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EFECTOS DEL CALENTAMIENTO EN LAS PROPIEDADES HIDRÁULICAS FOLIARES DE PLANTAS ANTÁRTICAS Y SU INFLUENCIA EN EL RENDIMIENTO FOTOSINTÉTICO: UN ANÁLISIS INTEGRADOR PARA PREDECIR POSIBLES EFECTOS DEL CAMBIO CLIMÁTICO

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TABLA DE CONTENIDO

ÍNDICE DE TABLAS

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

ÍNDICE DE ILUSTRACIONES

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

RESUMEN

Los modelos de cambio climático predicen una tendencia general de calentamiento en las regiones polares, con una mayor variabilidad climática, incluyendo alteraciones en los patrones de precipitación y una mayor frecuencia y duración de los ciclos de congelación y descongelación. Estos cambios ya son evidentes en la Antártica, incrementado el interés por entender los impactos biológicos en los ecosistemas terrestres antárticos, y especialmente en las dos únicas especies de plantas vasculares de la Península Antártica: *Deschampsia antarctica* Desv. (Poaceae) y *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae). Bajo estos escenarios de cambio climático, las plantas deben aclimatarse para asegurar su supervivencia, lo que implica, entre otras cosas, equilibrar el transporte de agua y el intercambio de gases a nivel foliar. Este equilibrio depende de varios ajustes que incluyen la morfoanatomía, fisiología y bioquímica de las hojas. En este contexto, surge la pregunta: ¿Cómo se ajustan las propiedades hidráulicas foliares de las especies vasculares antárticas frente a incrementos de temperatura y ciclos de congelación-descongelación, y cómo influyen estos ajustes en el intercambio de gases? Para responder a esta pregunta, se establecieron diferentes condiciones experimentales, tanto en condiciones controladas de laboratorio como en campo, realizando evaluaciones de rasgos hidráulicos y de intercambio de gases, además de análisis anatómicos estructurales y ultraestructurales. Los resultados indican que las plantas antárticas tienen una estrecha interdependencia entre el rendimiento hidráulico y fotosintético ante variaciones de temperatura y ciclos de congelación-descongelación, con ajustes anatómicos específicos en las vías xilemáticas y extra-xilemáticas.

ABSTRACT

Climate change models predict a general warming trend in polar regions, with increased climate variability, including alterations in precipitation patterns and more frequent and prolonged freeze-thaw cycles. These changes are already evident in Antarctica, increasing the interest in understanding the biological impacts on Antarctic terrestrial ecosystems, particularly on the only two vascular plant species in the Antarctic Peninsula: *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae). Under these climate change scenarios, plants must acclimate to ensure their survival, which involves, among other things, balancing water transport and gas exchange at the leaf level. This balance depends on various adjustments including leaf morphoanatomy, physiology, and biochemistry. In this context, the question arises: How do the foliar hydraulic properties of Antarctic vascular species adjust to temperature increases and freeze-thaw cycles, and how do these adjustments influence gas exchange? To answer this question, different experimental conditions were established, both in controlled laboratory conditions and in the field, conducting evaluations of hydraulic and gas exchange traits, along with structural and ultrastructural anatomical analyses. The results indicate that Antarctic plants have a close interdependence between hydraulic and photosynthetic performance in response to temperature variations and freeze-thaw cycles, with specific anatomical adjustments in xylem and extra-xylem pathways.

INTRODUCCIÓN GENERAL

La Antártida marítima y la Península Antártica se encuentran entre las regiones que han experimentado las más evidentes tendencias de calentamiento a nivel global (Turner *et al*., 2021, Chown *et al*., 2022). Lo anterior, se ha manifestado en un importante retroceso de glaciares y plataformas de hielo, lo que, sumado a temporadas de crecimiento más largas, temperaturas más altas y mayor frecuencia de lluvias, han proporcionado nuevas áreas disponibles para la colonización y propagación de poblaciones de plantas (Colesie *et al*., 2022). En consecuencia, se han informado aumentos tanto en el tamaño y número de poblaciones de las dos únicas especies de plantas vasculares que crecen en forma natural en la Antártica: *Deschampsia antarctica* Desv. (Poaceae) y *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) (Torres-Mellado *et al.*, 2011, Cannone *et al.*, 2016; 2021; 2022). Estos cambios a nivel poblacional se han asociado con una mayor capacidad reproductiva de ambas especies (Fowbert and Smith, 1994), así como con un mayor rendimiento fotosintético (Sáez *et al.*, 2017; 2018a; b; 2019). En relación con la fotosíntesis, Sáez y cols. profundizaron en los mecanismos implicados en su regulación, incluyendo las limitaciones anatómicas y bioquímicas, así como sus respuestas a diversas condiciones ambientales. En base a esto, se determinó que, bajo condiciones de campo, la asimilación de carbono (A_N) está fuertemente limitada por la conductancia del CO_2 a través del mesófilo foliar (g_m**)** (Sáez *et al.*, 2017). Esta limitación difusiva está asociada a las características fuertemente xerofíticas de las hojas, esto es: alta masa foliar por área (LMA) y alta densidad foliar (LD), permitiéndoles enfrentar el severo clima antártico, marcado por fuertes vientos, bajas temperaturas y radiación variable (Cavieres *et al.*, 2016). Para aliviar esta restricción en la difusión del CO_2 hacia los sitios de carboxilación en los cloroplastos, la enzima ribulosa-1,5-bisfosfato carboxilasa/oxigenasa (Rubisco) de las especies antárticas muestra una alta especificidad por el CO_2 ($S_{c/o}$) y altas tasas de recambio catalítico (k^{catc}) (Sáez *et al*., 2017).

Al evaluar el efecto del calentamiento *in situ* mediante el uso de cámaras de calentamiento pasivo tipo OTC, se observó que las especies antárticas responden diferencialmente a las condiciones más cálidas (Sáez *et al*., 2018a). *C. quitensis* ajusta su anatomía (menor LMA y LD), bioquímica (menor contenido de fibras) y ultraestructura (mayor proximidad de los cloroplastos a la pared celular y mayor superficie de mesófilo expuesta a espacios aeríferos), facilitando la transferencia de CO_2 de la hoja, lo que incrementa la g_m y A_N, promoviendo así una mayor ganancia de carbono y crecimiento de las plantas. Por el contrario, *D. antarctica* no muestra respuestas significativas al calentamiento en los parámetros estudiados por Sáez y cols. (2018a). Sin embargo, en condiciones de laboratorio, con mayores incrementos de temperatura (> 10°C), *D. antarctica* responde de manera similar a *C. quitensis* (Sáez *et al*., 2018b).

Dado que los cambios en la asimilación de carbono están directamente relacionados con cambios anatómicos foliares, y sabiendo que el CO_2 y el agua comparten parcialmente rutas de movilización en el mesófilo foliar (Sack and Holbrook, 2006; Flexas *et al*., 2013), Sáez y cols. propusieron que los ajustes anatómicos también se acompañan de modificaciones en el flujo de agua foliar en las especies vasculares antárticas.

Las temperaturas más cálidas pueden provocar un mayor déficit de presión de vapor, una mayor demanda de evaporación y mayores tasas de transpiración (McCulloh *et al.*, 2019), alterando el estado hídrico de las hojas. En consecuencia, si el sistema hidráulico no reemplaza suficiente agua, el mesófilo se desecaría y los estomas se cerrarían (Sack & Holbrook 2006). Los estudios sobre la hidráulica de las plantas han demostrado que el xilema puede ajustar su estructura a las variaciones ambientales mediante modificaciones del tamaño, número y distribución de los vasos (Hacke *et al.*, 2006). Según la ley de Hagen-Poiseuille, un ligero incremento en el diámetro de los vasos xilemáticos conlleva a un considerable aumento en la conductividad hidráulica, dado que la conductancia se incrementa exponencialmente a la cuarta potencia del diámetro del vaso (Tyree and Ewers, 1991). Varios estudios confirman que, bajo un régimen más cálido, las plantas tienen vasos más grandes y densos que permiten un mayor transporte de agua (Medek *et al.*, 2010, Hu *et al.*, 2014, He *et al.*, 2020). Si bien, reducir las pérdidas de agua es beneficioso en un ambiente seco y ventoso como el de la Antártica, mantener el flujo de agua a través de la planta es crucial para preservar su estado hídrico y permitir la asimilación de carbono y nutrientes. En general, en las especies que prosperan en ambientes fríos o secos, el diámetro promedio de los vasos conductores tiende a ser menor, ya que las heladas y la sequía aumentan el riesgo de cavitación, y los vasos más pequeños son más resistentes (Davis *et al.*, 1999; Lütz, 2010). Esta reducción en el tamaño de los vasos puede compensarse mediante un aumento en la densidad de ellos, lo que ayuda a mantener la conductividad hidráulica frente potenciales hídricos decrecientes, aumentando así la seguridad del xilema (García-Cervigón *et al.*, 2020). Debido a que la temperatura tiene efectos importantes sobre la anatomía de la hoja y, por lo tanto, sobre la eficiencia hidráulica foliar y de la planta completa, es esencial investigar cómo ésta influye en las propiedades hidráulicas de las especies vasculares antárticas.

En base a lo anteriormente expuesto, el primer capítulo de esta tesis se centró en investigar las propiedades hidráulicas y fotosintéticas de las especies antárticas en respuesta al aumento de temperatura (5, 8 y 15 °C) bajo condiciones controladas de laboratorio. En este capítulo, se planteó como hipótesis que: las modificaciones anatómicas foliares inducidas por el calentamiento en especies vasculares antárticas incrementan la conductividad hidráulica foliar. Este capítulo contribuyó significativamente a nuestro conocimiento sobre las propiedades hidráulicas de las especies antárticas y su respuesta frente a incrementos simulados de temperatura, abriendo paso a una nueva pregunta de investigación: ¿Cómo responden los rasgos hidráulicos de las especies antárticas a cambios en las condiciones ambientales *in situ*? Esta pregunta es especialmente relevante debido a los ajustes anatómicos específicos de las hojas descritos por Sáez y cols. (2018a), particularmente en *C. quitensis*. Para abordar este punto, el segundo capítulo de esta tesis investigó entonces las propiedades hidráulicas y fotosintéticas de las especies antárticas bajo condiciones experimentales de calentamiento *in situ*, proponiendo la siguiente hipótesis: las respuestas hidráulicas de las plantas vasculares antárticas al calentamiento *in situ* dependen de modificaciones en la anatomía del xilema foliar, y estas respuestas están coordinadas con el intercambio gaseoso foliar.

