1 **Title page**

2 **Title:** Elevated $[CO_2]$ alleviates the impacts of water deficit on xylem anatomy and

3 hydraulic properties of maize stems

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5 **Authors:** Junzhou Liu[1, 2], Shaozhong Kang[1, 2, 4], William J. Davies[3], Risheng Ding[1, 2]

6

7 **Contact Information:**

8 [1] Center for Agricultural Water Research in China, China Agricultural University,

9 Beijing 100083, China

10 [2] Shiyanghe Experimental Station for Improving Water Use Efficiency in Agriculture,

11 Ministry of Agriculture and Rural Affairs, Beijing 100125, China

12 [3] Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster, LA1 4YQ,

13 UK

14 [4] Corresponding author:

15 Prof. Shaozhong Kang

16 Center for Agricultural Water Research in China, China Agricultural University

17 No. 17 Tsinghua East Road, Haidian District, Beijing, 100083, China

18 Email: kangsz@cau.edu.cn

19

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Abstract:

Plants can modify xylem anatomy and hydraulic properties to adjust to water status. Elevated $[CO_2]$ can increase plant water potential via reduced stomatal conductance and water loss. This raises the question of whether elevated $[CO_2]$, which thus improves plant water status, will reduce the impacts of soil water deficit on xylem anatomy and hydraulic properties of plants? To analyze the impacts of water and $[CO_2]$ on maize stem xylem anatomy and hydraulic properties, we exposed potted maize plants to varying $[CO_2]$ levels (400, 700, 900, 1200 ppm) and water levels (full irrigation, deficit irrigation). Results showed that at current $[CO_2]$, vessel diameter, vessel roundness, stem cross-section area, specific hydraulic conductivity and vulnerability to embolism decreased under deficit irrigation; yet, these impacts of deficit irrigation were reduced at elevated $[CO_2]$. Across all treatments, midday stem water potential was tightly correlated with xylem traits and displayed similar responses. A distinct trade-off between efficiency and safety in stem xylem water transportation in response to water deficit was observed at current $[CO_2]$, but not observed at elevated $[CO_2]$. The results of this study enhance our knowledge of plant hydraulic acclimation under future climate environments and provide insights into trade-offs in xylem structure and function.

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53 **hydraulic properties of maize stems**

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58 and water loss. This raises the question of whether elevated [CO_2], which thus

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60 anatomy and hydraulic properties of plants? To analyze the impacts of water and

61 [CO_2] on maize stem xylem anatomy and hydraulic properties, we exposed potted

62 maize plants to varying [CO_2] levels (400, 700, 900, 1200 ppm) and water levels (full

63 irrigation, deficit irrigation). Results showed that at current [CO_2], vessel diameter,

64 vessel roundness, stem cross-section area, specific hydraulic conductivity and

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66 deficit irrigation were reduced at elevated [CO_2]. Across all treatments, midday stem

67 water potential was tightly correlated with xylem traits and displayed similar

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71 plant hydraulic acclimation under future climate environments and provide insights

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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 at 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 $[CO_2]$ significantly

119 increases the diameter of the xylem vessel, hydraulic conductivity, and vulnerability

120 to embolism (Atkinson & Taylor 1996; Kostiainen et al. 2014; Kim et al. 2015); yet,

121 some researchers have found that elevated $[CO_2]$ had no impact on these characters

122 or that their impact is insignificant (Kostiainen 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 $[CO_2]$ 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 $[CO_2]$, and the interaction with soil water status should be taken into

134 consideration.

135   There are many comprehensive reviews focused on the influence of $[CO_2]$ 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 $[CO_2]$. In recent years, several studies have

139 emphasized the interactive impacts of $[CO_2]$ and water availability on crops. Deficit

140 irrigation (DI), in particular, provokes more pronounced responses to elevated $[CO_2]$

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 $[CO_2]$ 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 $[CO_2]$ on soybean decreased as drought intensified (Gray et al. 2016).

