11 ± 0,75 *	13,33 ± 0,66	2,11 ± 0,28 *	23,94 ± 2,29	12,93 ± 1,23 *	22,16 ± 2,22	15,14 ± 1,85 *
CA	17,40 ± 1,03	13,64 ± 0,97 *	12,55 ± 0,49	8,07 ± 1,06 *	57,88 ± 3,88	56,84 ± 5,73	69,51 ± 2,23	72,85 ± 2,88
MA	41,33 ± 5,69	25,57 ± 2,63 *	33,13 ± 4,93	11,85 ± 0,97 *	60,54 ± 7,10	40,11 ± 2,82 *	63,86 ± 8,64	62,26 ± 8,38

Suc, sucrose; Gluc, glucose; Frut, fructose; CA, citric acid; MA, malic acid. Data are mean ± SEM (n = 5). \*, Significant differences (P < 0.05) between aCO<sub>2</sub> and eCO<sub>2</sub> treatments.

**Table 4.** Analysis of variance for the effect of eCO<sub>2</sub> and Fe-supply on sugar (µmol g FW<sup>-1</sup>), organic acid (µmol g FW<sup>-1</sup>), and mineral concentrations (µg g<sup>-1</sup>) in roots and leaves of soybean plants

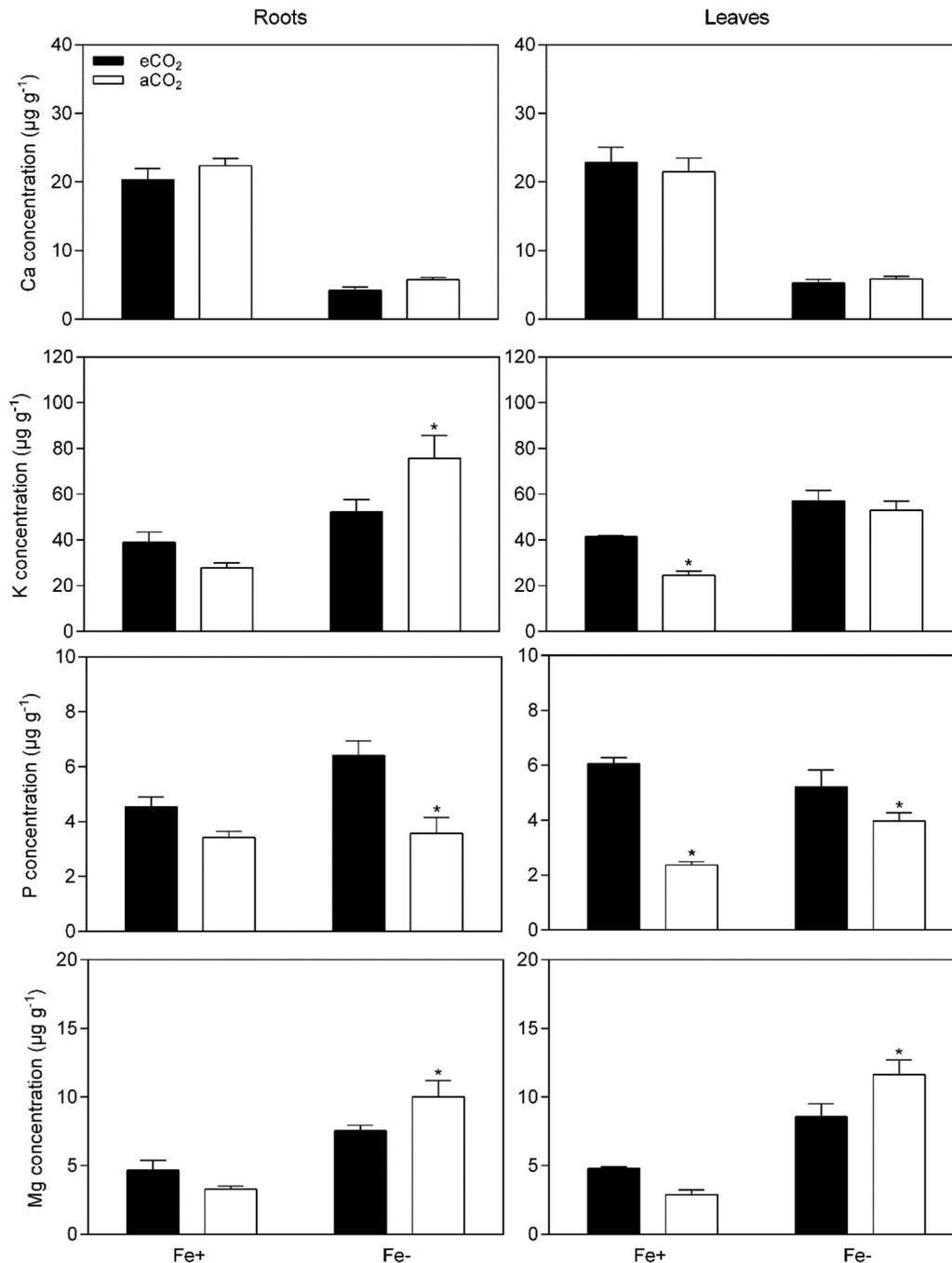
Parameters	Tissue	d.f.	CO <sub>2</sub>	Fe	CO <sub>2</sub> x Fe
Sucrose	Roots	1	0.056; 0.817	0.504; 0.493	4.311; 0.062
	Leaves	1	1.835; 0.209	0.020; 0.890	0.958; 0.353
Glucose	Roots	1	<b>7.533; 0.019</b>	0.635; 0.443	0.920; 0.358
	Leaves	1	3.903; 0.072	0.047; 0.833	2.677; 0.128
Fructose	Roots	1	<b>56.533; &lt;0.001</b>	4.549; 0.059	0.030; 0.866
	Leaves	1	<b>23.209; &lt;0.001</b>	0.013; 0.910	1.137; 0.311
Citric acid	Roots	1	<b>19.240; 0.001</b>	<b>30.839; &lt;0.001</b>	0.144; 0.711
