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Leaf coordination between petiole vascular development and water demand in response to elevated CO_2 in tomato plants

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Abstract

The rise in atmospheric CO_2 has a profound impact on plants physiology and performance. Stomatal gas exchange such as reduction in water loss via transpiration and higher photosynthetic rates are among the key plant physiological traits altered by the increase of CO_2. Water acquired in plant roots is transported via the xylem vessels to the shoots. Under conditions of elevated CO_2, water flux decreases due to higher water use efficiency and a decline in stomatal conductance. However, the mechanism by which the shoot vascular development is affected under elevated CO_2 is still largely unclear in herbaceous crops. In the current study, tomato plants were exposed to either 400 or 800 ppm of CO_2 and were analyzed for growth, leaf area, gas exchange rate, and petiole anatomy. Elevated CO_2 caused a reduction in metaxylem vessel diameter, which in turn, decreased leaf theatrical conductivity by 400% as compared with plants grown under ambient CO_2. This work links anatomical changes in the petioles to the rise in atmospheric CO_2 and water use. Plant water demand declined under elevated CO_2, while photosynthesis increased. Thus, the decrease in leaf specific conductivity was attributed to lower water consumption in leaf gas exchange and, by extension, to higher leaf water use efficiency. As the global climate changes and water scarcity becomes more common, such anatomical alterations caused by elevated CO_2 may affect plant response to water limitation. Further research on petiole anatomical alterations under conditions of combined climate change factors such as drought and heat with elevated CO_2 may assist in clarifying the responses expected by future climate scenarios.

KEYWORDS
carbon assimilation (A), elevated CO_2 (eCO_2), leaf specific conductivity (K_L), tomato (*Solanum lycopersicum*), transpiration rates (Tr), water use efficiency (WUE)

1 | INTRODUCTION

Since the beginning of the Industrial Revolution (mid-18th century), the use of fossil fuels and deforestation have caused a continuous increase in the atmospheric CO_2 concentration as compared with previous centuries. The current concentration is ~ 410 µmol mol^{-1}, while projections predict an increase to 530–970 µmol mol^{-1} by the end of the twenty-first century (Kant et al., 2012). This increase in atmospheric CO_2 levels has substantial effects on plant photosynthesis and growth in both managed and natural environments (Bloom et al., 2012).

Photosynthesis involves leaf gas exchange of CO_2 and water through the stomata; therefore, when the gradient of CO_2 between leaf intercellular spaces and the atmosphere increases, the rise in atmospheric CO_2 concentration helps plants to lower stomatal water loss per unit of CO_2 gained (Paudel et al., 2018).

Elevated CO_2 (eCO_2) simultaneously increases carbon assimilation (A) and decreases the transpiration rate (T) on leaf, plant, and ecosystem scales (Mohamed et al., 2013). Dividing A by T (A/T) generates the widely used term water use efficiency (WUE), which describes how efficiently carbon is assimilated per unit of water evaporated on the leaf scale (Blum, 2009; Medrano et al., 2015). Numerous studies have reported that eCO_2 increases WUE in C3 plants (Ainsworth & Long, 2005; Franzaring et al., 2011; Wu et al., 2004; Wullschleger et al., 2002). This higher WUE is attributed to the increase in frequent stomatal closure events due to higher leaf intercellular CO_2 concentrations (Leakey et al., 2009). Because eCO_2 increases net carbon gain, it was proposed that an increase in WUE under eCO_2 is beneficial for plants under limited water availability, particularly in dry lands (Wu et al., 2004). Heat and drought stresses are key climate change factors negatively impacting photosynthesis and crop productivity (Cohen, Zandalinas, Fritschi, & Mittler, 2020; Cohen, Zandalinas, Huck, et al., 2020). This positive impact of CO_2 fertilization on photosynthesis may partly off-set the negative impacts of climate change factors on plants in both managed and natural environments.

Water acquired by the root system is carried by the transpiration stream in the vascular tissue to the shoot, mainly for stomatal gas exchange (Leakey et al., 2009). Xylem is a vascular tissue found in all vascular plants and functions as a rapid conduit of water and a mechanical support for vertical growth (Růžička et al., 2015). Recently, it was shown that the transport of water through the xylem vessels is over 10^6 times more efficient than transport via the parenchyma cells or plasmodesmata (Qaderi et al., 2019).

A previous study observed that increasing the CO_2 levels caused alterations in the tomato root vascular system's ability to conduct water, especially when nitrogen availability is low (Cohen et al., 2019) or when nitrogen is applied as ammonium (Cohen et al., 2018). Water conductivity in xylem vessel obeys Hagen–Poisseuille's law, and the flow rate in the xylem is mostly determined by the sum of the diameters of each individual xylem vessel (Hochberg et al., 2015; Tyree et al., 1994). Accordingly, for any given leaf, an increase in the leaf petiole xylem vessel size results in higher water conductivity and greater leaf specific conductivity (K_L, hydraulic conductivity leaf

area^{-1}) (Rodríguez-Gamir et al., 2010). However, an increase in the stem and petiole xylem vessel diameter also raises the risk of cavitation (Cochard, 2002) and decreases embolism resistance. Thus, xylem size development is a trade-off between water conductivity and embolism resistance (Gleason et al., 2016; Wagner et al., 1998). It was suggested that crop plants are sensitive to drought-induced embolism because of the low cost of xylem tissue construction (Liu et al., 2020).

Moreover, genotypes with lower axial conductivity can maintain a more favorable hydration status under water limitation than high conductivity genotypes (Strock et al., 2020). In oaks (*Quercus robur* L.), it was shown that trees with larger xylem vessels displayed higher mortality under prolonged droughts (Qaderi et al., 2019).

