

1 **Title page**

2 **Title:** Elevated [CO<sub>2</sub>] alleviates the impacts of water deficit on xylem anatomy and  
3 hydraulic properties of maize stems

4

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22

23 **Abstract:**

24 Plants can modify xylem anatomy and hydraulic properties to adjust to water status.  
25 Elevated [CO<sub>2</sub>] can increase plant water potential via reduced stomatal conductance  
26 and water loss. This raises the question of whether elevated [CO<sub>2</sub>], which thus  
27 improves plant water status, will reduce the impacts of soil water deficit on xylem  
28 anatomy and hydraulic properties of plants? To analyze the impacts of water and  
29 [CO<sub>2</sub>] on maize stem xylem anatomy and hydraulic properties, we exposed potted  
30 maize plants to varying [CO<sub>2</sub>] levels (400, 700, 900, 1200 ppm) and water levels (full  
31 irrigation, deficit irrigation). Results showed that at current [CO<sub>2</sub>], vessel diameter,  
32 vessel roundness, stem cross-section area, specific hydraulic conductivity and  
33 vulnerability to embolism decreased under deficit irrigation; yet, these impacts of  
34 deficit irrigation were reduced at elevated [CO<sub>2</sub>]. Across all treatments, midday stem  
35 water potential was tightly correlated with xylem traits and displayed similar  
36 responses. A distinct trade-off between efficiency and safety in stem xylem water  
37 transportation in response to water deficit was observed at current [CO<sub>2</sub>], but not  
38 observed at elevated [CO<sub>2</sub>]. The results of this study enhance our knowledge of  
39 plant hydraulic acclimation under future climate environments and provide insights  
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41

42 **Keywords:** Xylem anatomy; hydraulic property; elevated [CO<sub>2</sub>]; water deficit;  
43 embolism; maize.

44

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53 **hydraulic properties of maize stems**

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75 embolism; maize.

76

## 77 **Introduction**

78 As the long-distance, low-resistance water transport pathway in terrestrial plants,  
79 xylem allows rapid and substantial movement of water from roots to leaves and is  
80 necessary to sustain gas exchange by leaves in a comparatively dry atmospheric  
81 environment. Xylem hydraulic characteristics have a primary control effect on the  
82 survival and productivity of plants (Brodribb 2009). These hydraulic properties are  
83 determined by the anatomical structure of the conduit units (vessel or tracheid) in  
84 xylem. According to the Hagen-Poiseuille law, xylem hydraulic conductivity (i.e., the  
85 water transport efficiency) is related to the vessel number and the fourth power of  
86 the vessel diameter (Tyree and Zimmermann 2002). The pore structure of the  
87 conduit and the negative pressure on the conduit walls means that the water  
88 transport in the xylem faces the threat of embolism caused by cavitation of water  
89 columns and wall collapse, which increases water transport resistance and can even  
90 lead to hydraulic failure (Zimmermann 1983; De Guzman et al. 2016).

91 Xylem anatomy and hydraulic properties in plants are strongly regulated by  
92 genetic control and show considerable variation across different species (Maherali  
93 et al. 2004; Choat et al. 2012). In addition, recent work has demonstrated xylem  
94 plasticity within single species, beyond genetic control (Stiller 2009; Plavcová and  
95 Hacke 2012; Hacke 2015; Wang et al. 2016). Soil drying is the most frequent and

96 primary environmental stress that plants face. Experiments conducted under  
97 natural conditions across climatic and environmental gradients indicate that xylem  
98 conduit diameter, conduit length, hydraulic conductivity, and vulnerability to  
99 embolism of tree branches were positively correlated with local precipitation or soil  
100 water availability (Villar-Salvador et al. 1997; Choat et al. 2012; Schreiber et al.  
101 2015). Similar findings have been recorded in experiments under controlled  
102 environment conditions. Trees and crops, in dryland environments, often have  
103 smaller stem diameter, conduit size, hydraulic conductivity, and less vulnerability to  
104 embolism. Increases in the thickness of xylem cell walls and the density of conduits  
105 have also been reported (Stiller 2009; Awad et al. 2010; Hudson et al. 2018). These  
106 reports have additionally shown that the more severe the drought is, the higher the  
107 impacts.

108       Rising atmospheric carbon dioxide concentration ( $[CO_2]$ ) and related climate  
109 changes are critical environmental problems in many parts of the world, mainly  
110 stemming from the rapid growth of population and increased manufacturing  
111 capacity since the industrial revolution (IPCC 2007). If the current high emission  
112 trend continues,  $[CO_2]$  is predicted to exceed 1000 ppm by the year 2100 (IPCC  
113 2014). Associated climate changes such as increased atmospheric vapour pressure  
114 deficit will likely increase water use in agriculture, thereby increasing soil drying  
115 leading to more severe regional or global drought and food shortage (IPCC 2014). As  
116 the most important substrate for photosynthesis, elevated  $[CO_2]$  has a profound  
117 impact on plant growth. There is little consensus on the impact of elevated  $[CO_2]$  on

118 stem xylem in woody plants. One perspective is that elevated [CO<sub>2</sub>] significantly  
119 increases the diameter of the xylem vessel, hydraulic conductivity, and vulnerability  
120 to embolism (Atkinson & Taylor 1996; Kostianen et al. 2014; Kim et al. 2015); yet,  
121 some researchers have found that elevated [CO<sub>2</sub>] had no impact on these characters  
122 or that their impact is insignificant (Kostianen et al. 2004; Kilpeläinen et al. 2007;  
123 Vaz et al. 2012). Hydraulic failure at severe drought has been associated with forest  
124 mortality worldwide (McDowell et al. 2008; Allen et al. 2010). In addition, species  
125 diversity of trees is more suitable for research on long-distance transport. Woody  
126 plants have received more attention than crop plants in the literature. Xylem  
127 hydraulics also play an essential role in modifying crop growth (Sperry et al. 2003;  
128 Stiller et al. 2003), and crops are sensitive to embolism by drought stress because of  
129 the relatively low cost of xylem construction (Neufeld et al. 1992; Holloway-Phillips  
130 and Brodribb 2011). Researches on the impacts of [CO<sub>2</sub>] on crop stem xylem are  
131 limited and results are often disputed (Medeiros & Ward 2013; Rico et al. 2013),  
132 therefore further study is necessary to investigate the response of crop stem xylem  
133 to elevated [CO<sub>2</sub>], and the interaction with soil water status should be taken into  
134 consideration.

135       There are many comprehensive reviews focused on the influence of [CO<sub>2</sub>] on  
136 crops (e.g., Lawlor and Mitchell 1991; Drake et al. 1997; Long et al. 2004; Leakey et  
137 al. 2009). In general, crops show increased photosynthetic rate, growth rate, yield,  
138 and water use efficiency under elevated [CO<sub>2</sub>]. In recent years, several studies have  
139 emphasized the interactive impacts of [CO<sub>2</sub>] and water availability on crops. Deficit

140 irrigation (DI), in particular, provokes more pronounced responses to elevated [CO<sub>2</sub>]  
141 when compared with sufficient irrigation (Kang et al. 2002; Wall et al. 2006; Qiao et  
142 al. 2010; Li et al. 2018). Drought stress, as a major abiotic stress, has negative  
143 impacts on the growth of crops, but elevated [CO<sub>2</sub>] may relieve this stress by  
144 increasing water use efficiency (Meng et al. 2014; Frank et al. 2015) and may  
145 alleviate the negative impacts of soil water deficit on crops partially or wholly  
146 (Robredo et al. 2007). However, conflicting views indicate the stimulation of yield by  
147 elevated [CO<sub>2</sub>] on soybean decreased as drought intensified (Gray et al. 2016).

