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RESEARCH PAPER

Leaf hydraulic properties of Antarctic plants: effects of growth temperature and its coordination with photosynthesis

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Abstract

One of the well-documented effects of regional warming in Antarctica is the impact on flora. Warmer conditions modify several leaf anatomical traits of Antarctic vascular plants, increasing photosynthesis and growth. Given that CO_2 and water vapor partially share their diffusion pathways through the leaf, changes in leaf anatomy could also affect the hydraulic traits of Antarctic plants. We evaluated the effects of growth temperature on several anatomical and hydraulic parameters of Antarctic plants and assessed the trait co-variation between these parameters and photosynthetic performance. Warmer conditions promoted an increase in leaf and whole plant hydraulic conductivity, correlating with adjustments in carbon assimilation. These adjustments were consistent with changes in leaf vasculature, where Antarctic species displayed different strategies. At higher temperature, *Colobanthus quitensis* decreased the number of leaf xylem vessels, but increased their diameter. In contrast, in *Deschampsia antarctica* the diameter did not change, but the number of vessels increased. Despite this contrasting behavior, some traits such as a small leaf diameter of vessels and a high cell wall rigidity were maintained in both species, suggesting a water-conservation response associated with the ability of Antarctic plants to cope with harsh environments.

Keywords: Antarctic plants, climate change, growth temperature, hydraulic, photosynthesis, warming.

Introduction

One focus of scientific research in Antarctica is the enigma that represents the natural existence of only two species of vascular plants: the Antarctic hair grass (*Deschampsia antarctica* É.Desv.) and the Antarctic pearlwort (*Colobanthus quitensis* (Kunth) Bartl.). Disentangling the ecophysiological traits enabling these two species to withstand the harsh Antarctic environment may help explain their exceptional geographic distribution. Furthermore, considering the fast regional warming documented in the Antarctic Peninsula (Turner *et al.*, 2021; Chown *et al.*, 2022), it is of pivotal importance to evaluate how the ecophysiology of these species will respond to the climatic changes already impacting the Antarctic ecosystems. Reports have indicated dramatic effects of regional warming on the Antarctic Peninsula flora (Parnikoza *et al.*, 2009; Torres-Mellado *et al.*, 2011; Cannone *et al.*, 2016, 2022).

Previously, we demonstrated that the *in situ* photosynthetic performance of *D. antarctica* and *C. quitensis* was determined by particular anatomical leaf traits (Sáez *et al.*, 2017). In both species, we found exceptionally thick and tightly packed mesophyll together with other xerophytic characteristics such as high leaf mass area (LMA) and leaf density (LD), which contribute to freezing and desiccation resistance. These adaptations impose notably low values on leaf mesophyll conductance to CO_2 (g_m), which reduces the CO_2 availability for the carboxylation enzyme Rubisco (Sáez *et al.*, 2017). When CO_2 is limited, the probability of O_2 binding instead of CO_2 at the catalytic site of Rubisco increases, with the subsequent reduction in net carbon gain due to photorespiration. To counteract this negative effect, Rubisco function in both Antarctic species has evolved towards high specificity for CO_2 ($S_{C/O}$, Sáez *et al.*, 2017). This combination of traits (low g_m and high $S_{C/O}$) appears to be a key feature for the successes of these two plant species in the Antarctic environment.

In theory, the response of photosynthesis to changes in temperature reflects a complex interaction between diffusive and biochemical processes (Salvucci and Crafts-Brandner, 2004). Regarding diffusive determinants, plants grown at lower temperatures tend to have smaller leaves with smaller and more tightly packed cells, which result in greater LMA due to both enhanced LD and enhanced thickness (Tardieu and Granier, 2000). In contrast, plants grown at higher temperatures present greater leaf expansion and lower leaf thickness, which can favor net assimilation rates (Niinemets *et al.*, 2009). In previous experiments, when Antarctic plants were grown *in situ* under warmer conditions, a significant decrease in LMA and LD was observed only in *C. quitensis*, whereas *D. antarctica* did not show major changes (Sáez *et al.*, 2018a). In fact, *D. antarctica* required higher temperatures to display similar changes to those observed in *C. quitensis* (Sáez *et al.*, 2018b, 2019). When growth temperature was increased to 10 °C, both species decreased LMA and LD, which promoted changes in g_m resulting in higher net photosynthetic rates (Sáez *et al.*, 2018b). Given that CO_2 and water vapor share in part their diffusion pathways through the leaf mesophyll (Evans *et al.*, 2009; Terashima *et al.*, 2011), changes in the leaf anatomy could also affect leaf hydraulic traits (Sack and Holbrook, 2006; Xiong *et al.*, 2017). However, despite the relevance of water limitations for Antarctic vascular plants (Kennedy, 1993; Block *et al.*, 2009; Sáez *et al.*, 2019), there are no studies on their hydraulic characteristics, which is an important gap in our knowledge of their adaptive traits.

To support carbon assimilation and growth, it is essential to maintain the integrity of the root-to-leaf water transport pathway (Meinzer and McCulloh, 2013). According to cohesion–tension theory, leaves draw water from the soil by a water potential gradient generated by evaporation at the leaf (Martínez-Vilalta *et al.*, 2002). The difference between soil and leaf water potential is therefore determined by the rate of water loss at the leaf, and the resistance to water flow imposed by the vasculature and non-xylem pathways radial to the vasculature (Brodribb *et al.*, 2005). Leaf hydraulic resistance is estimated to be between 25% and 80% of the whole plant resistance (Nardini *et al.*, 2001; Sack *et al.*, 2003), thereby representing a substantial part of the hydraulic resistance of the entire plant (Tyree *et al.*, 1993; Brodribb *et al.*, 2005). The leaf hydraulic conductivity (K_{leaf}), determined as the ratio between water flow rate and the gradient of water potential, is a measure of how efficiently water is transported through the leaf, from the petiole, through the xylem to the storage tissue to replace the water lost through the stomata (Franks, 2006; Sack and Holbrook, 2006). Given that stomatal conductivity correlates closely with the photosynthetic CO_2 assimilation rate, there is a direct relationship between the leaf hydraulic conductivity and carbon assimilation across a broad range of species (Brodribb *et al.*, 2007). This is also true in response to differences in leaf hydration status and incident light (Brodribb and Holbrook, 2003; Scoffoni *et al.*, 2008).

The assessment of the hydraulic properties in the leaves of Antarctic vascular plants and the coordination with photosynthesis can shed light on two important issues: the relevance of leaf xerophytic traits (such as high LMA and LD) for the control of water loss in the dry and windy environment of Antarctica, and the role of these xerophytic traits in determining the response of *D. antarctica* and *C. quitensis* to increased temperature. If some of the xerophytic traits of these species become altered by the increase in temperature, this may compromise not only a higher carbon assimilation rate (through altered g_m), but also the ability to resist freezing events (i.e. hydraulic failure). To maintain hydraulic homeostasis, vascular plants increase rates of photosynthetic gas exchange by increasing leaf hydraulic conductivity (Franks, 2006). Therefore, it can be hypothesized that the leaf anatomical modifications promoted in Antarctic vascular species in response to warmer conditions increase leaf hydraulic conductivity. To test this hypothesis, we evaluated the effects of growth temperature on several anatomical and hydraulic parameters of the Antarctic vascular plants and assessed the trait co-variation between these parameters and photosynthetic performance. This information is a novel contribution to Antarctic plant ecophysiology and is essential for making accurate predictions of the response of these exceptional species to climate change.