148 To further assess these possibilities, we evaluated the capacity of elevated

149 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ might have an indirect impact on stem xylem development

154 especially in plants under soil water deficit, as elevated $[CO_2]$ 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 $[CO_2]$ on stem xylem in

158 woody plants mentioned earlier.

159 Based on the effects of elevated $[CO_2]$ 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 $[CO_2]$. 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 $[CO_2]$ under different

164 moisture conditions were not compared in this review. Previous studies on the

165 impact of elevated $[CO_2]$, 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 $[CO_2]$, and the plants that showed significant responses

170 to elevated $[CO_2]$ suffered water stress, which provides additional support to our

171 hypothesis above. In this study, we evaluated the impacts of $[CO_2]$ 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

Materials and methods

175

Plant material and growth conditions

176

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/cm3; soil water holding capacity, 28% (m^3/m^3)), 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_2PO_4, and 0.65 g $Ca(H_2PO4)_2$ 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 μ mol $m^{-2} s^{-1}$ in

201   2016 and 461.7 μ mol $m^{-2} s^{-1}$ in 2017), the [CO_2] 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_{stem}, mm) were assessed with measuring tapes and callipers every five or

222 ten days after the treatments (both water and [CO_2] began). The total leaf area (total

223 A_{leaf}, 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_{leaf}, 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.

Leaf gas exchange

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

Water potential measurements

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

Hydraulic measurements

After 6 weeks in the greenhouse and 10-12 weeks under different irrigation and

[CO_2] of treatment, five plants representative of each treatment were randomly

harvested at the silking stage (the 16-17th weeks after sowing in 2016; the 18th

week after sowing in 2017); each plant was cut at the base of the stem and taken

back to the laboratory. Stem segments were prepared from the parts under the

ears, and cut to a length of 27.5cm with razor blades under boiled-degassed water,

making sure the cut face was smooth. Segments were typically 14–20 mm in

diameter. We use 20mM KCl solution (vacuum degassed and 0.22μm filtered) to

flush segments for 30 min under 100kPa to remove any possible native embolisms.

Maximum hydraulic conductivity (K_{max}, mg mm kPa^{-1} s^{-1}) was measured after

flushing by the gravity method outlined in Sperry et al. (1988). The hydraulic head

pressure was supplied by a raised glass bottle and maintained at 6 kPa. The

computer connected to the balance automatically recorded the weigh every 15s

and calculated the K_{max}. In order to prevent water leakage caused by asymmetrical

stem cross-sections, we referenced the operation of Li et al. (2009) to filling the

cavity at one end of the stem with poster mud and wrapping with Teflon tape,

making sure a close combination between stem and silicone tube. Stem specific

hydraulic conductivity (K_s, mg mm^{-1} kPa^{-1} s^{-1}) was subsequently calculated as the

K_{max} of a stem segment divided by the corresponding stem cross-section area.

The centrifuge method was first and widely used to determine the stem xylem

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 cavitated.

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_{stem}, 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, μm) and roundness

298   (R, μm μm^{-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   (longest diameter + shortest diameter)/2, R = shortest diameter/longest diameter.

302   Vascular bundle density (ρ , mm^{-2}) was calculated as N/A_{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_{hp}, mg mm^{-1} kPa^{-1} s^{-1}) of the

306   stem using the Hagen-Poisueille 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 [CO_2] treatments but also to attempt to capture a more accurate

313   theoretical value of K_{hp}, as most of the xylem vessels are not regular circles but

314   approximate to ellipses. We used formula (2) to calculate K_{hp} with an elliptical

315   correction.