148 To further assess these possibilities, we evaluated the capacity of elevated  
149 [CO<sub>2</sub>] to alleviate the impacts of soil water deficit on xylem anatomy and hydraulic  
150 function of maize stems. If these were to be the case, elevated [CO<sub>2</sub>] might have a  
151 direct impact on stem xylem development as the increasing of assimilate availability  
152 plays a critical role in cell expansion and growth (Steppe et al. 2015). Besides,  
153 elevated [CO<sub>2</sub>] might have an indirect impact on stem xylem development  
154 especially in plants under soil water deficit, as elevated [CO<sub>2</sub>] might significantly  
155 improve plant water status which has a regulatory effect on cell expansion and  
156 growth (Hsiao and Acevedo 1974; Sheriff and Whitehead 1984). These contrasting  
157 responses might explain the conflicting effects of elevated [CO<sub>2</sub>] on stem xylem in  
158 woody plants mentioned earlier.

159 Based on the effects of elevated [CO<sub>2</sub>] on plant water status, we hypothesized  
160 that the impact of soil water deficiencies on xylem anatomy and hydraulic  
161 properties of plant stem would be alleviated by elevated [CO<sub>2</sub>]. A previous review



162 summarized the effects of atmospheric carbon dioxide enrichment on plant  
163 hydraulics (Domec et al. 2017), but the effects of elevated [CO<sub>2</sub>] under different  
164 moisture conditions were not compared in this review. Previous studies on the  
165 impact of elevated [CO<sub>2</sub>], particularly to stem xylem, were selected from the review  
166 (Domec et al. 2017) and other publications, and we analyzed these studies to more  
167 thoroughly assess the hypothesis (Table 1). In most of these studies, xylem anatomy  
168 and hydraulic properties of woody plants in well water conditions were not  
169 significantly influenced by [CO<sub>2</sub>], and the plants that showed significant responses  
170 to elevated [CO<sub>2</sub>] suffered water stress, which provides additional support to our  
171 hypothesis above. In this study, we evaluated the impacts of [CO<sub>2</sub>] and water  
172 availability on the xylem anatomy and hydraulic properties of a plant stem. Maize, a  
173 staple crop across the globe, was used in this study.

174

## 175 **Materials and methods**

### 176 **Plant material and growth conditions**

177 The experiment was performed from April to August in 2016 and 2017, in a climate  
178 phytotron at Shiyanghe Experimental Station of China Agricultural University,  
179 located in Wuwei City, Gansu Province in Northwest China (N 37°52', E 102°50';  
180 altitude 1581 m). Maize (*Zea mays* L. cultivar Qiangsheng 51) was grown from seeds  
181 in 15-L (26.5 cm height, 21 cm bottom diameter and 33 cm top diameter) plastic  
182 pots, to achieve the precise control of soil water; each pot was filled with local  
183 sandy loam (formed by 12% clay, 60% silt, and 28% sand; soil bulk density,

184 1.55g/cm<sup>3</sup>; soil water holding capacity, 28% (m<sup>3</sup>/m<sup>3</sup>)), with little stones and fine  
185 sand spread over the bottom to act as a filtration layer, the surface of the soil was  
186 mixed with nutritive soil to reduce the impact of irrigation and avoid soil  
187 compacting. We chose local soil to enhance the relevance to field conditions. The  
188 local soil had been air-dried, crushed and sieved before use, to make the soil  
189 uniform and eliminate spatial differences. A wire-netting (8mm mesh size) was used  
190 to sieve to remove the small stones and residual plant roots and leaves, which  
191 should not have changed the composition and physical properties of the soil. The  
192 same fertilization scheme was applied in each pot, which was 0.86 g urea, 0.11 g  
193 KH<sub>2</sub>PO<sub>4</sub>, and 0.65 g Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> with water every time at the 1st, 4th, 8th, and 13th  
194 week after sowing.

195 For the first six weeks (seedling stage), all pots were placed in a greenhouse  
196 without water deficit (about 80% field water capacity), one plant from each pot was  
197 selected to remain in the greenhouse at the four-leaf stage during this time. The  
198 maize started to elongate at the 7th week after sowing, then they were moved into  
199 the climate phytotron which was supplied with natural light (the average of the  
200 photosynthetically active radiation in maize growth period is 459.2  $\mu$  mol m<sup>-2</sup> s<sup>-1</sup> in  
201 2016 and 461.7  $\mu$  mol m<sup>-2</sup> s<sup>-1</sup> in 2017), the [CO<sub>2</sub>] and moisture treatments  
202 commenced. The rooms in climate phytotron were set to 400 ppm and 700 ppm in  
203 2016, 400ppm, 700ppm, 900 ppm and 1200 ppm in 2017; the temperature was  
204 maintained at 27°C/18°C (day (8:00-18:00) / night (18:00-8:00)), and the humidity  
205 to 50%/80%. In each room, two soil moisture treatments were created: full

206 irrigation (FI, 95%-65% field water capacity) and deficit irrigation (DI, 75%-55% field  
207 water capacity in 2016, 65%-45% field water capacity in 2017). The experimental  
208 pots were weighed every one to five days by high precision weighing scales for the  
209 basis of the irrigation amount, plants in each soil moisture treatment were irrigated  
210 once those in 400 ppm room reached the lowest limit. Measuring cylinders were  
211 used for measuring the irrigation water manually. The variety of soil water content  
212 in the maize jointing stage was recorded (supporting information Fig. S1). The tops  
213 of the experimental pots in 2017 were covered with plastic film to prevent soil  
214 evaporation, so that we can calculate the water consumption by plant transpiration.  
215 Maize in different two water treatments was placed adjacent in each room. The  
216 equipment and the controls of the climate phytotron are given in detail by Li et al.  
217 (2018).

### 218 **Plant growth**

219 Three plants representative of each treatment were randomly selected to measure  
220 the plant growth. Leaf length, maximum leaf width, plant height (cm), and stem basal  
221 diameter ( $D_{\text{stem}}$ , mm) were assessed with measuring tapes and callipers every five or  
222 ten days after the treatments (both water and  $[\text{CO}_2]$  began). The total leaf area (total  
223  $A_{\text{leaf}}$ ,  $\text{m}^2$ ) was calculated by summing the leaf length multiplied by the maximum leaf  
224 width and then multiplied by a conversion factor of 0.74 (Li et al., 2008). Supported  
225 leaf area (supported  $A_{\text{leaf}}$ ,  $\text{m}^2$ ) is the leaf area of foliage located downstream from the  
226 stem segment for hydraulic measurement, and it was measured before the harvest  
227 for hydraulic measurement.

228 **Leaf gas exchange**

229 Gas exchange measurements were made twice (the 10th week and 14th week after  
230 sowing) during the jointing stage in 2017 with a photosynthesis system (Li 6400;  
231 LI-COR, USA). The third leaves of three representative plants undergoing the same  
232 treatment were randomly selected for measurement. The incoming air stream was  
233 not conditioned, and the environmental parameters in the gas exchange cuvette  
234 were recorded (see Table S1). The daily variations of the parameters, including net  
235 photosynthetic rate ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ), and  
236 transpiration rate ( $T_r$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), were recorded at two-hourly intervals from 8:00  
237 to 18:00, then we calculated the average value.

238 **Water potential measurements**

239 The midday (12:00-13:00) stem water potential ( $\Psi_{\text{stem}}$ , MPa) was measured twice  
240 (the 10th week and 14th week after sowing) on typically sunny days during the  
241 jointing stage in 2017, three or four plants per treatments were randomly selected in  
242 each measurement. Stem water potential was estimated by measuring the water  
243 potential of a non-transpiring (bagged) leaf (Begg and Turner 1970; Sack et al. 2003;  
244 Hillabrand et al. 2016). An aluminium bag was used to wrap the sample leaf (the  
245 nearest leaf under the ear of maize, close to the segment for anatomical  
246 measurement) 2 hours before harvest. We sample small round pieces from the  
247 leaves with a leaf punch and sealed them in sample cuvettes before brought back to  
248 the laboratory. A dew-point water potential meter (WP4-C; Decagon, USA) was used  
249 for the measurement of plant water status.