Recently, eCO_2 was found to alter root xylem development (Cohen et al., 2019), and other studies showed that stem xylem was also affected by eCO_2 (Kostiainen et al., 2004; Liu et al., 2020). However, the effects of eCO_2 on the size of petiole vessels and leaf specific conductivity (K_L) remain uncharacterized.

The current study hypothesized that the decrease in petiole metaxylem vessel size and K_L was due to a reduction in WUE caused by eCO_2.

The present study investigated the effects of eCO_2 on the development of xylem vessels in the petioles and on the leaf specific conductivity of tomato plants.

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

Tomato plants (*Solanum lycopersicum* cv. Brigade, Hishtil Co.; Ashkelon, Israel) were planted in an environmentally controlled greenhouse. Throughout the experiment, the plants were grown at day/night temperatures of 25/17°C, respectively, supplied with either ambient (400 µmol mol^{-1}) or elevated (800 µmol mol^{-1}) CO_2 concentrations and relative humidity of $\sim 45\%$. The plants were grown during April–May of 2017, with a day length of about 13 h under natural sunlight.

CO_2 levels in the two greenhouses were continuously monitored with an infrared gas analyzer sensor (Gascard NG; Edinburgh Sensors), and CO_2 was automatically resupplied on demand.

The nutrient solution for each treatment contained 6.5, 1.5, 2.6, 4, 5, and 4 mM of N, P, Mg, Ca, K, and S, respectively, and micronutrients (Cohen et al., 2019).

2.2 | Gas exchange measurements

Gas exchange measurements were conducted on young fully mature leaves prior to plant harvest. Measurements were taken during the mid-morning (10:00–12:00), and the net CO_2 assimilation rates (A), transpiration (T), and stomatal conductance (g$_s$) of the young fully mature leaves of each plant were measured using a portable Li-6400XT Infrared Gas Analyzer (Li-Cor Biosciences Inc.; NE, USA). The

open photosynthetic system was equipped with an external carbon dioxide source, in order to maintain the leaf chamber at a CO_2 concentration of either 400 or 800 μmol mol^{-1}. The chamber temperature was adjusted to 24°C, and the photosynthetic active radiation (PAR) rate was 1,000 μmol photons m^{-2} s^{-1} (Cohen et al., 2019).

Leaf instantaneous WUE was calculated from gas exchange measurements as the ratio between net carbon assimilation (A) and transpiration rate (T) (Medrano et al., 2015).

2.2.1 | Biomass measurement

At the end of the experiment, the aboveground portion of the plants was harvested and collected for fresh weight biomass analysis.

2.3 | Leaf area measurements

The leaf area measurements of whole young fully mature tomato leaves were taken by digital image analysis, using Easy Leaf Area software (Easlon & Bloom, 2014). The leaves were collected at the end of study, and the same leaves were later subjected to anatomical analysis. Photographs were taken with an iPhone 5 mobile phone and calibrated using a 4-cm^2 red calibration area, according to the software guidelines.

2.4 | Anatomy

At the end of the study, the harvested petioles of young fully mature leaves and preserved them in 70% ethanol till further processing. The samples were analyzed with cross sections made freehand. Sections were depigmented with commercial sodium hypochlorite (8%) in proportions of 1:1 hypochlorite and distilled water, which in a few seconds, removed the natural plant pigments. Subsequently, the samples were washed in distilled water and stained with aqueous solutions of Alcian Blue (1% w/v) and Safranin 1% (w/v) for 3 min each. Cross sections were placed on slides with glycerin 50% in distilled water, examined through an Axio ImagerA1 microscope with dark-field illumination (Zeiss; Goettingen, Germany), and photographed with an AxioCam HRC camera (Zeiss). The total number of xylem vessels in each petiole was counted, and the areas of individual metaxylem vessels and total xylem tissue were analyzed using image analysis software; we did not measured the area of individual protoxylem vessels (Digimizer version 4.2.5; Ostend, Belgium) as described by Kraus and Arduin (1997).

2.5 | Theoretical conductivity

Leaf theoretical conductivity was calculated as described in Shtein et al. (2016). Using the modified Hagen–Poisseuille's law, the current study divided the leaf area into the sum of the calculated theoretical conductivity $= \frac{\pi\rho}{128\eta} \times \left(d^4\right)$ of each individual xylem vessel, and the collective conductivity of all individual vessels in a given petiole was calculated (d is the vessel diameter, ρ is the fluid density [assumed to be $1,000\,kg\,m^{-3}$], and η is the viscosity [assumed to be 1×10^{-9} MPa × s]).

2.6 | Experimental design and statistical analyses

The experiment was designed in a random plot with eight replications, with CO_2 treatments as a single factor.

A statistical analysis was performed using Student's t test, with an α level of .05 using JMP 10 (SAS).

3 | RESULTS

eCO_2 significantly increased the aboveground biomass accumulation of tomato plants. The dry weights of the plants were 150 and 170 g for the ambient and eCO_2-grown plants, respectively (Figure 1a). This difference in dry biomass corresponds to a 14% increase in eCO_2-grown plants. Average leaf area slightly increased in response to CO_2 enrichment, displaying areas of 150 and 158 cm^2 for ambient and eCO_2-grown plants, respectively (Figure 1b).

The photosynthetic rates were 13.5 and 17 μmol CO_2 s^{-1} m^{-2} for the ambient and eCO_2-grown tomato plants, respectively. This significant difference in the leaf-based photosynthetic rate constituted a 26% increase in eCO_2-grown plants (Figure 2a). In addition to photosynthesis, transpiration also differed significantly between CO_2 treatments. Tomato plants exhibited transpiration rates of 0.62 and 0.5 mmol H_2O s^{-1} m^{-2} for the ambient and eCO_2 treatments, respectively. This difference corresponds to a 20% decrease in the transpiration of eCO_2-grown plants (Figure 2b). Stomatal conductance was also found to be significantly affected by eCO_2; tomato plants displayed stomatal conductance rates of 0.2 and 0.12 mmol s^{-1} m^{-2} in the ambient and eCO2-grown plants, respectively (Figure 2c). This difference corresponds to a 40% decrease in the displayed stomatal conductance of eCO_2-grown plants. As a result of the described alterations in leaf gas exchange rates, leaf instantaneous WUE was accordingly changed. Tomato plants displayed WUE rates of 2.8 and 3.8 for ambient and eCO_2-grown plants, respectively. This difference corresponds to a 35% increase in WUE for the eCO_2-grown plants (Figure 2d).