	Leaves	1	0.078; 0.785	<b>11.331; 0.006</b>	0.283; 0.605
Malic Acid	Roots	1	<b>22.169; &lt;0.001</b>	<b>7.762; 0.018</b>	0.491; 0.498
	Leaves	1	4.033; 0.068	<b>5.395; 0.039</b>	2.947; 0.112
Calcium	Roots	1	<b>3.734; 0.075</b>	<b>319.693; &lt;0.001</b>	0.084; 0.776
	Leaves	1	0.058; 0.814	<b>81.918; &lt;0.001</b>	0.266; 0.615
Potassium	Roots	1	0.881; 0.364	<b>21.721; &lt;0.001</b>	<b>6.980; 0.019</b>
	Leaves	1	<b>10.694; 0.006</b>	<b>47.637; &lt;0.001</b>	3.944; 0.067
Phosphorous	Roots	1	<b>19.763; &lt;0.001</b>	<b>5.220; 0.038</b>	3.704; 0.075
	Leaves	1	<b>48.860; &lt;0.001</b>	1.190; 0.295	<b>12.069; 0.004</b>
Magnesium	Roots	1	0.645; 0.437	<b>49.296; &lt;0.001</b>	<b>7.957; 0.014</b>
	Leaves	1	0.819; 0.383	<b>90.837; &lt;0.001</b>	<b>14.331; 0.003</b>
Manganese	Roots	1	2.336; 0.149	<b>26.449; &lt;0.001</b>	1.354; 0.264
	Leaves	1	<b>20.476; &lt;0.001</b>	<b>42.025; &lt;0.001</b>	<b>18.519; &lt;0.001</b>
Zinc	Roots	1	<b>20.406; &lt;0.001</b>	<b>18.340; 0.001</b>	<b>10.278; 0.008</b>
	Leaves	1	<b>36.354; &lt;0.001</b>	<b>5.300; 0.038</b>	1.057; 0.323
Iron	Roots	1	1.058; 0.319	<b>63.952; &lt;0.001</b>	0.074; 0.789
	Leaves	1	<b>26.087; &lt;0.001</b>	<b>111.620; &lt;0.001</b>	<b>23.688; &lt;0.001</b>

