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Increasing atmospheric [CO_2] from glacial through future levels affects drought tolerance via impacts on leaves, xylem and their integrated function

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Summary

- Changes in atmospheric carbon dioxide concentration ([CO_2]) affect plant carbon/water trade-offs, with implications for drought tolerance. Leaf-level studies often indicate that drought tolerance may increase with rising [CO_2], but integrated leaf and xylem responses are not well understood in this respect. In addition, the influence of low [CO_2] of the last glacial period on drought tolerance and xylem properties is not well understood.
- We investigated the interactive effects of a broad range of [CO_2] and plant water potentials on leaf function, xylem structure and function and the integration of leaf and xylem function in *Phaseolus vulgaris*.
- Elevated [CO_2] decreased vessel implosion strength, reduced conduit specific hydraulic conductance, and compromised leaf specific xylem hydraulic conductance under moderate drought. By contrast, at glacial [CO_2], transpiration was maintained under moderate drought via greater conduit specific and leaf specific hydraulic conductance in association with increased vessel implosion strength.
- Our study involving the integration of leaf and xylem responses suggests that increasing [CO_2] does not improve drought tolerance. We show that under glacial conditions changes in leaf and xylem properties could increase drought tolerance, while under future conditions greater productivity may only occur when higher water use can be accommodated.

Keywords

drought tolerance; elevated [CO_2]; glacial [CO_2]; leaf and xylem integration; xylem hydraulic conductance

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Supporting Information
Additional supporting information may be found in the online version of this article.
Table S1 Watering and nutrient regimes applied during the course of the experiment
Table S2 Regression co-efficients relating root surface area to dry biomass for each [CO_2] × watering regime combination
Methods S1 Detailed methods for implementation of the water transport model.

Introduction

Changes in atmospheric carbon dioxide concentration ([CO_2]) are known to alter tradeoffs between plant carbon gain and water loss, with implications for drought tolerance and productivity. Studies focused on leaf-level responses to changes in [CO_2] often suggest that drought tolerance may improve as [CO_2] rises. However, changes in [CO_2] can alter a wide variety of traits related to drought tolerance beyond leaf traits. Specifically, the effects of [CO_2] and drought on xylem structure and function and the integration of xylem with leaf-level responses is not well understood, hindering our ability to accurately predict whole-plant responses to changing [CO_2] and drought. Furthermore, until now, there have been few studies incorporating the effects of low [CO_2] that occurred during the last glacial period (20,000 yr ago). In this paper we investigate the effects of a broad continuum of [CO_2] and drought on a suite of plant traits including leaf-level responses, xylem structure and function, as well as patterns of allocation to leaves versus xylem and roots. The results of this work offer unique insights into the mechanisms that drive the fundamental trade-offs between plant water use and carbon gain over geologic and contemporary time scales.

A simultaneous change in both [CO_2] and water availability has occurred since the last glacial period, and rapid changes are expected to continue into the future. During the last glacial period, [CO_2] was among the lowest levels that occurred during the evolution of vascular plants (as low as 180 ppm; Berner, 2006). With the onset of anthropogenic activities during the current inter-glacial period, [CO_2] has risen from 270 to 390 ppm, and is expected to reach 700–1000 ppm by the end of this century (Meehl *et al.*, 2007). Concurrent changes in soil water availability have also occurred, with many regions experiencing more extreme drought during glacial periods compared to modern times (Jansen *et al.*, 2007). Further changes in water availability are also anticipated for the near future, with the length and severity of droughts expected to worsen (Christensen *et al.*, 2007; Sheffield & Wood, 2008). Thus, analysis of the interactive effects of changes in [CO_2] and water availability is important for understanding the impact of both past and future climates on plant productivity. Actual plant specimens dating to the last glacial period show strong support for leaf-level carbon limitation as evidenced by reduced CO_2 availability in the leaf intercellular space (Ward *et al.*, 2005; Gerhart & Ward, 2010; Gerhart *et al.*, 2011). In addition, leaf-level studies of modern plants often find that increasing [CO_2] from glacial through elevated future levels enhances carbon gain for C_3 plants, while at the same time allowing for decreased stomatal conductance (Gerhart & Ward, 2010; Vanaja *et al.*, 2011; Franks *et al.*, 2013). Furthermore, the relative increase in photosynthetic rates at elevated [CO_2] is often greatest under drought conditions (Franks *et al.*, 2013), such that the negative impact of drought may be ameliorated as [CO_2] increases from glacial to future predicted levels by improved carbon gain, even when stomatal closure occurs in response to drought.

Studies examining how changes in [CO_2] affect water use at the leaf-level do not provide a complete assessment of how drought tolerance may change as [CO_2] rises, however, mainly because [CO_2] affects many other plant components that dictate water use. In fact, studies that have examined whole plant responses to altered [CO_2] often find no positive effect of elevated [CO_2] on drought tolerance (Vaz *et al.*, 2012; Perry *et al.*, 2013). One reason for this may be that plants must also tolerate the damaging effects of drought on xylem water transport, but little is known about the interactive effects of [CO_2] and water availability on this aspect of plant function. Hydraulic conductance can be increased when plants are grown under elevated versus current (Tognetti *et al.*, 1999; Tognetti *et al.*, 2001) or current versus glacial [CO_2] (Quirk *et al.*, 2013). This may result because lower transpiration rates at elevated [CO_2] allow plants to maintain water potential farther from the point where xylem embolism begins. In some cases, though, hydraulic conductance decreases at elevated [CO_2] (Tognetti *et al.*, 2005), which may become particularly problematic for plants with poor stomatal control or for those that retain active physiologically functioning during drought. This may be attributable, at least in part, to the fact that an increase in xylem conduit size is often observed at elevated compared to current [CO_2], resulting in a reduced ratio of conduit wall thickness to diameter (Eguchi *et al.*, 2008; Kostiainen *et al.*, 2009). Furthermore, [CO_2] effects on water transport may be strongly linked to concurrent changes in leaf area (Gebauer & BassiriRad, 2011), since an increase in total leaf area under elevated [CO_2] could increase total canopy water demand (Ward *et al.*, 1999; Wullschleger *et al.*, 2002).

If canopy water demand changes with increasing [CO_2], then greater allocation to water transport may be necessary for plants to maintain similar water supply on a leaf area basis across a broad [CO_2] range. At elevated [CO_2], increased allocation of carbon to shoots and/or roots could improve leaf specific hydraulic conductance during drought, and thereby decrease the risk of hydraulic failure (Maseda & Fernández, 2006). Indeed, plants usually allocate fewer resources to aboveground structures as [CO_2] increases in both drought and non-drought conditions (Ward *et al.*, 2005; Inauen *et al.*, 2012). Under well-watered conditions, hydraulic conductance on a leaf area basis can be conserved as [CO_2] increases from pre-industrial to current (Phillips *et al.*, 2011), and from current to elevated levels (Maherali & DeLucia, 2000; Eguchi *et al.*, 2008; Phillips *et al.*, 2011). In other cases, however, leaf specific hydraulic conductance can also be reduced at elevated compared to current [CO_2] (Tognetti *et al.*, 2005; Eguchi *et al.*, 2008). Thus, the effects of changing [CO_2] on the interaction of leaf and xylem traits may provide a mechanistic explanation as to why, in some cases, the positive effect of increasing [CO_2] on leaf-level traits does not translate into higher growth rates (Ward *et al.*, 1999; Ghannoum *et al.*, 2010). Though such whole-plant effects ultimately determine drought performance and survival, data examining the combined effects of [CO_2] *and* drought on the integration of leaf and xylem traits are generally lacking from the literature.