The total area of individual petioles' xylem tissue was not significantly different between the two CO_2 treatments (as seen in Figure 3a). Similarly, the number of metaxylem vessels in each petiole was found to be similar in both treatments, ranging from 90–120 vessels per petiole (Figure 3b). In contrast, under eCO_2, the average diameter of an individual metaxylem vessel decreased by an average of 40% in comparison with that under ambient CO_2 conditions (Figure 3c). These differences in individual metaxylem vessel diameters are shown in Figure 3d,e. The distribution of vessel size diameter is presented in Figure 4a,b: The median vessel diameter observed was
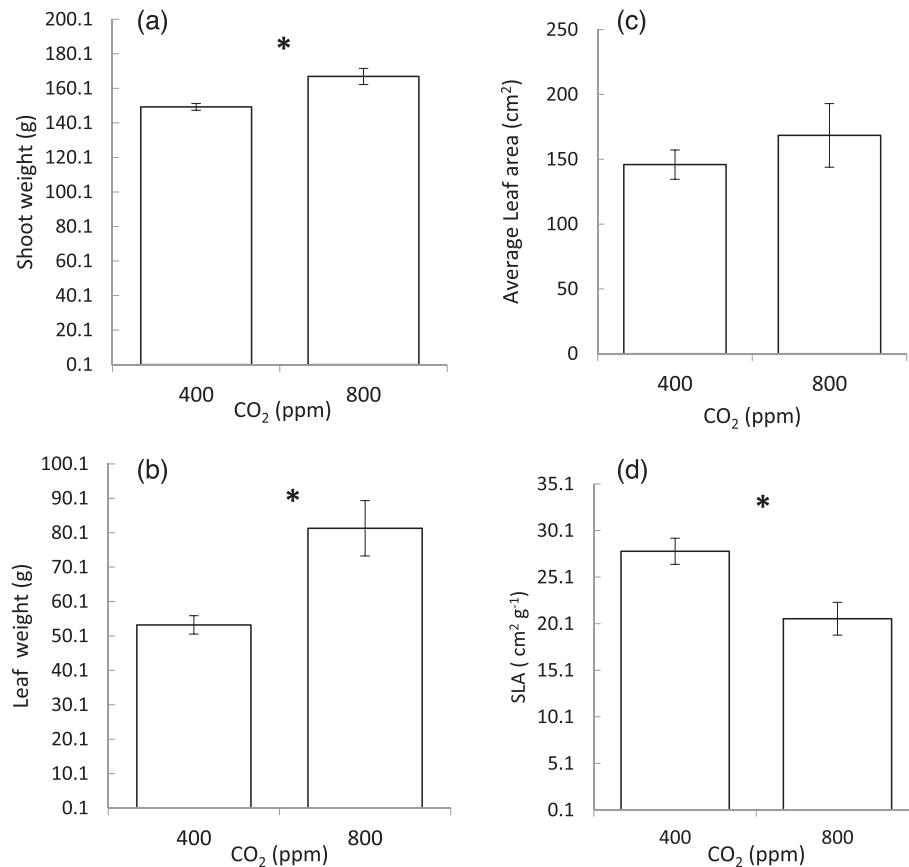
17.6 and 13.4 μm for ambient and eCO_2-grown plants, respectively. While xylem vessels demonstrated different responses to eCO_2, the average parenchyma cell diameter in the petiole was not different under eCO_2 conditions (Figure 3f).

Theoretical conductivity was calculated from the metaxylem vessel size and leaf area data. K_L significantly decreased in plants under eCO_2, from 0.042 to 0.01 ([kg s^{-1} m^{-1} MPa^{-1}] $\times 10^{-4}$); this response represents an over 400% decrease in theoretical conductivity for plants grown under eCO_2 compared with plants grown under ambient CO_2 (Figure 5).

4 | DISCUSSION

The current study demonstrates that tomato petiole vascular anatomy displays phenotypic plasticity under eCO_2 as compared with growth under ambient CO_2 conditions. This alteration coincides with the decrease in leaf transpiration rate and leaf specific conductivity. Vascular tissue development is balanced between the need to rapidly conduct water and the need to provide cavitation resistance (Hacke & Sperry, 2001). Previous studies showed a tight correlation between leaf stomatal conductance and petiole xylem water transport capacity (Brocious & Hacke, 2016). While other studies presented changes in plant vascular anatomy under eCO_2, these reports either focused on stress (Domec et al., 2015; Hao et al., 2018) or described changes in the phloem (Ainsworth & Lemonnier, 2018). Leaf area did not change

(Figure 2e), leaf thickness increased and specific leaf area decreased (Figure 2d) under eCO_2, which is an adaptation and acclimation mechanism to the increase of CO_2. Similar responses were shown in other C3 crops under eCO_2 (Gillespie et al., 2012), and as a consequence, this alteration could explain the reduction in stomatal conductance as well.