Además de los escenarios de calentamiento ya reportados para la Península Antártica (Siegert *et al.*, 2023; Chown *et al.*, 2022), los modelos de cambio climático anticipan un incremento en la frecuencia de los ciclos de congelación y descongelación en las regiones polares (IPCC, 2019; 2022; Liang *et al*., 2022). Esto plantea una nueva interrogante: ¿Cómo responderán las especies vasculares antárticas si el aumento en las temperaturas de crecimiento se combina con ciclos de congelación y descongelación sucesivos? Para abordar esta pregunta, el tercer capítulo de esta tesis evaluó en laboratorio el efecto de la temperatura de crecimiento (5 y 15 °C) y ciclos sucesivos de congelación y descongelación sobre las propiedades hidráulicas y fotosintéticas, planteando la siguiente hipótesis: el rendimiento hidráulico y fotosintético de las plantas vasculares antárticas se verá menos afectado por los ciclos repetidos de congelación y descongelación cuando crecen a temperaturas más altas en comparación con aquellas que crecen a temperaturas más bajas.

Para probar las hipótesis planteadas, se evaluaron una serie de rasgos hidráulicos (conductividad hidráulica foliar y de planta completa), fotosintéticos (tasa de asimilación de CO_2, conductancia estomática y conductancia del mesófilo) y morfoanatómicos asociado a los vasos xilemáticos y del mesófilo foliar, así como la covariación entre los rasgos hidráulicos y fotosintéticos.

CAPÍTULO I: 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} PhostrogenR (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 (Saez *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 (Saez *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.

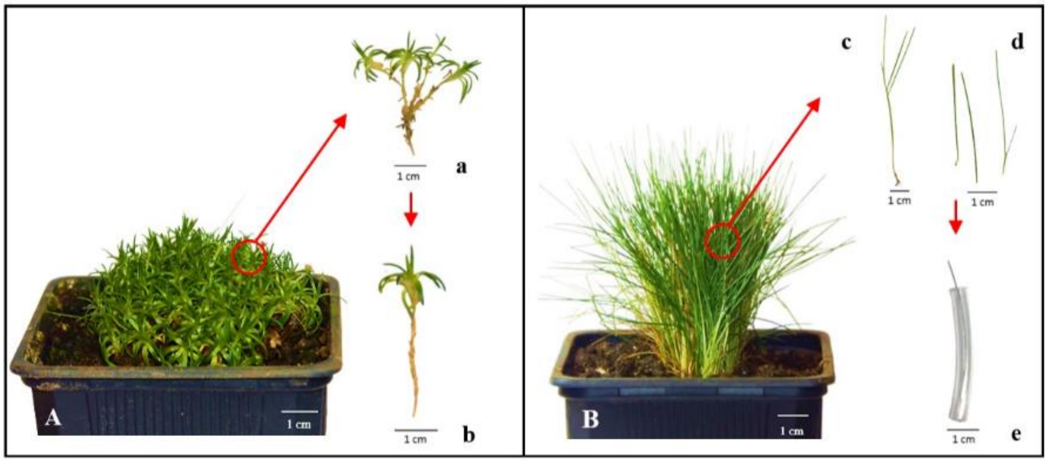

Figure 1. The Antarctic vascular species *Colobanthus quitensis* (A) and *Deschampsia antarctica* (B). Plant material used to perform the measurements of hydraulic properties: shoots (a) and rosettes (b) of *C. quitensis*; and leaves (c), tillers (d), and a leaf in a flexible silicone tube (e) of *D.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

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 $(W_f – W_{dry})/(W_{sat} – W_{dry})$. 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 (ε_{max}).

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:

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

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:

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

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. *C. 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):

$$K_h = \Sigma\ ((d_i{}^4 \pi \rho) / (128 \eta_w)),$$

where di 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):

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

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 ϕPSII represents the number of electrons transferred per photon absorbed by PSII, the electron transport rate (ETR) can be calculated as: ETR = $\phi PSII \times PPFD \times \alpha \times \beta$, 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):

$$g_m = A_N / (C_i - (\Gamma (ETR + 8 (A_N + R_L)) / (ETR - 4 (A_N + R_L)))) ,$$

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

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, nonsignificant 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).

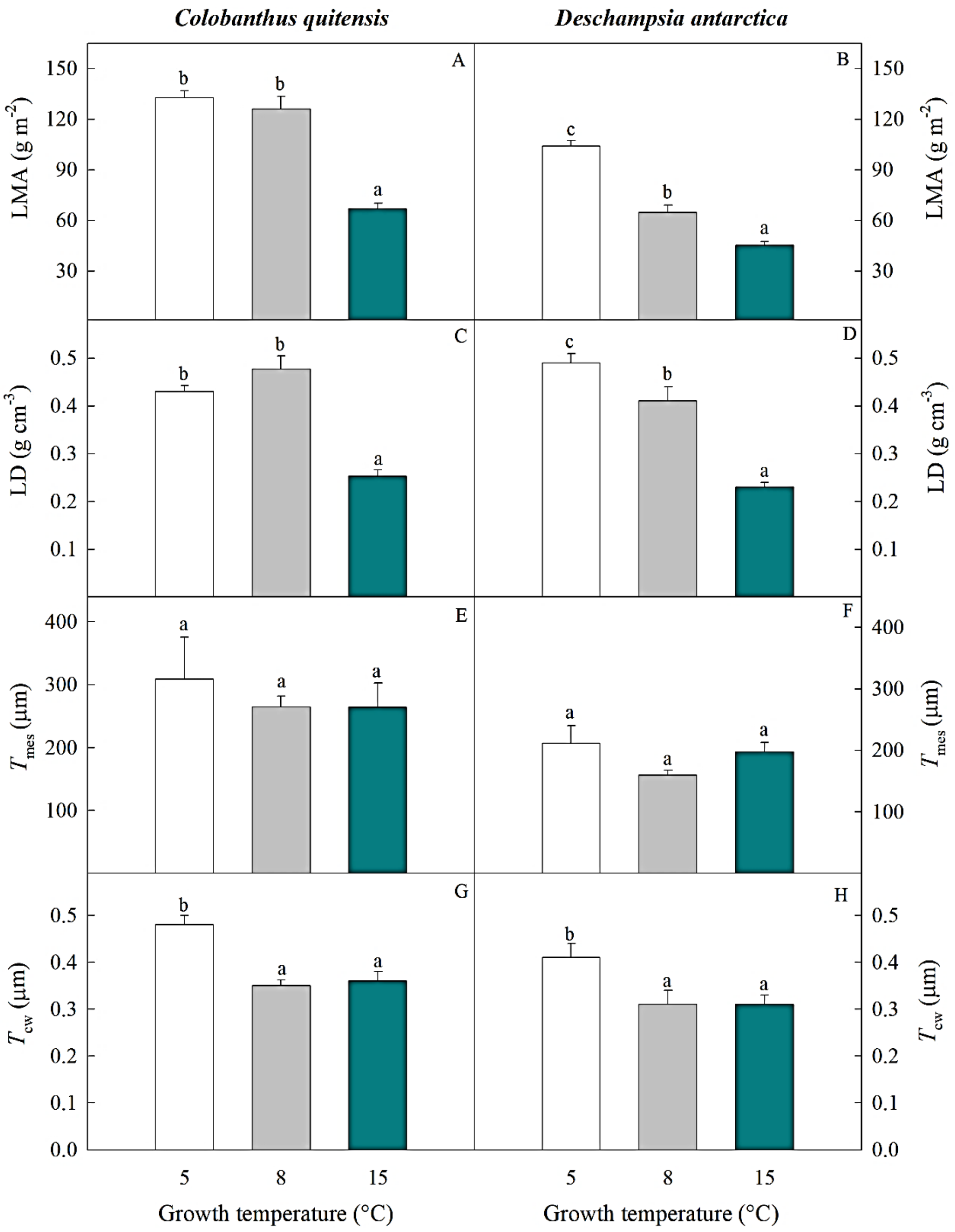

Figure 2. Temperature response of anatomical traits in Antarctic vascular plants: leaf mass area (LMA), leaf density (LD), mesophyll thickness (T_{mes}) and cell wall thickness (T_{cw}) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and

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 ($P < 0.05$).

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.

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

Parameters	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 ($P < 0.05$)

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).