Results from the analysis of variance with degrees of freedom (d.f.), F ratios and probabilities (P) for some parameters. Significant effects are shown in boldface.

plants (P < 0.05) and in leaves of Fe-sufficient plants (P < 0.05). Besides, fructose concentration increased in Fe-deficient and Fe-sufficient plants (P < 0.05) in both tissues analyzed due to CO<sub>2</sub> enrichment. The effect of Fe-supply was not statistically significant on the carbohydrate responses (P > 0.05), as well as the CO<sub>2</sub> x Fe interaction (P > 0.05, Table 4). The citric acid concentration increased by the exposure to eCO<sub>2</sub> from 13.64–17.40, and from 8.07–12.55 µmol g FW<sup>-1</sup> in roots of Fe-sufficient and Fe-deficient plants, respectively. In leaves, citric acid was not affected under eCO<sub>2</sub> (P > 0.05, Table 3). At the end of the growth period, eCO<sub>2</sub> increased malic acid concentration in roots from 25.57–41.33 in Fe-sufficient and from 11.85–22.13 µmol g FW<sup>-1</sup> in Fe-deficient plants. By comparison, leaves of soybean plants grown in the same nutrient conditions, eCO<sub>2</sub> only increased malic acid in Fe-sufficient plants from 40.11–60.54 µmol g FW<sup>-1</sup>. Moreover, the effect of Fe-supply was significant on citric and malic acid concentrations (P < 0.05, Table 4).

### 3.4. Effects of eCO<sub>2</sub> and Fe-deficiency on the mineral concentrations

The mineral-based responses under different Fe-supplies in soybean plants were also influenced by eCO<sub>2</sub>, as demonstrated in Figures 5 and 6. Elevation of atmospheric CO<sub>2</sub> concentration did not affect Ca concentration in root and leaf tissues, but it was significantly reduced in Fe-deficient plants (P < 0.05, Table 4). eCO<sub>2</sub> increased K concentration (P < 0.05) from 24.6–41.4 µg g<sup>-1</sup> in leaves of Fe-sufficient plants, and decreased K concentration (P < 0.05) from 75.7–52.3 µg g<sup>-1</sup> in roots of Fe-deficiency plants. The concentration of P was generally higher under eCO<sub>2</sub> conditions (P < 0.05), except in roots of Fe-sufficient plants. Furthermore, eCO<sub>2</sub> did not affect Mg concentration (P > 0.05) in Fe-sufficient plants, but a significant decrease was achieved in Fe-deficient plants (P < 0.05). Regarding to micronutrients, Mn concentration was not changed by CO<sub>2</sub> treatment



**Figure 5.** Effect of  $e\text{CO}_2$  on the macronutrient concentrations from soybean plants grown in Fe-sufficient ( $20 \mu\text{M}$  Fe-EDDHA) and Fe-deficient ( $0.5 \mu\text{M}$  Fe-EDDHA) conditions. Data are mean  $\pm$  SEM ( $n = 5$ ). \*, Significant differences ( $P < 0.05$ ) between  $a\text{CO}_2$  and  $e\text{CO}_2$  treatments.

in Fe-deficient and Fe-sufficient plants ( $P > 0.05$ ), apart from the increase in Mn concentration occurring in leaves of Fe-sufficient plants ( $P < 0.05$ ) from  $56.7$ – $155.8 \mu\text{g g}^{-1}$ .  $\text{CO}_2$  enrichment led to a significant increase in Zn concentration, except in roots of Fe-sufficient plants ( $P > 0.05$ ). The concentration of Fe was significantly higher in Fe-sufficient plants as expected and was not changed by the  $\text{CO}_2$  treatment, excluding the leaves of Fe-sufficient plants.