Materials and methods

Plant material and growth conditions

Deschampsia antarctica and *Colobanthus quitensis* plants were collected from King George Island, in the South Shetlands, near to the Henryk Arctowski Polish Antarctic Station (62°09′S, 58°28′W, February 2018). Plants were transferred to the laboratory and reproduced vegetatively in plastic pots (Fig. 1A, B) using a sterile soil and vermiculite mixture (3:1 v/v) and maintained at 15 °C in a growth chamber with irradiance of 150 μmol photons m^{-2} s^{-1} and 16/8 h light–dark photoperiod. Plants were watered to full soil capacity every 2–3 d and fertilized once a week with 0.2 g l^{-1} Phostrogen® (Distribuidora Yates Ltda, Chile). After 3 weeks, plants were randomly assigned to three different diurnal growth temperature regimes: 5 °C, 8 °C, and 15 °C. The first two temperatures were chosen because they are close to the mean maximum air temperature recorded *in situ* during the Antarctic summer in open field (4.83 ± 0.40 °C) and inside open-top-chamber passive warming systems (7.81 ± 0.50 °C), respectively (Sáez *et al.*, 2018a). The third temperature was chosen because 15 °C is the optimal photosynthetic temperature determined in *D. antarctica* and *C. quitensis* growing on King George Island (Sáez *et al.*, 2017). Soil and air temperatures were recorded every hour using a HOBO U-30 Station (Onset Computer Co., Bourne, MA, USA). The irradiance, irrigation, and fertilization were maintained throughout the experiment. Once the plants reached an adequate size (about 3 cm^2) for measurement under each temperature regime (at least 45 d), the following measurements were conducted.

Leaf mass area and leaf density

LMA was calculated as the ratio of dry mass to leaf area. For this, 10 individuals from each temperature regime were randomly selected and at least six leaves per individual were collected for measurements. Leaf area was determined with fresh leaves by analysing photos with ImageJ (Wayne Rasband/NIH, Bethesda, MD, USA). Then, the dry mass of these leaves was measured after oven-drying for 64 h at 70 °C. LD was determined by dividing LMA by leaf thickness. The leaf thickness was obtained from leaf cross-sections analysed by optical microscopy.

Pressure–volume curves

Pressure–volume (*P*–*V*) relationships were determined in leaves of *C. quitensis* and shoots of *D. antarctica* (Fig. 1) following the free-transpiration method described in previous studies (Corcuera *et al.*, 2002; Vilagrosa *et al.*, 2003). Water potential (Ψ) was measured using a Scholander pressure chamber, but, due to the lack of petioles and the morphology of *D. antarctica*, it was necessary to introduce its leaves in flexible silicone tubes (diameter 5 mm) sealed with slightly moistened cotton wool for better handling, to prevent gas leakage throughout the sealing ring of the chamber and to avoid dehydration caused by the gas flow (Fig. 1Be). For each species and temperature treatment, six *P*–*V* curves were conducted on six different plants. Due to the fragility and the small size of both plant species, it was also necessary to use 20–30 leaves or shoots of the same plant to obtain one *P*–*V* curve. First, well-watered plants were covered with plastic bags overnight to ensure full hydration. Next day, three to four series of 5–10 leaves or shoots per plant were cut, weighed using an analytical balance (MS105U, accuracy ±0.0001 g, Mettler-Toledo, Switzerland) to obtain the water full saturated weight (W_{sat}), and allowed to dry slowly at room temperature with a dark plastic cover. Eventually, Ψ and fresh weight (W_{f}) in these leaves or shoots were recorded during the dehydration process in order to complete the *P*–*V* curve. After the last record, leaves or shoots were oven dried for 72 h at 65 °C, obtaining the dry weight (W_{dry}). Then, the relative water content (RWC) for each point was calculated as $(W_{\text{f}}-W_{\text{dry}})/(W_{\text{sat}}-W_{\text{dry}})$. Finally, *P*–*V* curves were plotted and analysed to calculate osmotic potential at full turgor (π_{o}), leaf water potential at the turgor-loss point (Ψ_{tlp}), relative water content at the turgor-loss point (RWC_{tlp}) and maximum bulk modulus of elasticity ($\varepsilon_{\max}$).

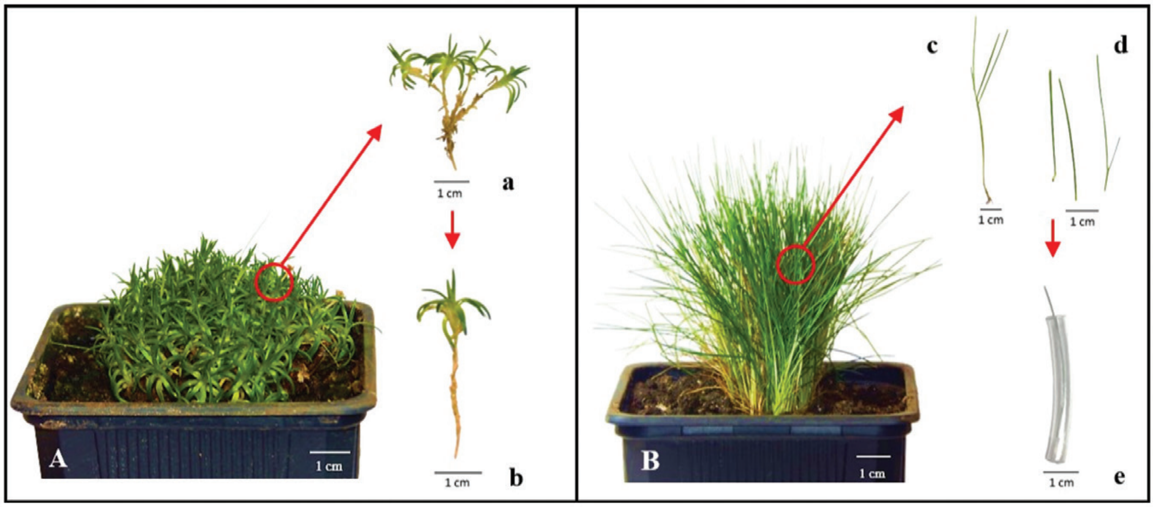

Fig. 1. The Antarctic vascular species *Colobanthus quitensis* (A) and *Deschampsia antarctica* (B). Plant material used to perform the measurements of hydraulic properties: shoots (a) and rosettes (b) of *C. quitensis*; and leaves (c), tillers (d), and a leaf in a flexible silicone tube (e) of *D. antarctica*.

Measurement of leaf hydraulic conductivity

Leaf hydraulic conductivity (K_{leaf}, mmol m^{-2} s^{-1} MPa^{-1}) was measured using the kinetic method of rehydration described by Brodribb and Holbrook (2003). Fully hydrated rosettes with three to four branches and fully hydrated tillers with four to six leaves were collected for *C. quitensis* (Fig. 1Ab) and *D. antarctica* (Fig. 1Be), respectively. Rosettes and tillers were enclosed in black plastic bags and were allowed to dry slowly at room temperature for 1 h approximately, ensuring the same water potential in all branches and leaves from the same rosette or tiller. Then, water potential was measured in one or two branches of *C. quitensis* and one or two leaves of *D. antarctica* obtaining values around −1 MPa. It is assumed that this is the water potential prior to rehydration (Ψ_0). Once this value was obtained, another branch or leaf was cut with a scalpel under distilled, filtered (0.22 µm), and degassed water, and allowed to take up water for 240 s for *C. quitensis* and 120 s for *D. antarctica*. Water potential after rehydration was subsequently measured (Ψ_f). The rehydration time (*t*) was chosen to allow the relaxation of the water potential to approximately half of the initial value. K_{leaf} was then calculated according to:

$$K_{leaf} = C \ln\left[\Psi_0/\Psi_f\right]/t$$

where *C* (mol m^{-2} MPa^{-1}) is the leaf capacitance of each species, determined as the initial slope of the *P*–*V* curves normalized by the leaf area (Tyree and Hammel, 1972; Brodribb *et al.*, 2005). For each species, K_{leaf} was measured on six to nine plants per temperature treatment.