250 **Hydraulic measurements**

251 After 6 weeks in the greenhouse and 10-12 weeks under different irrigation and  
252 [CO<sub>2</sub>] of treatment, five plants representative of each treatment were randomly  
253 harvested at the silking stage (the 16-17th weeks after sowing in 2016; the 18th  
254 week after sowing in 2017); each plant was cut at the base of the stem and taken  
255 back to the laboratory. Stem segments were prepared from the parts under the  
256 ears, and cut to a length of 27.5cm with razor blades under boiled-degassed water,  
257 making sure the cut face was smooth. Segments were typically 14–20 mm in  
258 diameter. We use 20mM KCl solution (vacuum degassed and 0.22µm filtered) to  
259 flush segments for 30 min under 100kPa to remove any possible native embolisms.  
260 Maximum hydraulic conductivity ( $K_{max}$ , mg mm kPa<sup>-1</sup> s<sup>-1</sup>) was measured after  
261 flushing by the gravity method outlined in Sperry et al. (1988). The hydraulic head  
262 pressure was supplied by a raised glass bottle and maintained at 6 kPa. The  
263 computer connected to the balance automatically recorded the weigh every 15s  
264 and calculated the  $K_{max}$ . In order to prevent water leakage caused by asymmetrical  
265 stem cross-sections, we referenced the operation of Li et al. (2009) to filling the  
266 cavity at one end of the stem with poster mud and wrapping with Teflon tape,  
267 making sure a close combination between stem and silicone tube. Stem specific  
268 hydraulic conductivity ( $K_s$ , mg mm<sup>-1</sup> kPa<sup>-1</sup> s<sup>-1</sup>) was subsequently calculated as the  
269  $K_{max}$  of a stem segment divided by the corresponding stem cross-section area.

270 The centrifuge method was first and widely used to determine the stem xylem  
271 cavitation vulnerability curve (Alder et al., 1997) and also reliable for non-woody

272 plants (e.g., Maize, Li et al. 2009; Sunflower, Rico et al. 2013; Phaseolus, Medeiros &  
273 Ward 2013; Arabidopsis, Tixier et al. 2013). We used a centrifuge (H2050R-1;  
274 Xiangyi, China) and a custom-built rotor modified to suit maize stem segments  
275 based on the design on the website of Sperry lab  
276 (<http://sperry.biology.utah.edu/methods.html>) in 2016. The centrifuge (H2100R;  
277 Xiangyi, China) and rotor modified based on Chinatron (Wang et al. 2014) were  
278 used in 2017. Segments were secured in the rotor and spun on the centrifuge, after  
279 spinning to induce the desired negative pressure at the stem centre for 3 min, the  
280 stems were removed, and hydraulic conductivity was again measured. The process  
281 was repeated at progressively higher spinning speeds (more negative pressure) until  
282 more than 90% of the  $K_{\max}$  was lost, and most of the xylem had been cavitating.  
283 Stem xylem vulnerability curves were expressed by plotting percentage loss of  
284 conductivity vs xylem pressure. The vulnerability curve was fitted using a cubic  
285 function ( $r^2 > 0.9$ ), and we calculated the water potential inducing 50% loss of  
286 hydraulic conductivity (P50, MPa). The laboratory was maintained at approximately  
287 25°C to reduce the influence of water viscosity change on conductance.

### 288 **Anatomical measurements**

289 The stem cross-sections between internodes were obtained from hydraulic samples,  
290 stained with safranin and then photographed by a digital optical microscope (BA210;  
291 Motic, China). In maize stem, xylem vessels are grown scattered in vascular bundles  
292 and distributed in the whole stem cross section. In general, every matured maize  
293 vascular bundle has two metaxylem vessels and one protoxylem vessel, but the

294 protoxylem vessel is considered to be damaged and lost its function during the  
295 elongation of stem (Lucas et al. 2013). The stem cross-section area ( $A_{\text{stem}}$ ,  $\text{mm}^2$ ) and  
296 the total number of vascular bundles (N) in this cross-section were measured and  
297 counted from the photographs. Metaxylem vessel diameter (D,  $\mu\text{m}$ ) and roundness  
298 (R,  $\mu\text{m} \mu\text{m}^{-1}$ ) based on a random selection of 24 vascular bundles were measured  
299 from the photograph by ImageJ software (NIH Image, Bethesda, MD, USA). The  
300 longest diameter and the shortest diameter of each vessel were recorded,  $D =$   
301  $(\text{longest diameter} + \text{shortest diameter})/2$ ,  $R = \text{shortest diameter}/\text{longest diameter}$ .  
302 Vascular bundle density ( $\rho$ ,  $\text{mm}^{-2}$ ) was calculated as  $N/A_{\text{stem}}$ . Vascular bundles were  
303 divided into central vascular bundles (subscript C) and peripheral vascular bundles  
304 (subscript P) according to their morphology and position.

305 We calculated a theoretic hydraulic conductivity ( $K_{\text{hp}}$ ,  $\text{mg} \text{mm}^{-1} \text{kPa}^{-1} \text{s}^{-1}$ ) of the  
306 stem using the Hagen-Poiseuille formula. This Hagen-Poiseuille conductivity was  
307 expressed per stem cross-sectional area for comparison with measured  
308 conductivity of the same stems. For this calculation, the vessel cross section was  
309 generally simplified into a circle as calculated in formula (1) (see Calkin et al. 1985;  
310 Li et al. 2009), and the resistance of perforation plate between vessels was ignored.  
311 We measured vessel roundness, not only to reveal the response of the vessel shape  
312 to water and  $[\text{CO}_2]$  treatments but also to attempt to capture a more accurate  
313 theoretical value of  $K_{\text{hp}}$ , as most of the xylem vessels are not regular circles but  
314 approximate to ellipses. We used formula (2) to calculate  $K_{\text{hp}}$  with an elliptical  
315 correction.

316 
$$K_{hp} = (\pi / 128\eta) \sum_{i=1}^n D_i^4 / A_{stem} \quad (1)$$

317 
$$K_{hp} = (\pi / 128\eta) \sum_{i=1}^n D_i^4 (2 / (R_i + 1 / R_i)) / A_{stem} \quad (2)$$

318 Where  $\eta$  is the viscosity of water (Pa·s), and  $n$  is the number of vessels.

### 319 **Statistical analysis**

320 SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A one-way  
321 ANOVA was applied to test the differences in means of treatments. Two-way  
322 ANOVA was also performed for all measured traits using [CO<sub>2</sub>] and irrigation level as  
323 two factors (results see supporting information Table. S2). The figures and the  
324 analysis of correlations between parameters were prepared with SigmaPlot 12.5  
325 (SPSS Inc., Chicago, IL, USA).

326

## 327 **Result**

### 328 **Plant growth**

329 We selected the maximum value of total  $A_{leaf}$ , height, and basal  $D_{stem}$  during the  
330 growth period of each plant to compare the growth of maize under different  
331 treatments. Elevated [CO<sub>2</sub>] stimulated the growth of maize plants, and water deficit  
332 had a negative impact on growth (Table 2). The total leaf area of maize increased at  
333 elevated [CO<sub>2</sub>]. In contrast, water deficit led to a significant reduction in total leaf  
334 area especially at 400 ppm, from  $0.55 \pm 0.01 \text{ m}^2$  to  $0.47 \pm 0.03 \text{ m}^2$  in 2016 and  $0.65$   
335  $\pm 0.03 \text{ m}^2$  to  $0.51 \pm 0.03 \text{ m}^2$  in 2017. The negative impact of water deficit decreased  
336 at elevated [CO<sub>2</sub>]. Plant height showed similar responses to water and [CO<sub>2</sub>],  
337 ranging from  $196 \pm 6 \text{ cm}$  to  $279 \pm 2 \text{ cm}$  in 400 ppm DI and 1200 ppm FI plants,



338 respectively. Basal stem diameter was smallest in 400 ppm DI ( $19.1 \pm 0.6$  mm) and  
339 largest in 1200 ppm FI ( $23.4 \pm 0.3$  mm).