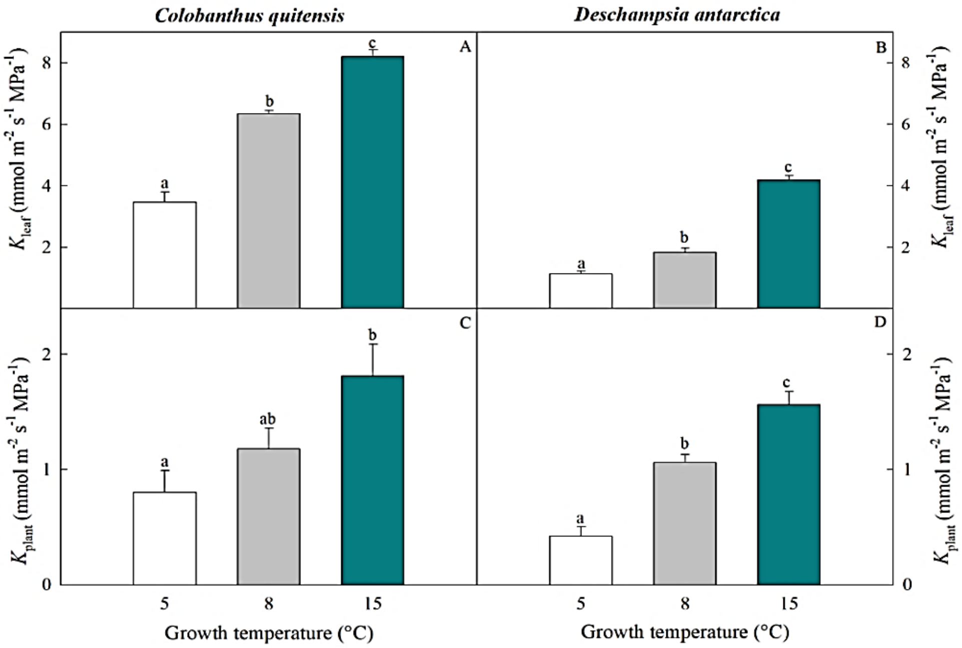

Figure 3. The leaf hydraulic conductivity (K_{leaf}) and the whole plant hydraulic conductivity (K_{plant}) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Values are means ±SE (n = 6–9 and n = 4–8, for K_{leaf} and K_{plant}, respectively). For each species,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

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).

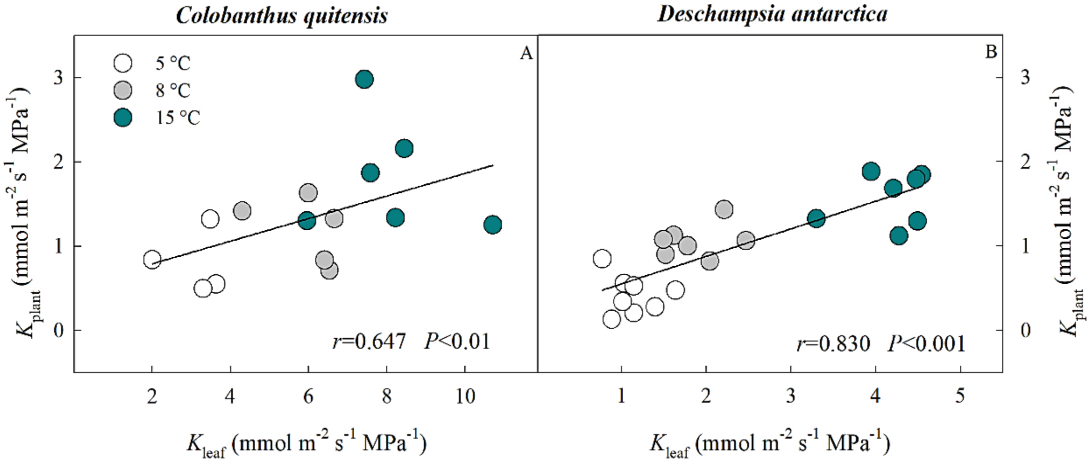

Figure 4. The relationship between the leaf hydraulic conductivity (K_{leaf}) and the whole plant hydraulic conductivity (K_{plant}) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Pearson's correlation coefficient and the

significance of the relationship are shown for each species considering all growth temperatures together.

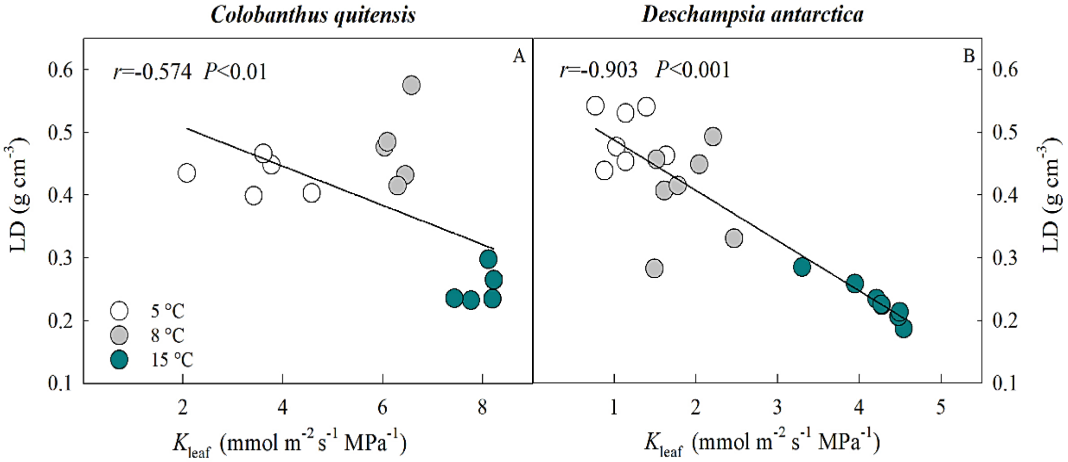

Figure 5. The relationship between leaf hydraulic conductivity (K_{leaf}) and leaf density (LD) for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. Pearson's correlation coefficient and the significance of the relationship are shown for each species considering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

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).

Table 2. The number of 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 °C, 8 °C and 15 °C. Values are means ± SE (*n* = 4-6). For each species, different letters indicate statistically significant differences among growth temperatures, according to Tukey ($P < 0.05$).

Parameters	5 °C	8 °C	15 °C
	Colobanthus quitensis		
N° 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		
N° vessels	8.64 ± 1.19a	11.56 ± 2.04ab	12.33 ± 2.93b
D_h (µm)	5.87 ± 0.61a	6.02 ± 0.59a	5.6 ± 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

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 (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).

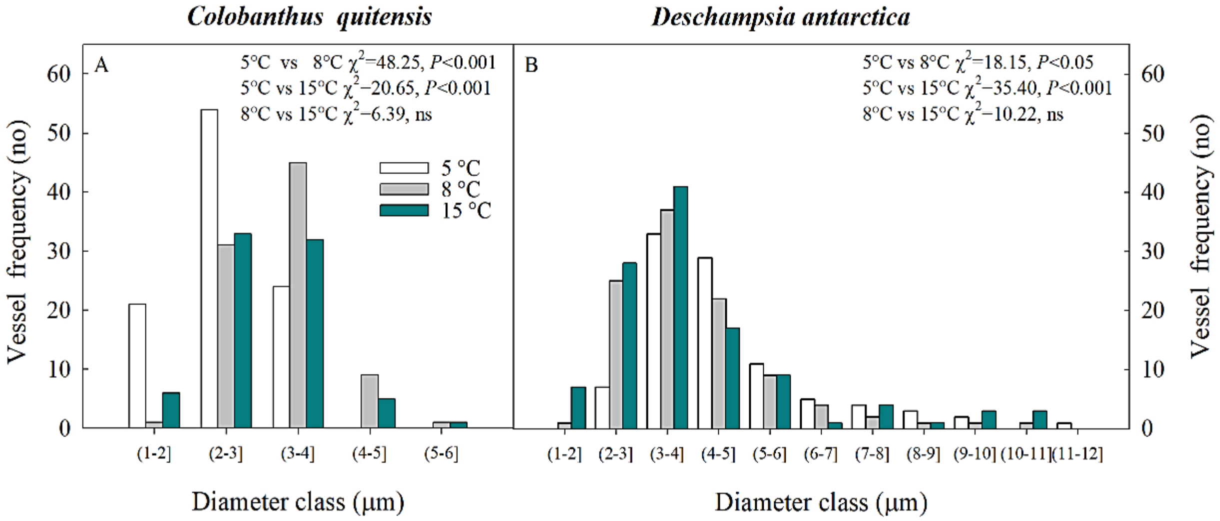

Figure 6. Frequency distribution of leaf xylem vessels diameters for *Colobanthus quitensis* and *Deschampsia antarctica* growing at 5 °C, 8 °C, and 15 °C. For each species, statistically significant differences in distribution among temperature treatments were analysed according to the χ^2 test ($P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

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.