We addressed this knowledge gap by determining the effects of a range of [CO_2] and plant water potentials on leaves, xylem and whole-plant function. Specifically, we hypothesized that increasing [CO_2] from glacial to future predicted levels would: reduce the impact of drought on productivity; and increase the capacity for xylem water transport to meet leaf water demand. We investigated these hypotheses using the annual *Phaseolus vulgaris*, which has been previously developed as a model system in [CO_2] studies (e.g. Sage & Reid, 1992; Cowling & Sage, 1998). Though drought does not typically limit *P. vulgaris* growth in irrigated cropping systems, varieties of *P. vulgaris* are commonly cultivated using dryland farming techniques in regions where extended droughts frequently limit their yield (Subbarao *et al.*, 1995). In addition, the effect of changing [CO_2] and drought on dryland agriculture is expected to intensify in the coming century, such that a more thorough understanding of the interactive effects of [CO_2] and drought will be crucial for determining future management practices in dryland agricultural regions (Jat *et al.*, 2012). We chose a drought tolerant variety, Bolita, providing a more conservative test of the effects of changing [CO_2] on drought tolerance. We grew plants under [CO_2] representing glacial to elevated conditions predicted for the future and drought levels ranging from none to severe. We measured biomass accumulation and allocation, leaf gas exchange, xylem hydraulic conductance, as well as xylem anatomical features related to embolism vulnerability and water transport capacity. In addition, we applied our data to a water transport model developed by Sperry *et al.* (1998) that quantifies the risk of runaway embolism during drought.

Materials and Methods

Plant material and growth conditions

Phaseolus vulgaris L. var. Bolita (hereafter *P. vulgaris*; seeds from Plants of the Southwest, Santa Fe, NM, USA) were grown for 28–31 d in a full factorial design with three [CO_2] treatments representing glacial (180 ppm), current (380 ppm), or elevated (700 ppm) conditions in combination with four water regimes, resulting in a range of water potentials representing the full operating range of this species (*c.* −0.2 to −1.8 MPa, Holste *et al.*, 2006). Each [CO_2] treatment was replicated in two different growth chambers (Conviron, BDR16, Winnipeg, Manitoba), and all reported measurements were made on the day of harvest. Plants in all treatments were grown in 3.8 l pots in a sandy clay loam soil (49% sand, 18% silt, 33% clay) for 29–31 d. Growth chamber temperature and RH were controlled at 25 : 18 °C and 50 : 65 % (day : night), respectively. Light was provided from 08:00 h to 22:00 h at 1048 (+/− 66) μmol m^{-2} s^{-1}. Within each growth chamber pots containing sown seeds were randomly assigned to one of four water availability regimes ranging from high to low (see Supporting Information Table S1 for detailed description of water regimes). For each plant, the level of drought was determined on the day of harvest as predawn plant water potential (Ψ_{PD}) measured using a pressure chamber (PMS, Model #1000, Corvalis, OR, USA). We also measured midday plant water potential (Ψ_{MD}) for use in the water transport model described later.

Since *P. vulgaris* forms a symbiotic relationship with nitrogen fixing bacteria, all soil was autoclaved before planting to eliminate nodulation and ensure equal access to nitrogen at all [CO_2]. Nitrogen demand is known to increase at elevated [CO_2] in *P. vulgaris* (Jifon & Wolfe, 2002), but high nutrient levels negatively impact hydraulic function and reduce transpiration (Ewers *et al.*, 2000). Therefore, we used preliminary experiments to identify a nutrient regime that maximized transpiration for plants grown at the control [CO_2] of 380 ppm (See Table S1 for description of nutrient regime).

Growth and allocation

Total plant dry biomass (leaves, stems and roots) on the day of harvest was determined after drying at 70°C for 72 h. Biomass allocation was determined as the ratio of absorbing root area to transpiring leaf area (A_R:A_L). We measured fresh leaf area using a leaf area meter (LiCor Biosciences, LI-3100, Lincoln, NE, USA). We estimated fresh absorbing root area for each [CO_2] × water availability combination using a regression co-efficient relating fresh root surface area to dry mass (detailed methods and co-efficient for each treatment combination presented in Table S2).

Leaf gas exchange

Gas exchange measurements were made at midday with an LI 6400 (LiCor Biosciences) on the day of harvest, using the upper most leaf large enough to fill the cuvette. For all plants in all treatments, this leaf had developed after the start of drought treatments. Cuvette conditions were controlled to be similar to growth conditions (T_{block} = 25 °C, T_{leaf} = 26 °C ± 1.3, RH_{sample} = 48% ± 4, VPD = 1.8 kPa ± 0.3, 1000 μmol $m^{-2}s^{-1}$ light intensity, and [CO_2] representing experimental treatments). Leaves were allowed to acclimate to cuvette conditions for a minimum of 10 and up to 20 min, or until stomatal conductance, photosynthesis and transpiration were fully stable for at least 1 min. Upon reaching steady state, we made five measurements over a 1-min period and report the average of these five measurements. Whole plant water use per unit of time was calculated by multiplying transpiration rate measured using the LI 6400 by total leaf area. Instantaneous water use efficiency (*WUE*) was calculated from photosynthetic and transpiration rates measured by the LI 6400.

Xylem function and anatomy

Immediately following gas exchange measurements on the day of harvest, we measured total stem hydraulic conductance (K_h) using an Ultra-Low Flow Meter (apparatus described in Tyree *et al.*, 2002). The precise methods used here are described in detail in Medeiros & Pockman (2011), except that in this study we removed each leaf at the base of the petiole, cut the stem underwater *c.* 1 cm above the root/shoot junction and then measured K_h for the entire above-ground portion of the plant stem. We calculated leaf-specific hydraulic conductance (K_l) by dividing K_h by leaf area measured as described above. Measurements of xylem anatomy were made using the same plants measured for K_h. Stem samples were cut from *c.* 1 cm above the root/shoot junction, embedded in resin (Electron Microscopy Sciences, Catalog #14300, Hatfield, PA, USA), sectioned, and then imaged using a digital camera (Photometrics, CoolSNAP ES, Tuscon, AZ, USA) connected to a microscope (Nikon Instruments, Eclipse 80i, Melville, NY, USA). We measured vessel diameter for all vessels in a single cross-section. From this, we calculated the hydraulically weighted diameter ($D_h = \Sigma\, D^5/\Sigma D^4$), and the conduit area specific hydraulic conductance ($K_c = K_h/$ total xylem conduit area), which both provide estimates of total water transport capacity. Based on the method described in Hacke *et al.* (2001), we also determined vessel implosion strength (t/b), or the ratio of the thickness (t) to the span (b) of the double wall between two adjacent vessels, which is strongly correlated with xylem vulnerability to drought embolism (Hacke *et al.*, 2001). Plants from only one chamber were used in this case for these exceptionally time-intensive measures.

Water transport model

Using the water transport model of Sperry *et al.* (1998) we estimate the critical transpiration rate at which runaway embolism begins, and the corresponding critical plant water potential (Ψ_{crit}). A brief description of our implementation is provided here, but complete methods are described in the supporting information for this article (Methods S1). The model uses a standard finite difference approach to solve Darcy’s law for the main portion of the hydraulic pathway from the bulk soil to the atmosphere. The model was parameterized for each [CO_2] treatment using maximum measured values of transpiration (E), K_l, Ψ_{PD} and Ψ_{MD}. Measurements of E, Ψ_{PD}, Ψ_{MD} and A_R:A_L for individual plants were used for model inputs, resulting in an output critical transpiration rate and Ψ_{crit} for each plant. The safety margin from runaway embolism was calculated as the difference between estimated critical transpiration rate and measured E. These model results provide a quantitative estimate of the *relative* risk of runaway embolism across [CO_2] treatments based on measured leaf and xylem traits, but should be interpreted with some caution since transpiration is typically over-estimated in a cuvette due to elimination of the boundary layer (LI-COR, 2012). We performed t-tests and regression analysis to determine the fit of the model. The fitted regression lines are presented here (Fig. 7a,b) and the results of the model fit analysis are presented in the supporting information for this article.

Data analysis

All data were analyzed using SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA). We performed two-way ANOVA to test for differences across treatments using PROC GLM with [CO_2], Ψ_{PD}, and [CO_2] × Ψ_{PD} as main effects with interactions (growth chamber was treated as a random variable). Because Ψ_{PD} was not continuously distributed (Fig. 1), it was necessary to treat it as a categorical variable in all analyses. Plants were assigned to one of four drought categories according to measured Ψ_{PD} (Fig. 1): none, mild, moderate or severe drought, with each [CO_2] × drought combination including 4–11 plants. To account for unequal sample sizes, Type III Sums of Squares were used to determine significance for all analyses. Overall, growth chamber effects were negligible, exhibiting a significant effect in only a small subset of measured characters (Table 1) and no significant interactions with

[CO_2] or drought level. Therefore, interactions with growth chamber were removed from all analyses to conserve model degrees of freedom. Gas exchange parameters were analyzed using MANOVA due to high levels of autocorrelation typical of these measures. Planned pairwise comparisons were made using the LSMEANS option. Because *t/b* and A_R:A_L are ratios, these data were transformed for analysis using an arcsin square root.

