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Research paper

Coordinated responses of plant hydraulic architecture with the reduction of stomatal conductance under elevated CO_2 concentration

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Stomatal conductance (g_s) generally decreases under elevated CO_2 concentration (eCO_2) and its sensitivity varies widely among species, yet the underlying mechanisms for these observed patterns are not totally clear. Understanding these underlying mechanisms, however, is critical for addressing problems regarding plant–environment interactions in a changing climate. We examined g_s, water transport efficiency of different components along the whole-plant hydraulic system and allometric scaling in seedlings of six tree species grown under ambient and eCO_2 treatments (400 and 600 ppm, respectively). Growth under eCO_2 caused g_s to decrease in all species but to highly variable extents, ranging from 13% (*Populus tremuloides Michx*.) to 46% (*Gymnocladus dioicus* (L.)). Accompanying this significant decrease in g_s, substantial changes in plant hydraulic architecture occurred, with root hydraulic conductance expressed both on leaf area and root mass bases overall exhibiting significant decreases, while stem and leaf hydraulic efficiency either increased or showed no consistent pattern of change. Moreover, significant changes in allometry in response to eCO_2 affected the whole-plant water supply and demand relations. The interspecific variation in g_s response among species was not correlated with relative changes in stem and leaf hydraulic conductance but was most strongly correlated with the relative change in the allometric scaling between roots and leaves, and to a lesser extent with the intrinsic root hydraulic conductance of the species. The results underscore that allometric adjustments between root and leaf play a key role in determining the interspecific sensitivity of g_s responses to eCO_2. Plant hydraulics and their associated allometric scaling are important changes accompanying g_s responses to eCO_2 and may play important roles in mediating the interspecific variations of leaf gas exchange responses, which suggests that mechanistic investigations regarding plant responses to eCO_2 need to integrate characteristics of hydraulics and allometric scaling in the future.

Keywords: allometry, elevated CO_2, plant hydraulics, root hydraulic conductance, vascular transport, xylem water transport.

Introduction

In assessing biosphere carbon and hydrologic cycles of the future, it is critical to understand factors that regulate plant water and carbon exchange with the atmosphere and how they might respond to the projected rise in atmospheric CO_2 concentration. Stomatal conductance (g_s) is a key regulator of these exchanges and has been observed to decrease at elevated CO_2 concentrations (eCO_2) but with substantial variations among species (Morison 1985, Field et al. 1995, Curtis and Wang 1998, Medlyn et al. 2001, Xu et al. 2016). Examining the general pattern of g_s response and the interspecific variability raises insightful questions about the underlying mechanisms as well as its implications for forest responses to eCO_2 in a mixed canopy. The decrease in g_s under eCO_2 and the interspecific variability of g_s responses that are generally found may reflect or be accompanied by concomitant adjustments in carbon metabolism and, critically, in hydraulic architecture that regulates water supply (Tyree and Alexander 1993, Tognetti et al. 1998, Kergoat et al. 2002, Schäfer et al. 2002, Wullschleger et al. 2002, Obrist and Arnone 2003, Domec et al. 2009*a*, 2009*b*, 2017). Both theoretical considerations and empirical evidence indicate a tight coupling of liquid and vapor phase water movements (e.g., Sperry and Pockman 1993, Fredeen and Sage 1999, Nardini and Salleo 2000, Meinzer 2002, Franks and Brodribb 2005, Domec et al. 2010). A recent study showed that reduced g_s in *Pinus taeda* and *Liquidambar styraciflua* at the canopy level under eCO_2 was an indirect effect of changed hydraulic architecture due to structural changes and increased leaf shading rather than a direct effect of eCO_2 on stomatal behavior (Tor-ngern et al. 2015). Knowledge of plant hydraulic responses to eCO_2 and its variability among different tree species is critical for understanding forest performance in a changing environment and forest–atmosphere gas exchange in the context of climate change, especially in mixed canopy systems.

Experimental studies of recent years have shown that CO_2 enrichments have great impact on plant hydraulics and water use, with the most representative results obtained from the long-term free-air CO_2 enrichment (FACE) experiments (e.g., Domec et al. 2009*b*, 2010, 2016, Warren et al. 2011, Tor-ngern et al. 2015). Based on measurements of the steady state sap flow and water potential gradients across the plant water transport pathway, long-term eCO_2 exposure has been found to consistently reduce whole-plant hydraulic conductance in different species (Domec et al. 2010, 2017). Studies focusing on different parts of the whole-plant water transport pathway showed that eCO_2 could affect hydraulic conductance of roots, stems and leaves to different extents and even in different directions as synthesized by Domec et al. (2017). However, no study has yet considered hydraulic adjustments to eCO_2 in root, stem and leaves at the same time using the same set of species. A major objective of this study is to simultaneously assess the responses of individual hydraulic components and relate their changes to net g_s responses to eCO_2, taking into account the interspecific variations of responses in both g_s and plant hydraulic architecture. In that regard, roots are particularly important because they offer a disproportionately large portion of the whole-plant resistance to water movement in the soil–plant–atmosphere continuum (Saliendra and Meinzer 1989, Radin 1990, Nobel and Cui 1992, Kramer and Boyer 1995, Tyree et al. 1998, Nardini et al. 2003, Domec et al. 2004, Trillo and Fernandez 2005, Wang et al. 2016). Compared with stems and leaves, many fewer studies have considered hydraulic conductance of the root system in the context of climate change (Bunce 1996, Bunce and Ziska 1998, Huxman et al. 1999, Domec et al. 2010, 2017, Gebauer and BassiriRad 2011). One of our main goals is to assess the coordinated responses of root hydraulic conductance (K_r) and g_s across multiple species to eCO_2.