Whole plant hydraulic conductivity

Whole plant hydraulic conductivity (K_{plant}, mmol m^{-2} s^{-1} MPa^{-1}) was calculated according to Brodribb and Cochard (2009). Plants watered to field capacity and installed in a chamber with the target temperature regimes (5 °C, 8 °C, and 15 °C). Plants were exposed during 3 h to a gradual increase in light intensity, from 150 to 900 µmol photons m^{-2} s^{-1} followed by a continuous exposure at 900 µmol photons m^{-2} s^{-1} using fluorescent tubes and LED panels (GP-180W, Innova-Led, Santiago, Chile). The soil and air temperature close to the plants were recorded using HOBO sensors (Onset Computer Co.) to ensure we had the same target temperatures. After 3 h of total light exposure, transpiration of a group of leaves (*E*, mmol m^{-2} s^{-1}) was measured using a portable photosynthesis system (Li-6400; LI-COR Inc., Lincoln, NE, USA). In parallel, leaf water potential (Ψ_{leaf}) was measured using a pressure chamber. K_{plant} was then calculated as:

$$K_{plant} = E/\left(\Psi_{soil} - \Psi_{leaf}\right),$$

where Ψ_{soil} is the soil water potential. Since the soil was well irrigated throughout the experiment, temperature had virtually no effect on Ψ_{soil} and therefore it was assumed that Ψ_{soil}=0 MPa (Cochard *et al.*, 2000). For each species, K_{plant} was measured on four to eight plants per temperature treatment.

Anatomical measurements

Central portions of leaves of each species (*n*=4) growing at each selected temperature were collected and fixed in formaldehyde, acetic acid, and ethanol, and stored at 4 °C. Tissues were fixed in paraffin and embedded in paraffin blocks. Leaf cross sections of both species were obtained from the blocks using a rotary microtome and deparaffinized. *Colobanthus quitensis* sections were analysed by spectral confocal microscopy (Zeiss; LSM780, Germany), and *D. antarctica* sections were stained with toluidine blue and analysed by optical microscopy (Olympus; CX31, Japan). Micrographs were randomly selected to measure the leaf mesophyll thickness (T_{mes}), cell wall thickness (T_{cw}), number of leaf xylem vessels and leaf xylem vessel lumen diameter (*d*). All images were analysed with image analysis software (ImageJ). Mean leaf hydraulic diameter (D_h, µm) was calculated according to Corcuera *et al.* (2012):

$$D_h = \Sigma d^5/\Sigma d^4.$$

Additionally, from the leaf vessel number and lumen diameter, we determined the theoretical hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}) according to the Hagen–Poiseuille law (Tyree and Zimmermann, 2002; Eguchi *et al.*, 2008):

$$K_h = \Sigma\left(\left(d_i^4\pi\rho\right)/\left(128\eta\mathbf{w}\right)\right),$$

where d_i is the diameter of a single lumen (m), ρ and *w* correspond to water density (kg m^{-3}) and viscosity (MPa s), respectively, normalized at 5 °C, 8 °C, and 15 °C. Finally, K_h was normalized to the xylem area to obtain the specific hydraulic conductivity (K_s):

$$K_s = K_h/A_{xyl}\left(\mathrm{kg\,m^{-1}\,s^{-1}\,MPa^{-1}}\right),$$

where A_{xyl} (m^2), is the xylem cross-sectional area.

Leaf gas exchange and chlorophyll fluorescence

Leaf gas exchange with chlorophyll *a* fluorescence measurement was recorded using a Li-6400XT with a Li-6400-40 leaf chamber (LI-COR). Six individuals from each growth temperature were randomly selected for the measurements. For each individual, the gas exchange measurements were performed on a group of leaves (as described in Sáez *et al.*, 2018a), trying to maximize the occupation of the infrared gas analyser's chamber area but avoiding leaf overlap. In those cases where the chamber area was not fully occupied, the actual leaf area in the chamber was estimated and used for measurement corrections.

The response of the net photosynthesis CO$_2$ uptake (A_N) to varying substomatal CO$_2$ concentration (C_i) was studied with the A_N–C_i curves in the same way as reported in Sáez *et al.* (2017). The leaf gas exchange measurements were performed at leaf temperatures of 5 °C, 8 °C, and 15 °C. Corrections for CO$_2$ leakage of the leaf chamber of the Li-6400XT were applied to all gas-exchange data as described in Flexas *et al.* (2007).

The quantum efficiency of the photosystem II (PSII)-driven electron transport was determined using the equation: $\phi_{PSII} = (F'_m - F_s)/F'_m$, where F_s is the steady-state fluorescence in the light (photosynthetic photon flux density (PPFD) 1000 µmol photons m^{-2} s^{-1}) and F'_m the maximum fluorescence obtained with a light-saturating pulse (8000 µmol photons m^{-2} s^{-1}). As ϕ_{PSII} represents the number of electrons transferred per photon absorbed by PSII, the electron transport rate (ETR) can be calculated as: ETR = $\phi_{PSII}\times\mathrm{PPFD}\times\alpha\times\beta$, where PPFD is the photosynthetic photon flux density, α is the leaf absorptance, and β is the distribution of absorbed energy between the two photosystems, assumed to be 0.5. The leaf absorptance was measured as described by Sáez *et al.* (2017). In *C. quitensis* leaf absorptance values were 0.76 ± 0.01 for plants growing at 5 °C, 0.83 ± 0.01 for plants growing at 8 °C, and 0.82 ± 0.01 for plants at 15 °C. In *D. antarctica*, the leaf absorptance values were 0.89 ± 0.01 for plants growing at 5 °C, 0.91 ± 0.01 for plants growing at 8 °C, and 0.81 ± 0.01 for plants at 15 °C.

The mesophyll conductance to CO$_2$ (g_m) was calculated as in Harley *et al.* (1992):

$$g_m = A_N/\left(C_i - \left(\Gamma^*\left(\mathrm{ETR} + 8\left(A_N + R_L\right)\right)/\left(\mathrm{ETR} - 4\left(A_N + R_L\right)\right)\right)\right),$$

where A_N and C_i were obtained from gas exchange measurements at saturating PPFD (1000 µmol photons m^{-2} s^{-1}). The rate of non-photorespiratory CO_2 evolution in the light (R_L) was assumed to be half of R_{dark}, and the chloroplast CO_2 compensation point (Γ^*) was calculated according to Brooks and Farquhar (1985) from the Rubisco specificity factor ($S_{C/O}$) measured *in vitro* (Sáez *et al.*, 2017). The values of g_m were used to calculate the chloroplast CO_2 concentration (C_c), converting A_N–C_i curves into A_N–C_c curves, as $C_c = C_i–(A_N/g_m)$.

The maximum carboxylation rate (V_{cmax}) was derived from A_N–C_c curves according to Farquhar *et al.* (1980) and using the *in vitro* Rubisco kinetic constants reported in Sáez *et al.* (2017) for both Antarctic vascular species.

Statistical analysis

A one-way ANOVA was performed to assess differences among growth temperature (5 °C, 8 °C, and 15 °C) on the hydraulic and photosynthetic parameters evaluated. When ANOVA was significant, differences among means were assessed by an *a posteriori* Tukey test ($P<0.05$). Pearson's correlation analysis was performed to assess the relationship between several anatomical and hydraulic parameters and to assess trait co-variation between these parameters and the photosynthetic performance. Chi square (χ^2) tests were used to assess the statistical differences among the distribution of xylem vessel diameters among temperature treatments. All these analyses were done using Statistica 7.0 (StatSoft Inc., Tulsa OK, USA).