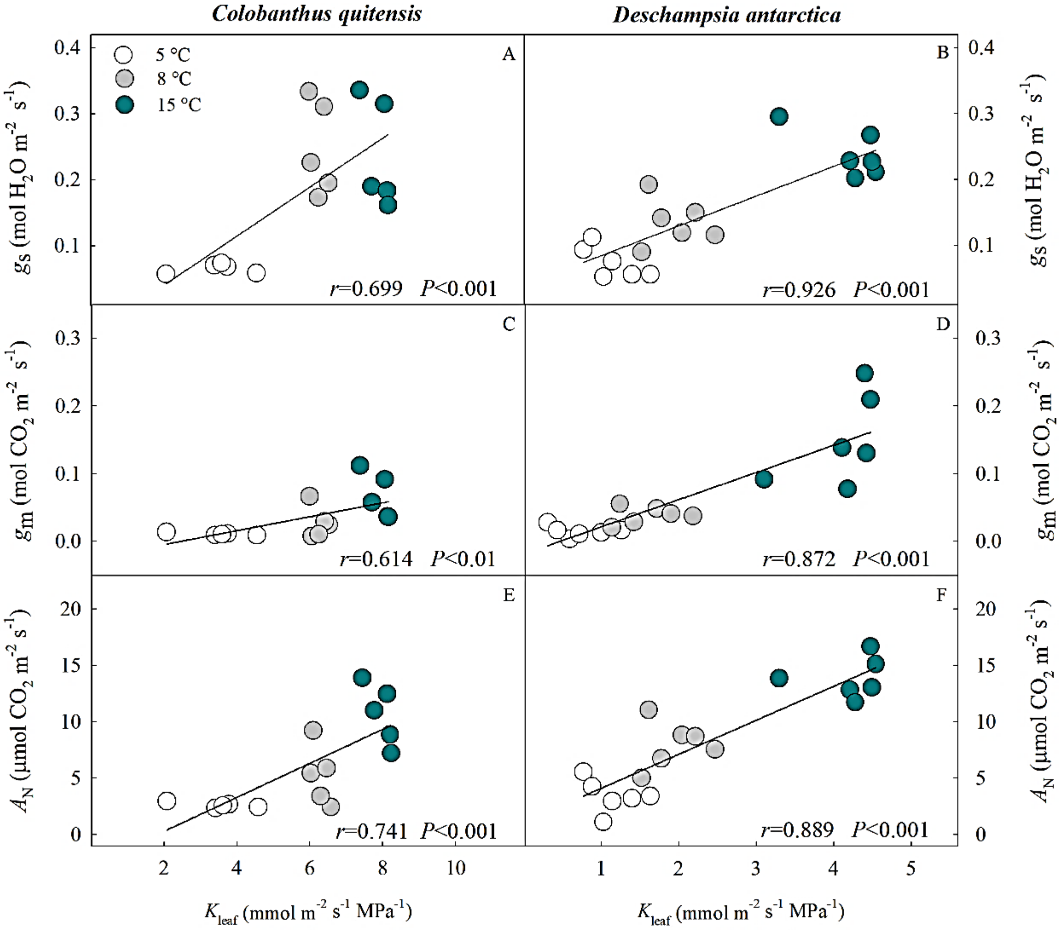

Figure 7. The relationship between the leaf hydraulic conductivity (K_{leaf}) with the stomatal conductance (g_s), the mesophyll conductance (g_m), and the net CO_2 assimilation rate (A_N) for Colobanthus quitensis and Deschampsia antarctica growing at 5 °C, 8 °C,

and 15 °C. Pearson's correlation coefficient and the significance of the relationship are shown for each species considering all growth temperatures together.

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 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 temperaturas (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 los point (π_{tlp}) (Supplementary Fig. S2). However, within each Antarctic species, we observed an inverse relationship between πo and π_{tlp}. Thus, changes in π_{tlp} with increased warming might not be associated with changes in πo. In this sense, πo 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). 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 C4-type anatomical specializations (Sonawane *et al.*, 2021). It has been described that both Antarctic species, despite being C3 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).

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 gs 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 gs 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.

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Anexo

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

Parameters	5 °C	8 °C	15 °C
		Colobanthus quitensis	
A_N (µmol CO_2 m^{-2} s^{-1})	2.60 ± 0.10a	5.29 ± 1.18a	10.69 ± 1.20b
g_s (mol H_2O m^{-2} s^{-1})	0.07 ± 0.00a	0.25 ± 0.03b	0.24 ± 0.04b
g_m (mol CO_2 m^{-2} s^{-1})	0.01 ± 0.00a	0.03 ± 0.01ab	0.07 ± 0.02b
C_c (µmol CO_2 mol^{-1} air)	95.04 ± 4.51a	115.62 ± 25.24a	154.19 ± 23.28a
V_{cmax} (µmol CO_2 m^{-2} s^{-1})	10.10 ± 0.75a	20.89 ± 3.22a	39.51 ± 5.51b
R_{dark} (µmol CO_2 m^{-2} s^{-1})	-1.06 ± 0.07a	-1.56 ± 0.06a	-1.76 ± 0.35a
		Deschampsia antarctica	
A_N (µmol CO_2 m^{-2} s^{-1})	3.43 ± 0.60a	7.96 ± 0.84b	13.89 ± 0.73c
g_s (mol H_2O m^{-2} s^{-1})	0.07 ± 0.01a	0.14 ± 0.01b	0.24 ± 0.02c
g_m (mol CO_2 m^{-2} s^{-1})	0.02 ± 0.00a	0.04 ± 0.01a	0.15 ± 0.03b
C_c (µmol CO_2 mol^{-1} air)	72.27 ± 7.97a	80.30 ± 5.52a	183.68 ± 11.57b
V_{cmax} (µmol CO_2 m^{-2} s^{-1})	18.90 ± 2.06a	48.50 ± 2.15b	44.77 ± 2.22b
R_{dark} (µmol CO_2 m^{-2} s^{-1})	-1.17 ± 0.09b	-1.14 ± 0.16b	-1.84 ± 0.24a

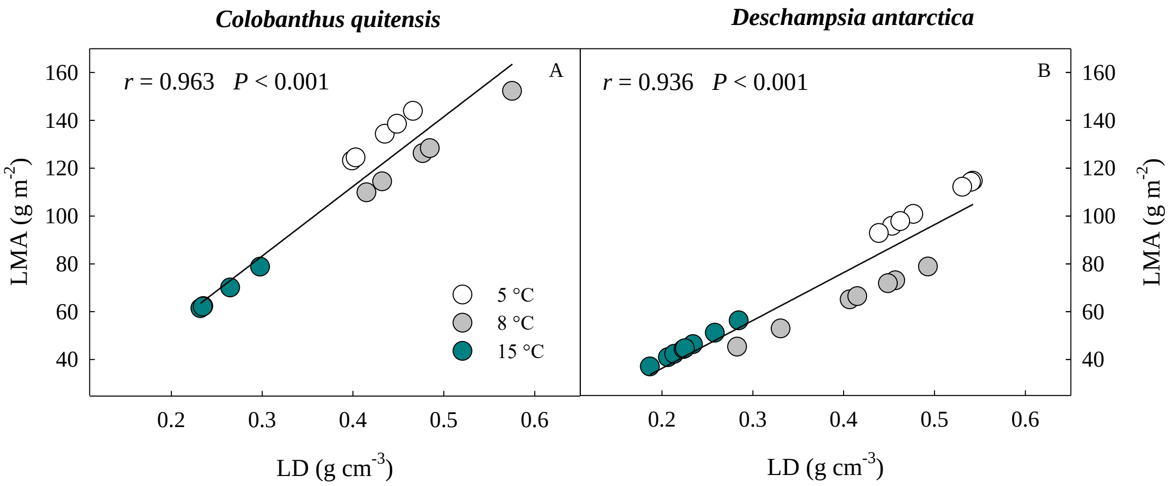

Figure S1. The relationship between the leaf mass area (LMA) and the leaf density (LD) for *Colobanthus quitensis* and *Deschampsia antarctica*.

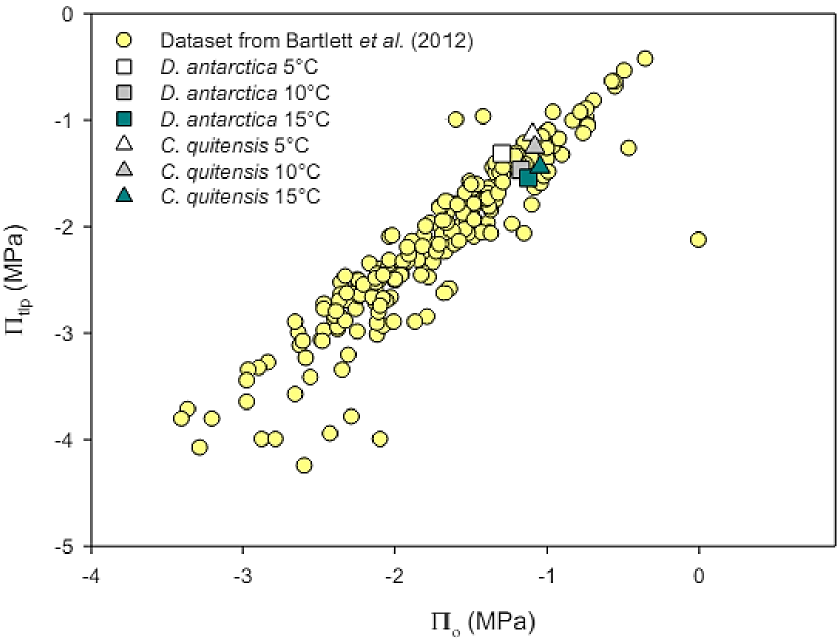

Figure S2. The relationship between the osmotic potential at full turgor (π_o) and the leaf water potential at the turgor loss point (π_{tlp}) for Antarctic plants and dataset from Bartlett *et al.* (2012).