It is well recognized that the leaf is also a major site of resistance to water flow (Yang and Tyree 1994, Nardini and Pitt 1999, Nardini and Salleo 2000, Nardini 2001, Brodribb et al. 2005, Sack and Holbrook 2006, Domec et al. 2009*a*, 2009*b*, Wang et al. 2016) and that leaf hydraulic conductance (K_{leaf}) is strongly coordinated with g_s across species (Brodribb and Holbrook 2003, Brodribb et al. 2005, Hao et al. 2010). In fact, it has been found that 30–80% of the whole-plant hydraulic resistance may reside in leaves (Sack and Holbrook 2006, Wang et al. 2016). Nonetheless, K_{leaf} response to eCO_2 has rarely been studied and, in fact, we know of only very few studies that have explicitly examined responses of K_{leaf} to eCO_2 and that together represent data for only two tree species and two crop species (Domec et al. 2009*b*, 2016, Locke et al. 2013, Rico et al. 2013, Locke and Ort 2015, Tor-ngern et al. 2015). Therefore, in addition to K_r, we assessed the potential role of K_{leaf} in affecting g_s responses to eCO_2. Stem hydraulics have also been found to be strongly coordinated with g_s (Meinzer and Grantz 1990, Aasamaa et al. 2001, Santiago et al. 2004, Hao et al. 2011, Bartlett et al. 2016), which suggests a potentially important role for it in regulating g_s responses to eCO_2. Faster stem growth and larger conduit lumen diameters in trees grown under eCO_2 (Domec et al. 2010, 2017, Watanabe et al. 2010, Sharma et al. 2014) may cause tree stems to be more vulnerable to drought-induced hydraulic failure, which would have great implications for forest health, given the projected increases in the risks of drought and extreme weather (IPCC 2012). However, studies on the impact of eCO_2 on stem resistance to drought-induced embolism are sparse and mixed results have been obtained (Domec et al. 2017). Stem xylem vulnerability to simulated drought stress was thus assessed in plants grown under both ambient and eCO_2 conditions in addition to stem hydraulic efficiency.

Besides the influence of each of the three hydraulic components (root, stem and leaf), the allometric adjustments among them in response to changes of environmental conditions can also significantly affect whole-plant hydraulic architecture and plant water economy (Meinzer 2002, Domec et al. 2009*b*, Buckley and Schymanski 2014). While the importance of adjustments in aboveground allometry under eCO_2, such as changes in leaf area index and sapwood area to leaf area ratio, in affecting plant hydraulic architecture and plant water use have often been recognized (e.g., Warren et al. 2011, Tor-ngern et al. 2015), there has been much less attention paid to the allometric relations between roots and shoots. Thus, another major objective of this study was to examine whether the changes in allometric partitioning between roots, stems and leaves accompany g_s responses to eCO_2. Specifically, we tested the following three hypotheses: (i) given the tight coupling between the hydraulic and vapor phase water flow through plants, the commonly observed reduction in g_s in response to eCO_2 would be accompanied by concomitant reductions in root, stem and leaf hydraulic conductances; (ii) coordinated reduction in allometric scaling between the water supply and demand organs would occur with a reduction in g_s under eCO_2; and (iii) interspecific variation in the magnitude of g_s response to eCO_2 can be explained at least partially by differences in these hydraulic and allometric adjustments.

Materials and methods

Plant materials and growth conditions

One-year-old seedlings of six broad-leaved tree species were purchased from Lawyer Nursery, Inc. (Plains, MT, USA) in March of 2012. Thus, seedlings were in their second full growing season during our experiments. Three of these species, namely *Acer rubrum* (L.) (red maple), *Betula pendula* (Roth) (silver birch) and *Populus tremuloides* (quaking aspen) have diffuse-porous wood and the other three, namely *Gymnocladus dioicus* (Kentucky coffee tree), *Quercus rubra* (L.) (red oak) and *Robinia pseudoacacia* (L.) (black locust), have ring-porous wood. The species are all common species native to North America. We selected our species to represent relatively large variation in phylogeny and functional type. Except for *R. pseudoacacia* and *G. dioicus* that belong to different subfamilies of Fabaceae, each of the species is from a different family.

Bare root seedlings were initially placed into 40 L Rubbermaid™ containers filled with vigorously aerated quarter-strength Hoagland solution for ~2 weeks until healthy seedlings with significant newly emerged roots and shoots were identified. Individual seedlings were then transplanted into 1 L rocket pots (Stuewe and Son, Tangent, OR, USA) containing washed river sand. The ratio of potting volume and plant size must be carefully selected to avoid a pot-binding effect (Poorter et al. 2012). Based on initial plant sizes, our previous experience with the seedling growth of these species and seedling management in our greenhouse (e.g., frequent watering and fertilizing), we are confident that the small pot used did not limit growth of the seedlings over the relatively short experimental period, i.e., one growing season. Additional subsequent observation convinced us further that pot size was not a confounding factor in this study. Those observations included the absence of rhizosheets at the pot periphery, gas exchange values comparable to those in large pots and field studies, lack of a correlation between changes in root mass and total biomass (intra- or inter-species), and lack of any correlations between changes in root mass and in g_s. The pot size and dimensions selected here were an ideal fit for the Scholander pressure chamber (see below) designed to measure root hydraulic conductance. To minimize the effect of ontogenetic variability, we chose seedlings of similar size and rooting vigor within each species. For the subsequent 6 months seedlings were grown in two greenhouse rooms set at either 400 or 600 (±25) ppm CO_2, herein referred to as ambient and eCO_2 levels, respectively. Environmental conditions were similar between the two rooms, i.e., light and relative humidity regimes tracked ambient conditions but temperature was controlled so that the daily maximum values did not exceed 28 °C. The CO_2 levels and plants were swapped weekly between the two greenhouse rooms to minimize any potential differences that may exist between them. Seedlings were located on mobile carts and the location and position of each cart within each room were changed twice a week to minimize the impact of environment heterogeneity within the rooms. Seedlings were fertilized with half-strength Hoagland solution to free drainage three times a week. On all other days, seedlings received deionized water to saturation to avoid soil drying as well as salt build-up. We used 25 seedlings of each species for both the control and eCO_2 treatments.

Leaf gas exchange and water potential measurements

Physiological and biomass variables were measured ~6 months after the start of the eCO_2 treatment. Leaf gas exchange was measured using a LI-6400 photosynthesis system (LI-COR, Inc., Lincoln, NE, USA) between 09:00 and 11:30 h during clear days. The photosynthetic photon flux density in the cuvette was set at $1000\ \mu mol\ m^{-2}\ s^{-1}$. Two to three sun-exposed mature leaves from each of six to nine seedlings for each species under each treatment were measured under their respective growth CO_2 concentration (400 ppm for the ambient treatment and 600 ppm for the eCO_2 treatment, respectively), temperature and ambient humidity. In the gas exchange measurements, average leaf temperature of these species differed by only 0.1–1.6 °C between ambient and eCO_2 treatments and average relative humidity varied only 3–9%. Leaf water potential (Ψ_L) was measured using a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) during two consecutive clear days. Sun-exposed mature leaves for all species were sampled within the same period as g_s measurements. After excision, leaves were sealed in zip-lock bags containing wet paper towels and kept in a cooler until Ψ_L was measured. Six leaves with each from a different seedling for each species and treatment were measured.