Results

Growth and allocation

For all [CO_2] treatments, leaf area was significantly reduced in response to increased drought intensity (Fig. 2a; Table 1a) and the response to drought was similar across [CO_2] (no significant [CO_2] × drought interaction). For plants grown at 180 ppm leaf area was reduced most dramatically in response to drought and was always significantly lower compared to plants grown at 380 and 700 ppm. By contrast, plants grown at 380 had leaf areas similar to, or larger than, those grown at 700 ppm at all levels of drought intensity.

Plants grown at 180 ppm exhibited the lowest root areas across all levels of drought (Fig. 2b), and there was a significant effect of [CO_2] on this character (Table 1a). For plants grown at the control level of 380 ppm, drought did not significantly affect root area, but for plants grown at 180 and 700 ppm, root area declined in response to greater drought intensity (significant [CO_2] × drought interaction).

For plants grown at the control level of 380 ppm, more intense drought resulted in a significant increase in A_R:A_L (Fig. 2c), while for plants grown at 180 and 700 ppm A_R:A_L was more similar across all levels of drought (significant [CO_2] × drought interaction; Table 1a). Under moderate and severe drought, A_R:A_L did not differ between plants grown at 380 and those grown at 700 ppm. A_R:A_L was significantly lower for plants grown at 180 ppm compared to those grown at 700 ppm, regardless of drought.

Total plant biomass was significantly reduced by the most severe drought treatment (Fig. 2d; Table 1), and the relative patterns across [CO_2] treatments were similar (no significant [CO_2] × drought interaction). Total plant biomass was always significantly lower for plants grown at 180 ppm compared to those grown at 380 and 700 ppm regardless of drought. By contrast, significant differences between plants grown at 700 and 380 ppm were only observed in the absence of drought.

Leaf gas exchange

In the absence of drought, A (net assimilation; Fig. 3a) increased with increasing [CO_2], while E (transpiration; Fig. 3b) and g_s (stomatal conductance; Fig. 3c) declined. MANOVA indicated a significant effect of [CO_2] on gas exchange parameters (F_{126} = 33.44, df = 6, $P <$ 0.0001). For plants grown at 700 ppm, mild drought did not affect A. However A, E and g_s were reduced significantly under moderate and severe drought for all plants (drought effect, $F_{153.48}$ = 41.45, df = 9, $P <$ 0.0001). The drop in net photosynthetic rates under drought was least dramatic for plants grown at 180 ppm, compared to those grown at 380 and 700 ppm. Thus, we did not detect a significant difference among the three [CO_2] treatments under moderate or severe drought, resulting in a significant [CO_2] × drought interaction ($F_{178.68}$ = 8.27, df = 18, $P <$ 0.0001). Similarly, E and g_s remained significantly higher for plants grown at 180 compared to those grown at 380 and 700 ppm under all but the most severe levels of drought. There was not a significant effect of growth chamber on gas exchange (F_{63} = 1.86, df = 3, P = 0.1461).

Total water use was reduced significantly as drought intensity increased (Fig. 4a; Table 1b), and the relative pattern across [CO_2] treatments was similar (no significant [CO_2] × drought interaction). Across all levels of drought intensity plants grown at 180 ppm exhibited the highest rates of total water use, resulting in a significant effect of [CO_2] on this character, though differences across [CO_2] were not significant under the most severe level of drought. Although in the absence of drought total water use was higher for plants grown at 380 compared to those grown at 700 ppm, there was not a significant difference among these plants under any level of drought.

Plants grown at 180 ppm exhibited the lowest *WUE* at all levels of drought (Fig. 4b), and there was a significant effect of [CO_2] on this measure (Table 1b). *WUE* did not increase significantly at higher levels of drought for plants grown at 180 ppm. By contrast, *WUE* increased under moderate and severe drought for plants grown at 380 and 700 ppm, and this increase was greatest for plants grown at 700 ppm (significant [CO_2] × drought interaction).

Xylem hydraulic function

In the absence of drought, K_h was significantly higher for plants grown at 180 and 380 compared to those grown at 700 ppm (Fig. 5a). Moderate and severe drought significantly reduced K_h for all [CO_2] treatments (significant drought effect, Table 1c), although K_h was reduced more dramatically for plants grown at 700 ppm compared with those grown at180 and 380 ppm (significant [CO_2] × drought interaction). Similarly, in the absence of drought K_l was significantly higher for plants grown at 180 and 380 compared to those grown at 700 ppm (Fig. 5b). There were also striking differences among the [CO_2] treatments in the response of K_l to moderate and severe drought. For plants grown at 180 ppm, K_l increased under moderate and severe drought, while it remained similar for plants grown at 380 ppm, and decreased for plants grown at 700 ppm (significant [CO_2] × drought interaction). We also found a significant effect of [CO_2] on K_c, with plants grown at 180 and 380 ppm maintaining higher K_c than plants grown at 700 ppm regardless of drought intensity (Fig. 5c). Though there was a trend for K_c to decline with increasing drought for all [CO_2], we did not detect a significant effect of drought or [CO_2] × drought interaction on this character.

Xylem anatomy

There was not a significant effect of drought or a significant [CO_2] × drought interaction for any of the measured xylem anatomical traits (Table 1d). Therefore, for simplicity, only the [CO_2] effect is shown in Fig. 6. Increasing [CO_2] resulted in a significant increase in mean xylem vessel diameter (Fig. 6a). Vessel density decreased significantly as [CO_2] increased from 180 to 700 ppm (Fig. 6b). There was also a significant increase in D_h as [CO_2] rose from 180 to 380 ppm, although plants grown at 380 and 700 ppm did not differ in this measure (Fig. 6c). In addition, *t/b* (Fig. 6d) decreased significantly as [CO_2] increased from 180 to 700 ppm. There were no significant differences across [CO_2] in xylem cell wall thickness (*t*; Table 1, Mean *t* 180 ppm = 4.24 ± 0.43, 380 ppm = 4.45 ± 0.41 and 700 ppm = 4.26 ± 0.23).

Water transport model

Based on the measurements of A_R:A_L, plant water potential, gas exchange, and xylem hydraulic conductance made in the current study, the water transport model predicted lower Ψ_{crit} for plants grown at 180 and 380 compared with those grown at 700 ppm, regardless of Ψ_{PD} (Fig. 7a). At higher Ψ_{PD}, the safety margin from runaway embolism was predicted to be lower for plants grown at 180 ppm than for plants grown at 380 and 700 ppm (Fig. 7b). Under severe drought, however, plants grown at 180 ppm were predicted to have the largest safety margin relative to plants grown at 380 and 700 ppm, respectively. By contrast, a larger safety margin was always predicted for plants grown at 380 compared to those grown at 700 ppm.

Discussion

A number of leaf-level studies have now indicated that increasing [CO_2] from glacial through future predicted levels may improve drought tolerance by enhancing photosynthesis while at the same time allowing for reduced transpiration (e.g. Vanaja *et al.*, 2011; Franks *et al.*, 2013). Yet, [CO_2] can affect a number of additional components relating to drought tolerance, including xylem function and allocation to leaves, roots and/or xylem. Until now, however, the impact of glacial and elevated [CO_2] on the co-ordination of leaf and xylem function has not been adequately addressed. We hypothesized that increasing [CO_2] from glacial through future predicted levels would reduce the negative effects of drought on productivity and increase the capacity for xylem water transport to meet leaf water demand. We investigated these hypotheses using *P. vulgaris* grown across a range of [CO_2] from glacial through elevated levels under drought conditions ranging from none to severe. Surprisingly, we found that the hydraulic function of plants grown at glacial [CO_2] was least affected by drought. This was associated with reduced leaf area and changes in xylem structure in response to growth at low [CO_2], which together allowed higher transpiration rates under moderate drought. By contrast, plants grown at elevated [CO_2] exhibited a reduced ability of xylem water transport capacity to meet leaf water demand and stronger drought-induced limitations on transpiration.