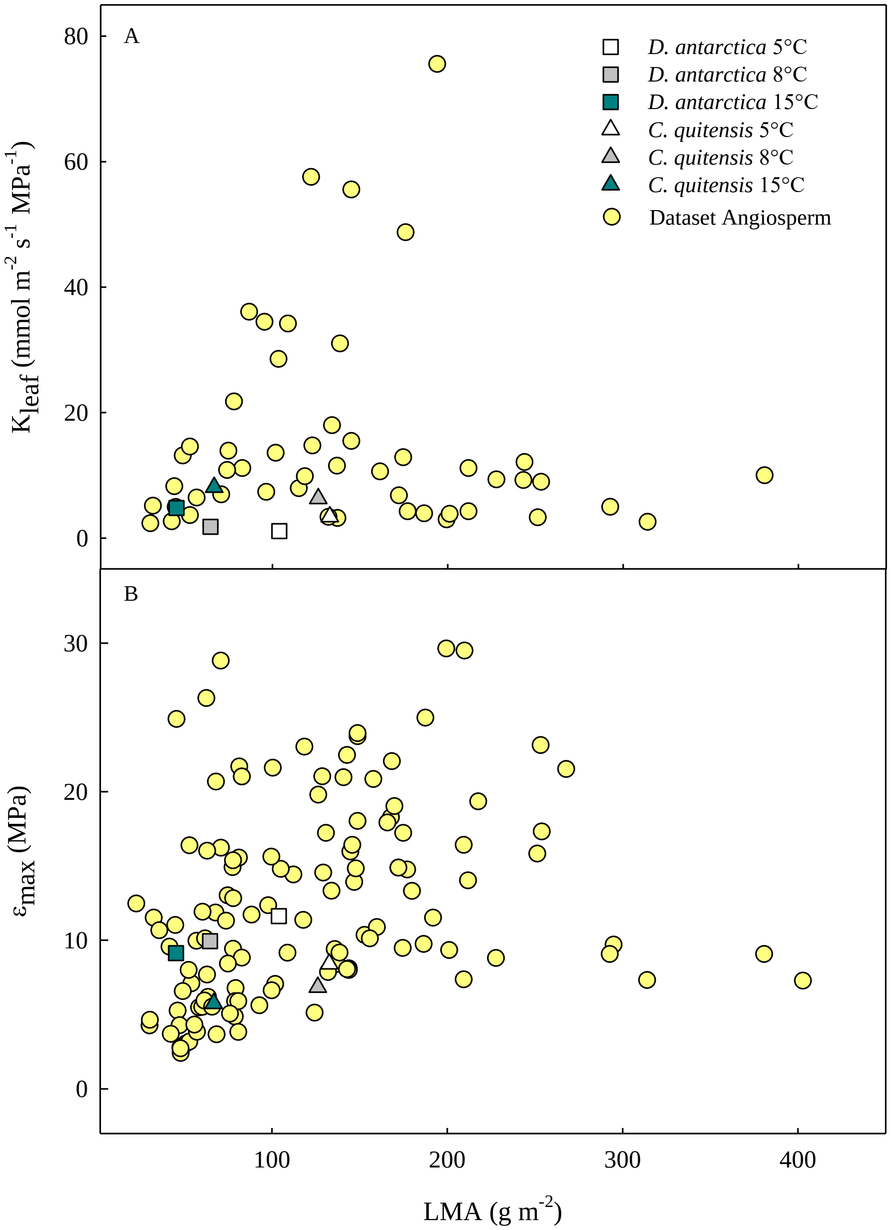

Figure S3. The leaf hydraulic conductivity and the bulk of elasticity *vs*. leaf mass area of Antarctic plants. Data for other Angiosperms from Nadal *et al.* (2018).

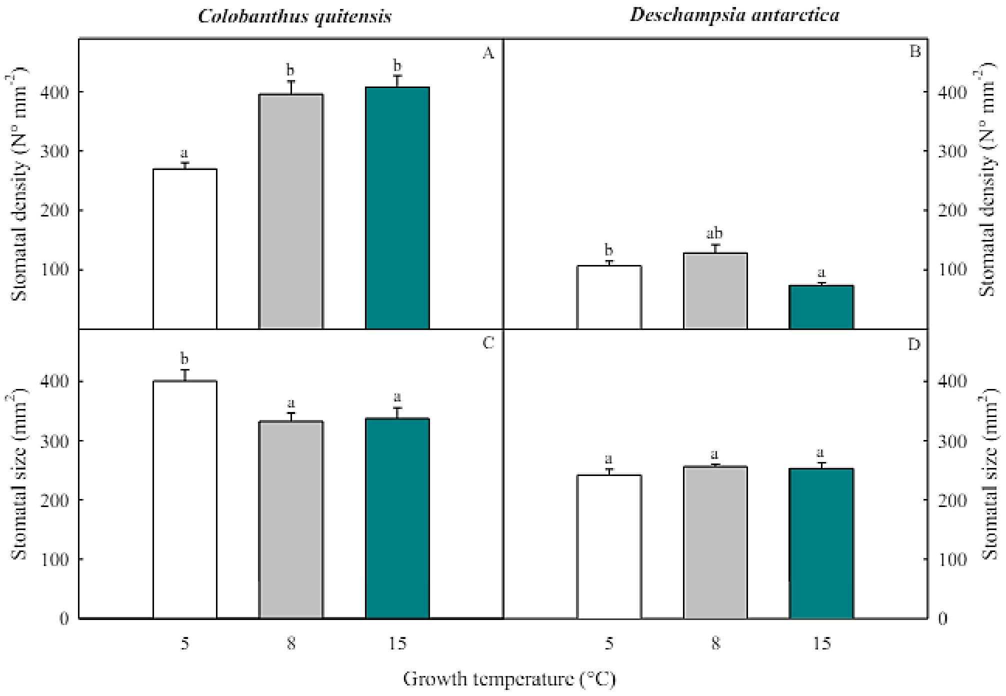

Figure S4. The stomatal density and the stomatal size for *Colobanthus quitensis* and *Deschampsia antarctica.*

CAPÍTULO II: Leaf hydraulic responds differentially between Antarctic vascular plant species to *in situ* environmental changes, but always in a coordinated way with photosynthesis

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Abstract

The hydraulic efficiency of Antarctic vascular plants and their interaction with photosynthetic performance under *in situ* environmental changes remain unexplored. We evaluated both processes *in situ* for plants growing under natural conditions (OAs) and with continuous passive warming (OTCs). In OAs, both species showed hydraulic conductivity within the range of herbaceous plants and with the lowest values of leaf diameter vessels. After seven years in OTCs, hydraulic and photosynthetic responses showed divergence between species. This could be due to the OTCs experiencing more hours below freezing than expected, negatively affecting *Deschampsia antarctica* but favoring *Colobanthus quitensis*. Regarding this, each species showed specific adjustments in morphoanatomy (leaf and vascular) and water traits, which support the co-variation between both processes. From co-variation results, two key findings emerged (i) outside-xylem modulation leading to downregulation in *D. antarctica,* while (ii) the modulus of elasticity supported better water transport and CO_2 diffusion in *C. quitensis*. Additionally, *D. antarctica* showed higher photosynthetic responsiveness over time than the initial three years in OTCs, but its overall photosynthetic performance declined. This decline may be due to a senescence program (yellow leaves) triggered by freeze events, allowing nutrient recycling to generate new leaves under improved conditions. Conversely, *C. quitensis* consistently maintained higher photosynthesis in OTCs over time, likely benefiting from cumulative positive effects on leaf and vascular anatomy. In addition, their cushion shape could help conserve higher temperatures inside the plant and mitigate the negative impacts of freeze events on photosynthetic and hydraulic performance. Overall, both species adjust their physiological processes based on environmental conditions, which is crucial for survival in extreme habitats.

Keywords: Antarctic vascular plants, *in situ* environmental changes, hydraulic conductivity, photosynthetic performance.

Introduction

The warming trend in the Antarctic Peninsula has been reported as the second fastest on Earth during the last century, with temperature records in 2015 and 2020 (Turner *et al.*, 2021; Gorodetskaya *et al.*, 2023; Siegert *et al.*, 2023). Several reports have indicated that the increase in the number and size of the populations of vascular plants in Antarctica is the product of this accelerated warming (Parnikoza *et al.*, 2009; Torres-Mellado *et al.*, 2011; Cannone *et al.*, 2016; 2022). In a detailed comparison of the cover and number of localities of both Antarctic species in the Argentine Islands, it was reported that between 1960 and 2009, the Antarctic hair grass (*Deschampsia antarctica* É. Desv., Poaceae) increased its cover and number of localities by 191% and 104%, respectively. By contrast, the Antarctic pearlwort (*Colobanthus quitensis* (Kunth) Bartl, Caryophyllaceae) showed increases of 208% and 35% in its cover and number of localities, respectively (Cannone *et al.*, 2016). Thus, the only two native flowering Antarctic species responded quite differently to the increase in temperature; while warming promotes *D. antarctica* colonization rather than its growth, and hence cover, the opposite is observed in *C. quitensis*.

Different responses have also been observed under *in situ* warming experiments. In a short-term study (two growing seasons), Day *et al.* (1999) showed that the vegetative growth of *C. quitensis* increased with warming, whilst in *D. antarctica* it decreased. A greater above ground biomass was also found in *C. quitensis* after four growing seasons with warmer conditions in the Antarctic Peninsula, but non-significant changes were detected in *D. antarctica* (Day *et al.*, 2008). Above ground tissue had a greater C/N ratio under warmer conditions, and water use efficiency was greater in both species (Day *et al.*, 2008). Warming has an accumulative effect and, in the second growth season, improved sexual reproduction in both species, increasing the number of seeds by 20 and 15% for *C. quitensis* and *D. antarctica*, respectively (Day *et al.*, 1999). Sáez *et al.* (2018a) also observed contrasting responses between the Antarctic species after growing for three years with *in-situ* warming conditions exerted by open top chambers (OTCs). For instance, *C. quitensis* growing inside OTCs increased the net photosynthesis (A_N), dark respiration (R_{dark}), and growth due to an improvement in leaf carbon gain, where A_N was comparatively more favored by temperature than R_{dark}. Changes in A_N were strongly determined by changes in the diffusive and biochemical determinants (mesophyll conductance and Rubisco carboxylation rate, respectively). On the other hand, no responses in those traits were observed in *D. antarctica* (Sáez *et al.*, 2018a). In the same experimental set-up, Sierra-Almeida *et al.* (2018) reported that *C. quitensis* particularly decreased the freezing resistance when growing in warmer conditions. On the other hand, *D. antarctica* exhibited a slight reduction in freezing tolerance, and only in one out of the three studied sites. It should be noted that plant responses to *in situ* manipulative experiments could be variable. It has been found that photosynthetic adjustments are relatively rapid and sensitive to warming (Kremers *et al.*, 2015), while adjustments in leaf morphology and anatomy seem to be less sensitive (Zhou *et al.*, 2019). In this sense, photosynthesis and anatomy variations could depend on the warming experiments' duration (Hudson and Henry, 2010; Schollert *et al.*, 2015; Zhou *et al.*, 2019).

The mesophyll conductance (g_m) constitutes the major limitation for the photosynthesis of Antarctic vascular plants, regardless of the provenance (Sáez *et al.*, 2017), temperature (Sáez *et al.*, 2018a; b), or water availability (Sáez *et al.*, 2019). However, an equally important constraint for photosynthesis is to keep leaf cell turgor and a continuous water flow to replace the water lost under the high evaporative demand generated for CO_2 uptake in the leaves. This process requires higher hydraulic efficiency at both the leaf (K_{leaf}) and whole plant (K_{plant}) levels. In a recent study, Sáez *et al.* (2024) observed that increases in growth temperature have significant effects on the hydraulics conductivities of both Antarctic vascular species, correlating with adjustments in A_N and vascular leaf traits, where both species display different strategies. At higher growth temperature, *D. antarctica* deploys a more conservative strategy, maintaining the leaf xylem vessel size, but increasing the number of them. On the contrary, *C. quitensis* displays a decrease in the number of leaf xylem vessels but with a significantly higher size, which is associated with a notable increase in A_N (Sáez *et al.*, 2024).