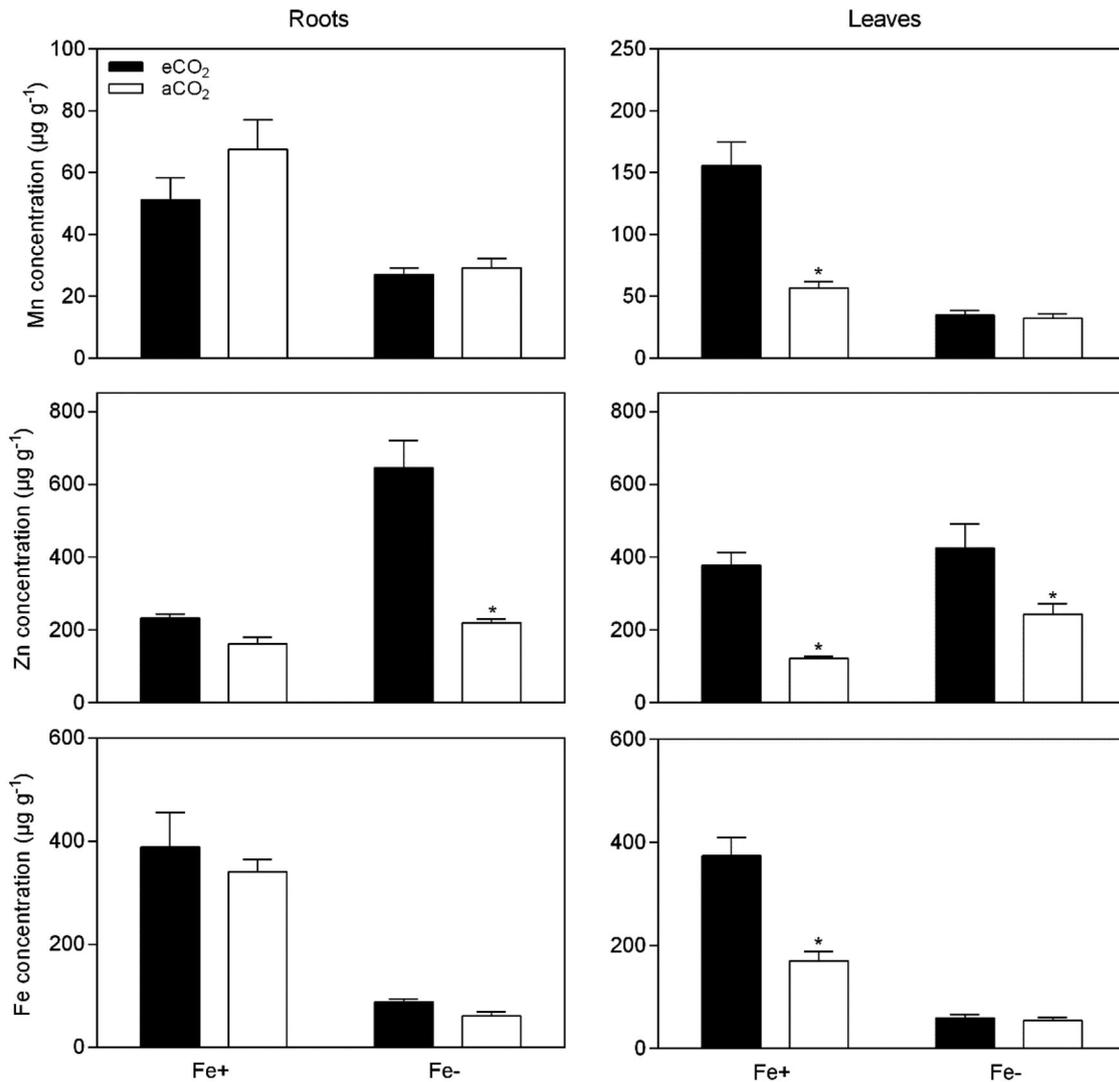
### 3.5. Effects of $e\text{CO}_2$ and Fe-deficiency on the expression of genes involved in Fe-uptake mechanisms