When plants are grown under eCO_2, their WUE increases since, for each carbon molecule obtained, plants evaporate fewer water molecules. In tomato plants, the current study showed that doubling the CO_2 concentrations resulted in an approximately 35% increase in WUE (Figure 2d), which was the result of a simultaneous increase ($\sim$20%) in CO_2 assimilation and a decrease ($\sim$30%) in transpiration. Similarly, due to their capacity to concentrate CO_2, in the bundle-sheath cells, C4 plants function as if they were under constant eCO_2 conditions (Chaves & Oliveira, 2004; Rachmilevitch et al., 1999). Accordingly, as a result of their higher WUE, it was previously suggested that C4 plants display smaller vessel diameters and lower K_L than C3 plants grown in similar habitats, as shown by Kocacinar and Sage (2004).

Moreover, the current study indicates that eCO_2 did not affect the number of vessels per petiole, the total xylem tissue area, or the size of parenchyma cells. In contrast, the individual metaxylem vessels were significantly smaller in the eCO_2-grown plants, demonstrating that the metaxylem vessel size, not the actual number of vessels, was affected by CO2 concentrations. In *Pinus radiate*, eCO_2 was shown to decrease the lignin content and the area occupied by xylem vessels,
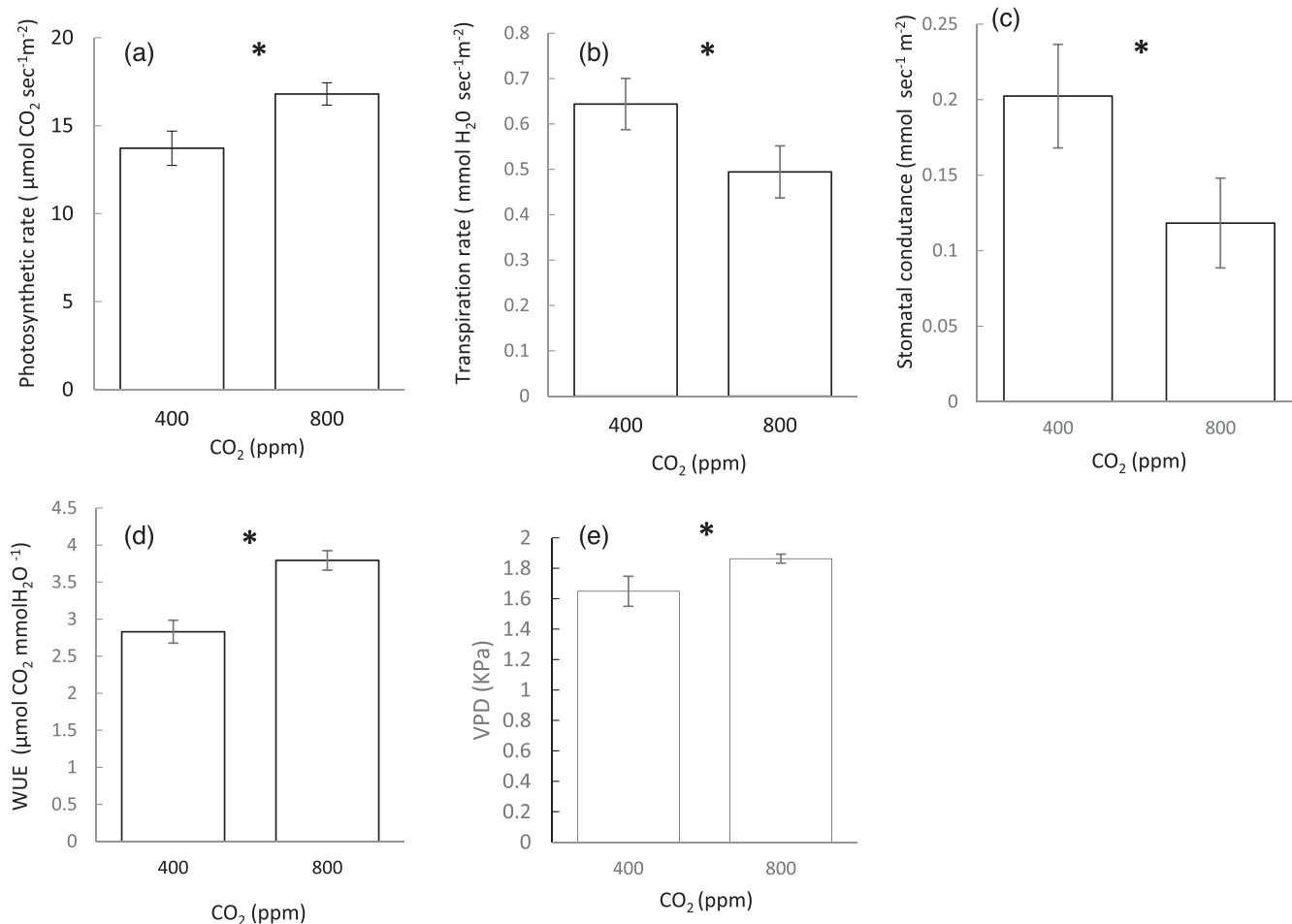
FIGURE 2 Tomato leaf carbon assimilation (a), transpiration rate (b), stomatal conductance (c), water use efficiency (d), and leaf vapor pressure deficit (VPD) (e) as affected by CO_2 treatment. Results are expressed as means $\pm$ standard error. Bars labeled with an asterisk are significantly different ($p < .05$)

indicating an increase in sapwood density at eCO_2 due to thicker tracheid walls (Atwell et al., 2003). In addition, eCO_2 reduced stem hydraulic conductivity in oaks and eucalyptus, which was attributed to a decrease in vessel diameter (Atwell et al., 2007; Heath et al., 1997).