#### 340 **Leaf gas exchange**

341 For all [CO<sub>2</sub>] treatments, net photosynthetic rate ( $P_n$ ; Fig. 1a) was reduced in  
342 response to water deficit and significantly under 400 ppm and 900 ppm. At 400  
343 ppm,  $P_n$  was  $19.5 \pm 6.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $11.1 \pm 4.3 \mu\text{mol m}^{-2} \text{s}^{-1}$  under FI and DI,  
344 respectively. 700 ppm [CO<sub>2</sub>] significantly increased  $P_n$  under DI but not FI, and with  
345 [CO<sub>2</sub>] supplementation to 900 ppm and 1200 ppm, the variations of  $P_n$  under DI or  
346 FI were not significant. [CO<sub>2</sub>] supplementation and water deficit both led to  
347 decreases in stomatal conductance ( $g_s$ ; Fig. 1b), ranging from  $0.162 \pm 0.093 \text{ mol m}^{-2}$   
348  $\text{s}^{-1}$  in 400 ppm FI plants to  $0.029 \pm 0.007 \text{ mol m}^{-2} \text{s}^{-1}$  in 1200 ppm DI plants. Similarly,  
349 transpiration rate ( $T_r$ ; Fig. 1c) was largest in 400 ppm FI ( $2.28 \pm 0.96 \text{ mmol m}^{-2} \text{s}^{-1}$ )  
350 and smallest in 1200 ppm DI ( $0.76 \pm 0.17 \text{ mmol m}^{-2} \text{s}^{-1}$ ). The decreases in  $P_n$ ,  $g_s$ , and  
351  $T_r$  under water deficit were smaller for plants grown at 700, 900, and 1200 ppm,  
352 when compared with those grown at 400 ppm [CO<sub>2</sub>]. Increases in  $P_n$  and the  
353 decreases in  $T_r$  resulted in significant increases in leaf water use efficiency ( $WUE_{\text{leaf}}$ ,  
354  $P_n/T_r$ ; Fig. 1d) at elevated [CO<sub>2</sub>].

#### 355 **Plant water relations**

356 Because of the lower transpiration rate, most of the plants under elevated [CO<sub>2</sub>]  
357 were irrigated at the level close to but did not reach the lowest limit of soil water  
358 content when those under 400 ppm [CO<sub>2</sub>] reached. Thus the averages of soil water  
359 content under elevated [CO<sub>2</sub>] were close to or non-significantly higher than those

360 under 400 ppm conditions during the jointing stage (Fig. S1). Maize midday stem  
361 water potential ( $\Psi_{\text{stem}}$ , Table 2) at jointing stage was sensitive to soil water content  
362 at the current 400 ppm  $[\text{CO}_2]$ , and  $\Psi_{\text{stem}}$  significantly decreased from  $-1.07 \pm 0.09$   
363 MPa under FI to  $-1.55 \pm 0.14$  MPa under DI. With  $[\text{CO}_2]$  rising to 700 and 900 ppm,  
364  $\Psi_{\text{stem}}$  increased under both FI and DI. Then  $\Psi_{\text{stem}}$  had a slight and non-significant  
365 decrease at 1200 ppm  $[\text{CO}_2]$  under FI, and a significant decrease under DI when  
366 compare with 900ppm  $[\text{CO}_2]$  plants. Elevated  $[\text{CO}_2]$  reduced the difference in  $\Psi_{\text{stem}}$   
367 between FI and DI, and  $\Psi_{\text{stem}}$  under DI treatment at elevated  $[\text{CO}_2]$  was increased  
368 and showed no significant difference with  $\Psi_{\text{stem}}$  under 400 ppm FI treatment.

### 369 **Xylem anatomy**

370 The impacts of water and  $[\text{CO}_2]$  treatments on xylem vessel shape and vascular  
371 bundle distribution are shown in Table 3. Among the two types of vascular bundles,  
372 central vascular bundles have larger diameter vessels and were found in greater  
373 numbers, suggesting that central vascular bundles are more critical in the xylem  
374 water transportation when compared with periphery vascular bundles.

375 For the central vascular bundles, water deficit significantly reduced vessel  
376 diameter ( $D_c$ ) at 400 ppm  $[\text{CO}_2]$ , from  $53.3 \pm 5.3$   $\mu\text{m}$  under FI to  $47.6 \pm 2.8$   $\mu\text{m}$  under  
377 DI in 2016, and from  $57.1 \pm 2.6$   $\mu\text{m}$  under FI to  $48.4 \pm 1.7$   $\mu\text{m}$  under DI in 2017.  
378 Elevated  $[\text{CO}_2]$  significantly increased  $D_c$  under DI but not significant under FI.  
379 Similarly, vessel roundness ( $R_c$ ) was  $0.76 \pm 0.03$   $\mu\text{m} \mu\text{m}^{-1}$  under FI and  $0.67 \pm 0.03$   
380  $\mu\text{m} \mu\text{m}^{-1}$  under DI at 400 ppm  $[\text{CO}_2]$  in 2016, and  $0.82 \pm 0.01$   $\mu\text{m} \mu\text{m}^{-1}$  under FI and  
381  $0.77 \pm 0.01$   $\mu\text{m} \mu\text{m}^{-1}$  under DI at 400 ppm  $[\text{CO}_2]$  in 2017. Elevated  $[\text{CO}_2]$  had

382 significantly increased  $R_c$ , especially under DI. Moreover,  $R_c$  showed no significant  
383 difference between water treatments at a specific elevated  $[\text{CO}_2]$ . Peripheral  
384 vascular bundles either showed similar responses with central vascular bundles or  
385 were little affected by water and  $[\text{CO}_2]$  treatments.

386 Stem segment cross-section area ( $A_{\text{stem}}$ ) significantly decreased under water  
387 deficit, and  $A_{\text{stem}}$  increased with  $[\text{CO}_2]$  rising under both FI and DI. The number of  
388 central vascular bundles ( $N_c$ ) also significantly increased with  $[\text{CO}_2]$   
389 supplementation, but water deficit had no impact on this variable, with the  
390 result that their density ( $\rho_c$ ) showed no significant difference between  $[\text{CO}_2]$   
391 treatments but an increase in response to water deficit in 2017. Similar responses  
392 were found in the number and the density of peripheral vascular bundles.

393  $D_c$  was significantly correlated with  $\Psi_{\text{stem}}$  (Fig. 2a;  $r^2=0.70$ ,  $P=0.009$ ), and  $R_c$   
394 was also significantly correlated with  $\Psi_{\text{stem}}$  (Fig. 2b;  $r^2=0.71$ ,  $P=0.009$ ), moreover,  
395  $A_{\text{stem}}$  was significantly correlated with  $\Psi_{\text{stem}}$  (Fig. 2c;  $r^2=0.67$ ,  $P=0.013$ ).

### 396 **Xylem hydraulic properties**

397 At the current 400 ppm  $[\text{CO}_2]$ , water deficit significantly reduced stem specific  
398 hydraulic conductivity ( $K_s$ ; Fig. 3a) from  $0.59 \pm 0.10 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$  under FI to  
399  $0.51 \pm 0.09 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$  under DI in 2016, and significantly reduced  $K_s$  from  
400  $0.74 \pm 0.07 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$  under FI to  $0.52 \pm 0.05 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$  under DI in  
401 2017. Similarly, theoretical Hagen-Poiseuille hydraulic conductivity ( $K_{\text{hp}}$ ; Fig. 3b) was  
402  $0.87 \pm 0.34 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$  under FI and  $0.55 \pm 0.10 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$  under DI in  
403 2016, and  $1.10 \pm 0.17 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$  under FI and  $0.75 \pm 0.14 \text{ mg mm}^{-1} \text{ kPa}^{-1} \text{ s}^{-1}$

404 under DI in 2017. Elevated [CO<sub>2</sub>] increased K<sub>s</sub> and K<sub>hp</sub> under DI, but not under FI. At  
405 elevated [CO<sub>2</sub>], the drops in K<sub>s</sub> or K<sub>hp</sub> under water deficit were smaller when  
406 compared with plants at 400 ppm [CO<sub>2</sub>]. Differences between these assessments  
407 can mainly be attributed to the fact that the Hagen-Poiseuille formula ignores the  
408 resistance caused by the unsmooth inner wall surface and the perforation plate  
409 between vessels, K<sub>hp</sub> showed similar responses with K<sub>s</sub> to water and [CO<sub>2</sub>]  
410 treatments but with more substantial values. K<sub>s</sub> was also tightly correlated with D<sub>c</sub>  
411 (Fig. 4; r<sup>2</sup>=0.96, P < 0.001). K<sub>hp</sub> with ellipses corrected was 1.2%-4.4% lower than the  
412 results provided by formula (1) and was closer to the values of K<sub>s</sub> (data not shown).  
413 However, the error generated by simplifying the ellipse into a circle was limited and  
414 can be ignored.