We found evidence that steeper drought-induced declines in net photosynthesis under increasing [CO_2] observed in this study (Fig. 3a) and in others (André & Du Cloux, 1993; Engel *et al.*, 2004) could be driven by negative effects of elevated [CO_2] on water transport. In our study, K_h was reduced during drought for plants grown at current [CO_2] (Fig. 5a), but simultaneous declines in leaf area (Fig. 2a) resulted in similar K_l as drought progressed (Fig. 5b). By contrast, at elevated [CO_2] significantly steeper reductions in K_h combined with similar reductions in leaf area produced a mismatch between water supply and demand under moderate and severe drought (reduced K_l). In conjunction with this, plants grown at elevated [CO_2] exhibited no difference in biomass accumulation during drought compared to plants grown in the current [CO_2] treatment (Fig. 2d). In addition, we saw that plants grown at glacial [CO_2] exhibited a significantly smaller decline in K_h in response to more intense drought compared to plants grown at elevated [CO_2], and had the greatest relative reduction in leaf area. Thus, K_l went up during drought for glacial [CO_2]-grown plants. Furthermore, this was associated with higher transpiration rates and a smaller decrease in net photosynthetic rates under mild drought compared to plants grown at current and elevated [CO_2] (Fig. 3).

It is worth noting here that simultaneous, and opposing effects of [CO_2] on leaf area (increased with [CO_2]) and K_h (decreased at elevated [CO_2]) resulted in an overall effect that was different than would be predicted from measurement of leaf function, allocation to leaf area, or hydraulic function alone. Consequently, the integration of all factors was critical for a clear understanding the impact of [CO_2] on plant drought tolerance. Using this approach we found that the effects of [CO_2] under well-watered conditions likely negatively impacted the response of plants to drought: K_l was significantly and negatively related to [CO_2]. Published data for a wide range of plant taxa ranging from trees and crops to lianas suggests that K_l is often reduced at elevated [CO_2] under well-watered conditions (Fig. 8a). Interestingly though, xeric shrubs appear to maintain K_l at elevated [CO_2] (Fig. 8b), while fast growing tree species like *Eucalyptus* and *Quercus sp*. show a relative increase in K_l (Fig. 8c) as [CO_2] rises. Thus, the ability of plants to maintain coordinated leaf and xylem function under changing [CO_2] may vary according to traits such as drought tolerance or life history strategy. Furthermore, our work extends our understanding of the effects of [CO_2] on whole plant hydraulic function by providing evidence that K_l can become increasingly compromised during drought for plants grown at elevated [CO_2]. The commonality of this remains to be seen, however, since other studies concerning the combined effects of drought and [CO_2] on K_l are lacking in the current literature.

One possible explanation for the drought responses we observed is that reduced K_l at elevated [CO_2] under non-drought conditions caused plants to experienced an increase in drought embolism. We found several lines of evidence in support of this. First, we saw that the differences in K_h and K_l between non-drought and drought plants were greatest for plants grown under elevated [CO_2]. Second, we found that for a given conduit size relative investment in cell walls declined significantly as [CO_2] increased (lower *t/b*, Fig. 6d). Across a wide variety of woody plants reduced *t/b* is correlated with increased embolism vulnerability (Hacke *et al*., 2001), though the applicability of this relationship to herbaceous plants has not been experimentally justified. However, our results with glacial [CO_2] do extend and support observations that *t/b* is reduced at elevated [CO_2] in both herbaceous (Davey *et al*., 2004) and tree species (Kilpeläinen *et al*., 2007). Third, an increased risk of embolism with increasing [CO_2] is further suggested by the results of the water transport model, which predicted the highest Ψ_{crit} for plants grown at elevated [CO_2] (Fig. 7a). The model also predicted a smaller safety margin from runaway embolism for plants grown at elevated compared to current [CO_2], regardless of water availability (Fig. 7b). A global data set examining 191 forest species (Choat *et al*., 2012) indicates that in comparison with most other angiosperms *P. vulgaris* var. Bolita has a similar or lower Ψ_{crit} (at current [CO_2] Ψ_{crit} in our study was below −2.5 MPa), suggesting that our results using the drought tolerant Bolita variety of *P. vulgaris* are possibly applicable to a wide range of species.

We also found evidence, however, that the negative effect of increasing [CO_2] on K_l was due at least in part to a change in saturated water transport capacity. In support of this, we saw that K_c was *c*. 50% lower for plants grown at elevated compared to those grown at glacial and current [CO_2], regardless of water availability (Fig. 5c). It is possible that K_c was compromised at elevated [CO_2] due to greater embolism even under non-drought conditions, but the differences in K_c across [CO_2] did not increase as drought progressed, so this possibility cannot be discerned under the current study design. We also saw that the significant positive effect of elevated [CO_2] on xylem water transport capacity estimated from xylem conduit diameter (D_h; Fig. 6c) was not associated with an increase in either K_h or total canopy water use at elevated [CO_2] (Fig. 4a). Taken together, these results raise the intriguing possibility that the hydraulic and subsequent drought effects of [CO_2] we observed were driven by not only be changes in embolism rate, but also by changes in xylem anatomical traits related to saturated water transport capacity, such as vessel length. Recent evidence for substantial within-species variation in vessel length across a broad taxonomic range of woody plants (Jacobsen *et al*., 2012) suggests that this character may display plasticity in response to environmental conditions, but the impact of [CO_2] on vessel length has not been previously determined. Though our study cannot provide direct support for an impact of [CO_2] on vessel length, our whole-plant approach did allow us to more narrowly define the impact of [CO_2] on plant hydraulic function and drought tolerance, and highlights the need for more studies of this nature.

Despite the negative effect of increasing [CO_2] on hydraulic function, we observed several characteristics of plants grown at current and elevated [CO_2] that could increase their drought tolerance compared to those grown at glacial [CO_2]. First, we saw that plants grown at current and elevated [CO_2] always accumulated more biomass than glacial [CO_2]-grown plants, regardless of drought (Fig. 2d), which could improve drought survival and recovery (Ward *et al*., 1999; Wullschleger *et al*., 2002). Second, we saw that plants grown at glacial [CO_2] had the highest total water use in most cases (Fig. 4a) and did not significantly increase water use efficiency (*WUE*) in response to drought (Fig. 4b). By contrast, *WUE* increased significantly during drought for those grown at current, and even more so for those grown at elevated [CO_2]. Still, it is important to note here that our data concerning xylem hydraulic function (Fig. 5), xylem structure (Fig. 6) as well as Ψ_{crit} and safety margin (Fig. 7) suggest that lower WUE in glacial plants was accommodated without increasing the risk of hydraulic failure, an important component of drought tolerance.

We also found some evidence that growth at elevated [CO_2] could improve drought tolerance compared to current [CO_2], but in this case the benefits did not extend beyond mild drought. First, though total water use was significantly lower for plants grown at elevated compared to current [CO_2] in the absence of drought (Fig. 4a), we did not find a significant difference between current and elevated [CO_2] under drought. Second, we observed significantly greater root area (Fig. 2b) and higher A_R:A_L (Fig. 2c) with increasing [CO_2] under well-watered and mild drought conditions. This pattern has been observed in a large number of previous studies (e.g. Inauen *et al.*, 2012) and could improve access to soil water resources. But, again, under moderate and severe drought the advantage of elevated compared to current [CO_2] was diminished. Plants grown under elevated [CO_2] had significantly lower root area compared to those grown under current conditions, and we saw no significant difference between plants grown at current and elevated [CO_2] in A_R:A_L. Taken together these data indicate that elevated [CO_2] may provide some protection from the onset of drought conditions and/or from the negative effects of mild drought, but the opposite may be true under moderate and severe drought.

Our observations ultimately have broad implications for productivity at both future and past [CO_2]. Reduced K_l observed in our study (Fig. 5b) and others (Fig. 8a) suggests that elevated [CO_2] could compromise productivity under moderate and severe drought by limiting stomatal opening. In addition, the results of the water transport model suggest that cessation of growth may occur at higher plant water potentials as [CO_2] rises in the future. In addition, our investigation of xylem structure and function indicates that productivity may be reduced at glacial [CO_2], not only as a result of low substrate availability and increased photorespiration (Ward, 2005), but also due to the need for relatively greater investment in xylem cell walls (higher *t/b*). In our study this came at a cost of reduced water transport capacity (Fig. 6c), which in turn can reduce productivity (Pittermann *et al.*, 2006). Though this could lower the risk of xylem embolism, it also increases carbon inputs into construction of xylem (Hacke *et al.*, 2001). Based on this one might predict that carbon limitation at glacial [CO_2] would result in reduced investment in cell walls, nevertheless, optimal carbon use efficiency may be achieved in some cases when conduits remain functional (Holtta *et al.*, 2011).