The effects of  $e\text{CO}_2$  and Fe-deficiency on genes related to the Fe-uptake mechanisms – DMT1, FER, FRO2, IRT1, and ferritin – were analyzed in soybean roots using qPCR. Overall,

higher gene expression levels were associated with Fe-deficiency responses as demonstrated in Figure 7. In the case of the FRO2 gene, the expression differed between treatments, with higher expression levels observed in Fe-deficiency plants, and this change was higher under  $e\text{CO}_2$  conditions. Similar findings were obtained in DMT1 and FER gene expression levels. Moreover, IRT1 gene expression was higher in Fe-deficient plants, but  $e\text{CO}_2$  did not affect the expression levels. Contrastingly, the ferritin gene expression was detected only in Fe-sufficient plants, and this change was higher under  $e\text{CO}_2$  conditions.

## 4. Discussion

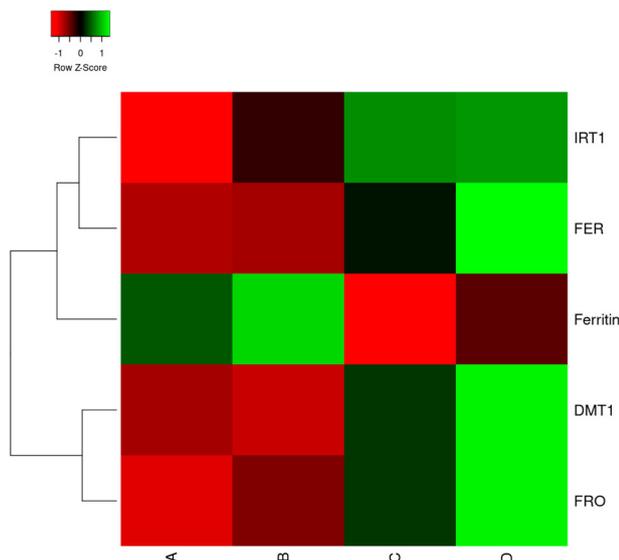
The beneficial effects of  $e\text{CO}_2$  on stimulating plant growth and biomass accumulation of several crops have been widely



**Figure 6.** Effect of eCO<sub>2</sub> on the micronutrient concentrations from soybean plants grown in Fe-sufficient (20 µM Fe-EDDHA) and Fe-deficient (0.5 µM Fe-EDDHA) conditions. Data are mean ± SEM (n = 5). \*, Significant differences (P < 0.05) between aCO<sub>2</sub> and eCO<sub>2</sub> treatments.

studied (Ainsworth et al. 2002; Long et al. 2004; Ziska and Bunce 2007; Bunce 2008; Vasconcelos et al. 2014; Bunce 2015; Santos et al. 2015; Bishop et al. 2015b; Bunce 2016;

Kimball 2016). However, there is a lack of information about the influence of eCO<sub>2</sub> on the changing demands for Fe-supply and is crucial to consider how eCO<sub>2</sub> affects and might alleviate the Fe-deficiency responses. In this study, we treated soybean plants with limited (0.5 µM) and sufficient (20 µM) Fe-supply under eCO<sub>2</sub> conditions. Supplying soybean plants with 0.5 µM Fe is sufficiently low to induce FCR activity. Furthermore, soybean plants revealed clear Fe chlorosis symptoms in young leaves at aCO<sub>2</sub> but not at eCO<sub>2</sub> conditions, which affected biomass accumulation and growth (Figure 1). An increase from 0.5–20 µM Fe in hydroponic solution alleviated the chlorosis symptoms in soybean plants. As reported for other species (Wu et al. 2004; Bourgault et al. 2016; Bunce 2016; Bourgault et al. 2017), soybean growth increased at eCO<sub>2</sub> conditions, as well as the requirement for nutrients and water, to sustain such CO<sub>2</sub>-based stimulation. Consequently, more nutrients will need to be applied, or plants will have to become more effective at obtaining those nutrients from the soil (Jin et al. 2009). In this study, eCO<sub>2</sub> led to an increase in biomass, even at reduced Fe-supply, similar to plants grown at Fe-sufficient conditions (Figure 3). Therefore, in Fe-sufficient plants, root and shoot dry weight increased by 66.4% and 40.1%, respectively, whereas in Fe-deficient plants increased by 119.7% and 157.4% due to eCO<sub>2</sub> conditions (Figure 3).