415 The supported A<sub>leaf</sub> scaled linearly with stem's capacity to transport water,  
416 including A<sub>stem</sub> (Fig. 5a; r<sup>2</sup>=0.54, P=0.007) and K<sub>max</sub> (Fig. 5b; r<sup>2</sup>=0.87, P<0.001).

417 Under FI treatment, elevated [CO<sub>2</sub>] slightly increased the stem xylem  
418 vulnerability to embolism (Fig. 6). Under the DI treatment, maize showed the  
419 lowest vulnerability at 400 ppm [CO<sub>2</sub>] and showed an increase in vulnerability at  
420 700 ppm and 900 ppm [CO<sub>2</sub>] but slightly decreased at 1200 ppm [CO<sub>2</sub>]. We  
421 compared the water potential inducing 50% loss of conductivity (P50, Table 4)  
422 calculated from the vulnerable curves between treatments. At 400 ppm [CO<sub>2</sub>], P50  
423 dropped from -0.87 ± 0.26 MPa under FI to -1.21 ± 0.16 MPa under DI in 2016, and  
424 from -1.83 ± 0.19 MPa under FI to -2.56 ± 0.22 MPa under DI in 2017. At elevated  
425 [CO<sub>2</sub>], P50 under DI significantly increased, and there was no significant difference

426 between water treatments.

427 There was a strong correlation between P50 and  $\Psi_{\text{stem}}$  under different  
428 treatments in 2017 (Fig. 7a;  $r^2=0.84$ ,  $P<0.001$ ). There was a very weak correlation  
429 between P50 and  $K_s$  in 2017 (Fig. 7b;  $r_1^2=0.42$ ,  $P=0.08$ ), if we analyze the data apart  
430 from 400 ppm DI plants, there was no correlation between P50 and  $K_s$  (Fig. 7b;  
431  $r_2^2=0.06$ ,  $P=0.58$ ).

432

### 433 **Discussion**

434 Xylem facilitates the rapid movement of substantial volumes of water from roots to  
435 transpiring leaves, which have a primary control effect on plant water status and  
436 photosynthetic capacity of plants (Brodribb 2009). Until now, however, the effects  
437 of elevated  $[\text{CO}_2]$  on xylem anatomy and hydraulic traits have not been adequately  
438 addressed. We hypothesized that the impact of soil water deficiencies on xylem  
439 anatomy and hydraulic properties of the plant stem would be alleviated by elevated  
440  $[\text{CO}_2]$ , due to the effects of elevated  $[\text{CO}_2]$  on plant water status.

#### 441 **Xylem anatomy**

442 At the current atmosphere  $[\text{CO}_2]$ , xylem anatomy showed a significant response to  
443 water deficit in maize stems, with decreases in vessel diameter ( $D_c$ ), vessel  
444 roundness ( $R_c$ ), and stem cross-section area ( $A_{\text{stem}}$ ) (Table 3). Studies of many trees  
445 and crops report similar long-term response to water stress (Arend and Fromm  
446 2007; Stiller 2009; Awad et al. 2010; Plavcová and Hacke 2012; Schreiber et al. 2015;  
447 Hudson et al. 2018). Photosynthetic capacity is associated with xylem formation

448 because assimilation is crucial not only for the synthesis of cell-wall products but  
449 also for maintaining turgor pressure in the enlarging cells (Ray et al. 1972; Larcher  
450 2003; Steppe et al. 2015). Elevated [CO<sub>2</sub>] can stimulate plant photosynthetic rate  
451 and which was observed in our study (Figure 1). However, this direct impact of  
452 elevated [CO<sub>2</sub>] (increasing assimilate) on xylem anatomy was not obvious, as the  
453 increases of  $D_c$ ,  $R_c$ ,  $A_{stem}$  under FI were not significant in our study. Besides  
454 increased photosynthetic rate, stomatal conductance is also the direct impacts of  
455 elevated [CO<sub>2</sub>] on plants (Morison 1998). Plants can improve their internal water  
456 status and maintain high water potential and turgor pressure when face with water  
457 deficiency (e.g. Robredo et al. 2007). An indirect impact of elevated [CO<sub>2</sub>] on xylem  
458 anatomy by stomatal control was observed in this study. In this study, elevated [CO<sub>2</sub>]  
459 alleviates the impacts of deficit irrigation on xylem anatomy, due to the indirect  
460 impact of elevated [CO<sub>2</sub>]. The data in 2017 showed that at 400 ppm [CO<sub>2</sub>],  $D_c$  under  
461 DI was 16.8% lower than FI, and with [CO<sub>2</sub>] supplementation,  $D_c$  under DI was  
462 11.8%, 12.4%, and 11.9% lower than FI, at 700, 900, 1200 ppm [CO<sub>2</sub>] respectively;  
463 DI treatment significantly reduced  $R_c$  at 400 ppm [CO<sub>2</sub>], and  $R_c$  was not significantly  
464 different between water treatments at elevated [CO<sub>2</sub>];  $A_{stem}$  under DI was 24.8%,  
465 22.7%, 20.1%, 18.0% lower than FI at 400, 700, 900, 1200 ppm respectively (Table  
466 3). The drops of  $D_c$ ,  $R_c$ , and  $A_{stem}$  by DI were also less dramatic for plants at elevated  
467 [CO<sub>2</sub>] in 2016. Water deficit can significantly decrease  $D_c$ ,  $R_c$ , and  $A_{stem}$ , but these  
468 impacts of water deficit were relieved at elevated [CO<sub>2</sub>], which support our  
469 hypothesis.

470 In our study, maize stems grew rapidly during the jointing stage, and xylem  
471 conduit cells were differentiated during this process, through a complex process  
472 encompassing cell-type determination, cell division, cell differentiation, cell  
473 expansion and programmed cell death (Ye 2002). Plant water status and assimilate  
474 availability play a critical role in cell expansion and growth (Hsiao and Acevedo 1974;  
475 Steppe et al. 2015). Xylem anatomy traits, including  $D_c$ ,  $R_c$ , and  $A_{stem}$ , were  
476 significantly correlated with the midday stem water potential ( $\Psi_{stem}$ , Fig. 2).  $\Psi_{stem}$   
477 was measured at jointing stage on the leaves that were close to the segments for  
478 later anatomical measurements; thus we use  $\Psi_{stem}$  to characterize the water status  
479 of differentiated stem xylem. Turgor pressure is the driving force of cell expansion  
480 during xylem differentiation, and it irreversibly stretches the plasticized cell wall  
481 because of the water potential gradient drawing water into the cell (Lockhart 1965,  
482 Hacke et al. 2017). It is difficult for cells to absorb water from the surroundings to  
483 maintain turgor and expand when plant tissue becomes dehydrated under low  
484 water potential (Hsiao and Acevedo 1974; Sheriff and Whitehead 1984). We have  
485 not measured the turgor pressure of differentiated xylem, but we can estimate it  
486 from  $\Psi_{stem}$ . Because unless it is altered by an osmotic adjustment, the turgor  
487 pressure of cells in vascular meristems will decline proportionally with xylem water  
488 potential (Fereres et al. 1978; Boyer and Silk 2004). So 400 ppm DI plants  
489 experienced significant water stress with reduced  $\Psi_{stem}$  presumably decreasing the  
490 turgor pressure, resulting in a significant decrease in  $D_c$ . At the jointing stage, the  
491 increases of  $P_n$  and the decreases of  $T_r$  result in a significant increase of  $WUE_{leaf}$  at