In conclusion, our study involving whole-plant responses highlights the fact that a change in [CO_2] elicits simultaneous changes in xylem structure and function, allocation to leaves and roots as well as leaf function, and it is the collective impact of these changes that governs plant responses to drought. We provide evidence that growth under low [CO_2] conditions could have increased the drought tolerance of some C_3 plants during glacial times compared to the present. Our work also provides mechanistic support for studies indicating that water availability will remain an important factor C_3 limiting productivity as [CO_2] increases (Linares *et al.*, 2009; Vaz *et al.*, 2012; Perry *et al.*, 2013). Furthermore, we show that increased productivity at elevated [CO_2] may only occur under conditions where higher water use can be accommodated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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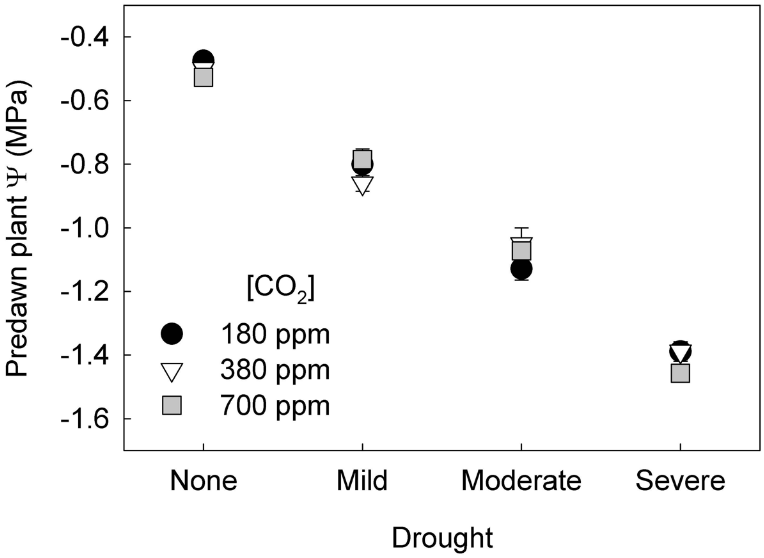

Fig. 1.

Plant predawn water potential at the four levels of drought for *Phaseolus vulgaris* var. Bolita grown at glacial (180 ppm; circles), current (380 ppm; triangles) and elevated (700 ppm; squares) [CO_2]. Measurements were taken on the final day of plant growth, just before measurement of biomass and allocation, gas exchange and xylem hydraulic conductance. Error bars represent ± 1 SE.

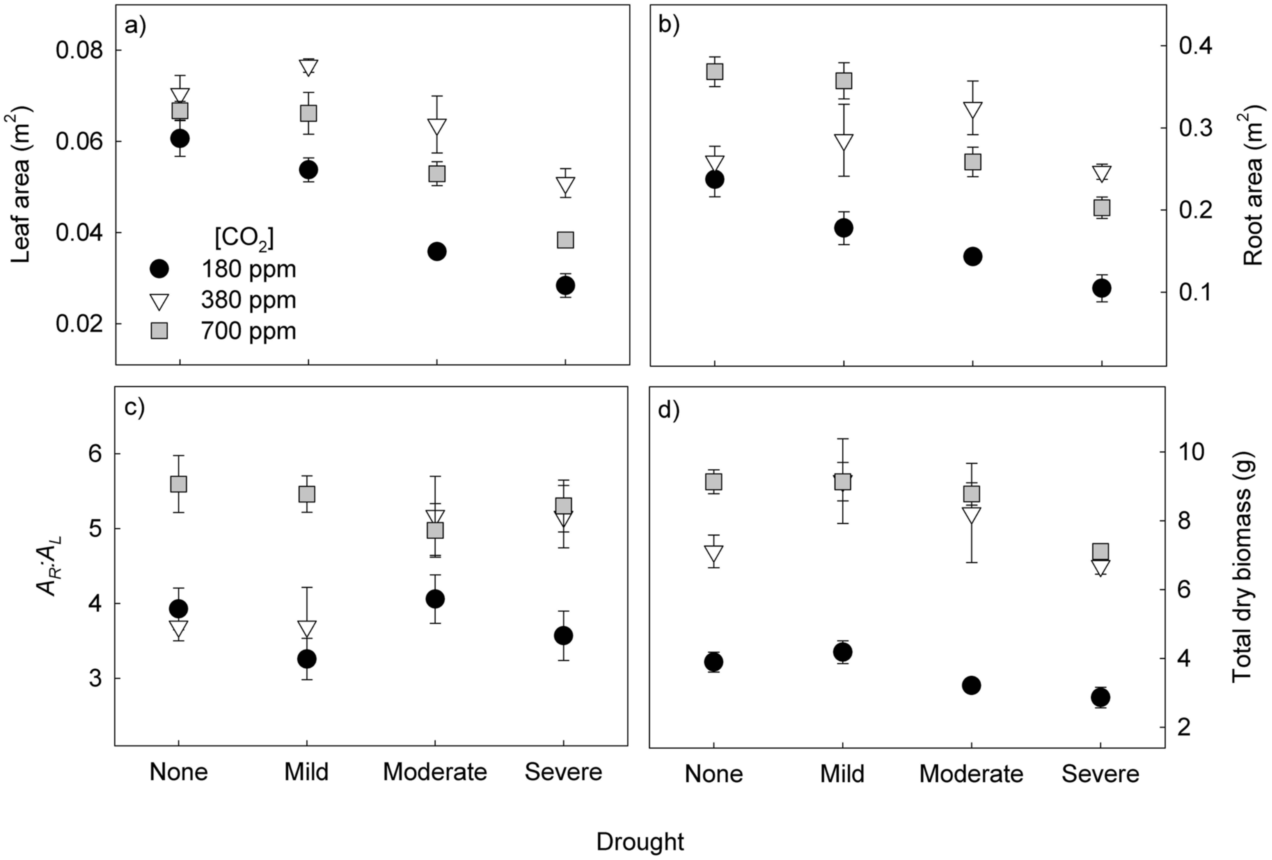

Fig. 2.

Growth and allocation of *Phaseolus vulgaris* var. Bolita grown under four levels of drought at glacial (180 ppm; circles), current (380 ppm; triangles) and elevated (700 ppm; squares) [CO$_2$] including: (a) leaf area, (b) root area, (c) the ratio between absorbing root area and transpiring leaf area (A_R:A_L), and d) total plant biomass. Error bars represent ± 1 SE.