**Figure 7.** Heatmap of the expression profiles of *DMT1*, *FRO2*, *ferritin*, *FER* and *IRT1* genes in roots of soybean plants grown at Fe-sufficient and aCO<sub>2</sub> (A), Fe-sufficient and eCO<sub>2</sub> (B), Fe-deficient and aCO<sub>2</sub> (C), Fe-deficient and eCO<sub>2</sub> (D) from three independent replicates.



Similar findings were reported in tomato (Jin et al. 2009) and barley (Haase et al. 2008) grown at  $e\text{CO}_2$  with or without Fe-supply. The root/shoot ratio increased in Fe-sufficient plants due to  $e\text{CO}_2$  and was not affected in Fe-deficient plants, suggesting that the biomass accumulation changed with Fe-supply. The reason proposed might be that under  $e\text{CO}_2$  and Fe-supply, upregulation of the root over the shoot growth was observed appearing in a higher root/shoot ratio. Also,  $e\text{CO}_2$  alleviated the Fe-deficiency chlorosis symptoms, as demonstrated by the increase in chlorophyll concentration (Figure 1). The driving force for increased soybean growth at  $e\text{CO}_2$  is a higher  $A$  (Ainsworth et al. 2002). We noticed that the  $A$  was not changed due to  $\text{CO}_2$  enrichment and could not predict the magnitude of biomass increase. However, the photosynthetic capacity was affected by Fe-supply ( $P < 0.001$ , Table 1), with a concomitant decrease in Fe-deficient plants of about 20% (Table 2). Therefore, under  $e\text{CO}_2$  photosynthetic acclimation was observed and attributed to a decrease in  $V_{c,\text{max}}$  and  $J_{\text{max}}$ . Nevertheless, despite acclimation of photosynthetic capacity, biomass production is greater in plants exposed to  $e\text{CO}_2$ . It is generally accepted that Fe-deficiency decreased photosynthesis in higher plants (Pestana et al. 2001 Jiang et al. 2007), since the Fe-status is a crucial parameter that affects metabolic changes, photosynthesis, and consequently plant growth at  $e\text{CO}_2$  (Briat et al. 2015).  $e\text{CO}_2$  reduces the transpiration-driven mass flow due to the stomatal closure (Leakey et al. 2009; Prior et al. 2010). In our study,  $e\text{CO}_2$  decreased the  $g_s$  by 60–64.9% and  $T_r$  by 44.7–52.9% compared to  $a\text{CO}_2$  conditions (Table 2). The decrease in  $g_s$  at  $e\text{CO}_2$  was associated with higher WUE and consistent with previous studies (Prior et al. 2010; Zheng et al. 2020). Our results suggested that soybean plants may have increased drought tolerance under  $e\text{CO}_2$  conditions. The decrease in the  $T_r$  might be a modification to maximize the carbon fixation under  $e\text{CO}_2$  conditions (Jauregui et al. 2015). The carbohydrate accumulation, mainly glucose and fructose, which are assimilates from photosynthesis, exhibited significant variation with  $e\text{CO}_2$  in leaf and root tissues resulting in differential carbon allocation, as demonstrated in Table 3. Therefore,  $e\text{CO}_2$  increased carbohydrate accumulation independently of Fe-supply, while an increase of photosynthesis capacity was not confirmed. A possible reason for this phenomenon, at  $e\text{CO}_2$ , is that plants exceed what they are capable of using or distributing to sinks, increasing the carbohydrates levels, and leading to feedback inhibition of photosynthesis (Thompson et al. 2017).