The current study results suggest that the reduction in leaf water requirement under eCO_2 led to a decrease in the petiole metaxylem vessel diameter, similarly to what occurs under other environmental conditions that limit water conductivity in the foliage, such as drought and salinity (Baum et al., 2000; Hochberg et al., 2015; Jerszurki et al., 2020; Lynch et al., 2014). Since stomatal closure and water conductivity are coupled, it is likely that changes in one will also affect the other (Qaderi et al., 2019). When water consumption is low, there is less need to produce larger vessels, which are prone to embolism. Previous studies in different plant species suggested that eCO_2 either increased (Liu et al., 2020), decreased (Medeiros & Ward, 2013; Rico et al., 2013), or had minimal impact on xylem vessel diameter (Kostiainen et al., 2004). Additionally, it was suggested that under mild drought, smaller vessels can be beneficial in improving drought tolerance, not just due to lower embolism risk, but also because of the lower axial water transportation, which may create a more favorable

soil water status (Liu et al., 2020; Medeiros & Ward, 2013). Due to the intense use of fossil fuels, atmospheric CO_2 levels are steadily rising, and drought and heat episodes are projected to increase in frequency in the near future due to climatic changes (Lobell & Gourdji, 2012). The current study results are supported by the previous work of Kocacinar et al. (2008), which showed that plants operated in elevated-CO_2-like conditions (C4 plants) developed smaller metaxylem vessels than C3 plants from the same habitats. It was also demonstrated that increasing CO_2 from subambient to ambient results in higher (~20%) cavitation resistance (Rico et al., 2013). This suggests that a rise in atmospheric CO_2 may partially mitigate the negative effects of drought on plants, both directly due to reduced water losses and indirectly by increasing the resistance to cavitation due to small xylem size. The decrease in theoretical conductivity under eCO_2 indicates coordination between vascular plasticity to environmental factors that affect water consumption, such as the rise in atmospheric CO_2, and the way in which plants balance water conductivity with the ability to resist cavitation. In addition, stomatal conductance is sensitive to changes in leaf VPD (Dai et al., 1992). In our study, leaf VPD differed among the CO_2 treatments; therefore, the reduction in
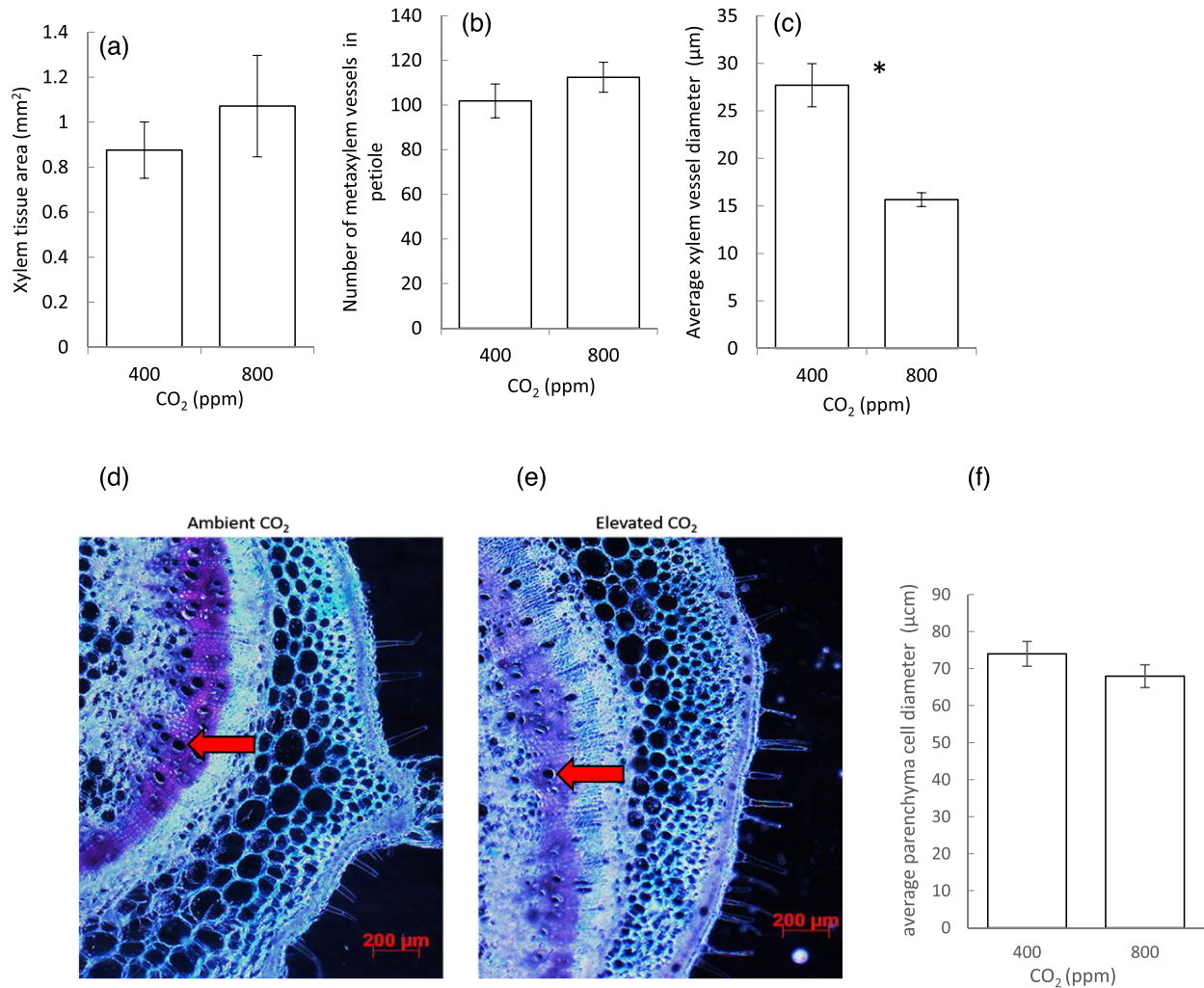
FIGURE 3   Average number of xylem vessels in petioles (a), xylem tissue area (b) ($n = 8$), average area of individual metaxylem vessel (c) ($n = 100$–110), transverse sections of plant petioles under ambient CO_2 (d) and elevated CO_2 (e), and average parenchyma cell diameter (f). Arrows show metaxylem vessels as affected by CO_2 treatment. Results are expressed as means $\pm$ standard error. Bars labeled with an asterisk are significantly different ($p < .05$)
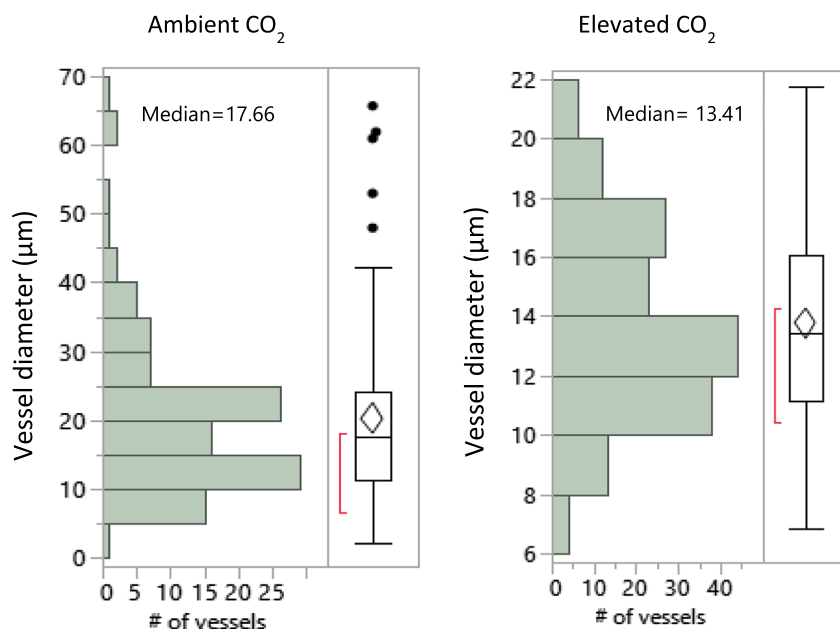
FIGURE 4   Distribution of vessel diameters in petioles of tomato plants grown at either ambient (a) or elevated (b) CO_2
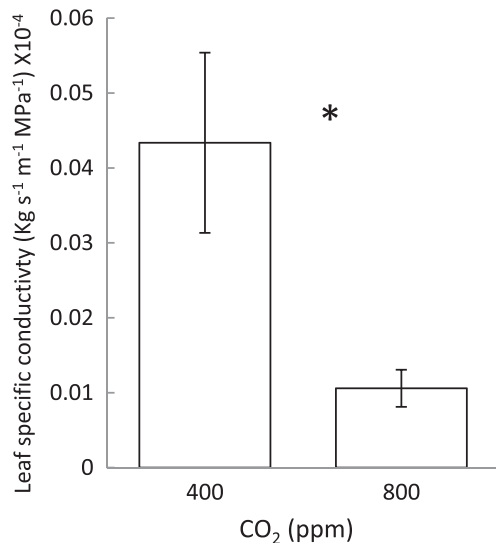
FIGURE 5 Leaf theoretical conductivity as affected by CO_2 treatment ($n = 8$). Results are expressed as means $\pm$ standard error. Bars labeled with an asterisk are significantly different ($p < .05$)