Hydraulic measurements in roots, stems and leaves

Root hydraulic conductance, K_r, was estimated by measuring sap exudation rate of a detopped whole root system using the pressure-chamber method (Gebauer and BassiriRad 2011). To minimize native embolism and avoid induced embolism caused by cutting the stem under tension (Wheeler et al. 2013), seedlings were watered to saturation and drained 1 h before measurements. Following the free drainage, shoots were cut off under water at about 6 cm above the root/shoot junction. After trimming off the top of the plastic pot above the soil surface, the pot containing the intact whole root system was sealed in a 30-cm-tall pressure chamber (Soil Moisture Equipment Corp.) with the stem extruding through the hole of a rubber stopper. The pressure of the chamber was then increased to 0.4 MPa and kept constant. The sap exuded from the stem cutting point under the constant pressure was collected for the first 5 min and weighed. The K_r was calculated as the rate of sap exudation (J, mg s^{-1}) divided by the applied pressure (P, MPa) and then normalized by root dry weight (K_{rDW}, in mg s^{-1} MPa^{-1} g^{-1}) or by total leaf area (K_{rLA}, in mg s^{-1} MPa^{-1} m^{-2}). Previous to the experiment, for each species J was measured in one seedling under different pressures (0, 0.05, 0.1, 0.3, 0.4, 0.5 MPa) in a stepwise increasing manner. We found that for all the species 0.4 MPa safely falls in the linear portion of J vs P curve and thus was used as the applied pressure for K_r estimation for later measurements.

The main stems of seedlings sampled from the same plants for K_r measurements were used for measuring stem hydraulic conductivity with a tubing system (Zwieniecki and Holbrook 1998). Briefly, the flow rate of degassed and ultra-filtered (particle size <0.2 μm) KCl solution (20 mM) through a stem segment under a known hydrostatic pressure was measured in real time by measuring the weight loss of the source reservoir using a Sartorius balance (CPA225D, Sartorius, Göttingen, Germany) controlled by LabVIEW software (National Instruments Corp., Woburn, MA, USA). Hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}) was calculated as:

$$K_h = J_v / (\Delta P / \Delta L) \qquad (1)$$

where J_v is flow rate through the segment (kg s^{-1}), ΔP is the pressure difference between the two ends of the stem segment (MPa) and ΔL is the length of the segment. After K_h measurement, 0.1% Toluidine Blue solution was perfused through two 1-cm pieces of stem cut from both ends of the segment and the average stained cross-sectional area was taken as the sapwood area. Total leaf area distal to the stem segment was measured using a LI-3000 leaf area meter (LI-COR, Inc.). Sapwood-specific hydraulic conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) and leaf-specific hydraulic conductivity (K_L, kg m^{-1} s^{-1} MPa^{-1}) were calculated by dividing K_h with sapwood area and leaf area, respectively. Sapwood to leaf area ratio (SA/LA) was calculated as sapwood area, averaged over both ends of the stem segment, divided by total leaf area distal to the segment used for hydraulic conductivity measurement.

Leaf hydraulic conductance was determined according to Zwieniecki et al. (2007), which is based on kinetics of sap flux from petiole of a fully rehydrated leaf following an abrupt increase in its surrounding air pressure. Leaves were cut under water from well-watered plants in early morning and allowed to rehydrate for about 1 h in deionized water. The petiole of a target leaf was then connected to a polypropylene tubing fitting and the connection was then tightly wrapped in parafilm. The whole leaf wrapped in a wet paper towel was then sealed into a PVC chamber, the pressure of which was monitored continuously using an electronic pressure transducer and the data were logged every second using LabVIEW. The water-filled tubing connected to the leaf petiole led to a water reservoir seated on an analytical balance (model CPA225D, Sartorius). The whole system was allowed to equilibrate for a few minutes and then the pressure inside the leaf chamber was abruptly increased to ~0.2 MPa. During the whole process, water flux to the reservoir and the pressure inside the chamber were measured simultaneously every second for 15–40 min. After a final stable constitutive flux rate was extracted, a double exponential model was fitted to the leaf water release rate following the pressure increase (Zwieniecki et al. 2007). The residual constant water release was not related to the leaf water potential change and was subtracted before further calculation of K_{leaf}. Resistance of water flow in the leaf (inverse of K_{leaf}) was calculated according to a capacitance–time constant theory based on analogy to an electrical capacitor discharge:

$$\tau = RC \qquad (2)$$

where τ is the time constant (s), the time required to discharge the capacitor through the resistor by 63.2% of its full charge, R is resistance (mol^{-1} MPa s) and C is leaf water storage capacitance (mol MPa^{-1}), which was calculated based on the total amount of water released caused by the abrupt pressure increase. Total leaf area of a seedling was determined on fresh leaves using a LI-3000 leaf area meter (LI-COR Inc.). After the root hydraulic conductance measurements, the roots were carefully washed free of soil and thoroughly rinsed with deionized water. All tissues were oven-dried to a constant weight at 70 °C prior to dry mass determination.

All methods used here are well established in the literature for calculating the efficiency of water transport in different plant parts and are sensitive enough to detect relative changes in response to eCO_2. It may not be very useful to directly compare the absolute values of hydraulic conductance of roots, stems and leaves since different techniques were used; however, these parameters are useful indicators of relative sensitivities to eCO_2 in liquid water fluxes along the whole-plant water transport pathway.