Results

Anatomical traits of the Antarctic vascular species under studied temperature regimes

There were no differences in LMA between *C. quitensis* plants grown at 5 °C and 8 °C (Fig. 2A), while plants grown at 15 °C showed a 50% decrease compared with the values at 5 °C and 8 °C. A similar trend for a decrease in LMA at higher growth temperature was observed in *D. antarctica*, except that statistically significant differences ($P<0.05$) were found for plants grown at 5 °C and 8 °C (Fig. 2B). With respect to LD, non-significant differences between 5 °C and 8 °C, and lower values for plants grown at 15 °C were again observed in *C. quitensis* (Fig. 2C), while significant differences were found among the three temperature regimes in the case of *D. antarctica* (Fig. 2D). As a result, a positive linear correlation between LMA and LD was found in both species when combining data from the three growth temperatures (Supplementary Fig. S1).

The leaf mesophyll thickness (T_{mes}) was the only leaf anatomical parameter with non-significant differences among growth temperatures in either of the two species (Fig. 2E, F). The two species showed similar T_{cw} response patterns to growth temperature, consisting of higher values measured in plants grown at 5 °C, and non-significant differences between 8 °C and 15 °C (Fig. 2G, H).

Pressure–volume curve parameters

The response of P–V curve parameters to the different growth temperatures was similar for both Antarctic vascular species. The leaf capacitance (C) increased with growth temperature in both species, being the highest values measured at 15 °C, 3.74 and 0.63 mol m^{-2} MPa^{-1} for *C. quitensis* and *D. antarctica*, respectively (Table 1). The relative water content at turgor-loss point (RWC_{tlp}) and the leaf water potential at the turgor-loss point (Ψ_{tlp}) decreased in both species at higher growth temperatures. Finally, the osmotic potential at full turgor (π_o) and the maximum bulk modulus of elasticity (ε_{max}) showed no differences among temperature treatments in any of the two species.

Leaf and whole plant hydraulic conductivity under different growth temperatures

Consistent with the effect of temperature on the leaf capacitance, the increase in growth temperature induced an increase in the leaf hydraulic conductivity (K_{leaf}) in both species (Fig. 3). Hence, the lowest K_{leaf} values were measured in plants grown at 5 °C, being 3.47 and 1.13 mmol m^{-2} s^{-1} MPa^{-1} for *C. quitensis* and *D. antarctica*, respectively (Fig. 3A, B).

The same trend was observed for the whole plant hydraulic conductivity (K_{plant}), which progressively increased with the increase in the growth temperature in both species (Fig. 3C, D). In *C. quitensis*, the values ranged between 0.80 and 1.81 mmol m^{-2} s^{-1} MPa^{-1}, in plants grown at 5 °C and 15 °C, respectively. In *D. antarctica*, K_{plant} ranged between 0.42 and 1.56 mmol m^{-2} s^{-1} MPa^{-1}, in plants grown at 5 °C and 15 °C, respectively. Although it was outside our objectives to make a comparison between the two species, it is remarkable that, *C. quitensis* presented K_{leaf} values 2- to 3-fold higher than *D. antarctica*, while K_{plant} differences between the two species were minimal with the increase in growth temperature.

When combining data measured on plants grown at the three temperature regimes, a positive relationship between K_{leaf} and K_{plant} was found in both species (Fig. 4). In turn, K_{leaf} showed a negative relationship with LD in the two species (Fig. 5).

Leaf vascular anatomy changes in response to temperature

The growth temperature also had significant effects on the leaf vascular traits of both Antarctic species, although in contrast with previous analyses, contrasting patterns of change were observed between species (Table 2).

The number of midvein xylem vessels in the leaves of *C. quitensis* decreased at higher growth temperature, from 24 vessels at 5 °C to 20 vessels at 15 °C. Thus, when *C. quitensis* grew at low temperature, it had a larger number of vessels mainly distributed in small diameter classes (Fig. 6A). In contrast, at higher temperatures, there was a decrease in the number of vessels, but these were larger vessels in diameter classes from 4 to 6 μm, not observed at 5 °C (Fig. 6A). The increase in the size of vessels coincided with the increase in the mean hydraulic diameter (D_h), the theoretical hydraulic conductivity (K_h), and the specific hydraulic conductivity

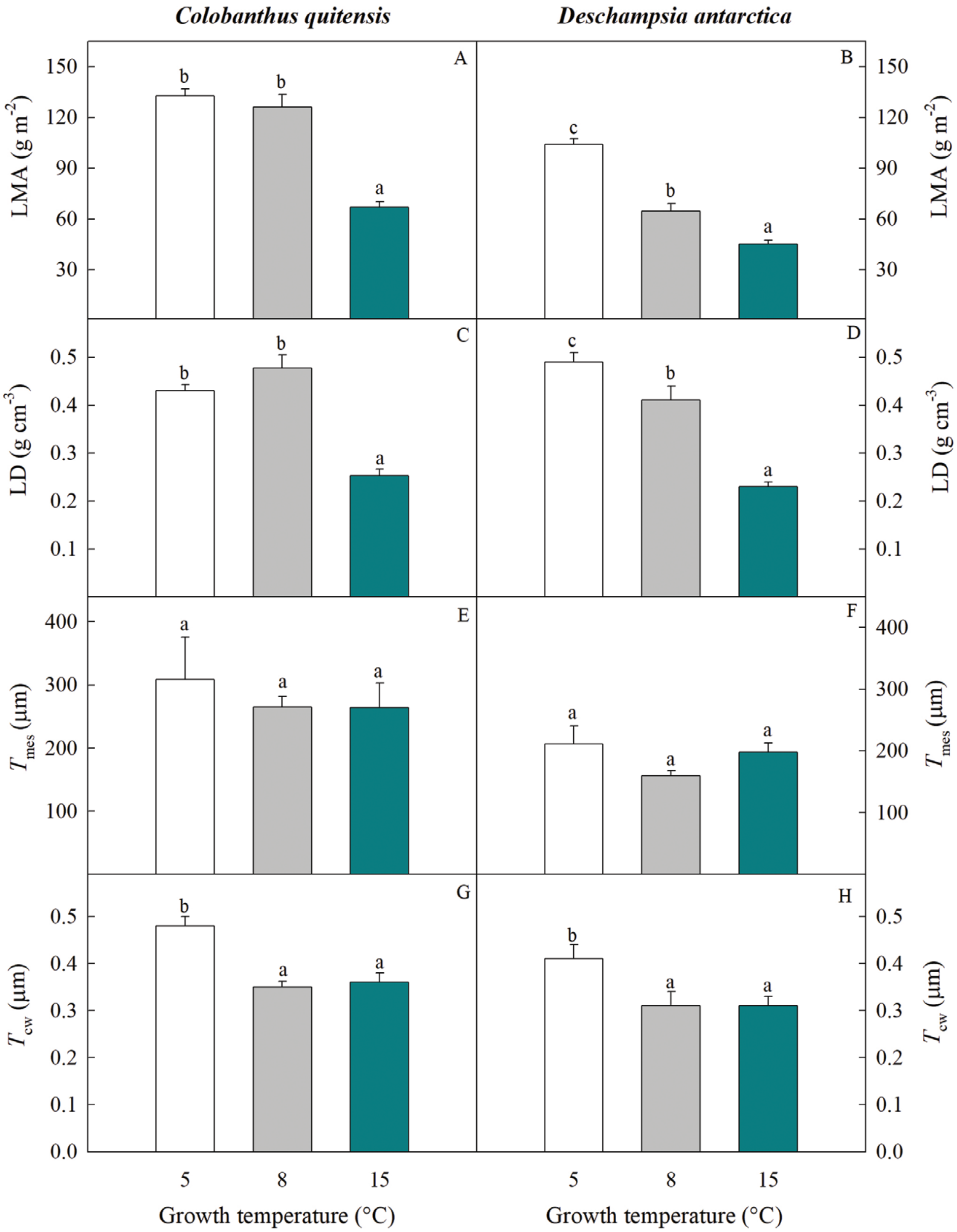

Fig. 2. Temperature response of anatomical traits in Antarctic vascular plants: leaf mass area (LMA), leaf density (LD), mesophyll thickness (T_{mes}) and cell wall thickness (T_{cw}) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Values are means ±SE (*n*=6–10). For each species, different letters indicate statistically significant differences among growth temperatures, according to the Tukey test ($P<0.05$).