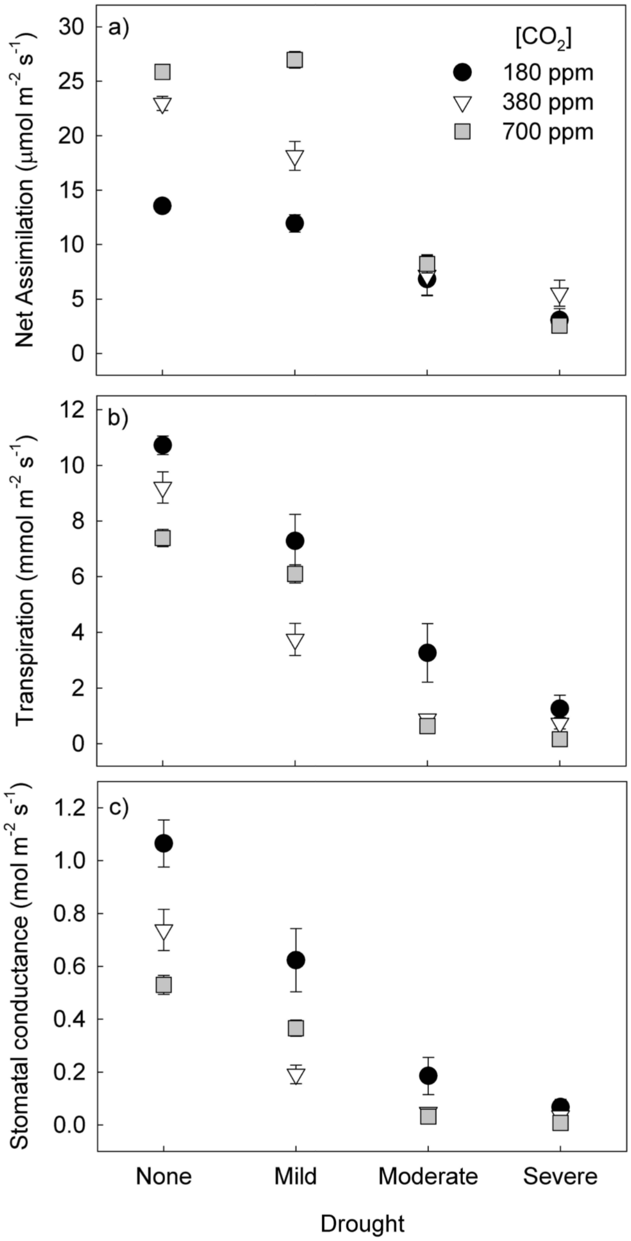

Fig. 3.

Leaf-level gas exchange of *Phaseolus vulgaris* var. Bolita grown under four levels of drought at glacial (180 ppm; circles), current (380 ppm; triangles) and elevated (700 ppm; squares) [CO$_2$]. Error bars represent ± 1 SE.

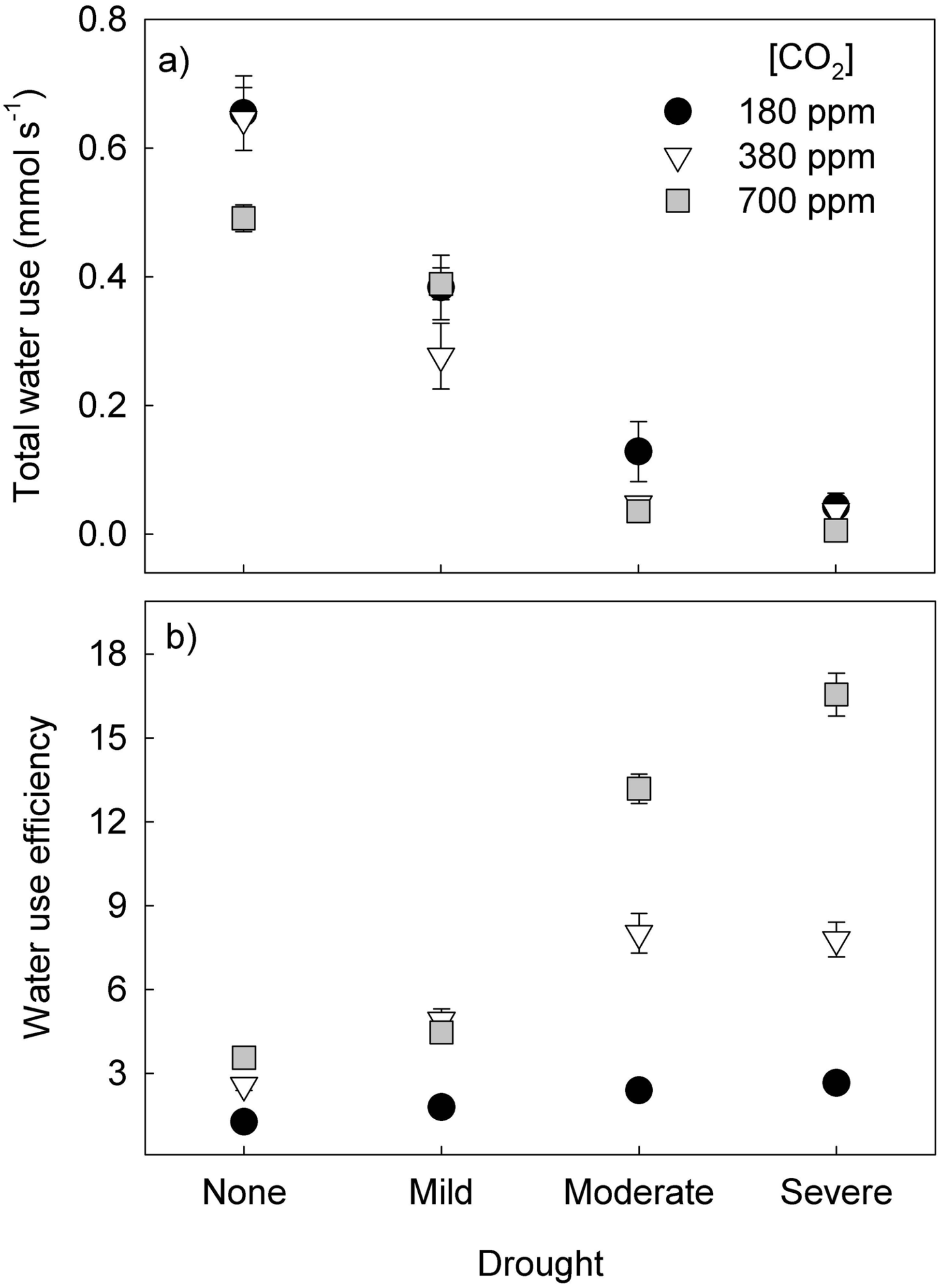

Fig. 4.

Whole-plant water use and water use efficiency of *Phaseolus vulgaris* var. Bolita grown under four levels of drought at glacial (180 ppm; circles), current (380 ppm; triangles) and elevated (700 ppm; squares) [CO_2]. Error bars represent ± 1 SE.

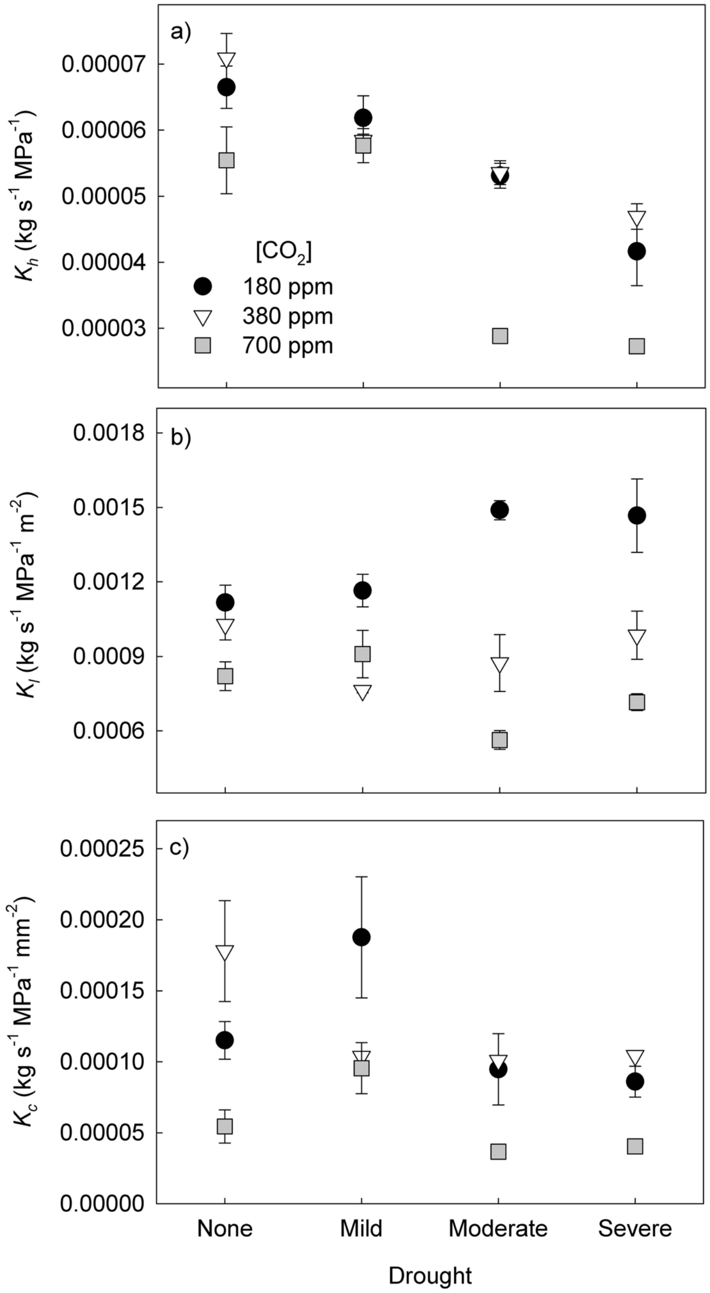

Fig. 5.