Recent studies have demonstrated a link between leaf hydraulic conductivity and various anatomical traits, including the mesophyll (S_m/S) and chloroplast (S_c/S) surface areas that face intercellular air spaces per leaf area, as well as the fraction of intercellular air space (f_{ias}) (Xiong *et al.*, 2017; Xiong and Nadal, 2020). These anatomical traits mainly contributed to understanding the coordination between g_m and K_{leaf}. Previous studies found that both Antarctic species, especially *C. quitensis*, adjusted S_m, S_c, and f_{ias} in response to *in situ* warming and increased temperatures in the laboratory. These observations suggest that the divergent responses in key leaf anatomical traits between *D.*

antarctica and *C. quitensis* may have significant implications for the water and CO_2 transport efficiency, a critical aspect for their survival in Antarctica and their adaptability to changing environmental conditions. In addition, both species have shown differences in their sclerophylly and cell wall chemical components in response to *in situ* warming, with direct consequences on g_m (Sáez *et al.*, 2018a). In part, contrasting pattern of g_m, could be related to differences in bulk tissue elasticity, with potential effects on water transport and CO_2 diffusion (Nadal *et al.*, 2018). On this basis, we provide the first field study on the main leaf hydraulic traits of the two Antarctic vascular plant species to investigate: 1) How do leaf hydraulic properties respond to *in situ* changes in environmental conditions? and 2) are these responses coordinated with photosynthesis? We hypothesized that 1) hydraulic responses of Antarctic vascular plants to *in situ* warming depends on modifications in the leaf xylem anatomy, and (2) hydraulic responses always occur in coordination with leaf gas exchange.

Materials and methods

Study site and microclimatic conditions

The study was carried out at King George Island, near the Henryk Arctowski Polish Antarctic Station (62° 9′49.15″S; 58°28′9.60″W), where *D. antarctica* and *C. quitensis* coexist (Kozeretska *et al.*, 2010; Cavieres *et al.*, 2016). Specifically, we used an *in situ* experiment installed in December 2012, where open top chambers (hereafter OTCs) and open areas as control plots (hereafter OAs) were assigned (see further details in Sáez *et al.*, 2018a). For each growing condition, air temperature at 5 cm above ground level was recorded every hour using HOBO®-U-30 Station (Onset Computer Co, Bourne, MA, USA). We analyzed the mean, maximum, and minimum daily air temperature (6:00 h to 20:00 h) (77 days distributed from 1 January 2018 to 15 February 2019). The number of hours below 0 °C was estimated considering the day and night. Additionally, we estimated growing degree days as in Sierra-Almeida *et al.* (2018). $GDD = (T_{max} + T_{min}) / 2 - T_{base}$, where T_{max} and T_{min} are the daily maximum and minimum temperatures, respectively, and T_{base} is the base temperature (5 °C in this case).

During the growing season 2019 (December 2018 to March 2019), 7 yrs. after OTCs installation, individuals of both Antarctic species growing in OAs and inside OTCs were randomly selected for leaf hydraulic and gas exchange measurements and leaf anatomical characterization. Prior to any hydraulic and photosynthetic measurement, plants collected in OAs and OTCs were immediately taken to the laboratory in the Antarctic Polish Station and placed in a room at 10 °C and 15 °C, respectively. This temperature was chosen to follow similar conditions used in Sáez *et al.* (2018a). Each collected plant was measured within 24 hours after collection.

Pressure-volume curves

To obtain pressure-volume (P-V) curves, six individuals were tested for each species and growing condition using the free-transpiration method (Corcuera *et al.*, 2002; Vilagrosa *et al.*, 2003), with minor adjustments for the Antarctic species (see Sáez *et al.*, 2024 for details). We used 20-30 leaves and rosettes for each species to perform the P-V curves from different tillers and shoots of *D. antarctica* and *C. quitensis*, respectively. First, well-watered plants were covered with plastic bags overnight to ensure full hydration. The next day, 3-4 series of 5-10 leaves and 1-3 rosettes per individual of *D. antarctica* and *C. quitensis*, respectively, were cut, weighed with an analytical balance (MS105U, accuracy ± 0.0001 g, Mettler-Toledo, Swiss) to obtain the water full saturated weight (W_{sat}), and allowed to dry slowly at room temperature with a dark plastic cover. Eventually, to obtain points of the P-V curve, one leaf or rosette was randomly selected during the dehydration process to record its leaf water potential (Ψ_{leaf}) and fresh weight (W_f). We measured Ψ_{leaf} using a pressure chamber (PM600, USA), obtaining values ranging from close to zero to 0.01 MPa. After the last record, plant material was 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 $(W_f\text{-}W_{dry})/(W_{sat}\text{-}W_{dry})$. Finally, P-V curves were plotted and analyzed to calculate 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 (π_{tlp}), apoplastic water content (a_f), and maximum bulk modulus of elasticity (ε_{max}).

Leaf hydraulic conductivity

Leaf hydraulic conductivity (K_{leaf}, mmol m^{-2} s^{-1} MPa^{-1}) was measured on six plants for species and growing conditions using the kinetic method of rehydration described by Brodribb and Holbrook (2003). Tillers with four to six leaves and shoots with three to four rosettes for *D. antarctica* and *C. quitensis* were collected to obtain water potential before and after rehydration. The time chosen for rehydration was the same as previously reported by Sáez *et al.* (2024). K_{leaf} was then calculated as:

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

where C (mol m^{-2} MPa^{-1}) is the leaf capacitance of each species, determined from the initial slope of the P-V curves initial slope normalized by leaf area (Tyree and Hammel, 1972; Brodribb and Holbrook, 2005), Ψ_0 and Ψ_f is the water potential before rehydration and after rehydration (MPa), respectively, and t is the rehydration time (s).

Whole plant hydraulic conductivity

Whole plant hydraulic conductivity (K_{plant}, mmol m^{-2} s^{-1} MPa^{-1}) was measured on six plants per species and growing condition, according to Brodribb and Cochard *et al.* (2009). Plants watered to field capacity were subjected to a gradual increase in light intensity over 3 hours, starting from 150 and reaching 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 weather stations (HOBO®-U-30, Onset Computer Co, Bourne, MA, USA). After 3 h light exposure, leaf transpiration rate (E, mmol m^{-2} s^{-1}) was measured using a portable photosynthesis system (Li-6400; LI-COR Inc., Lincoln, NE, USA). Concurrently, leaf water potential (Ψ_{leaf}) was measured using a pressure chamber. K_{plant} was then calculated as:

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

where Ψ_{soil} is the soil water potential. Since the soil was well irrigated throughout the experiment, and the temperature had virtually no effect on $\Psi_{soil,}$ and therefore it was assumed that $\Psi_{soil} = 0$ MPa (Cochard *et al.*, 2000).

Leaf xylem anatomy

Central portions of the leaves of each species and growing condition were collected (n = 5), fixed in formaldehyde, acetic acid, and ethanol, and stored at 4 °C. Following standard methods, these tissues were embedded in paraffin to obtain cross-sections (Ruzin, 1999). Briefly, paraffin blocks were cut at 10 µm thickness using a rotary microtome mounted on the glass slide. Cross-sections were deparaffinized in xylene and rehydrated in a graded series of ethyl alcohols and distilled water (ddH$_2$O). Two procedures were employed for each species to study leaf xylem anatomy. For *D. antarctica,* cross-sections were stained with 0.5 % toluidine blue solution and analyzed by optical microscopy (CX31, Olympus, Japan). For *C. quitensis,* cross-sections were directly observed under a confocal spectral microscope (LSM780; Zeiss; Germany) in the 488 nm excitation wavelength and emission range 490-555 nm. The images were acquired using the Z-stack mode and later processed using Zen software to generate a single overlay image of the entire Z-stack (maximum intensity projection). Micrographs were randomly selected to measure the number of leaf xylem vessels and leaf xylem vessel lumen diameter (d). In *D. antarctica*, vessels from both the main and lateral veins were considered, while in *C. quitensis*, only vessels from the main vein. All the images were analyzed using ImageJ (ImageJ; Wayne Rasband/NIH, Bethesda, MD, USA). The mean 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 Hagen-Poiseuille's law (Tyree and Zimmermann, 2002; Eguchi *et al.*, 2008):

$$K_h = \Sigma\ ((d_i^4\pi\rho) / (128\eta_w)),$$

where d_i is the diameter of a single lumen (m) ρ corresponds to water density (kg m^{-3}) and viscosity (MPa s), respectively, normalized at 10 °C and 15 °C. Subsequently, we determined specific hydraulic conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) by dividing K_h by the conductive xylem area (m^2). Finally, the leaf-specific conductivity (LSC, kg m^{-1} s^{-1} MPa^{-1}) was obtained by dividing K_h by the leaf area (m^2) supported by the measured segment.

Leaf mass area and leaf density

Leaf mass area (LMA) was calculated as the ratio of dry mass to leaf area. Six individuals from each growing condition were randomly selected, and at least fifteen leaves per individual were measured. Leaf area was determined in fresh leaves using ImageJ software (Wayne Rasband/NIH, Bethesda, MD, USA). The dry mass of these leaves was determined after oven dried for 72 h at 65 °C. Leaf density (LD) was calculated by dividing LMA by leaf thickness (LT) (Niinemets, 1999). The leaf thickness and mesophyll thickness (T_{mes}) were obtained from leaf cross-sections analyzed by optical microscopy (CX31, Olympus, Japan).