stomatal conductance and transpiration is probably due to both a reduction in metaxlyem diameter and an increase in leaf VPD. Thus, understanding the inverse impacts of drought and eCO_2 on plant growth and development may be more significant than ever, especially since, globally, C3 plants constitute 90% of plant species (Ibrahim et al., 2008). This study demonstrated that the rise in CO_2 will also induce the development of smaller xylem vessels in petioles that potentially have a great impact on sensitivity to embolism.

5 | CONCLUSIONS

Plants plastically in response to climate change factors occur at all levels, and in our particular case, the vascular system. Our data indicate that tomato, a C3 model crop, displayed a plastic response in xylem vessel size and theoretical conductivity under eCO_2, and this reduction in xylem vessel size may be associated with lower leaf water consumption under eCO_2. The impact of combined climate change factors (heat, drought, and eCO_2) on the development of the plant's vascular system needs further attention, especially in forest trees, where massive cavitation negatively affects trees' ability to recover and increase the chances of wildfires.

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REFERENCES

Ainsworth, E. A., & Lemonnier, P. (2018). ScienceDirect phloem function: A key to understanding and manipulating plant responses to rising atmospheric [CO_2]? *Current Opinion in Plant Biology*, 43, 50–56. https://doi.org/10.1016/j.pbi.2017.12.003

Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free air CO_2 enrichment (FACE)? A meta-analytic review of the response of photosynthesis, canopy properties and plant production to rising CO_2. *The New Phytologist*, 179(2), 351–372. https://doi.org/10.1111/j.1469-8137.2004.01224.x

Atwell, B. J., Henery, M. L., Rogers, G. S., et al. (2007). Canopy development and hydraulic function in eucalyptus tereticornis grown in drought in CO_2-enriched atmospheres. *Functional Plant Biology*, 34(12), 1137–1149.

Atwell, B. J., Henery, M. L., & Whitehead, D. (2003). Sapwood development in *Pinus radiata* trees grown for three years at ambient and elevated carbon dioxide partial pressures. *Tree Physiology*, 23, 13–21. https://doi.org/10.1093/treephys/23.1.13

Baum, S. F., Tran, P. N., & Silk, W. K. (2000). Effects of salinity on xylem structure and water use in growing leaves of sorghum. *The New Phytologist*, 146, 119–127. https://doi.org/10.1046/j.1469-8137.2000.00625.x

Bloom, A. J., Asensio, J. S. R., Randall, L., Rachmilevitch, S., Cousins, A. B., & Carlisle, E. A. (2012). CO_2 enrichment inhibits shoot nitrate assimilation in C3 but not C4 plants and slows growth under nitrate in C3 plants. *Ecology*, 93, 355–367. https://doi.org/10.1890/11-0485.1

Blum, A. (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *F Crop Res*, 112, 119–123. https://doi.org/10.1016/j.fcr.2009.03.009

Brocious, C. A., & Hacke, U. G. (2016). Stomatal conductance scales with petiole xylem traits in Populus genotypes. *Functional Plant Biology*, 43(6), 553–562.