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

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

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

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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$. In contrast, water deficit led to a significant reduction in total leaf

334 area especially at 400 ppm, from 0.55 ± 0.01 m^2 to 0.47 ± 0.03 m^2 in 2016 and 0.65

335 ± 0.03 m^2 to 0.51 ± 0.03 m^2 in 2017. The negative impact of water deficit decreased

336 at elevated $[CO_2]$. Plant height showed similar responses to water and $[CO_2]$,

337 ranging from 196 ± 6 cm to 279 ± 2 cm in 400 ppm DI and 1200 ppm FI plants,

338   respectively. Basal stem diameter was smallest in 400 ppm DI (19.1 ± 0.6 mm) and

339   largest in 1200 ppm FI (23.4 ± 0.3 mm).

340   **Leaf gas exchange**

341   For all $[CO_2]$ 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 ± 6.0 μmol m^{-2} s^{-1} and 11.1 ± 4.3 μmol m^{-2} s^{-1} under FI and DI,

344   respectively. 700 ppm $[CO_2]$ significantly increased P_n under DI but not FI, and with

345    $[CO_2]$ supplementation to 900 ppm and 1200 ppm, the variations of P_n under DI or

346   FI were not significant. $[CO_2]$ supplementation and water deficit both led to

347   decreases in stomatal conductance (g_s; Fig. 1b), ranging from 0.162 ± 0.093 mol m^{-2}

348   s^{-1} in 400 ppm FI plants to 0.029 ± 0.007 mol m^{-2} s^{-1} in 1200 ppm DI plants. Similarly,

349   transpiration rate (T_r; Fig. 1c) was largest in 400 ppm FI (2.28 ± 0.96 mmol m^{-2} s^{-1})

350   and smallest in 1200 ppm DI (0.76 ± 0.17 mmol m^{-2} 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 $[CO_2]$. Increases in P_n and the

353   decreases in T_r resulted in significant increases in leaf water use efficiency (WUE$_{leaf}$,

354   P_n/T_r; Fig. 1d) at elevated $[CO_2]$.

355   **Plant water relations**

356   Because of the lower transpiration rate, most of the plants under elevated $[CO_2]$

357   were irrigated at the level close to but did not reach the lowest limit of soil water

358   content when those undrt 400 ppm $[CO_2]$ reached. Thus the averages of soil water

359   content under elevated $[CO_2]$ 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 (Ψ_{stem}, Table 2) at jointing stage was sensitive to soil water content

362   at the current 400 ppm [CO_2], and Ψ_{stem} significantly decreased from -1.07 ± 0.09

363   MPa under FI to -1.55 ± 0.14 MPa under DI. With [CO_2] rising to 700 and 900 ppm,

364   Ψ_{stem} increased under both FI and DI. Then Ψ_{stem} had a slight and non-significant

365   decrease at 1200 ppm [CO_2] under FI, and a significant decrease under DI when

366   compare with 900ppm [CO_2] plants. Elevated [CO_2] reduced the difference in Ψ_{stem}

367   between FI and DI, and Ψ_{stem} under DI treatment at elevated [CO_2] was increased

368   and showed no significant difference with Ψ_{stem} under 400 ppm FI treatment.

369   **Xylem anatomy**

370   The impacts of water and [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 [CO_2], from 53.3 ± 5.3 µm under FI to 47.6 ± 2.8 µm under

377   DI in 2016, and from 57.1 ± 2.6 µm under FI to 48.4 ± 1.7 µm under DI in 2017.

378   Elevated [CO_2] significantly increased D_c under DI but not significant under FI.

379   Similarly, vessel roundness (R_c) was 0.76 ± 0.03 µm µm^{-1} under FI and 0.67 ± 0.03

380   µm µm^{-1} under DI at 400 ppm [CO_2] in 2016, and 0.82 ± 0.01 µm µm^{-1} under FI and

381   0.77 ± 0.01 µm µm^{-1} under DI at 400 ppm [CO_2] in 2017. Elevated [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 [CO_2]. Peripheral

384 vascular bundles either showed similar responses with central vascular bundles or

385 were little affected by water and [CO_2] treatments.