Stem vulnerability curves

Stem vulnerability curves were constructed using the centrifugal force method (Alder et al. 1996). Briefly, vulnerability to tension-induced xylem cavitation was measured as the reduction in K_h in response to stepwise increasing xylem tension (−0.5, −1, −2, −3, −4, −5, −6 MPa) that was generated by spinning a 14.5 cm stem segment under different speeds using a RC-5B Sorvall centrifuge (Du Pont Instruments, Wilmington, DE, USA). Percentage loss of conductivity (PLC) following each spin was calculated as PLC = $100[(K_{max} - K_h)/K_{max}]$, where K_h is the hydraulic conductivity after each spin and K_{max} is the maximum hydraulic conductivity. Vulnerability curves were fitted with an exponential sigmoid model (Pammenter and Vander Willigen 1998):

$$\mathrm{PLC} = \frac{100}{1 + \exp(a(T - b))} \qquad (3)$$

where T is tension (negative pressure), a is a measure of the degree that conductivity responds to change of xylem tension (curve slope) and b represents tension at 50% loss of hydraulic conductivity. T was calculated as $-0.25\rho\omega^2R^2$, where ρ is the density of water, ω is the angular velocity of spinning and R is the radius of the stem segment measured from the axis of the rotation to the reservoir water surface during the spinning (Alder et al. 1996). The use of a rotor with a relatively small diameter may potentially cause measurement artifacts due to the problem of open vessels, which may result in 'r' shaped vulnerability curves showing artificially low resistance to drought-induced embolism in long-vesseled species (e.g., Choat et al. 2010, Cochard et al. 2013, but see Sperry et al. 2012). The small size of the seedlings used here did not allow us to do the measurements with a rotor of larger diameter; however, the open vessel issue was unlikely to have been a problem here. First, the 2-year-old seedlings used here may have relatively short vessels as it has been found that both maximum and average vessel lengths are much shorter in 1- and 2-year-old branches than in older ones (Cochard and Tyree 1990, Martín et al. 2013). In addition, our vulnerability curves, even those of the ring-porous species, are all 's' shaped, further confirming that artifact due to open vessels was not a problem of concern for our materials.

Statistical analyses

Stem vulnerability curves were fitted to the exponential sigmoid model using Sigmaplot v6.0 (Systat Software, Inc., San Jose, CA, USA). Two-way ANOVAs were performed for all measured traits using CO_2 level and species as two factors (SPSS software, SPSS Inc.). Comparisons between ambient and eCO_2 conditions for each species and each trait measured were performed using one-way ANOVAs. A Pearson's correlation was performed across species on percentage change of values in response to eCO_2 for all functional traits measured. The correlations between percent change in g_s and functional traits were assessed using the best-fit model.

Results

Under ambient CO_2 level, g_s varied by as much as two-fold among the studied species, while the direction of response to eCO_2 was universally negative (Table 1 and Figure 1a). Across species, g_s dropped by an average of 30% but the magnitude of this response varied substantially among species (Table 1; $0.05 < P < 0.1$, two-way ANOVA; Figure 1a) ranging from 13% in *Populus* to 46% in *Gymnocladus*. The decrease in g_s in response to eCO_2 coincided with an overall increase in leaf water potential ($P < 0.01$, two-way ANOVA; Table 1 and Figure 1b).

Overall, plants grown under eCO_2 had significantly reduced root hydraulic conductance expressed on both root mass and leaf area bases (Table 1; $P < 0.05$ and $0.05 < P < 0.1$, two-way ANOVA). Although root hydraulic conductance showed an overall decrease in response to eCO_2, the magnitude of change varied substantially among the six studied species (Figures 2a and d). The intrinsic

Table 1. Effects of growth CO_2 level on various physiological and allometric parameters in seedlings of six tree species. Values are means of six replications and means followed by a different letter are significantly different from each other at $P < 0.1$ level. The last three columns represent results of two-way ANOVAs assessing the significances of the main treatment effects (CO_2 levels and species) as well as their interactions. ns, $P > 0.1$; *, $0.05 \le P \le 0.1$; **, $0.01 \le P < 0.05$; ***, $P < 0.01$. A, ambient; E, elevated; g_s, stomatal conductance; Ψ_L, leaf water potential; K_{rLA} and K_{rDW}, root hydraulic conductance expressed on leaf area and root dry weight bases; K_L and K_S, leaf area-specific and sapwood area-specific stem hydraulic conductivities; K_{leaf}, leaf hydraulic conductance; BM, total biomass; RM, root mass; SA/LA, sapwood to leaf area ratio; RM/LA, root mass to leaf area ratio.

	CO_2 level	*Acer*	*Betula*	*Gymnocladus*	*Populus*	*Quercus*	*Robinia*	CO_2	Spp.	$CO_2 \times$ Spp.
g_s ($mol\ m^{-2}\ s^{-1}$)	A	0.210 a	0.351 a	0.168 a	0.285 a	0.174 a	0.216 a	*	*	***
	E	0.162 b	0.251 b	0.091 b	0.247 a	0.124 b	0.127 b			
Ψ_L (MPa)	A	−0.50 a	−1.07 a	−0.93 a	−1.20 a	−0.72 a	−0.99 a	***	***	**
	E	−0.55 a	−0.68 b	−0.76 a	−1.36 a	−0.61 a	−0.58 b			
K_{rLA} ($mol\ m^{-2}\ s^{-1}\ MPa^{-1}$)	A	1.03 a	3.33 a	0.60 a	3.75 a	0.79 a	0.32 a	*	***	ns
	E	0.78 a	2.12 b	0.19 b	2.51 b	0.68 a	0.37 a			
K_{rDW} ($\mu mol\ g^{-1}\ s^{-1}\ MPa^{-1}$)	A	54.1 a	242.1 a	7.94 a	227.0 a	10.86 a	22.3 a	**	***	ns
	E	37.5 b	193.6 a	4.49 b	119.5 b	11.16 a	22.1 a			
K_L ($10^{-4}\ kg\ m^{-1}\ s^{-1}\ MPa^{-1}$)	A	0.87 a	1.15 a	1.74 a	2.03 a	0.93 a	2.12 a	**	***	ns
	E	1.46 b	1.51 b	3.11 b	4.15 b	0.99 a	2.15 a			
K_S ($kg\ m^{-1}\ s^{-1}\ MPa^{-1}$)	A	0.84 a	1.07 a	1.22 a	1.97 a	0.80 a	3.88 a	**	***	ns
	E	1.09 a	1.74 b	2.18 b	2.88 b	0.79 a	3.33 a			
K_{leaf} ($mol\ m^{-2}\ s^{-1}\ MPa^{1}$)	A	0.79 a	2.95 a	1.66 a	3.10 a	3.38 a	1.81 a	ns	***	ns
	E	1.05 a	2.55 a	1.76 a	1.69 b	2.50 b	1.67 a			
BM (g)	A	16.9 a	6.23 a	21.4 a	11.7 a	12.9 a	21.7 a	***	***	ns
	E	19.9 b	13.7 b	23.4 a	16.1 b	19.9 b	26.5 b			
SA/LA ($mm^2\ m^{-2}$)	A	108.1 a	110.7 a	131.9 a	102.8 a	118.8 a	54.0 a	**	***	ns
	E	134.3 b	243.4 b	155.2 a	145.0 b	123.5 a	67.9 b			
RM/LA ($g\ m^{-2}$)	A	17.1 a	14.6 a	155.7 a	17.1 a	66.0 a	15.8 a	ns	***	ns
	E	16.3 a	12.1 a	83.4 b	19.9 a	64.9 a	14.7 a			