(K_s) (Table 2), which supported the increase in K_{leaf} with growth temperature (Fig. 3). In *D. antarctica* the number of leaf vessels increased at higher growth temperature (Table 2), but there was a higher frequency of vessels in the smaller diameter classes, resulting in a more than 2-fold increase in the number of vessels with 1 and 2 µm diameters (Fig. 6B). No significant changes were detected in D_h, K_h, and K_s in *D. antarctica* (Table 2).

Table 1. Parameters derived from the pressure–volume curves for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5, 8, and 15 °C.

	5 °C	**8 °C**	**15 °C**
Colobanthus quitensis			
C (mol m^{-2} MPa^{-1})	1.48 ± 0.16a	2.18 ± 0.28a	3.74 ± 0.30b
π_o (MPa)	−1.10 ± 0.08a	−1.08 ± 0.01a	−1.05 ± 0.04a
RWC_{tlp}	0.94 ± 0.01b	0.91 ± 0.01a	0.88 ± 0.01a
Ψ_{tlp} (MPa)	−1.14 ± 0.08b	−1.25 ± 0.03ab	−1.44 ± 0.04a
ε_{max} (MPa)	8.42 ± 1.54a	6.84 ± 1.37a	5.74 ± 0.13a
Deschampsia antarctica			
C (mol m^{-2} MPa^{-1})	0.22 ± 0.03a	0.43 ± 0.01b	0.63 ± 0.04c
π_o (MPa)	−1.30 ± 0.16a	−1.16 ± 0.02a	−1.13 ± 0.06a
RWC_{tlp}	0.96 ± 0.00c	0.92 ± 0.01b	0.89 ± 0.01a
Ψ_{tlp} (MPa)	−1.31 ± 0.09b	−1.47 ± 0.03ab	−1.54 ± 0.02a
ε_{max} (MPa)	11.63 ± 0.64a	9.94 ± 0.80a	9.15 ± 1.36a

Leaf capacitance (*C*), osmotic potential at full turgor (π_o), relative water content at the turgor-loss point (RWC_{tlp}), leaf water potential at the turgor-loss point (Ψ_{tlp}), and maximum bulk modulus of elasticity (ε_{max}). Values are means ±SE (*n*=6). For each species, different letters indicate statistically significant differences among growth temperatures, according to the Tukey test ($P<0.05$).

Trait co-variation between photosynthetic and hydraulic parameters at different growth temperatures

The net CO_2 assimilation rate (A_N) increased in plants grown at higher temperature (Supplementary Table S1). In both plant species, the enhancement in A_N occurred because of the combined increase in diffusive (g_s and g_m) and biochemical (V_{cmax}) factors. Trait co-variation was observed between the main hydraulic and photosynthetic parameters. Specifically, K_{leaf} correlated positively with g_s, g_m, and A_N (Fig. 7). However, both species tended to be grouped into low and high growth temperatures. *Colobanthus quitensis* showed higher values in both K_{leaf} and photosynthesis when it was grown at 8 °C and 15 °C. Meanwhile, *D. antarctica* increased these parameters mainly at 15 °C.

Discussion

Research on plant hydraulic properties has historically focused on woody species. While over the last few years there has been an increase in the study of herbaceous species, there remains

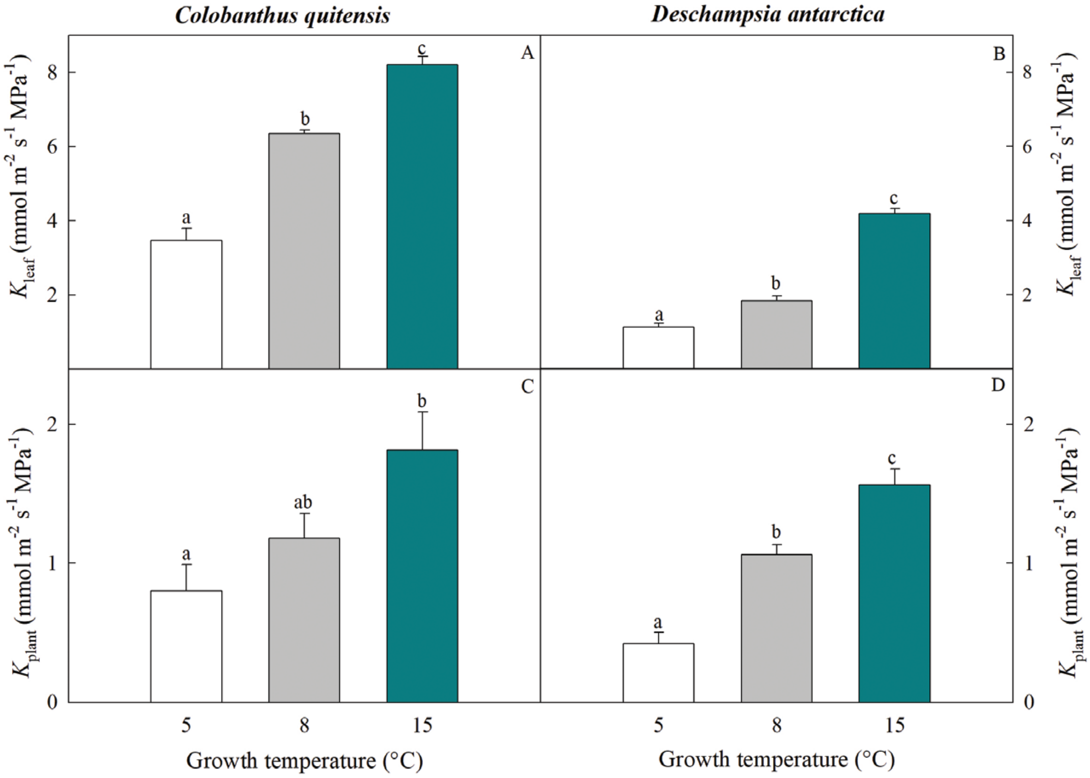

Fig. 3. The leaf hydraulic conductivity (K_{leaf}) and the whole plant hydraulic conductivity (K_{plant}) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Values are means ±SE (*n*=6–9 and *n*=4–8, for K_{leaf} and K_{plant}, respectively). For each species, different letters indicate statistically significant differences among growth temperatures, according to the Tukey test ($P<0.05$).

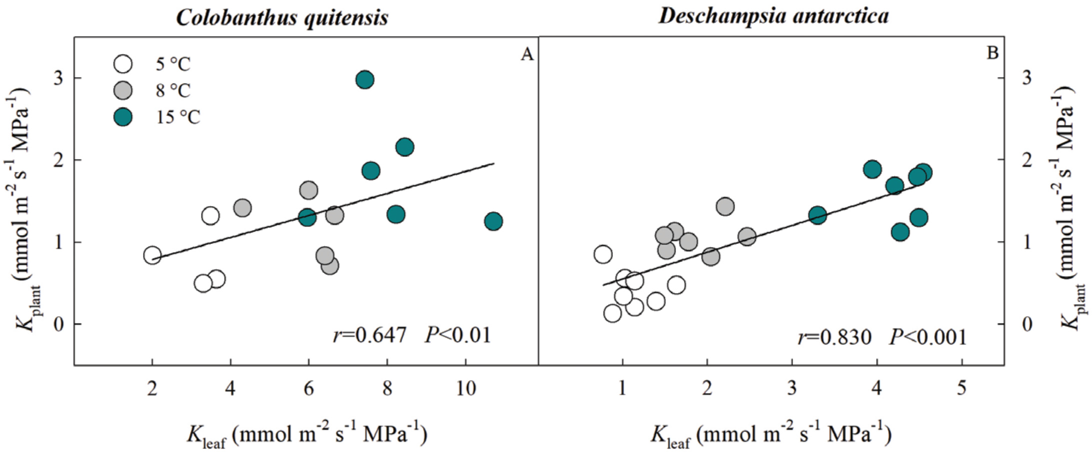

Fig. 4. The relationship between the leaf hydraulic conductivity (K_{leaf}) and the whole plant hydraulic conductivity (K_{plant}) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Pearson's correlation coefficient and the significance of the relationship are shown for each species considering all growth temperatures together.