492 elevated [CO<sub>2</sub>] (Fig. 1) and increased  $\Psi_{\text{stem}}$  under DI (Table 2), but the saturated  
493 water conditions under FI limited the increases of  $\Psi_{\text{stem}}$  and reduced the  
494 differences between water treatments at elevated [CO<sub>2</sub>].  $\Psi_{\text{stem}}$  of DI plants had  
495 more significant increases with [CO<sub>2</sub>] supplementation when compared with FI  
496 plants, presumably accompanied by an increase in the turgor pressure, which  
497 diminished the differences in vessel diameter under two water treatments at  
498 elevated [CO<sub>2</sub>]. Maize stem is composed of many single cells where cell size is  
499 regulated by turgor pressure, so  $A_{\text{stem}}$  had similar responses to  $\Psi_{\text{stem}}$ .  $R_c$  might also  
500 be regulated by turgor pressure; however, it is difficult for a cell to maintain a full  
501 and round shape with low turgor pressure under water stress. Previous studies have  
502 also indicated that xylem anatomy and hydraulic traits of plants were associated  
503 with the plant water potential they have experienced (Woodruff et al. 2008;  
504 Blackman et al. 2010; Vinya et al. 2013). It is worth noting here that the chemical  
505 signals (e.g. hormone and ion concentration) are also involved in the xylem  
506 differentiation (Sorce et al. 2013; Smet & Rybel 2016; Takahashi & Shinozaki  
507 2018).

#### 508 **Hydraulic traits**

509 Similarly, at the current atmosphere [CO<sub>2</sub>], maize stem water transport efficiency  $K_s$   
510 showed a significant decrease to respond to water deficit (Fig. 3a). The direct  
511 impact of elevated [CO<sub>2</sub>] on  $K_s$  was not observed, as  $K_s$  showed no significant  
512 difference respond to elevated [CO<sub>2</sub>] under full irrigation in our study. However,  $K_s$   
513 under DI was 29.1%, 19.6%, 23.0 %, and 16.7% lower than FI at 400, 700, 900, and



514 1200 ppm [CO<sub>2</sub>], respectively. The drop of K<sub>s</sub> by DI was also less dramatic for plants  
515 at elevated [CO<sub>2</sub>] in 2016. Taking into consideration the Hagen-Poiseuille law, it is  
516 not hard to understand why K<sub>s</sub> was proportional to D<sub>c</sub>, with a very high correlation  
517 (Fig. 4). So due to the indirect impact of elevated [CO<sub>2</sub>] on D<sub>c</sub>, elevated [CO<sub>2</sub>]  
518 alleviated the impact of water deficiency on K<sub>s</sub>.

519 The decrease of K<sub>s</sub> under DI was significant under the current [CO<sub>2</sub>] but not  
520 under elevated [CO<sub>2</sub>], so that we can get the conclusion that the impact of soil  
521 water deficiencies on K<sub>s</sub> was alleviated by elevated [CO<sub>2</sub>]. However, there was no  
522 significant interactive effect between [CO<sub>2</sub>] and irrigation on K<sub>s</sub> according to the  
523 analysis of two-way ANOVA (Table S2). Most of the gas exchange, xylem anatomy  
524 and hydraulic parameters were in the similar situations, so the information we can  
525 get from two-way ANOVA was limited. In that case, our analysis and conclusion  
526 primarily based on one-way ANOVA.

527 We observed that A<sub>stem</sub> and also K<sub>max</sub> was correlated with the supported A<sub>leaf</sub>  
528 across all treatments (Fig. 5). Water transport through the stem xylem is essential  
529 for replacing water loss during leaf transpiration, so it is often assumed that greater  
530 leaf area as a result of environmental change can increase the transport capacity of  
531 the xylem to meet water demand (Atkinson & Taylor 1996; Gebauer & BassiriRad,  
532 2011; Plavcová & Hacke 2012; Medeiros & Ward 2013). Conversely, assimilation  
533 can drive leaf development until a limitation on stem development limits further  
534 leaf area development. In addition, as A<sub>stem</sub> represents not only the principal  
535 anatomical basis of a stem's capacity to transport water but also the capacity to

536 provide physical support for the leaves, hence the size of a stem determines the leaf  
537 area it can support (Brodribb & Field 2010). Elevated [CO<sub>2</sub>] significantly increased  
538 maize leaf area, but not the water demand, actually plant water consumption  
539 decreased at elevated [CO<sub>2</sub>] (Table 2) due to the decrease of g<sub>s</sub>. In this case, the  
540 hydraulic system tends to be more redundant at elevated [CO<sub>2</sub>].

541 Besides efficiency, the safety of the conducting system is also essential for  
542 crop survival. DI treatment decreased stem xylem vulnerability to embolism at 400  
543 ppm [CO<sub>2</sub>] with a significant decrease in P50, and elevated [CO<sub>2</sub>] significantly  
544 increased the P50 of DI plants to a level with no significant difference from FI (Fig. 6;  
545 Table 4). Thus, elevated [CO<sub>2</sub>] also alleviated the impact of water deficiency on  
546 embolism vulnerability. Numerous studies have demonstrated that cavitation  
547 resistance was associated with the plant water potential that plants experience  
548 (Hacke et al. 2000; Sperry and Hacke 2002; Blackman et al. 2010; Vinya et al. 2013),  
549 and we observed a tight correlation between P50 and  $\Psi_{\text{stem}}$  (Fig. 7a). In general,  
550 small conduits are more resistant to cavitation than large ones. Because the  
551 probability of large pores in a small conduit decreases as pit-surface-area was  
552 reduced (Cai and Tyree 2010). Besides, a smaller diameter also increases the cell  
553 wall structural strength to prevent collapse (Cochard et al. 2004). Both scenarios  
554 make xylem with small conduits have a low probability of embolism. Our result  
555 showed that lower D<sub>c</sub> significantly decreased P50 at the current atmospheric [CO<sub>2</sub>],  
556 but elevated [CO<sub>2</sub>] alleviated the impact of water deficit by decreasing D<sub>c</sub> and  
557 permitting P50 to increase.

558           There is often assumed to be a distinct trade-off between efficiency and safety  
559 in the xylem water transportation between or within species. Decreases in the  
560 average diameter of xylem vessels lower xylem hydraulic conductivity but may  
561 prevent embolism, as smaller vessels are less susceptible to cavitation  
562 (Zimmermann 1983). However, several previous works do not unequivocally  
563 support this trade-off (Martinez-Vilalta et al. 2009; Award et al. 2010; Plavcová and  
564 Hacke 2012). Our results showed that at the current [CO<sub>2</sub>], maize suffered water  
565 stress with a decrease in hydraulic conductivity and an increase in embolism  
566 resistance, indicating that a clear trade-off between safety and efficiency was  
567 established. However, at elevated [CO<sub>2</sub>], maize stems under the DI treatment have  
568 smaller conduits and lower hydraulic conductivity, but similar embolism resistance  
569 when compared with FI treatment. There seems to be a very weak correlation  
570 between P<sub>50</sub> and K<sub>s</sub> (Fig. 7b;  $r_1^2=0.39$ ,  $P=0.098$ ). P<sub>50</sub> of maize under 400 ppm DI  
571 was significantly lower than other treatments, and if we only analyze the data  
572 without 400 ppm DI, there was no correlation between P<sub>50</sub> and K<sub>s</sub> (Fig. 7b;  $r_2^2=0.06$ ,  
573  $P=0.592$ ). A distinct trade-off between efficiency and safety in the xylem water  
574 transportation under soil water and [CO<sub>2</sub>] changes were not observed.