root hydraulic conductance also exhibited large variations among species (Table 1). *Populus* seedlings had the highest K_r, both expressed on root mass and on leaf area bases, whereas *Robinia* and *Gymnocladus* showed the lowest values (Table 1). Despite the relatively large variation in root hydraulic conductance in responses to eCO_2 among species, there was no significant interactive effect between CO_2 and species as factors (Table 1; $P > 0.1$, two-way ANOVA). The decrease in K_r was almost universal and the pairwise mean comparisons indicated that the negative effect of eCO_2 on K_{rLA} and K_{rDW} was significant in three out of the six species tested ($P < 0.1$, *t*-test; Figures 2a and d). Even though g_s and root hydraulic conductance both showed a general trend of decrease in response to eCO_2, the extents of the responses were not significantly correlated between these two traits (Table 2).

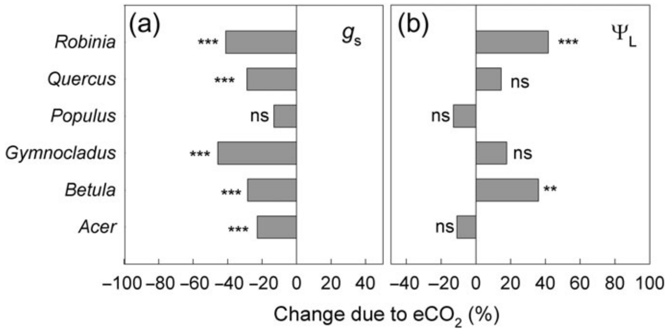

Figure 1. The effects of CO_2 enrichment (eCO_2) on (a) stomatal conductance (g_s) and (b) leaf water potential (Ψ_L) in seedlings of six tree species. Data are expressed as percentage changes of trait values. Differences between ambient and elevated CO_2 treatments for each species are compared using Student's *t*-tests. The significance levels are designated as follows: ns, $P > 0.1$; **, $0.01 \leq P < 0.05$; ***, $P < 0.01$.

Sapwood-specific hydraulic conductivity and leaf-specific hydraulic conductivity of stems also exhibited substantial interspecific variability, but unlike K_r and g_s, both of these stem hydraulic traits generally increased in response to eCO_2 (Table 1; two-way ANOVA, $P < 0.05$; Figures 2b and e). We used both Pearson correlation and linear regression analyses to assess the extent to which g_s responses to eCO_2 depended on species ability to adjust stem hydraulics and allometric scaling among roots, stems and leaves. We found no evidence that the sensitivity of stem hydraulics to eCO_2 was correlated with the sensitivity of g_s response to eCO_2 across the species studied (Table 2). We also found that eCO_2 had no significant overall effect on K_{leaf} (Table 1; $P > 0.1$, two-way ANOVA; Figure 2c) even though the comparisons indicated that in two of the species eCO_2 caused a significant decrease in K_{leaf} (Figure 2c). Moreover, the vulnerability of stem xylem conduits to drought-induced embolism did not respond to eCO_2; neither the qualitative pattern of vulnerability curves nor P_{50} values showed significant changes in all the six studied species ($P > 0.1$, *t*-tests; Figure 3).

The eCO_2 treatment showed significant effects on seedling growth and the scaling between sapwood area and leaf area (Table 1 and Figure 4). Moreover, in contrast to the hydraulic traits, the allometric relationship between root mass and leaf area (RM/LA) provided a better explanation for the pattern of the variability in g_s responses to eCO_2 across species. More specifically,

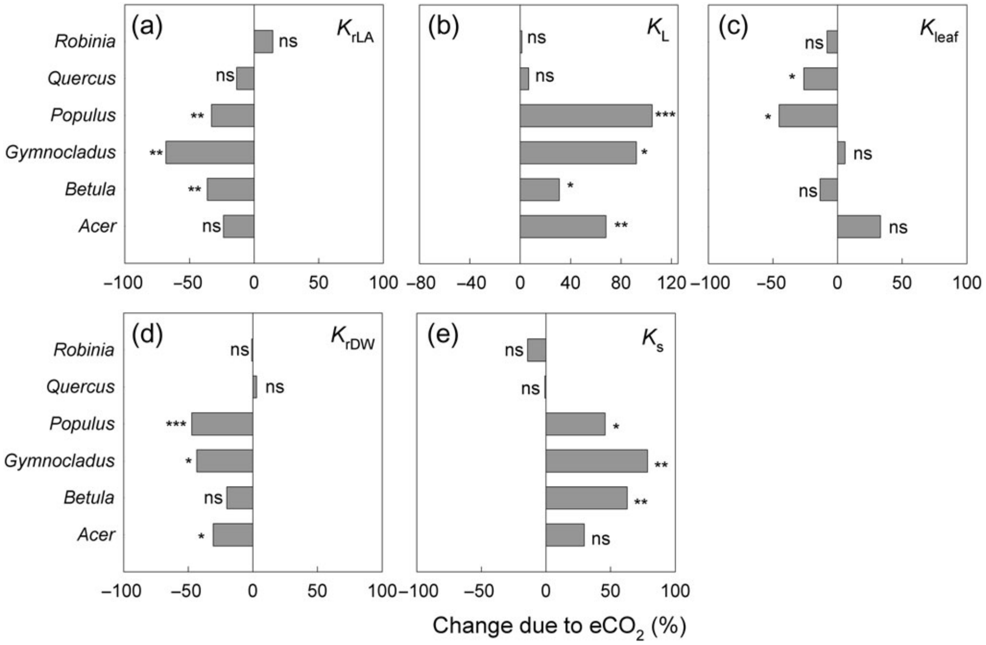

Figure 2. The effects of CO_2 enrichment (eCO_2) on (a) root hydraulic conductance expressed on leaf area (K_{rLA}) and (d) dry weight (K_{rDW}) bases, (b) leaf area-specific (K_L) and (e) sapwood-specific (K_s) hydraulic conductivities of stems, and (c) leaf hydraulic conductance (K_{leaf}) in seedlings of six tree species. Data are expressed as percentage changes of trait values. Differences between ambient and elevated CO_2 treatments for each species are compared using Student's *t*-tests. The significance levels are designated as follows: ns, $P > 0.1$; *, $0.05 \leq P \leq 0.1$; **, $0.01 \leq P < 0.05$; ***, $P < 0.01$.