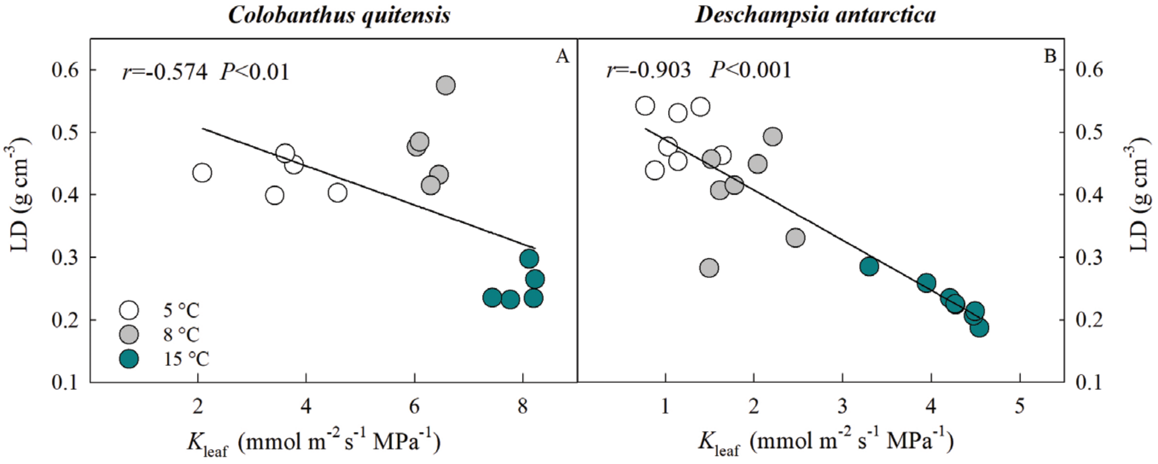

Fig. 5. The relationship between leaf hydraulic conductivity (K_{leaf}) and leaf density (LD) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Pearson's correlation coefficient and the significance of the relationship are shown for each species considering all growth temperatures together.

limited information on the hydraulic traits of grass species, particularly those inhabiting cold environments. This is partly due to the technical challenge that involves measurement of small and fragile tissues. Here, we present the first study that examines the leaf hydraulic properties in the only two Antarctic vascular plant species, providing new insights about the coordinated relationship between leaf hydraulic and photosynthetic processes at varying growth temperatures.

Consistent with previous studies on the Antarctic vascular plants (Sáez *et al.*, 2018a, b; Clemente-Moreno *et al.*, 2020a, b), several leaf anatomical (LMA, LD, and T_{cw}) and functional (photosynthesis and its determinants) traits displayed significant adjustments when exposed to different growth temperatures (Fig. 2; Supplementary Table S1). Thus, the current study corroborates that warmer temperature modifies leaf anatomical traits, increasing carbon diffusion and resulting in higher photosynthetic rates. Because CO_2 and water fluxes share part of their path within the leaf tissues, these responses also imply a potential effect on water transport processes and leaf hydraulic parameters.

The leaf hydraulic properties of Antarctic vascular species and their responses to an increase in growth temperature

The analysis of *P–V* curve parameters suggests that both Antarctic species behave like water-saver plants, especially at low temperatures (Table 1). Even though there is some acclimatization of the hydraulic parameters to higher growth temperature, their responses are generally conservative, tending to maintain their capability to resist low temperature and low water availability. This is consistent with the wide recognition of the importance of desiccation tolerance as a strategy in Antarctic terrestrial habitats (Block *et al.*, 2009; Wharton and Marshal 2009; Everatt *et al.,* 2014).

The water parameters obtained for the Antarctic plants in our study are in accordance with the global dataset found in Bartlett *et al.* (2012), where our values are positioned in the top part of the relationship between the osmotic potential at full turgor (π_o) and the leaf water potential at the turgor loss point (π_{tlp}) (Supplementary Fig. S2). However, within each Antarctic species, we observed an inverse relationship between π_o and π_{tlp}. Thus, changes in π_{tlp} with increased warming might not be associated with changes in π_o. In this sense, π_o maintains low values regardless of the growth temperature, even when Ψ_{tlp} decreases when plants grow at higher temperatures. Furthermore, the highest values of Ψ_{tlp} measured at 5 °C suggest that plants grown at low temperatures are more sensitive to water loss, thereby closing the stomata to avoid water loss. When plants grow at higher temperatures, Ψ_{tlp} decreases in both species, the plants having more margin to keep the stomata open and carry out photosynthesis (Supplementary Table S1). In addition, the Antarctic plants present low cell-wall elasticity (i.e. high ε_{max}) irrespective of the temperature. Cells with less elastic walls experience a greater loss in turgor for a relatively small decrease in water content. Consequently, they can maintain a higher relative water content at a lower turgor loss point, at which stomata are closed in most plants. Some cell wall mechanical properties may also contribute to the propagation of extracellular ice and to the avoidance of intracellular freezing (Solecka *et al.*, 2008).

Table 2. The number of leaf xylem vessels (N° vessels), the mean hydraulic diameter (D_h), the theoretical hydraulic conductivity (K_h) and the specific conductivity (K_s) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5, 8, and 15 °C.

	5 °C	**8 °C**	**15 °C**
Colobanthus quitensis			
No. of vessels	24.00 ± 2.32b	21.75 ± 2.06b	19.50 ± 0.87a
D_h (µm)	2.95 ± 0.02a	3.53 ± 0.16ab	3.66 ± 0.02b
K_h ($\times 10^{-11}$ kg m s^{-1} MPa^{-1})	2.17 ± 0.29a	4.84 ± 0.96ab	5.59 ± 1.24b
K_s (kg m^{-1} s^{-1} MPa^{-1})	0.13 ± 0.01a	0.29 ± 0.02b	0.44 ± 0.04c
Deschampsia antarctica			
No. of vessels	8.64 ± 1.19a	11.56 ± 2.04ab	12.33 ± 2.93b
D_h (µm)	5.87 ± 0.61a	6.02 ± 0.59a	5.61 ± 1.08a
K_h ($\times 10^{-10}$ kg m s^{-1} MPa^{-1})	1.46 ± 0.52a	1.43 ± 0.52a	1.56 ± 0.67a
K_s (kg m^{-1} s^{-1} MPa^{-1})	0.70 ± 0.12a	0.67 ± 0.10a	0.63 ± 0.11a

Values are means ±SE (*n*=4–6). For each species, different letters indicate statistically significant differences among growth temperatures, according to the Tukey test (*P*<0.05).

Despite the above, due to the significant changes in the leaf structure, the hydraulic capacitance (*C*; Table 1) increased at higher growth temperature, in line with the reduction in cell wall thickness and LMA (Fig. 2). Higher *C* and lower Ψ_{tlp}, together with less dense leaves, help to mobilize more water at the leaf and the whole plant levels (Fig. 3). Having said this, K_{leaf} of the Antarctic species ranged between 1.1 and 8.2 mmol m^{-2} s^{-1} MPa^{-1}, which are relatively low values compared with those reported for other angiosperms (Supplementary Fig. S3). K_{leaf} is a complex trait influenced by both leaf xylem (K_x) and outside-xylem (K_{ox}) conductance to water. The former, theoretically influenced by the diameter of xylem conduit and the water viscosity, could be strongly associated with our results. However, K_{ox} may also contribute greatly to K_{leaf}, particularly in species with C_4-type anatomical specializations (Sonawane *et al.*, 2021). It has been described that both Antarctic species, despite being C_3 plants, have two bundle-sheaths and mestome as a functional specialization to optimize photosynthesis under the harsh Antarctic conditions (Vieira and Mantovani, 1995; Romero *et al.*, 1999).