Gas exchange and fluorescence measurements

To evaluate the coordination of leaf water relations and gas exchange, photosynthesis characterization was performed as described by Sáez *et al.* (2018a), using a Li-6400XT, Li-6400-40 leaf chamber (LI-COR Inc., Lincoln, NE, USA). Six plants from each growth condition were randomly selected for the measurements. The response of net photosynthesis CO_2 uptake (A_N) to varying substomatal CO_2 concentration (C_i) was studied with A_N-C_i curves as reported in Sáez *et al.* (2018a) at leaf temperatures of 10 ºC and 15 ºC for OAs and OTCs, respectively. CO_2 leakage was corrected following Flexas *et al.* (2007).

The quantum efficiency of the photosystem II (ϕPSII) was calculated according to Genty *et al.* (1989) as follows: $\phi PSII = (F'_m - F_s)/F'_m$. The electron transport rate (ETR) was then calculated as: $ETR = \Phi PSII \times PPFD \times \alpha\beta$, 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. The leaf absorptance was measured, as described by Sáez *et al.* (2017).

Mesophyll conductance (g_m) and the chloroplastic CO_2 concentration (C_c) were calculated as in Harley *et al.* (1992). The non-photorespiratory CO_2 evolution rate 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). The maximum carboxylation rate (V_{cmax}) was estimated according to Farquhar *et al.* (1980) after converting A_N–C_i to A_N–C_c curves using the estimated g_m. We used previously published

Rubisco specificity factors ($S_{c/o}$) and Rubisco kinetic values for each species reported by Sáez *et al*. (2017).

The approach of Tomás *et al*. (2013) was used for anatomical modeling of g_m. Central portions of leaves were fixed in glutaraldehyde 2.5 % with cacodylate buffer for optical and transmission electron microscopy (Libra 120 plus, Carl Zeiss Microscopy, Germany). Micrographs were randomly selected to measure the cell wall thickness (T_{cw}); chloroplast thickness (T_{chl}); chloroplast length (L_{chl}); the average distance between the chloroplasts and the cell wall (Δ_{Lcyt}); mesophyll (S_m/S) and chloroplast (S_c/S) surface area facing intercellular air spaces per leaf area and the fraction of intercellular air space (f_{ias}). All micrographs were analyzed using ImageJ. Finally, a quantitative photosynthesis limitations analysis was performed (Grassi and Magnani, 2005) to obtain the relative stomatal (l_s), mesophyll (l_m), and biochemical (l_b) limitations.

Statistical analyses

The effects of growing conditions (OAs and OTCs) on hydraulic (i.e., P-V curves, conductivities, and leaf xylem anatomy) and photosynthetic parameters (i.e., anatomy, morphology, and gas exchange) were assessed for each plant species with the Student's *t*-test. Regression analyses were performed to examine the relationship between cell wall thickness and modulus of elasticity. Pearson’s correlation analyses were performed to examine the relationships among hydraulic (i.e., leaf conductivity, anatomy, and water traits) and photosynthetic (i.e., leaf gas exchange and ultrastructure) parameters. Analyses were performed using the software InfoStat/L (FCA-UNC, Argentina).

Results

Microclimatic conditions during the growing season

For the growing season 2019, significant differences were recorded in the microclimatic condition between OAs and OTCs (Table 1). Daytime mean air temperature (T_{mean}) was significantly higher outside (OAs) than inside OTCs (5.12 ± 0.22 °C and 4.30 ± 0.26 °C, respectively), although the maximum air temperature was recorded inside OTCs (8.45 ± 0.43 °C). The mean minimum air temperature (T_{min}) was higher in OAs (2.95 ± 0.18 °C) than inside OTCs (0.92 ± 0.16 °C). Regarding the growing degree days above 5 °C (GDD_5), was significantly higher inside OTCs (85 °C day^{-1}) than OAs (53 °C day^{-1}). The number of hours (day-night hours) below 0 °C was 32 h in OAs and 309 h inside OTCs, and the number of hours with temperature above 10 °C was 31 h for OAs and 80 h for OTCs (Table 1). Additionally, we included daily air temperature parameters from six consecutive growing seasons, from 2013 until 2019 (Table S1).

Table 1. Daily air temperature parameters for open areas (OAs) and open top chambers (OTCs) between December 1st, 2018, and February 15th, 2019. Mean air temperature (T_{mean}), mean maximum air temperature (T_{max}), mean minimum air temperature (T_{min}), absolute maximum temperature ($T_{max\text{-}asbolute}$), absolute minimum temperature ($T_{min\text{-}absolute}$), time below 0 °C ($T_{ime < 0 °C}$), growing degree days (GDD_5), time in the ranges > 5 °C ($T_{ime >5 °C}$) and > 10 °C ($T_{ime >10 °C}$). Values are means ± S.E. * indicates statistically significant differences between OAs and OTCs according to Student's t-test ($P < 0.05$).

Parameters	OAs	OTCs
T_{mean} (°C)	5.12 ± 0.22	4.30 ± 0.26*
T_{max} (°C)	7.49 ± 0.30	8.45 ± 0.43
T_{min} (°C)	2.95 ± 0.18	0.92 ± 0.16*
$T_{max\text{-}absolute}$ (°C)	13.23	16.25
$T_{min\text{-}absolute}$ (°C)	-1.7	-2.9
GDD_5 (°C day^{-1})	53	85*
$T_{ime < 0 °C}$ (h)	32	309
$T_{ime >5°C}$ (h)	677	427
$T_{ime >10 °C}$ (h)	31	80

Pressure-volume parameters

In *D. antarctica*, no significant differences between OAs and OTCs were found for leaf capacitance (*C*), apoplastic water content (a_f), osmotic potential at full turgor (π_o), relative water content at the turgor lost point (RWC_{tlp}), leaf water potential at the turgor-loss point (Ψ_{tlp}) and maximum bulk modulus of elasticity (ε_{max}) (Table 2). On the contrary, in *C.*

quitensis OTCs exerted significant effects on several of those parameters (Table 2). The leaf capacitance and a_f were lower in plants growing in OAs (3.36 ± 0.16 mol m^{-2} MPa^{-1} and 41 ± 6.06 %, respectively), compared with plants growing inside OTCs (5.24 ± 0.34 mol m^{-2} MPa^{-1} and 64 ± 6.72 %, respectively). No differences were observed in π_o and RWC_{tlp}, with values close to -0.84 MPa and 93 %, respectively. Finally, Ψ_{tlp} and ε_{max} were higher in plants growing in OAs (-0.90 ± 0.04 MPa and 9.27 ± 0.75 MPa, respectively), compared with plants growing inside OTCs (-1.08 ± 0.05 MPa and 5.83 ± 0.53 MPa, respectively).

Table 2. Parameters derived from the pressure-volume curves for *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas (OAs) and inside open top chambers (OTCs): leaf capacitance (*C*), apoplastic water content (a_f), 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 ± S.E. ($n = 5$). * indicates significant differences between OAs and OTCs for each species according to Student's t-test ($P < 0.05$).

Parameters	*D. antarctica*		*C. quitensis*	
	OAs	OTCs	OAs	OTCs
C (mol m^{-2} MPa^{-1})	0.59 ± 0.06	0.45 ± 0.05	3.36 ± 0.16	5.24 ± 0.34*
a_f (%)	56 ± 4.72	64 ± 1.38	41 ± 6.05	64 ± 6.72*
π_o (MPa)	-1.01 ± 0.03	-1.00 ± 0.03	-0.84 ± 0.06	-0.83 ± 0.05
RWC_{tlp} (%)	94 ± 0.42	93 ± 0.99	93 ± 0.45	92 ± 0.72
Ψ_{tlp} (MPa)	-1.13 ± 0.05	-1.26 ± 0.06	-0.90 ± 0.04	-1.08 ± 0.05*
ε_{max} (MPa)	6.35 ± 0.60	6.20 ± 0.43	9.27 ± 0.75	5.83 ± 0.53*

Hydraulic conductivities and leaf vascular anatomy

In *D. antarctica*, the leaf hydraulic conductivity (K_{leaf}) was higher in plants of OAs than OTCs (4.70 ± 0.32 and 1.57 ± 0.06 mmol m^{-2} s^{-1} MPa^{-1}, respectively; Fig. 1a). Consequently, the whole plant hydraulic conductivity (K_{plant}) showed the same trend (1.70 ± 0.08 and 0.97 ± 0.09 mmol m^{-2} s^{-1} MPa^{-1} in OAs and OTCs, respectively; Fig. 1b). By contrast, in *C. quitensis* K_{leaf} was lower in OAs (7.91 ± 0.15 mmol m^{-2} s^{-1} MPa^{-1}) compared with OTCs (11.42 ± 0.36 mmol m^{-2} s^{-1} MPa^{-1}) (Fig. 1c), and no significant differences were detected in K_{plant} (Fig. 1d). Regardless of the differences observed, in both species, K_{leaf} showed a linear and positive relationship with K_{plant} (Fig. S1).