we found that g_s sensitivity to eCO_2 was significantly correlated with percent changes in RM/LA ($P < 0.05$, $R^2 = 0.67$; Figure 5a). Across these species, sapwood to leaf area ratio (SA/LA) was significantly affected by eCO_2 (Table 1), but percent changes in this allometric trait were not significantly correlated with relative g_s changes (Table 2). The intrinsic values of K_{rDW} and K_{rLA} measured in plants grown under ambient CO_2 level showed relatively strong positive correlations with the percentage change of g_s in response to eCO_2 (Figures 5b and c; $P = 0.098$ and $P = 0.07$, respectively).

Discussion

Our results showed that eCO_2 had a significant impact on the hydraulic architecture of tree seedlings even if the exposure was only for one growing season. Consistent with the decrease of g_s under eCO_2, root hydraulic conductance also decreased in response to CO_2 enrichment, indicating coupled responses between liquid and vapor phase water flow through plants. Differently, stem hydraulic conductivity and leaf hydraulic conductance either increased under eCO_2 or showed no consistent pattern of change. The magnitude of g_s response to eCO_2 showed relatively large interspecific variations, which is accompanied by large interspecific variations in the degrees of hydraulic and allometric adjustments. Most notably, the allometric scaling between root mass and leaf area emerged as an important factor in explaining the interspecific variability in stomatal response to eCO_2.

Concomitant changes of hydraulic architecture in response to eCO_2

Our observations that eCO_2 overall causes a significant decrease in g_s and that the magnitude of change has a large interspecific

Table 2. Pearson correlation coefficients (*r*) among percentage changes of functional trait values in response to CO_2 elevation. Values presented in boldface indicate statistical significance at $P < 0.05$ level. Functional trait name abbreviations are as defined in Table 1.

	Δg_s	$\Delta \Psi_L$	ΔK_{rLA}	ΔK_{rDW}	ΔK_L	ΔK_s	ΔK_{leaf}	ΔBM	ΔSA/LA
$\Delta \Psi_L$	−0.71								
ΔK_{rLA}	0.07	0.28							
ΔK_{rDW}	−0.28	0.60	**0.76**						
ΔK_L	0.43	**−0.76**	−0.68	**−0.98**					
ΔK_s	0.00	−0.18	**−0.95**	**−0.80**	0.68				
ΔK_{leaf}	−0.36	−0.03	−0.11	−0.06	−0.01	0.10			
ΔBM	0.26	−0.38	−0.01	−0.23	−0.31	0.19	−0.35		
ΔSA/LA	0.20	0.34	−0.16	−0.10	−0.02	0.44	−0.14	0.85	
ΔRM/LA	**0.82**	−0.44	0.59	0.15	0.03	−0.53	−0.49	0.09	−0.04

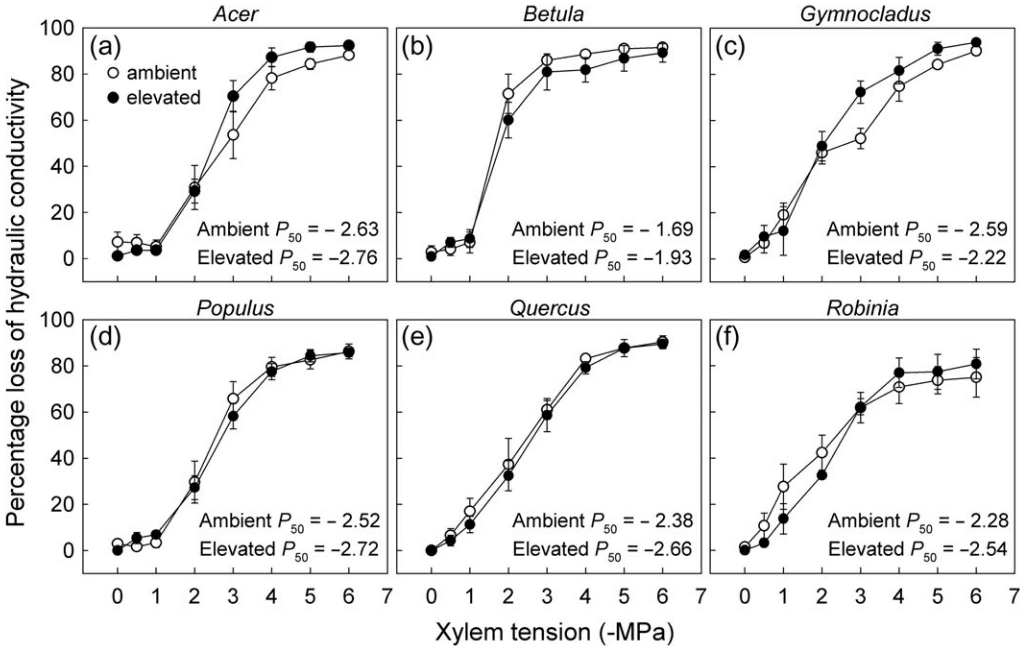

Figure 3. Percent loss of hydraulic conductivity as a function of increased xylem tension in stem segments of seedlings of six tree species grown under ambient or eCO_2 conditions (a–f). The values are means ± SE ($n = 6$). Numbers in each panel show tension at which 50% loss of conductivity (P_{50}) occurs for each species grown under ambient or eCO_2.