575

## 576 **Conclusion**

577 Our study revealed maize stem hydraulic acclimation under future climate  
578 environments. Elevated [CO<sub>2</sub>] alleviated the negative impact of water deficit to  
579 decrease water transport efficiency, with our maize showing lower resistance to

580 long-distance water transport, which is beneficial to the growth of maize due to the  
581 close correspondence between assimilation rate and hydraulic conductance  
582 (Brodribb 2009). On the other hand, elevated [CO<sub>2</sub>] alleviated the impact of water  
583 deficit to decrease vulnerability to embolism and our maize plants under DI showed  
584 lower resistance to embolism at elevated [CO<sub>2</sub>]. It might be disadvantageous to the  
585 growth of maize, since xylem embolism vulnerability may be related to drought  
586 resistance of plants (Cochard et al. 2008; Li et al. 2009). However, maize stems at  
587 elevated [CO<sub>2</sub>] usually maintain relatively high water potential potentially can avoid  
588 the formation of embolism and showed a redundant hydraulic system which can  
589 ensure water supply. The use of deficit irrigation is a promising approach to both  
590 save water and induce plant physiological regulations (Du et al. 2015), our results  
591 showed that deficit irrigation might benefit crops more in future agriculture under  
592 climate change as elevated [CO<sub>2</sub>] will reduce some negative impacts of soil water  
593 deficit on photosynthesis and growth of plants. A limitation of our study is that our  
594 maize was planted in pots in glasshouse conditions, but not in the field. Large-scale  
595 studies particularly free-air CO<sub>2</sub> enrichment (FACE) studies (Ainsworth & Long  
596 2005) are under natural conditions without enclosure, which could make the result  
597 more reliable.

598 Here, we showed that elevated [CO<sub>2</sub>] alleviated the impacts of water  
599 deficiency on xylem anatomy and hydraulic properties; moreover, we also  
600 demonstrated that these impacts of elevated [CO<sub>2</sub>] were not direct but indirect.  
601 The hypothesis and results are logical and based on well-known phenomena, yet

602 different species may have different adjustment strategies in the face of elevated  
603 [CO<sub>2</sub>] (Domec et al. 2017), and the magnitudes of responses may vary in other  
604 maize cultivars or soil types, which require further exploration. However, our  
605 results provide another perspective to assess the impact of elevated [CO<sub>2</sub>] to plant  
606 xylem anatomy and hydraulics, that soil water status and other environmental  
607 factors must be taken into consideration.

608

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616

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844 **Table. 1** Previous studies on the impact of elevated [CO<sub>2</sub>] to stem xylem.

Species	[CO <sub>2</sub> ]	Description of water conditions	Impact of elevated [CO <sub>2</sub> ] to stem xylem	References
Quercus and Prunus	350, 700 ppm	Acclimated to lower humidities in covered micropropagators	The vessel lumen diameter, total vessel lumen cross-sectional area and branch hydraulic conductivity significantly increased at elevated [CO <sub>2</sub> ].	Atkinson & Taylor 1996
Ponderosa pine	350, 550, 750, 1100 ppm	Watered to field capacity every 2d	Elevated [CO <sub>2</sub> ] had minimal effects on K <sub>s</sub> and K <sub>L</sub> .	Maherali & DeLucia 2000
Norway spruce	360, 720 ppm	nonlimiting soil water	Elevated [CO <sub>2</sub> ] had only minor effects on tracheid lumen diameter, tracheid length and cell wall thickness.	Kostiainen et al. 2004
Scotts pine	present [CO <sub>2</sub> ], 700 ppm	Mean annual precipitation is 740 mm	Elevated [CO <sub>2</sub> ] had little impact on xylem anatomy.	Kilpeläinen et al. 2007
Quercus and Alnus	360, 720 ppm	Pots were kept in trays with water to avoid desiccation.	Total vessel area, mean vessel area and hydraulic mean vessel diameter were not significantly influenced by [CO <sub>2</sub> ].	Watanabe et al. 2008
Cork oak trees	350, 700 ppm	First 12 months watered twice a week until field capacity, then subjected to a water stress	No significant changes in stem diameter, vessel lumen diameter, or vulnerability to embolism at different [CO <sub>2</sub> ].	Vaz et al. 2012
Phaseolus vulgaris	180, 380, 700 ppm	Four water regimes ranging from high to low	Elevated [CO <sub>2</sub> ] significantly increased vessel diameter and decreased embolism resistance under both water deficit and good water conditions. The hydraulic traits decreased under elevated [CO <sub>2</sub> ].	Medeiros & Ward 2013
Helianthus annuus	290, 390, 480 ppm	Watered daily to saturation	Elevated [CO <sub>2</sub> ] significantly decreased vessel diameter and vulnerability to embolism under good water conditions.	Rico et al. 2013
Populus and Betula	present [CO <sub>2</sub> ], + 200 ppm	Different annual precipitation in 10 years	Vessel diameter and wall thickness increased at elevated [CO <sub>2</sub> ], and significant differences occurred in the years with relatively less precipitation.	Kostiainen et al. 2014
Six broad-leaved tree species	400, 600 ppm	Received deionized water to saturation to avoid soil drying	Stem hydraulic efficiency either increased or showed no consistent pattern of change to elevated [CO <sub>2</sub> ]. The vulnerability to drought-induced embolism did not respond to elevated [CO <sub>2</sub> ].	Hao et al. 2018



845 **Table. 2** Plant growth and water relations under different water and [CO<sub>2</sub>] treatments in 2016 and 2017. A<sub>leaf</sub>, leaf area; D<sub>stem</sub>, stem basal  
 846 diameter;  $\Psi_{stem}$ , midday stem water potential; Transpiration, maize transpiration in 47 days (7th-15th weeks) during jointing stage; FI, full  
 847 irrigation; DI, deficit irrigation.

Year	[CO <sub>2</sub> ]	Water treatment	Height (cm)	Total A <sub>leaf</sub> (m <sup>2</sup> )	D <sub>stem</sub> (mm)	$\Psi_{stem}$ (MPa)	Transpiration (kg)
2016	400 ppm	FI	263±8b	0.553±0.012b	20.4±0.4	-	-
		DI	235±3c	0.466±0.032c	19.5±0.8	-	-
	700 ppm	FI	276±5a	0.644±0.026a	21.5±1.1	-	-
		DI	271±5ab	0.651±0.027a	20.5±1.2	-	-
2017	400 ppm	FI	256±3b	0.651±0.027cd	20.8±0.1cd	-1.07±0.08cd	14.2±0.6a
		DI	196±6d	0.513±0.034f	19.1±0.6e	-1.55±0.14e	6.6±0.4d
	700 ppm	FI	271±3a	0.675±0.068bcd	21.6±0.3bc	-0.90±0.10a	13.1±0.6b
		DI	240±2c	0.587±0.030de	19.7±0.6e	-1.15±0.07d	5.7±0.7d
	900 ppm	FI	273±1a	0.737±0.021ab	22.8±0.2a	-0.81±0.09a	11.0±0.5c
		DI	243±6c	0.626±0.019de	20.2±0.6ed	-1.01±0.06bc	4.9±0.4e
	1200 ppm	FI	279±2a	0.790±0.031a	23.4±0.3a	-0.92±0.09ab	11.6±0.7c
		DI	248±3bc	0.704±0.024bc	22.6±0.7ab	-1.16±0.05d	6.0±0.5d

848 Mean ± SD is shown. Plant growth, n=3;  $\Psi_{stem}$ , n=7=n<sub>1</sub>+n<sub>2</sub>, n<sub>1</sub>=3 (samples in the 10th week), n<sub>2</sub>=4 (samples in the 14th week); Transpiration,  
 849 n=7. Values on each line and in the same year followed by the different letters are significantly different at the level of P<0.05 (one-way  
 850 ANOVA).