Chaves, M. M., & Oliveira, M. M. (2004). Mechanisms underlying plant resilience to water deficits: Prospects for water-saving agriculture. *Journal of Experimental Botany*, 55, 2365–2384. https://doi.org/10.1093/jxb/erh269

Cochard, H. (2002). Xylem embolism and drought-induced stomatal closure in maize. *Planta*, 215, 466–471. https://doi.org/10.1007/s00425-002-0766-9

Cohen, I., Halpern, M., Yermiyahu, U., Bar-Tal, A., Gendler, T., & Rachmilevitch, S. (2019). CO_2 and nitrogen interaction alters root anatomy, morphology, nitrogen partitioning and photosynthetic acclimation of tomato plants. *Planta*, 250, 1423–1432. https://doi.org/10.1007/s00425-019-03232-0

Cohen, I., Rapaport, T., Berger, R. T., & Rachmilevitch, S. (2018). The effects of elevated CO_2 and nitrogen nutrition on root dynamics. *Plant Science*, 272, 294–300. https://doi.org/10.1016/j.plantsci.2018.03.034

Cohen, I., Zandalinas, S. I., Fritschi, F. B., & Mittler, R. (2020). The impact of water deficit and heat stress combination on the molecular response, physiology, and seed production of soybean. *Physiologia Plantarum*, 172(1–12), 41–52. https://doi.org/10.1111/ppl.13269

Cohen, I., Zandalinas, S. I., Huck, C., Fritschi, F. B., & Mittler, R. (2020). Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia Plantarum*, 1–11, 66–76. https://doi.org/10.1111/ppl.13203

Domec, J., Smith, D. D., & Mcculloh, K. (2015). A synthesis of the effects of atmospheric carbon dioxide enrichment on plant hydraulics: implications for whole-plant water use efficiency and resistance to drought. *Plant, Cell & Environment*, 40, 921–937. https://doi.org/10.1111/pce.12843

Easlon, H. M., & Bloom, A. J. (2014). Easy leaf area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in Plant Sciences*, 2, 1400033. https://doi.org/10.3732/apps.1400033

Franzaring, J., Weller, S., Schmid, I., & Fangmeier, A. (2011). Growth, senescence and water use efficiency of spring oilseed rape (*Brassica napus* L. cv. Mozart) grown in a factorial combination of nitrogen supply and elevated CO_2. *Environmental and Experimental Botany*, 72, 284–296. https://doi.org/10.1016/j.envexpbot.2011.04.003

Gillespie, K. M., Xu, F., Richter, K. T., et al. (2012). Greater antioxidant and respiratory metabolism in field-grown soybean exposed to elevated O3 under both ambient and elevated CO_2. *Plant, Cell and Environment*, 35, 169–184. https://doi.org/10.1111/j.1365-3040.2011.02427.x

Gleason, S. M., Westoby, M., Jansen, S., Choat, B., Hacke, U. G., Pratt, R. B., & Zanne, A. E. (2016). Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *The New Phytologist*, 209, 123–136. https://doi.org/10.1111/nph.13646

Hacke, U. G., & Sperry, J. S. (2001). Functional and ecologycal xylem anatomy. *Perspect Plant Ecol Evol Syst*, 4, 97–115. https://doi.org/10.1007/978-3-319-15783-2

Hao, G.-Y., Holbrook, N. M., Zwieniecki, M. A., Gutschick, V. P., & BassiriRad, H. (2018). Coordinated responses of plant hydraulic architecture with the reduction of stomatal conductance under elevated CO_2 concentration. *Tree Physiology*, 2, 1041–1052. https://doi.org/10.1093/treephys/tpy001

Heath, J., Kerstiens, G., & Tyree, M. T. (1997). Stem hydraulic conductance of European beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.) grown in elevated CO_2. *Journal of Experimental Botany*, 48(7), 1487–1489.

Hochberg, U., Degu, A., Gendler, T., Fait, A., & Rachmilevitch, S. (2015). The variability in the xylem architecture of grapevine petiole and its contribution to hydraulic differences. *Functional Plant Biology*, 42, 357–365. https://doi.org/10.1071/FP14167

Ibrahim, D. G., Gilbert, M. E., Ripley, B. S., & Osborne, C. P. (2008). Seasonal differences in photosynthesis between the C3 and C4 subspecies of Alloteropsis semialata are offset by frost and drought. 31, 1038–1050. https://doi.org/10.1111/j.1365-3040.2008.01815.x

Jerszurki, D., Sperling, O., Parthasarathi, T., Lichston, J. E., Yaaran, A., Moshelion, M., Rachmilevitch, S., & Lazarovitch, N. (2020). Wide vessels sustain marginal transpiration flux and do not optimize inefficient gas exchange activity under impaired hydraulic control and salinity. *Physiologia Plantarum*, 170, 60–74. https://doi.org/10.1111/ppl.13107

Kant, S., Seneweera, S., Rodin, J., Materne, M., Burch, D., Rothstein, S. J., & Spangenberg, G. (2012). Improving yield potential in crops under elevated CO_2: Integrating the photosynthetic and nitrogen utilization efficiencies. *Frontiers in Plant Science*, 3, 1–9. https://doi.org/10.3389/fpls.2012.00162

Kocacinar, F., & Sage, R. F. (2004). Photosynthetic pathway alters hydraulic structure and function in woody plants. *Oecologia*, 139, 214–223. https://doi.org/10.1007/s00442-004-1517-3

Kostiainen, K., Kaakinen, S., Saranpää, P., Sigurdsson, B. D., Linder, S., & Vapaavuori, E. (2004). Effect of elevated [CO_2] on stem wood properties of mature Norway spruce grown at different soil nutrient

availability. *Global Change Biology*, 10, 1526–1538. https://doi.org/10.1111/j.1365-2486.2004.00821.x

Kraus, J.E.; Arduin M (1997) *Manual basico de metodos em morfologia vegetal*. EDUR.