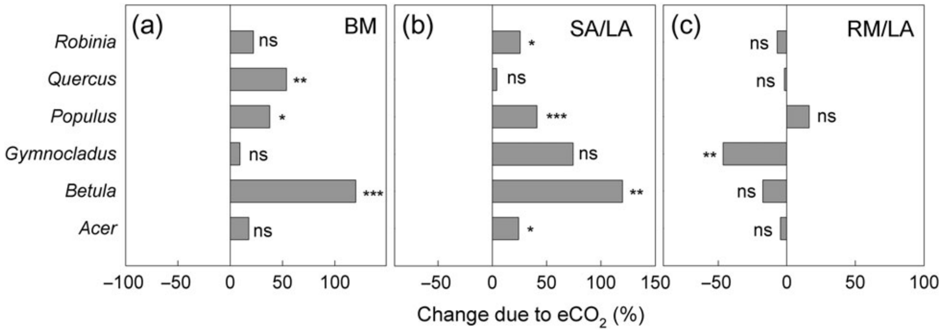

Figure 4. The effects of CO$_2$ enrichment (eCO$_2$) on (a) total biomass (BM), (b) sapwood to leaf area ratio (SA/LA) and (c) root mass to leaf area ratio (RM/LA) in seedlings of six tree species. Data are expressed as percentage changes of trait values. Differences between ambient and elevated CO$_2$ treatments for each species are compared using Student's *t*-tests. The significance levels are designated as follows: ns, $P > 0.1$; *, $0.05 \leq P \leq 0.1$; **, $0.01 \leq P < 0.05$; ***, $P < 0.01$.

variability are consistent with numerous other reports (Morison 1985, Field et al. 1995, Curtis and Wang 1998, Medlyn et al. 2001, Mencuccini 2003, Leakey et al. 2009). The concomitant changes in plant hydraulics observed here accompanying the significant g_s response to eCO$_2$ are in line with the tight coupling between liquid and vapor phases water flow through plants, which has been shown both by studies not involving the impact of CO$_2$ level change (Meinzer 2002, Wullschleger et al. 2002, Mencuccini 2003, Atwell et al. 2009) and recently by CO$_2$ enrichment studies (Domec et al. 2009*b*, Ward et al. 2013, Tor-ngern et al. 2015, Domec et al. 2016, 2017). In a recent study by Tor-ngern et al. (2015), using stepwise changes of CO$_2$ levels in the final phase of a 17-year FACE experiment, it was demonstrated that the tree canopy level decrease in g_s is the result of an indirect structural acclimation (long-term effect) of the whole-tree hydraulic system (i.e., decreased leaf hydraulic conductance and increased leaf are index) rather than a direct effect of eCO$_2$ on stomatal opening (short-term effect). Although the effect of structural change in the hydraulic system on plant–water relations can take several years to manifest in large trees, considering the time needed for tissues formed under the new CO$_2$ regime to dominate in functioning (Li et al. 2003, Wang et al. 2005), our study indicates that substantial structure-based hydraulic architecture changes can occur in tree seedlings over a relatively short period. This is likely due to the fact that newly formed tissues, even from a single growing season, can account for a major part of the hydraulic system and play a major role in functioning in tree seedlings with small initial sizes. The high susceptibility of hydraulic architecture response to eCO$_2$ in seedlings may have great implications for forest dynamics in a changing climate regime through influences on seedling survival and growth.

One of the most important patterns found in the present and previous studies regarding hydraulic responses to eCO$_2$ is the lack of a consistent pattern among different parts of the whole-plant water transport pathway, which is in clear contrast to the predominant decrease of g_s (Domec et al. 2017). This discrepancy can at least be partially reconciled by the fact that root, stem and leaf hydraulic systems account for very different proportions of the whole-plant resistance to water flow (Frensch and Steudle 1989, Tyree et al. 1998, Wang et al. 2016). Of the different components of hydraulic pathway, K_r has been often considered as the most prominent one that is tightly coordinated with g_s response (Nardini et al. 2003, Pratt et al. 2010), which is likely associated with the fact that K_r has a more significant control over g_s than other hydraulic components due to a 'bottle-neck' role it plays in the liquid water transport pathway (Nardini and Pitt 1999, Meinzer 2002, Nardini et al. 2003, Sack and Holbrook 2006, Domec et al. 2009*a*, 2009*b*, Wang et al. 2016). Consistent with previous studies (Huxman et al. 1999, Atwell et al. 2009, Gebauer and BassiriRad 2011), we found that similarly to g_s, K_r generally decreased under eCO$_2$ and that the extent of response was also highly variable among species. Although the magnitudes in g_s and K_r responses to eCO$_2$ appeared to be decoupled, the intrinsic K_r is modestly correlated with the g_s responses to eCO$_2$ (Figure 5). Cautiously interpreted, this correlation suggests that species with inherently high K_r are better able to maintain g_s under eCO$_2$. This relationship is intriguing but the exact mechanism requires further investigation.

The significantly higher stem hydraulic conductivity under eCO$_2$ likely mainly resulted from increased carbohydrate availability for stem growth due to the 'fertilization effect' of eCO$_2$. It has been shown that long-term exposure to eCO$_2$ stimulates cellulose and pectin biosynthesis in the vascular cambium (May et al. 2013) and usually contributes to thicker cell wall and larger xylem conduit size (e.g., Domec et al. 2010, Watanabe et al. 2010, Sharma et al. 2014). Although increase in conduit size under eCO$_2$ is usually associated with increased stem specific hydraulic conductivity (Gartner et al. 2003, Domec et al. 2010, Phillips et al. 2011), its influences on the whole-plant hydraulic conductance can be limited due to the fact that stem accounts for a disproportionally small portion of the whole-plant hydraulic resistance. For example, in *Fraxinus mandshrica* seedlings more than 80% of the whole-plant resistance to water flow is located in the root system and leaves