Furthermore, the results showed a differential variation of sugars in plant tissues, but this variation was independent of Fe-supply ( $P > 0.05$ , Table 4). Therefore, variation in nutrient uptake mechanisms should be less or partially influenced by sugars under Fe-deficiency. However, there are new insights on sugar sensing and signaling pathways of how plants are influenced by the higher sugars content produced under  $e\text{CO}_2$  conditions (Thompson et al. 2017). Sugars are recognized to crosstalk with hormones (e.g. auxin, nitric oxide and ethylene) and act on gene regulation and therefore modify nutrient uptake and transport, among other functions (Thompson et al. 2017). Hindt and Guerinot (2012) proposed a model of the Fe-deficiency response, where auxin, ethylene, and nitric oxide were involved as positive regulators of the Fe acquisition genes FIT, FRO2, and IRT1. A recent study of Lin et al. (2016) proposed sucrose as the

upstream signaling molecule of auxins, causing an increase in auxin and a subsequent increase in nitric oxide, ultimately causing FIT-mediated transcriptional regulation of FRO2 and IRT1 genes and inducing Fe-uptake. In this study, soybean plants allocate greater amounts of sugars (mainly glucose and fructose) to root and leaf tissues during exposure to  $e\text{CO}_2$  conditions, which might cause an imbalance in carbon and nitrogen metabolism. Therefore, Fe-deficiency signals with sugar variation induced by  $e\text{CO}_2$  conditions might act in concert and affect Fe-uptake mechanisms. Besides, if these genes are regulated by the increase of sugars, then it stands to reason that an increase in sugar content in roots mediated by exposure to  $e\text{CO}_2$  might also induce Fe-deficiency induced responses. The levels of organic acids increased at  $e\text{CO}_2$  in roots, and remained almost unchanged in leaves. The increased levels of carbohydrates and organic acids in plants exposed to  $e\text{CO}_2$  indicates a higher energy condition capable of maintaining the greater carbon allocation (Jauregui et al. 2015). Moreover, Pavlovic et al. (2013) demonstrated that increased concentration of citrate and malate in the root tissues of cucumber was also important in alleviating Fe deficient responses. Since  $e\text{CO}_2$  and sugars increase plant root growth, it was expected greater uptake of nutrients, thus alleviating nutrient deficiencies, however, there are other mechanisms that are affected by  $e\text{CO}_2$  that lead to nutrient deficiencies (Thompson et al. 2017). The influence of  $e\text{CO}_2$  and Fe-deficiency, on plant mineral concentrations, were also addressed. The roots and leaves were differentially affected by  $e\text{CO}_2$  and Fe-supply, as described in Figures 5 and 6. Overall,  $e\text{CO}_2$  increased K, P, Mn, Zn, and Fe concentrations in leaves of Fe-sufficient plants, and P and Zn in Fe-deficient plants but did not affect the other minerals. In roots of Fe-sufficient plants, the mineral concentrations were not affected by  $e\text{CO}_2$ , whereas in Fe-deficient plants,  $e\text{CO}_2$  increased P and Zn, and Ca, Mn, and Fe remained unchanged. Consequently, our results suggested that  $e\text{CO}_2$  can induce the accumulation of minerals particularly in soybean leaves and at Fe-sufficient conditions probably indicating that the mineral uptake and upward transport by soybean roots was not restricted. As also reported by others (Haase et al. 2008; Jin et al. 2009; Guo et al. 2015)  $\text{CO}_2$  enrichment could considerably increase the mineral uptake by the roots promoting the transport from soil to leaves. Also, it is recognized that increase in biomass at  $e\text{CO}_2$  changes the demands for nutrients (Seneweera 2011). In this study, with a consistent supply of nutrients under flooded conditions, the bioavailability was relatively higher to maintain that nutrient supply under  $e\text{CO}_2$ . Thus, the increase in the root/shoot ratio, mainly in Fe-sufficient plants, under  $e\text{CO}_2$  can promote nutrient uptake and possibly lead to higher nutrient concentrations under  $e\text{CO}_2$  conditions. Also, we found a decrease in K (roots) and Mg (roots and leaves) in Fe-deficient plants. This reduction could be attributed to the unbalanced mineral translocation from the roots to the leaves or by differential mineral uptake.