Leakey, A. D. B., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., & Ort, D. R. (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. *Journal of Experimental Botany* 60:2859–2876. https://doi.org/10.1093/jxb/erp096

Liu, J., Kang, S., Davies, W. J., & Ding, R. (2020). Elevated [CO_2] alleviates the impacts of water deficit on xylem anatomy and hydraulic properties of maize stems. *Plant, Cell & Environment*, 43, 563–578. https://doi.org/10.1111/pce.13677

Lobell, D. B., & Gourdji, S. M. (2012). The influence of climate change on global crop productivity. *Plant Physiology*, 160, 1686–1697. https://doi.org/10.1104/pp.112.208298

Lynch, J. P., Chimungu, J. G., & Brown, K. M. (2014). Root anatomical phenes associated with water acquisition from drying soil: Targets for crop improvement. *Journal of Experimental Botany*, 65, 6155–6166. https://doi.org/10.1093/jxb/eru162

Medeiros, J. S., & Ward, J. K. (2013). Increasing atmospheric [CO_2] from glacial to future concentrations affects drought tolerance via impacts on leaves, xylem and their integrated function. *New Phytologist*, 199(3), 738–748.

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J. M., & Bota, J. (2015). From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. *Crop J*, 3, 220–228. https://doi.org/10.1016/j.cj.2015.04.002

Mohamed, S. J., Jellings, A. J., & Fuller, M. P. (2013). Positive effects of elevated CO_2 and its interaction with nitrogen on safflower physiology and growth. *Agronomy for Sustainable Development*, 33, 497–505. https://doi.org/10.1007/s13593-013-0137-x

Paudel, I., Halpern, M., Wagner, Y., Raveh, E., Yermiyahu, U., Hoch, G., & Klein, T. (2018). Elevated CO_2 compensates for drought e ff ects in lemon saplings via stomatal downregulation, increased soil moisture, and increased wood carbon storage. *Environmental and Experimental Botany*, 148, 117–127. https://doi.org/10.1016/j.envexpbot.2018.01.004

Qaderi, M. M., Martel, A. B., & Dixon, S. L. (2019) Environmental factors influence plant vascular system and water regulation. *Plants*, 8, 1–23. https://doi.org/10.3390/plants8030065

Rachmilevitch, S., Reuveni, J., Pearcy, R. W., & Gale, J. (1999). A high level of atmospheric oxygen, as occurred toward the end of the Cretaceous period, increases leaf diffusion conductance. *Journal of Experimental Botany*, 50, 869–872. https://doi.org/10.1093/jxb/50.335.869

Rico, C., Pittermann, J., Polley, H. W., et al. (2013). The effect of subambient to elevated atmospheric CO_2 concentration on vascular function in Helianthus annuus: Implications for plant response to climate change. *New Phytologist*, 199(4), 956–965.

Rodríguez-Gamir, J., Intrigliolo, D. S., Primo-Millo, E., & Forner-Giner, M. A. (2010). Relationships between xylem anatomy, root hydraulic conductivity, leaf/root ratio and transpiration in citrus trees on different rootstocks. *Physiologia Plantarum*, 139, 159–169. https://doi.org/10.1111/j.1399-3054.2010.01351.x

Růžička, K., Ursache, R., Hejátko, J., & Helariutta, Y. (2015). Xylem development - from the cradle to the grave. *The New Phytologist*, 207, 519–535. https://doi.org/10.1111/nph.13383

Shtein, I., Hayat, Y., Munitz, S., Harcavi, E., Akerman, M., Drori, E., Schwartz, A., & Netzer, Y. (2016). From structural constraints to hydraulic function in three Vitis rootstocks. *Trees*, 1–11, 851–861. https://doi.org/10.1007/s00468-016-1510-6

Strock, C. F., Burridge, J. D., Niemiec, M. D., Brown, K. M., & Lynch, J. P. (2020). Root metaxylem and architecture phenotypes integrate to

regulate water use under drought stress. *Plant, Cell & Environment*, *44*(1), 49–67. https://doi.org/10.1111/pce.13875

Tyree, M. T., Davis, S. D., & Cochard, H. (1994). Biophysical perspectives of xylem evolution: Is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *IAWA Journal*, *15*, 335–360. https://doi.org/10.1163/22941932-90001369

Wagner, K. R., Ewers, F. W., & Davis, S. D. (1998). Tradeoffs between hydraulic efficiency and mechanical strength in the stems of four co-occurring species of chaparral shrubs. *Oecologia*, *117*, 53–62. https://doi.org/10.1007/s004420050631

Wu, D.-X., Wang, G.-X., Bai, Y.-F., & Liao, J.-X. (2004). Effects of elevated CO_2 concentration on growth, water use, yield and grain quality of wheat under two soil water levels. *Agriculture, Ecosystems and Environment*, *104*, 493–507. https://doi.org/10.1016/j.agee.2004.01.018

Wullschleger, S. D., Tschaplinski, T. J., & Norby, R. J. (2002). Plant water relations at elevated CO_2—Implications for water-limited environments. *Plant, Cell and Environment*, *25*, 319–331. https://doi.org/10.1046/j.1365-3040.2002.00796.x