386   Stem segment cross-section area (A_{stem}) significantly decreased under water

387 deficit, and A_{stem} increased with [CO_2] rising under both FI and DI. The number of

388 central vascular bundles (N_c) also significantly increased with [CO_2]

389 supplementation, but water deficit had no impact on this variable, with the

390 result that their density (ρ_c) showed no significant difference between [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 Ψ_{stem} (Fig. 2a; r^2=0.70, P=0.009), and R_c

394 was also significantly correlated with Ψ_{stem} (Fig. 2b; r^2=0.71, P=0.009), moreover,

395 A_{stem} was significantly correlated with Ψ_{stem} (Fig. 2c; r^2=0.67, P=0.013).

396 **Xylem hydraulic properties**

397 At the current 400 ppm [CO_2], water deficit significantly reduced stem specific

398 hydraulic conductivity (K_s; Fig. 3a) from 0.59 ± 0.10 mg mm^{-1} kPa^{-1} s^{-1} under FI to

399 0.51 ± 0.09 mg mm^{-1} kPa^{-1} s^{-1} under DI in 2016, and significantly reduced K_s from

400 0.74 ± 0.07 mg mm^{-1} kPa^{-1} s^{-1} under FI to 0.52 ± 0.05 mg mm^{-1} kPa^{-1} s^{-1} under DI in

401 2017. Similarly, theoretical Hagen-Poiseuille hydraulic conductivity (K_{hp}; Fig. 3b) was

402 0.87 ± 0.34 mg mm^{-1} kPa^{-1} s^{-1} under FI and 0.55 ± 0.10 mg mm^{-1} kPa^{-1} s^{-1} under DI in

403 2016, and 1.10 ± 0.17 mg mm^{-1} kPa^{-1} s^{-1} under FI and 0.75 ± 0.14 mg mm^{-1} kPa^{-1} s^{-1}

404   under DI in 2017. Elevated $[CO_2]$ increased K_s and K_{hp} under DI, but not under FI. At

405   elevated $[CO_2]$, the drops in K_s or K_{hp} under water deficit were smaller when

406   compared with plants at 400 ppm $[CO_2]$. 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_{hp} showed similar responses with K_s to water and $[CO_2]$

410   treatments but with more substantial values. K_s was also tightly correlated with D_c

411   (Fig. 4; r^2=0.96, P < 0.001). K_{hp} 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_s (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_{leaf} scaled linearly with stem's capacity to transport water,

416   including A_{stem} (Fig. 5a; r^2=0.54, P=0.007) and K_{max} (Fig. 5b; r^2=0.87, P<0.001).

417   Under FI treatment, elevated $[CO_2]$ slightly increased the stem xylem

418   vulnerability to embolism (Fig. 6). Under the DI treatment, maize showed the

419   lowest vulnerability at 400 ppm $[CO_2]$ and showed an increase in vulnerability at

420   700 ppm and 900 ppm $[CO_2]$ but slightly decreased at 1200 ppm $[CO_2]$. 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 $[CO_2]$, 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    $[CO_2]$, P50 under DI significantly increased, and there was no significant difference

426 between water treatments.

427   There was a strong correlation between P50 and Ψ_{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 [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 [CO_2], due to the effects of elevated [CO_2] on plant water status.

441 **Xylem anatomy**

442 At the current atmosphere [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_{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 at 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 $[CO_2]$ can stimulate plant photosynthetic rate

451   and which was observed in our study (Figure 1). However, this direct impact of

452   elevated $[CO_2]$ (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 $[CO_2]$ 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 $[CO_2]$ on xylem

458   anatomy by stomatal control was observed in this study. In this study, elevated $[CO_2]$

459   alleviates the impacts of deficit irrigation on xylem anatomy, due to the indirect

460   impact of elevated $[CO_2]$. The data in 2017 showed that at 400 ppm $[CO_2]$, D_c under

461   DI was 16.8% lower than FI, and with $[CO_2]$ supplementation, D_c under DI was

462   11.8%, 12.4%, and 11.9% lower than FI, at 700, 900, 1200 ppm $[CO_2]$ respectively;