Xylem hydraulic function of *Phaseolus vulgaris* var. Bolita grown under four levels of drought at glacial (180 ppm; circles), current (380 ppm; triangles) and elevated (700 ppm; squares) [CO$_2$] including: (a) total stem hydraulic conductance (K_h), (b) leaf specific hydraulic conductance (K_l) and (c) conduit specific hydraulic conductance (K_c). Error bars represent ± 1 SE.

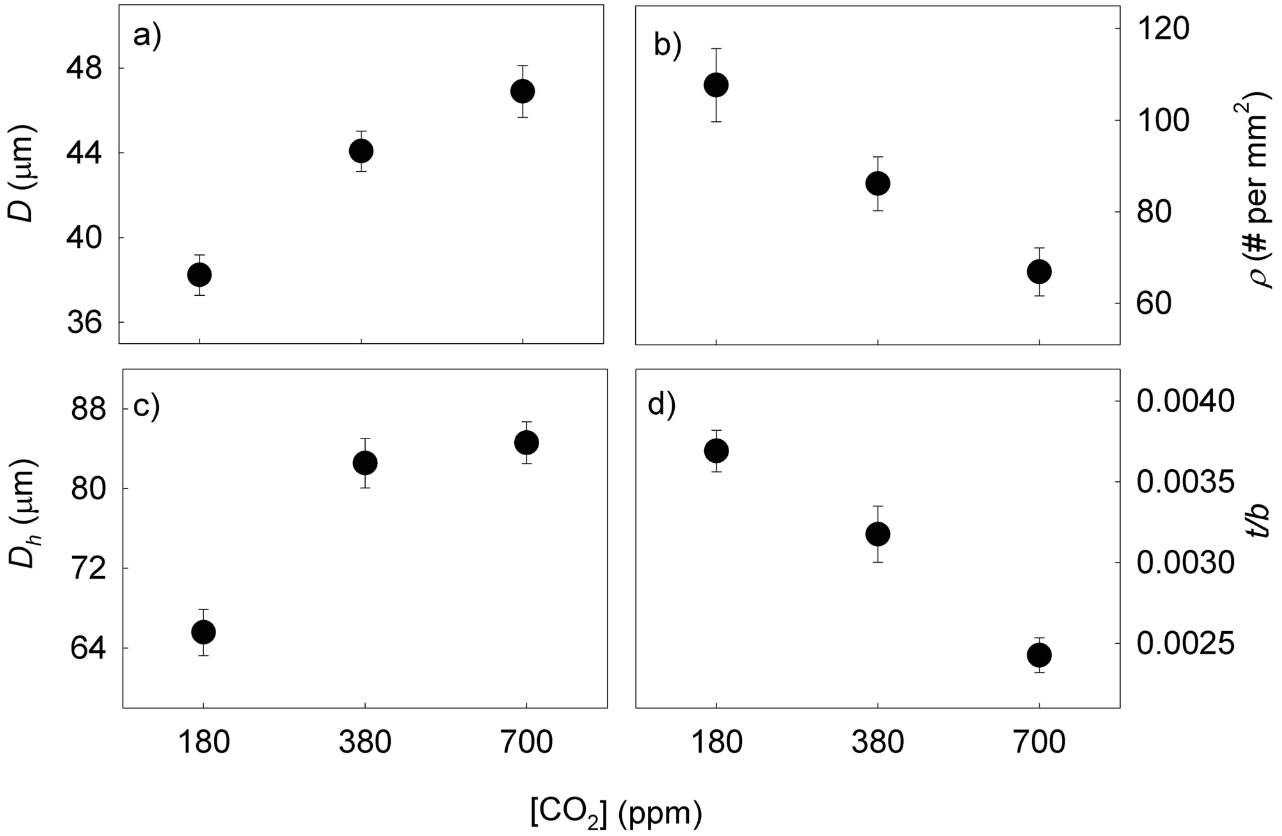

Fig. 6.
Xylem structural characters of *Phaseolus vulgaris* var. Bolita grown at glacial (180 ppm; circles), current (380 ppm) and elevated (700 ppm) [CO$_2$] including: (a) vessel diameter, (b) vessel density (ρ), (c) hydraulically weighted diameter (D_h), and (d) implosion strength (t/b). Error bars represent ± 1 SE.

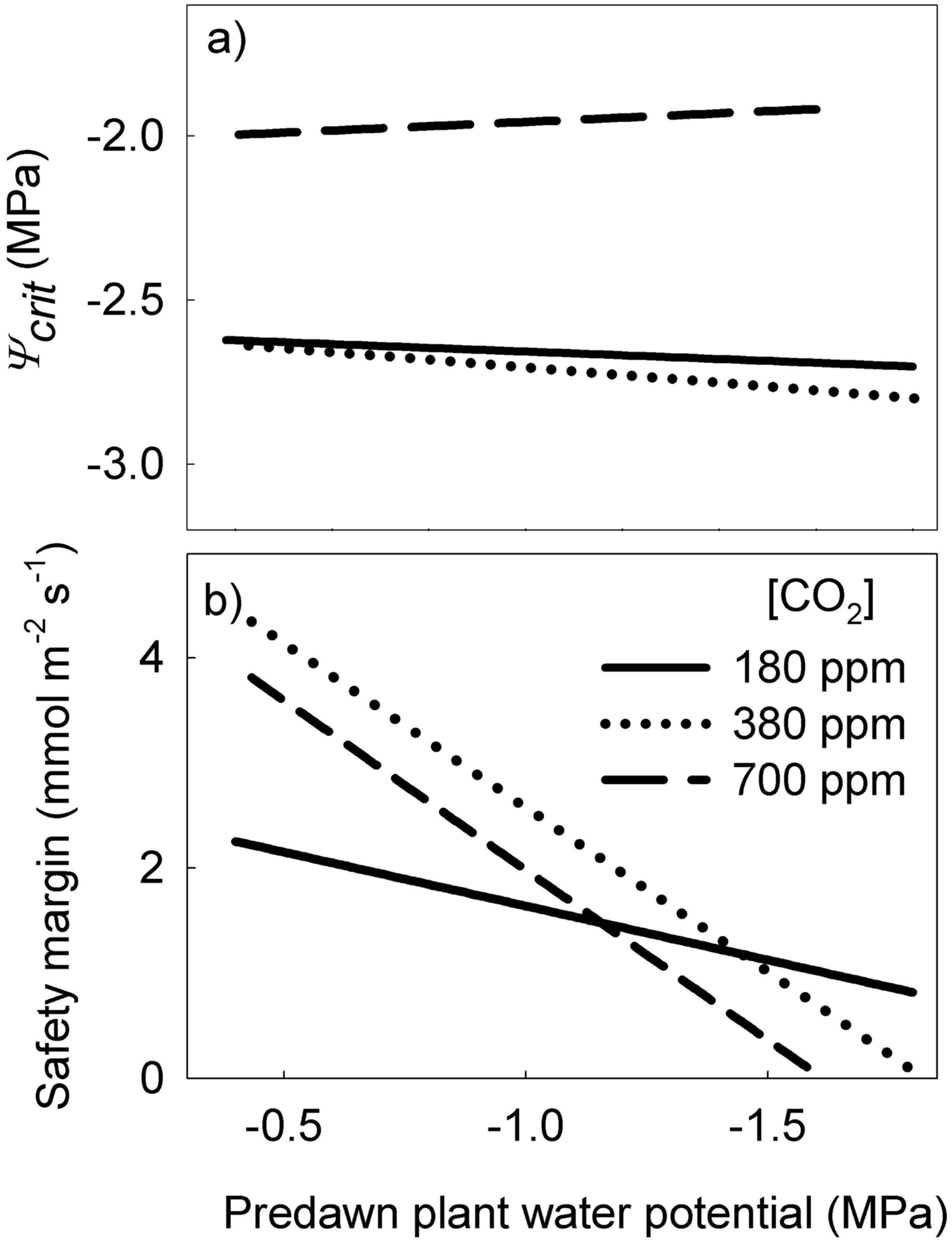

Fig. 7.