The adaptation of soybean plants to Fe-deficiency under  $e\text{CO}_2$  conditions was also demonstrated by evaluating the expression of genes involved in Fe-uptake and the activity of root FCR. It was reported in tomato and Arabidopsis, that the *FER* gene is upregulated by Fe-deficiency and encodes a root-specific bHLH transcription factor regulating the expression of some Fe-responsive genes, such as the ferric chelate reductase *FRO2* and the transporter *IRT1* (Connolly et al.

2003 Jin et al. 2009; Paolacci et al. 2014). In the present study, we found typical Fe-deficiency responses in soybean plants grown with different Fe-supplies at eCO<sub>2</sub> under controlled conditions. In agreement with previous findings (Jin et al. 2009), we found that FCR activity increased in response to Fe-deficiency. Moreover, *DMT1*, *FER*, *FRO2*, and *IRT1* gene expression levels were also upregulated by Fe-deficiency, and this phenomenon usually increased by exposure to eCO<sub>2</sub> (Figure 7). Graziano and Lamattina (2007) argued that these are crucial Fe-deficiency responses, but they are not sufficient to significantly increase Fe concentration in Fe-deficient plants (Figure 6). Furthermore, expression of the *ferritin* protein gene increased in Fe-sufficient plants, particularly at eCO<sub>2</sub> conditions. Iron homeostasis is strongly dependent on *ferritin* expression levels produced in response to Fe as previously demonstrated (Wei and Theil 2000; Arnaud et al. 2006; Briat et al. 2010). Fe homeostasis has to be precisely controlled, to prevent starvation that impairs the metabolism, and to avoid excess that may lead to cell death (Arnaud et al. 2006). Therefore, at eCO<sub>2</sub> conditions Fe-sufficient plants have more Fe content in their roots (Figure 6), and this could be a strategy (i.e. increasing *ferritin* expression) that plants use to control the Fe excess.

## 5. Conclusion

We have demonstrated that changes in CO<sub>2</sub> concentration and Fe-supply can induce a set of morphological, physiological, and molecular responses in soybean plants. CO<sub>2</sub> enrichment has a decisive influence on the adaptation to Fe-deficiency in soybean plants. According to our results, biomass accumulation was significantly increased by eCO<sub>2</sub> irrespective of Fe-supply. Therefore, our results suggest that eCO<sub>2</sub> alleviated symptoms of Fe-deficiency and might support biomass increase in plants grown in calcareous soils, at least during plant's earlier developmental stages, which is usually the most sensitive to Fe-deficiency. However, it would be interesting to replicate the responses under eCO<sub>2</sub> of soybean plants grown on alkaline and calcareous soils which can induce Fe-deficiency chlorosis. Improved acquisition of several minerals under sufficient Fe-supply may suggest that eCO<sub>2</sub> can support the unbalanced translocation of minerals to the leaves. eCO<sub>2</sub> also mitigates the Fe-deficiency-induced chlorosis and enhances Fe-acquisition mechanisms but they are not sufficient to increase the Fe-status of plants grown in Fe-deficient conditions. It will be meaningful to understand if these observed effects will be sustained under a longer-term exposure and what will be the reported influence of eCO<sub>2</sub> on nutritional value of soybean grains.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Data availability statement

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

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