463   DI treatment significantly reduced R_c at 400 ppm $[CO_2]$, and R_c was not significantly

464   different between water treatments at elevated $[CO_2]$; 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     $[CO_2]$ 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 $[CO_2]$, 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 (Ψ_{stem}, Fig. 2). Ψ_{stem}

477   was measured at jointing stage on the leaves that were close to the segments for

478   later anatomical measurements; thus we use Ψ_{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 Ψ_{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 Ψ_{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_2] (Fig. 1) and increased Ψ_{stem} under DI (Table 2), but the saturated

493   water conditions under FI limited the increases of Ψ_{stem} and reduced the

494   differences between water treatments at elevated [CO_2]. Ψ_{stem} of DI plants had

495   more significant increases with [CO_2] 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_2]. Maize stem is composed of many single cells where cell size is

499   regulated by turgor pressure, so A_{stem} had similar responses to Ψ_{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_2], 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_2] on K_s was not observed, as K_s showed no significant

512   difference respond to elevated [CO_2] 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_2], respectively. The drop of K_s by DI was also less dramatic for plants

515   at elevated [CO_2] in 2016. Taking into consideration the Hagen-Poiseuille law, it is

516   not hard to understand why K_s was proportional to D_c, with a very high correlation

517   (Fig. 4). So due to the indirect impact of elevated [CO_2] on D_c, elevated [CO_2]

518   alleviated the impact of water deficiency on K_s.

519   The decrease of K_s under DI was significant under the current [CO_2] but not

520   under elevated [CO_2], so that we can get the conclusion that the impact of soil

521   water deficiencies on K_s was alleviated by elevated [CO_2]. However, there was no

522   significant interactive effect between [CO_2] and irrigation on K_s 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_{stem} and also K_{max} was correlated with the supported A_{leaf}

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_{stem} 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 $[CO_2]$ significantly increased

538   maize leaf area, but not the water demand, actually plant water consumption

539   decreased at elevated $[CO_2]$ (Table 2) due to the decrease of g_s. In this case, the

540   hydraulic system tends to be more redundant at elevated $[CO_2]$.

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 $[CO_2]$ with a significant decrease in P50, and elevated $[CO_2]$ significantly

544   increased the P50 of DI plants to a level with no significant difference from FI (Fig. 6;

545   Table 4). Thus, elevated $[CO_2]$ 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 Ψ_{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_c significantly decreased P50 at the current atmospheric $[CO_2]$,

556   but elevated $[CO_2]$ alleviated the impact of water deficit by decreasing D_c 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_2], 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_2], 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 P50 and K_s (Fig. 7b; $r_1^2=0.39$, P=0.098). P50 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 P50 and K_s (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_2] changes were not observed.

575

Conclusion

577   Our study revealed maize stem hydraulic acclimation under future climate

578   environments. Elevated [CO_2] 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 $[CO_2]$ 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 $[CO_2]$. 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 $[CO_2]$ 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 $[CO_2]$ 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_2 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 $[CO_2]$ alleviated the impacts of water

599   deficiency on xylem anatomy and hydraulic properties; moreover, we also

600   demonstrated that these impacts of elevated $[CO_2]$ were not direct but indirect.

601   The hypothesis and results are logical and based on well-known phenomena, yet

different species may have different adjustment strategies in the face of elevated [CO_2] (Domec et al. 2017), and the magnitudes of responses may vary in other maize cultivars or soil types, which require further exploration. However, our results provide another perspective to assess the impact of elevated [CO_2] to plant xylem anatomy and hydraulics, that soil water status and other environmental factors must be taken into consideration.

Acknowledgements

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844   **Table. 1** Previous studies on the impact of elevated [CO_2] to stem xylem.