Model predictions quantifying the risk of runaway embolism as a function of predawn plant water potential for *Phaseolus vulgaris* var. Bolita grown at glacial (180 ppm; solid line), current (380 ppm; dotted line) and elevated [CO_2] (700 ppm; dashed line) including, (a) critical plant water potential (Ψ_{crit}, MPa), and (b) safety margin from runaway embolism (mmol $m^{-2}s^{-1}$).

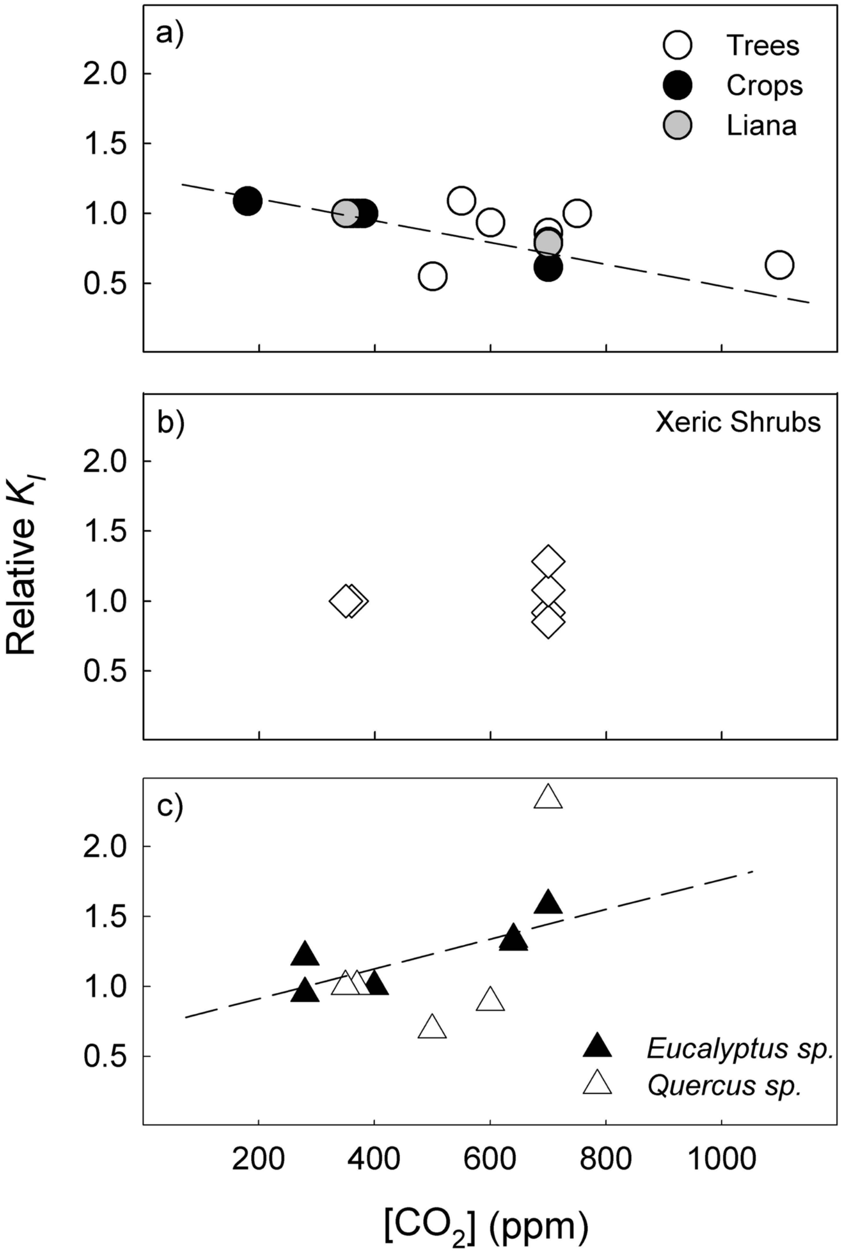

Fig. 8.

Relative leaf specific hydraulic conductance (K_l) as a function of increasing $[CO_2]$ for well-watered plants from a variety of plant taxa. Relative K_l was computed as the ratio of K_l under ambient $[CO_2]$ to that measured under a given low or elevated $[CO_2]$ treatment. For many types of plants (a), relative K_l declines as $[CO_2]$ increases from glacial to future predicted values (trees, *Betula maximowicziana* (Eguchi *et al.*, 2008), *Fagus sylvatica* (Heath *et al.*, 1997), *Prunus avium* × *pseudocerasus* (Atkinson & Taylor, 1996) and *Pinus ponderosa* (Maherali & DeLucia, 2000); crops, *Phaseolus vulgaris* var. Bolita (data from this study) and *Helianthus annuus* (Huxman *et al.*, 1999); liana, *Vitis vinifera* (Tognetti *et al.*, 2005); dotted line represents a significant negative relationship for crops, relative change in $K_l = -0.0008\ [CO_2] + 1.26$, $R^2 = 0.87$, $P = 0.0216$). For xeric shrubs (b), however, there appears to be no change in K_l with increasing $[CO_2]$ (*Larrea tridentata* (Huxman *et al.*, 1999), *Myrtus communis*, *Juniperus communis* and *Erica arborea* (Tognetti *et al.*, 2001)). By contrast, *Eucalyptus sp.* and *Quercus sp.* (c), exhibit relatively higher K_l as [CO_2] increases (*Eucalyptus sideroxylon*, *E. saligna* (Phillips *et al.*, 2011) and *E. pauciflora* (Atwell et al., 2009), *Quercus robur* (Atkinson & Taylor, 1996; Heath *et al.*, 1997) and *Q. mongolica* (Eguchi *et al.*, 2008); dotted line represents a significant positive relationship for *Eucalyptus sp.*, relative change in K_l = 0.0011 [CO_2] + 0.66, R^2 = 0.69, P = 0.0104). For studies that measured more than one time point, only the last time point was included. For studies that altered nitrogen availability, only the low or ambient treatment was included. For studies that altered temperature only treatments in the range of 25–26 °C were included. For studies that included sun and shade leaves, only sun leaves were included.

Table 1

F-statistics from ANOVAs testing for the effects of [CO_2], drought level and growth chamber on *Phaseolus vulgaris* var. Bolita physiological and anatomical characteristics including: (a) biomass and allocation (A_R:A_L, ratio of absorbing root area to transpiring leaf area), (b) whole plant water use and water use efficiency (*WUE*), (c) xylem hydraulic conductance (K_h, total xylem hydraulic conductance, K_l, leaf specific xylem hydraulic conductance and K_c, conduit area specific xylem hydraulic conductance), and (d) xylem anatomy (D_h, hydraulic diameter; t, width of the double wall and t/b, implosion strength)

Dependent variable	Drought df = 3	[CO_2] df = 2	[CO_2] × drought df = 6	Growth chamber df = 1
a) Biomass production and allocation				
Leaf area	**56.21*****	**43.84*****	1.86	**10.01****
Root area	**16.32*****	**53.48*****	**5.39*****	2.93
A_R:A_L	1.59	**23.08*****	**2.82***	0.00
Total plant biomass	**9.86*****	**161.40*****	1.99	**4.93***
b) Whole plant water use				
Total water use	**122.02*****	**3.12***	1.36	3.40
WUE	**258.89*****	**591.36*****	**95.24*****	2.67
c) Xylem hydraulic conductance				
K_h	**35.83*****	**22.40*****	**2.22***	3.60
K_l	0.95	**54.21*****	**5.44*****	0.02
K_c	2.75	**7.61****	1.98	-
d) Xylem anatomy				
Vessel diameter	2.97	**20.08*****	2.15	-
Vessel density	1.03	**7.96****	0.97	-
D_h	0.66	**14.38****	0.86	-
t	0.07	0.46	0.39	-
t/b	1.80	**26.87*****	2.20	-

Bold text indicates a significant treatment effect.

$^{*}P < 0.05$;

$^{**}P < 0.01$;

$^{***}P < 0.0001$,