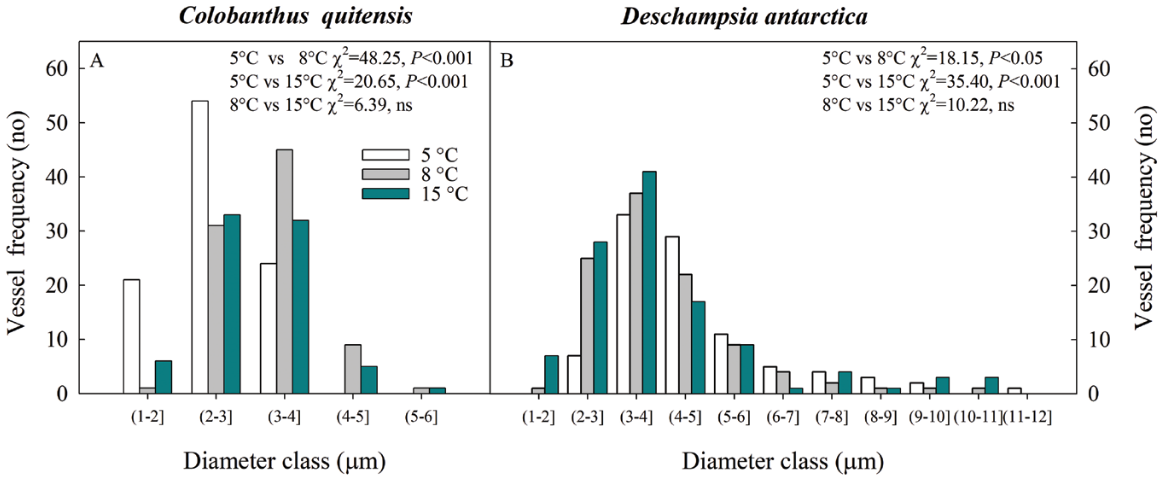

Fig. 6. Frequency distribution of leaf xylem vessels diameters for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. For each species, statistically significant differences in distribution among temperature treatments were analysed according to the χ^2 test (*P*<0.05).

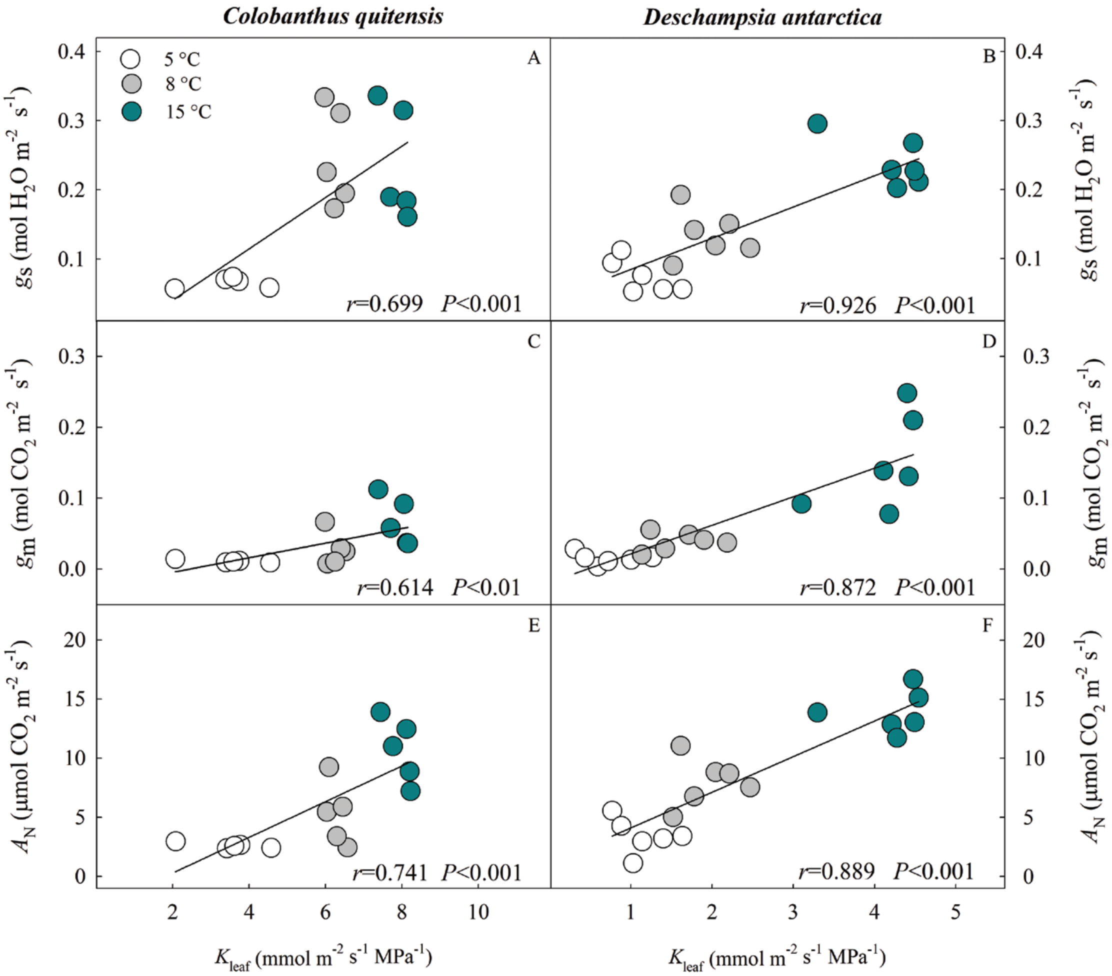

Fig. 7. The relationship between the leaf hydraulic conductivity (K_{leaf}) with the stomatal conductance (g_s), the mesophyll conductance (g_m), and the net CO_2 assimilation rate (A_N) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Pearson's correlation coefficient and the significance of the relationship are shown for each species considering all growth temperatures together.

The leaf hydraulic changes are supported by contrasting vascular modifications between Antarctic species

Both Antarctic species had small leaf vessel size, with most vessels not exceeding 6 µm in diameter (Fig. 6). This fact, added to other traits like the presence of a high percentage of unsaturated fatty acids in the cell membranes, a constitutive high activity of antifreeze proteins (Bravo and Griffith, 2005) and the high concentration of different non-structural carbohydrates (for more see Cavieres *et al.*, 2016) result in the greatest resistance to freezing described in plants (Bravo *et al.*, 2001, 2009) and a remarkable resistance to freeze–thaw-induced embolism (Sakai and Larcher, 1987; Day *et al.*, 1999; Medek, 2008). However, smaller conduits are less efficient at transporting water and would tend to support lower rates of gas exchange for a given stem diameter. This trade-off between freezing protection and photosynthetic productivity finally constrains resource allocation to growth. Despite this, when Antarctic plants are grown at warmer temperatures, there is a notable increase in the growth rate (Sáez *et al.*, 2018a). This increased growth was associated with a higher net photosynthetic rate, via modifications of leaf anatomy, which according to our hypothesis, promotes increases in the leaf hydraulic conductivity. Consistent with this, the Hagen–Poiseuille law states that a small increase in vessel diameter results in a large increase in hydraulic conductivity, because the conductivity scales to the fourth power of the diameter of the vessel (Tyree and Ewers, 1991). In some cases, these modifications are accompanied by a modification in the number of vessels (McCulloh *et al.*, 2004). Both adjustments were observed in the Antarctic species in response to higher growth temperatures, albeit there was a differential response between the two species (Fig. 6; Table 2).