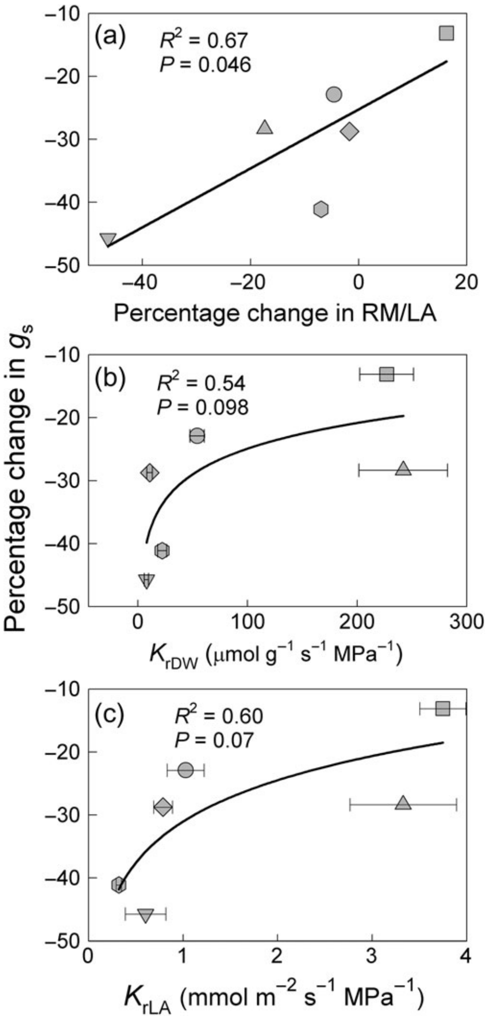

Figure 5. The cross-species correlations between mean percent change in stomatal conductance (g_s) and that in root mass to leaf area ratio (RM/LA) in response to eCO_2 (a); and the cross-species correlations between mean percent change in g_s and intrinsic values of root dry mass and total leaf area-weighted root hydraulic conductance (K_{rDW} and K_{rLA}) measured under ambient CO_2 level (b and c). Symbols: *Acer* (○), *Betula* (△), *Gymnocladus* (▽), *Populus* (□), *Quercus* (◇), *Robinia* (⬡). The relationship was fitted to a linear model in panel a ($y = -25.25 + 0.468x$). The regression between relative changes in g_s and intrinsic K_{rDW} and K_{rLA} was best fitted to a logarithmic model in (b) ($y = -52.04 + 5.89 \ln x$) and (c) ($y = -31.05 + 9.48 \ln x$).

(Wang et al. 2016), which lends the stem hydraulic system very small leverage in influencing the whole-plant hydraulic conductance (Meinzer 2002). In tall trees with a long stem water flow path or under circumstances that lead to a high level of embolism formation in stems, however, stem hydraulics can have a much greater influence on whole-plant hydraulic architecture (Meinzer 2002, Domec et al. 2008) and thus potentially play an important role in mediating hydraulic responses to eCO_2 at the whole-plant level. Although eCO_2 did not show a significant impact on P_{50}, the tendency that it led to higher water-use efficiency and less negative leaf water potentials may likely result in a larger hydraulic safety margin under field conditions.

Despite the fact that the leaf hydraulic system is a major bottleneck in the whole-plant water transport pathway (Meinzer 2002, Sack and Holbrook 2006, Brodribb and Jordan 2008), we found that across species K_{leaf} was not significantly affected by eCO_2 and hence it offered little explanation for decrease of g_s and the species-specific differences in g_s responses. This is in contrast to results from long-term FACE studies, which showed significant decreases in K_{leaf} in *Pinus taeda* and *L. styraciflua* (Domec et al. 2009*b*, 2016, Tor-ngern et al. 2015). These inconsistencies may indicate that different tree species can have different responses in leaf hydraulics to eCO_2 or they can result from the differences in experimental treatment (FACE vs greenhouse) and techniques used for K_{leaf} determination (rehydration kinetics vs pressurization). Besides the potential influence of eCO_2 on individual leaf hydraulic properties, what is more important might be the influence of eCO_2 on total leaf area production and the changed timing of leaf senescence especially under drought stressed conditions, which strongly affect the whole-plant water transport efficiency (Meinzer 2002, McCarthy et al. 2007, Warren et al. 2011, Tor-ngern et al. 2015). The apparent lack of coordination between K_{leaf} and g_s responses to CO_2 enrichment observed here may not necessarily mean a decoupling of responses at the whole-plant level between liquid and gaseous water transport through leaves. Future investigations on coordinated responses of liquid- and gas- phase water transport through leaves would benefit from measurements of leaf hydraulic conductance at the whole-plant or whole-shoot levels (Wang et al. 2016, Song et al. 2017) in combination with measurement of sap flow-based crown canopy conductance (Oren et al. 1998, Domec et al. 2009*b*).

Concomitant allometric adjustments in responses to eCO_2

Our study shows that the relative responses of g_s to eCO_2 and RM/LA were positively correlated across species, indicating that allometric adjustment between root and leaf played an important role in controlling the g_s responses to eCO_2. Despite the straightforwardness of this conclusion, this is the first time that adjustment in allometric scaling between roots and shoots, i.e., between water supply and demand organs, has emerged as a unifying variable to explain species-specific variability in g_s responses to eCO_2. Functional allometry between root and leaf has been recognized as an important plastic trait that could have a strong impact on plant hydraulics (Meinzer 2002, 2003). Allometric shifts are caused by relatively long-term exposure to changes in environmental conditions and they may pose different influences on whole-plant hydraulic conductance from those dictated by short-term physiological responses. Mencuccini (2003) suggested that the increase in biomass allocation to absorbing and conducting organs commonly observed in eCO_2-treated plants (Curtis and Wang 1998, Madhu and Hatfield 2013, but see Poorter and Nagel 2000, Ward et al. 2013) is likely associated with a reduced efficiency of the whole-plant hydraulic system (Domec et al. 2017).

Although the ratio of sapwood cross-sectional area to leaf area did respond significantly to eCO_2 and this trait has been recognized to affect plant hydraulics and g_s (Maherali and DeLucia 2001, Buckley and Roberts 2005, Bhaskar et al. 2007), we found that the percentage change in g_s did not correlate with this allometric adjustment in response to eCO_2. Our results suggest that further investigations aimed at improving the understanding, and hence, the prediction of vapor phase water transport responses to long-term eCO_2 exposure must include species plasticity in allometry between various components of the whole-plant hydraulic system, particularly between roots and leaves.

Concluding remarks

This study shows that the suppressive effect of CO_2 enrichment on g_s of tree seedlings was overall accompanied by substantial changes in hydraulic architecture, indicating a strong coordination between vapor and liquid phase water transport through plants despite the relatively large variations between plant organs and species. Plant hydraulics and their associated allometric scaling are important in regulating g_s responses to eCO_2 and in explaining the interspecific variations of the response. Studies aimed at investigating the underlying mechanisms of g_s response to eCO_2 need to integrate characteristics of hydraulics and allometric scaling in the future. Due to the dramatic ontogenetic changes trees have to experience from seedlings to mature sizes, results based on the seedling experiment of the present study may not readily be used to interpret responses of large trees or forests. However, the finding that seedlings are highly susceptible to changes in hydraulic architecture even after only one growing season exposure would have great implications on seedling establishment and recruitment and thus on forest dynamics in a changing CO_2 regime.

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Conflict of interest

None declared.

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