Species	[CO_2]	Description of water conditions	Impact of elevated [CO_2] 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_2].	Atkinson & Taylor 1996
Ponderosa pine	350, 550, 750, 1100 ppm	Watered to field capacity every 2d	Elevated [CO_2] had minimal effects on K_S and K_L.	Maherali & DeLucia 2000
Norway spruce	360, 720 ppm	nonlimiting soil water	Elevated [CO_2] had only minor effects on tracheid lumen diameter, tracheid length and cell wall thickness.	Kostiainen et al. 2004
Scotts pine	present [CO_2], 700 ppm	Mean annual precipitation is 740 mm	Elevated [CO_2] 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_2].	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_2].	Vaz et al. 2012
Phaseolus vulgaris	180, 380, 700 ppm	Four water regimes ranging from high to low	Elevated [CO_2] significantly increased vessel diameter and decreased embolism resistance under both water deficit and good water conditions. The hydraulic traits decreased under elevated [CO_2].	Medeiros & Ward 2013
Helianthus annuus	290, 390, 480 ppm	Watered daily to saturation	Elevated [CO_2] significantly decreased vessel diameter and vulnerability to embolism under good water conditions.	Rico et al. 2013
Populus and Betula	present [CO_2], + 200 ppm	Different annual precipitation in 10 years	Vessel diameter and wall thickness increased at elevated [CO_2], 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_2]. The vulnerability to drought-induced embolism did not respond to elevated [CO_2].	Hao et al. 2018

845 **Table. 2** Plant growth and water relations under different water and [CO_2] treatments in 2016 and 2017. A_{leaf}, leaf area; D_{stem}, stem basal

846 diameter; Ψ_{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_2]	Water treatment	Height (cm)	Totle A_{leaf} (m^2)	D_{stem} (mm)	Ψ_{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; Ψ_{stem}, n=7=n_1+n_2, n_1=3 (samples in the 10th week), n_2=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_2] treatments in 2016

852   and 2017. VB, vascular bundles; subscript C, central vascular bundles; subscript P, peripheral vascular bundles; A_{stem}, stem cross-section area; FI,

853   full irrigation; DI, deficit irrigation.

Year	2016				2017							
CO_2 concentration	400 ppm		700 ppm		400 ppm		700 ppm		900 ppm		1200 ppm	
Water treatment	FI	DI	FI	DI	FI	DI	FI	DI	FI	DI	FI	DI
Vessel diameter (µm)												
Central VB (D_C)	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_P)	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^{-1})												
Central VB (R_C)	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_P)	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_C)	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_P)	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^2)												
Stem (A_{stem})	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^{-2})												
Central VB (d_C)	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_P)	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 $[CO_2]$ treatments in 2016 and 2017. FI, full irrigation; DI, deficit

858 irrigation.

Year	$[CO_2]$	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 $[CO_2]$

863 treatments in 2017, including net assimilation (P_n) (a), stomatal conductance (g_s) (b),

864 transpiration rate (T_r) (c), and leaf water-use efficiency (WUE_{leaf}) (d). Mean ± SD is

865 shown ($n=6=n_1+n_2$, $n_1=3$ (samples in the 10th week), $n_2=3$ (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_s) and conduit

870 diameter (D_c) (a), conduit roundness (R_c) (b), and stem cross-section area (A_{stem}) (c) of

871 maize grown under different water and $[CO_2]$ 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_S) and (b) stem theoretical

875 Hagen-Poiseuille hydraulic conductivity (K_{HP}) of maize under different water and $[CO_2]$

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_s) and vessel

881 diameter (D_c) of maize grown under different water and $[CO_2]$ 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_{leaf}) and stem water

885   transport capacity measured as (a) stem cross-sectional area (A_{stem}), and (b) maximum

886   hydraulic conductivity (K_{max}) of maize grown under different water and [CO_2]

887   treatments. Mean ± 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 [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 ± 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 (P50) and (a) midday stem water potential (Ψ_{stem}), and (b) stem specific

897   hydraulic conductivity (K_S) of maize grown under different water and [CO_2] treatments

898   in 2017. Mean ± SD is shown. Significant effects, ***, $p < 0.001$.