At higher growth temperatures, *C. quitensis* decreased the number of leaf vessels but increased vessel diameter, concomitant with an increase in the efficiency of the xylem (higher specific hydraulic conductivity, K_s), the mean hydraulic diameter (D_h), and consequently, the theoretical hydraulic conductivity

(K_h). The increase in vessel diameter constitutes one way to improve xylem hydraulic capacity, thereby decreasing the hydraulic resistivity (Pittermann *et al.*, 2006), provided that the tracheid diameter shift does not jeopardize the resistance of the xylem to forming a freeze–thaw embolism (Pittermann and Sperry, 2006; Mayr and Sperry, 2010). Thus, if the vessel diameter increases at the expense of decreasing freezing tolerance, then leaves with bigger conduit diameters would be expected to have higher hydraulic conductivity and, correspondingly, higher stomatal conductance (Sack and Frole, 2006). Accordingly in *C. quitensis*, at higher growth temperature both the stomatal conductance (g_s) and the stomatal density increase, while the stomatal size is reduced (Supplementary Fig. S4). These results support the idea that smaller stomata may allow increasing A_N (Supplementary Table S1) and a rapid closure to minimize water loss (Li *et al.*, 2021). This latter trait may be important to reduce the risk of embolisms, counteracting the negative effects that warmer temperatures could have on the freezing resistance capacity.

On the other hand, although *D. antarctica* also increased K_{leaf} when grown at higher temperature, this was not due to an enhancement of D_h, but rather to an increase in the number of leaf vessels (Table 2). Interestingly, the increase in the number of vessels was observed only in the small diameter classes (Fig. 6B), maybe as a conservative measure to avoid hydraulic failure and reducing the probability of freezing-induced embolism. It should be noted that, despite sharing the same habitats, the Antarctic plant species display several differential responses to resist the same stressors (for the details of differential responses, see Xiong *et al.*, 2000; Bravo *et al.*, 2001; Pérez-Torres *et al.*, 2004a, b, 2007; Sáez *et al.*, 2018a; Sanhueza *et al.*, 2022), but with the same goal, to grow and reproduce in one of the most adverse climates on the planet.

According to the Antarctic Climate Change and the Environment report (Chown *et al.*, 2022), warmer temperatures will continue reducing snow cover duration, increasing plant exposure to sudden freezing events during the growing season. Under these conditions, changes to the leaf vasculature, such as those particularly observed in *C. quitensis*, may alter the susceptibility to freezing-induced cavitations. Narrower conducts require lower temperatures for ice nucleation (Sack *et al.*, 2004). In this sense, in a warming experimental field, Sierra-Almeida *et al.* (2018) reported that *C. quitensis* decreased its freezing resistance to a risky limit when grown at warmer temperatures. In the case of *D. antarctica*, however, only one of the three studied sites exhibited a slight reduction in freezing tolerance. Warming enhances the reproduction and growth of Antarctic vascular species (Cannone *et al.*, 2016; Sáez *et al.*, 2018a), but could reduce their survival ability, making them more susceptible to damage by freezing temperatures.

Coordination between leaf hydraulic conductivity and gas exchange

The coordination of K_{leaf} and A_{max} follows the assumption that leaves are the bottleneck of the plant hydraulic system, and that the stomatal conductance is the main determinant for photosynthesis (Brodribb *et al.*, 2007). The ability to keep the stomata open depends on the plant's capacity to replace the water lost through stomata. Hence, the whole plant's hydraulic conductivity should match g_s to maximize photosynthesis (Xiong and Flexas, 2022). Thus, at low temperatures, when the soil water availability is reduced, both Antarctic species seem to adopt a safety scheme maintaining a high stomatal resistance, at the cost of low leaf hydraulic conductivity and decreased photosynthetic rate. At higher growth temperatures, g_s, K_{leaf}, and A_N increase, with positive correlations among these parameters, consistent with data previously described for other plant species (Nardini and Saleo, 2003; Brodribb *et al.*, 2005; Franks, 2006; Xiong *et al.*, 2017).

Values of K_{leaf} in Antarctic species are relatively low (Supplementary Fig. S3) considering their A_N (Fig. 7), especially in *D. antarctica*, since it presents low K_{leaf} values but notably high values for A_N. These two traits are coupled due to the effect of K_{leaf} on g_s and therefore, the CO_2 uptake (Brodribb *et al.*, 2005; Xiong and Flexas, 2022). However, in Antarctic plants, stomatal opening and, especially, g_m is limited by a leaf structure intended to prevent water loss in an arid environment. Under these conditions, Antarctic plants have evolved towards a highly specific Rubisco for CO_2 with no penalty for photosynthetic rate despite a low total conductance (Sáez *et al.*, 2017), depending to a lesser extent on K_{leaf} to achieve positive rates of CO_2 assimilation.

K_{leaf} and g_m are determined by leaf anatomical traits, among them the cell wall thickness, the surface area of the water vapor transmission of the mesophyll cells (S_m), and the area between cells (f_{ias}) (Xiong *et al.*, 2017). The Antarctic plants showed, in general, low values of these traits when grown at low temperatures, and increase them at higher growth temperature (Sáez *et al.*, 2018a), supporting the positive correlation between K_{leaf} and g_m. In addition, we found a negative correlation between K_{leaf} and LD (Fig. 5). In both Antarctic species, LD strongly correlates with LMA (Supplementary Fig. S1), suggesting an intrinsic reduction in tissue density with the increase in growth temperature. It is likely that the higher growth temperature, which reduces LMA and LD, induces an increase in the leaf area for carbon fixation. Thus, the increase in K_{leaf} ensures a large amount of water is transported to leaves for transpiration to maintain open stomata and to the whole plant, achieving higher photosynthetic rates.

Concluding remarks

This study provides novel insights about the hydraulic properties of the two Antarctic vascular plants and the acclimation of photosynthesis- and water transport-related traits to different growth temperatures. Our results suggest that increases in growth temperature have significant effects on the leaf and whole plant hydraulic conductivity, correlating with adjustments in carbon assimilation. These adjustments are consistent with anatomical changes at the vascular leaf level, where the two Antarctic species display different strategies to support the increase in K_{leaf}. At higher growth temperatures, *C. quitensis* displays a decrease in the number of leaf vessels but with higher vessel diameter. In contrast, in *D. antarctica* the diameter does not change, but the number of vessels increases at higher growth temperatures. As compared with other angiosperms, the vessel diameters of both Antarctic species are remarkably small, which combined with other traits, such as the rigidity of cell wall, constitutes a water conservation mechanism associated with their ability to cope with the harsh Antarctic environment.

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. The relationship between the leaf mass area (LMA) and the leaf density (LD) for *Colobanthus quitensis* and *Deschampsia antarctica*.

Fig. S2. The relationship between the osmotic potential at full turgor (π_o) and the leaf water potential at the turgor loss point (π_{tlp}) for Antarctic plants and dataset from Bartlett *et al.* (2012).

Fig. S3. The leaf hydraulic conductivity and the bulk of elasticity *vs.* leaf mass area of Antarctic plants (data for other Angiosperms from Nadal *et al.*, 2018).

Fig. S4. The stomatal density and the stomatal size for *Colobanthus quitensis* and *Deschampsia antarctica*.

Table S1. The leaf photosynthetic parameters for *Colobanthus quitensis* and *Deschampsia antarctica*.

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Author contributions

PLS conceived of the study and wrote the article; VV, CR, PLS, and DSK performed the measurement and data analysis. PLS, LAC, JPP, DSK, EGP, LAB, and JG carried out the interpretation of the data and reviewed the manuscript.

Conflict of interest

The authors declare they have no conflicts of interest.

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Data availability

Data will be shared on request to the corresponding author.

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