851 **Table. 3** Xylem conduit shape, vascular bundle distribution, and stem cross-section area under different water and [CO<sub>2</sub>] treatments in 2016  
 852 and 2017. VB, vascular bundles; subscript C, central vascular bundles; subscript P, peripheral vascular bundles; A<sub>stem</sub>, stem cross-section area; FI,  
 853 full irrigation; DI, deficit irrigation.

Year	2016				2017							
	400 ppm		700 ppm		400 ppm		700 ppm		900 ppm		1200 ppm	
	FI	DI	FI	DI	FI	DI	FI	DI	FI	DI	FI	DI
Vessel diameter (µm)												
Central VB (D <sub>C</sub> )	53.3±5.3ab	47.6±2.8b	54.8±3.3a	54.0±4.0a	57.1±2.6a	48.4±1.7c	56.8±2.0a	52.7±1.3b	57.0±1.7a	51.3±0.8b	58.5±2.4a	53.0±2.0b
Peripheral VB (D <sub>P</sub> )	35.2±1.0	34.6±2.4	34.3±1.9	34.9±2.2	34.4±2.4ab	31.7±2.3b	34.6±3.2ab	33.5±0.7ab	34.3±2.8ab	32.3±3.0b	35.5±2.3a	32.8±1.8ab
Vessel roundness (µm µm <sup>-1</sup> )												
Central VB (R <sub>C</sub> )	0.76±0.03a	0.67±0.03b	0.76±0.03a	0.74±0.04a	0.82±0.01a	0.77±0.01b	0.82±0.01a	0.82±0.01a	0.83±0.01a	0.82±0.02a	0.83±0.02a	0.83±0.02a
Peripheral VB (R <sub>P</sub> )	0.79±0.01a	0.75±0.03b	0.82±0.03a	0.79±0.03a	0.85±0.02	0.83±0.03	0.85±0.01	0.85±0.04	0.85±0.02	0.86±0.03	0.85±0.03	0.84±0.02
VB number												
Central VB (N <sub>C</sub> )	334±15bc	319±16c	372±16a	355±18ab	336±8c	337±22c	341±16bc	349±12abc	360±8a	355±7ab	366±7a	366±19a
Peripheral VB (N <sub>P</sub> )	165±11	159±16	160±17	150±7	116±7b	119±15b	120±4b	125±9ab	140±5a	131±4ab	136±8a	137±8a
Cross-section area (mm <sup>2</sup> )												
Stem (A <sub>stem</sub> )	251±12ab	230±26b	269±31a	255±20ab	189±10b	149±9e	190±6b	171±18cd	197±9b	163±10d	211±7a	184±17bc
VB density (mm <sup>-2</sup> )												
Central VB (d <sub>C</sub> )	1.33±0.09	1.41±0.15	1.40±0.14	1.40±0.10	1.77±0.04c	2.23±0.19a	1.80±0.06c	2.14±0.2ab	1.81±0.07c	2.18±0.12ab	1.70±0.08c	2.03±0.18b
Peripheral VB (d <sub>P</sub> )	0.66±0.05	0.70±0.15	0.60±0.13	0.59±0.05	0.61±0.03b	0.79±0.09a	0.64±0.03b	0.79±0.05a	0.67±0.05b	0.80±0.04a	0.64±0.05b	0.78±0.10a

854 Mean ± SD is shown. 2016, n=5; 2017, n=6-7. Values on each line and in the same year followed by the different letters are significantly  
 855 different at the level of P<0.05 (one-way ANOVA).

856 **Table. 4** The water potential inducing 50% loss of hydraulic conductivity (P50) under  
 857 different water and [CO<sub>2</sub>] treatments in 2016 and 2017. FI, full irrigation; DI, deficit  
 858 irrigation.

Year	[CO <sub>2</sub> ]	Water treatment	P50 (MPa)
2016	400ppm	FI	-0.80±0.16a
		DI	-1.30±0.09b
	700ppm	FI	-0.78±0.28a
		DI	-0.77±0.15a
2017	400ppm	FI	-1.83±0.19ab
		DI	-2.56±0.22c
	700ppm	FI	-1.61±0.20a
		DI	-1.72±0.20ab
	900ppm	FI	-1.62±0.15a
		DI	-1.73±0.10ab
	1200ppm	FI	-1.81±0.10ab
		DI	-1.91±0.18b

859 Mean ± SD is shown. 2016, n=3-4; 2017, n=5. Values in the same year followed by the  
 860 different letters are significantly different at the level of P<0.05 (one-way ANOVA).  
 861

862 **Figure 1.** Leaf-level gas exchange of maize grown under different water and [CO<sub>2</sub>]  
863 treatments in 2017, including net assimilation (P<sub>n</sub>) (a), stomatal conductance (g<sub>s</sub>) (b),  
864 transpiration rate (T<sub>r</sub>) (c), and leaf water-use efficiency (WUE<sub>leaf</sub>) (d). Mean ± SD is  
865 shown (n=6=n<sub>1</sub>+n<sub>2</sub>, n<sub>1</sub>=3 (samples in the 10th week), n<sub>2</sub>=3 (samples in the 14th week)).  
866 Boxes followed by the different letters are significantly different at the level of P<0.05  
867 (one-way ANOVA). FI, full irrigation; DI, deficit irrigation.

868

869 **Figure 2.** The relationship between midday stem water potential (Ψ<sub>s</sub>) and conduit  
870 diameter (D<sub>c</sub>) (a), conduit roundness (R<sub>c</sub>) (b), and stem cross-section area (A<sub>stem</sub>) (c) of  
871 maize grown under different water and [CO<sub>2</sub>] treatments in 2017. Mean ± SD is shown.  
872 Significant effects, \*, 0.01<P<0.05; \*\*, 0.001<P<0.01.

873

874 **Figure 3.** (a) Stem specific hydraulic conductivity (K<sub>s</sub>) and (b) stem theoretical  
875 Hagen-Poiseuille hydraulic conductivity (K<sub>HP</sub>) of maize under different water and [CO<sub>2</sub>]  
876 treatments in 2016 and 2017. Mean ± SD is shown (2016, n=3-4; 2017, n=5). Boxes  
877 followed by the different letters are significantly different at the level of P<0.05  
878 (one-way ANOVA). FI, full irrigation; DI, deficit irrigation.

879

880 **Figure 4.** Relationship between stem specific hydraulic conductivity (K<sub>s</sub>) and vessel  
881 diameter (D<sub>c</sub>) of maize grown under different water and [CO<sub>2</sub>] treatments in 2016 and  
882 2017. Mean ± SD is shown. Significant effects, \*\*\*, p<0.001.

883

884 **Figure 5.** Relationship between supported leaf area (supported  $A_{\text{leaf}}$ ) and stem water  
885 transport capacity measured as (a) stem cross-sectional area ( $A_{\text{stem}}$ ), and (b) maximum  
886 hydraulic conductivity ( $K_{\text{max}}$ ) of maize grown under different water and  $[\text{CO}_2]$   
887 treatments. Mean  $\pm$  SD is shown. Significant effects, \*\*,  $0.01 < p < 0.001$ ; \*\*\*,  $p < 0.001$ .

888

889 **Figure 6.** Maize stem xylem vulnerability curves under different water and  $[\text{CO}_2]$   
890 treatments in 2016 and 2017. Curves show percentage loss of hydraulic conductivity  
891 (PLC) with decreasing xylem pressure. (a) Curves in 2016; (b) curves under full  
892 irrigation in 2017; (c) curves under deficit irrigation in 2017. Means  $\pm$  SD is shown.  
893 2016,  $n=3-4$ ; 2017,  $n=5$ .

894

895 **Figure 7.** Relationship between the water potential inducing 50% loss of hydraulic  
896 conductivity ( $P_{50}$ ) and (a) midday stem water potential ( $\Psi_{\text{stem}}$ ), and (b) stem specific  
897 hydraulic conductivity ( $K_s$ ) of maize grown under different water and  $[\text{CO}_2]$  treatments  
898 in 2017. Mean  $\pm$  SD is shown. Significant effects, \*\*\*,  $p < 0.001$ .