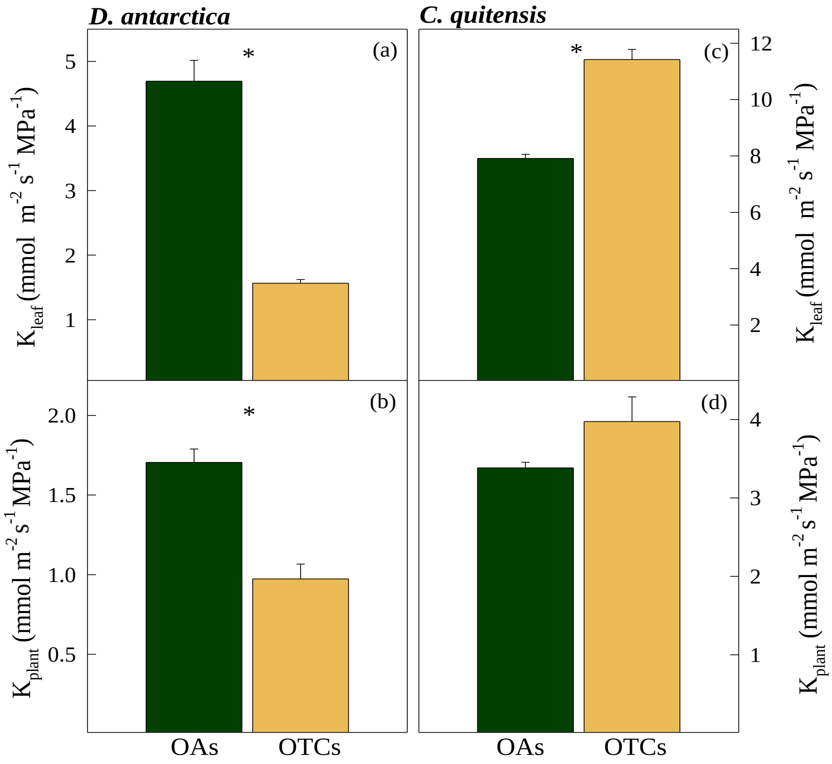


Figure 1. Leaf hydraulic conductivity (K_{leaf}) and whole plant hydraulic conductivity (K_{plant}) for *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas

(OAs) and inside open top chambers (OTCs). Values are means ± S.E. (*n* = 8 - 9). * indicated significant differences between OAs and OTCs for each species according to Student's t-test ($P < 0.05$).

Regarding leaf xylem vascular traits, both species showed differences between the *in situ* growing conditions (Table 3, Fig. 2). *D. antarctica* did not show significant differences in the number of leaf xylem vessels between plants growing in OAs and OTCs (Table 3) or in the frequency distribution of leaf xylem vessels (Fig. 2a). The lowest diameters (1-2 µm) were observed only inside OTCs, and the highest (8-10 µm) were mainly observed in OAs. The variability in size vessels resulted in a significantly higher mean hydraulic diameter (D_h) in plants growing in OAs (6.13 ± 0.49 µm) (Table 3). On the other hand, all theoretical conductivities tended to be higher in plants growing in OAs, but only K_s and LSC were statistically different (Table 3). In *C. quitensis*, significant differences between plants growing in OAs and OTCs were found in almost all parameters evaluated, being higher in plants growing inside OTC, except for the number of leaf vessels (Table 3). Regarding the distribution of leaf xylem vessels, plants growing inside OTCs showed a higher frequency of vessels in the highest diameter classes (Fig. 2b). In both species, vascular anatomy (D_h) and theoretical conductivities (D_h, K_h, K_s, and LSC) showed a positive relationship with K_{leaf} and K_{plant} (Table S2).

Table 3. Leaf hydraulic traits for *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas (OAs) and inside open top chambers (OTCs). The number of leaf xylem vessels, the mean hydraulic diameter (D_h), the theoretical hydraulic conductivity (K_h), specific hydraulic conductivity (K_s), and leaf-specific hydraulic conductivity (LSC). Values are means ± S.E. (n = 56-80 for vessels and *n* = 4-8 for D_h, K_s, K_h, LSC). * indicate significant differences between OAs and OTCs for each species according to Student's t-test ($P < 0.05$).

Parameters	*D. antarctica*		*C. quitensis*	
	OAs	OTCs	OAs	OTCs
Vessels (N°)	11 ± 2.22	9 ± 1.99	14 ± 1.08	16 ± 0.71
D_h (µm)	6.13 ± 0.49	4.68 ± 0.38*	2.87 ± 0.19	3.54 ± 0.11*
K_h ($x10^{-11}$ kg m s^{-1} MPa^{-1})	2.23 ± 0.66	0.73 ± 0.31	1.40 ± 0.33	3.95 ± 0.61*
K_s (kg m^{-1} s^{-1} MPa^{-1})	0.92 ± 0.12	0.51 ± 0.10*	0.19 ± 0.03	0.30 ± 0.02*
LSC ($x10^{-5}$ kg m^{-1} s^{-1} MPa^{-1})	4.6 ± 1.38	0.87 ± 0.35*	0.60 ± 0.07	1.61 ± 0.13*

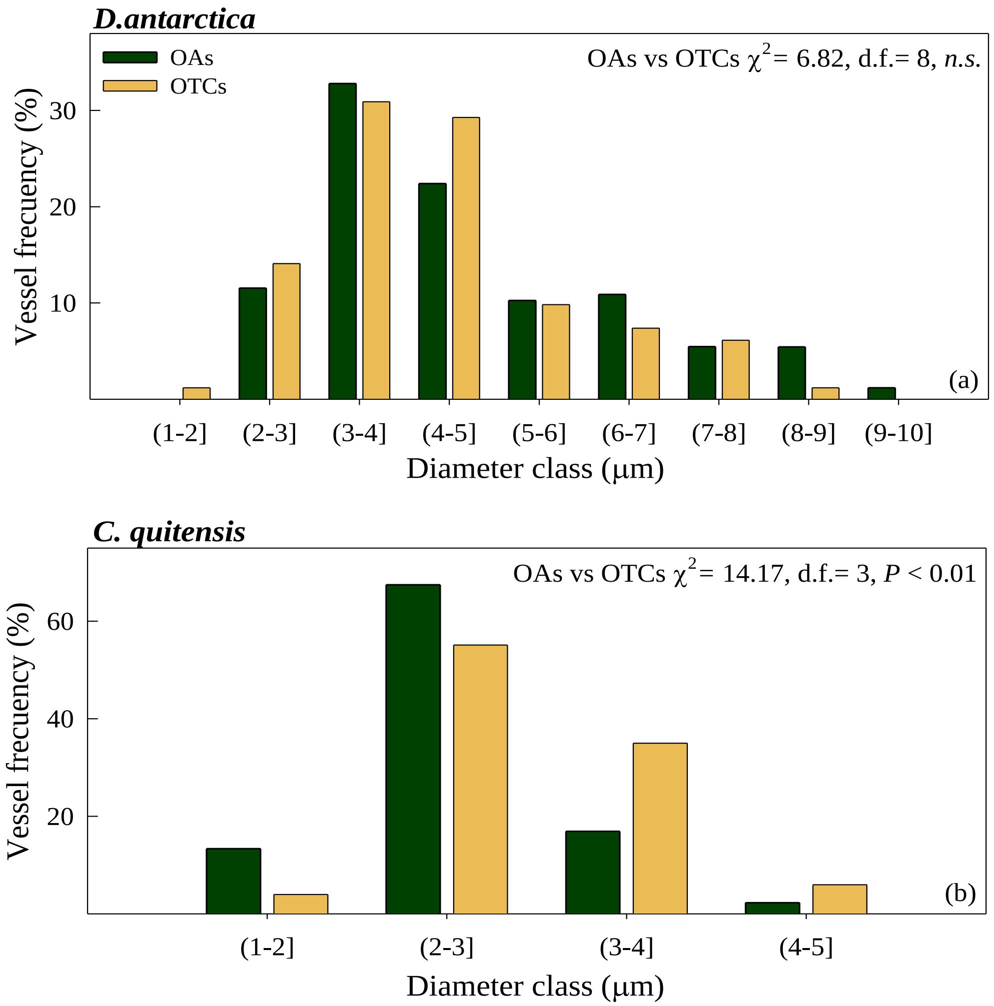


Figure 2. Frequency distribution of leaf xylem vessel diameters of *Deschampsia antarctica* and Colobanthus quitensis growing in open areas (OAs) and inside open top chambers (OTCs). The significance of the distributions is shown for each species considering both growing conditions together, according to the Chi-square (χ^2) test ($P < 0.05$). *n.s.* not significant.

Photosynthetic characterization

In *D. antarctica*, the net photosynthesis (A_N) and the mesophyll conductance (g_m) on a mass basis higher in OAs compared with OTCs (93.5 ± 8.94 to 59.4 ± 7.73 µmol CO_2 kg^{-1} s^{-1} and 0.53 ± 0.06 to 0.27 ± 0.05 mol CO_2 kg^{-1} s^{-1}, respectively) (Table 4). In contrast, the Rubisco carboxylation rate (V_{cmax}) increased inside OTC, while the growing conditions did not affect stomatal conductance (g_s). The opposite response was observed in *C. quitensis*; with few exceptions, photosynthetic traits increase inside OTCs (Table 4). Irrespective of the contrasted responses inside OTCs, both species deploy a positive relationship between A_N and g_m as with total leaf conductance to CO_2 (g_{tot}), indicative that photosynthesis was mainly limited by CO_2 diffusion across the leaf mesophyll (Fig. S2). Indeed, the quantitative limitation analysis of photosynthesis reaffirms A_N was mainly limited by the leaf mesophyll (l_m) rather than stomatal (l_s) or biochemical limitations (l_b) in both species (Table 4).

Table 4. Photosynthetic parameters and quantitative analysis limitation of photosynthetic CO_2 assimilation of *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas (OAs) and inside open top chambers (OTCs). Net photosynthetic CO_2 assimilation rate (A_N), stomatal conductance (g_s), leaf mesophyll conductance obtained through Harley's method (g_m), chloroplast CO_2 concentration (C_c), maximum Rubisco carboxylation rate (V_{cmax}), stomatal limitation (l_s), limitation due to mesophyll (l_m) and biochemistry (l_b). Values are means ± S.E. ($n = 5$). * indicate significant differences between OAs and OTCs for each species according to Student's t-test ($P < 0.05$).

Parameters	*D. antarctica*		*C. quitensis*	
	OAs	OTCs	OAs	OTCs
A_N (µmol CO_2 kg^{-1} s^{-1})	93.5 ± 8.94	59.4 ± 7.73*	58.1 ± 3.8	83.92 ± 6.52*
g_m (mol CO_2 kg^{-1} s^{-1})	0.53 ± 0.06	0.27 ± 0.05*	0.23 ± 0.02	0.36 ± 0.05
V_{cmax} (µmol CO_2 kg^{-1} s^{-1})	495.06 ± 37.33	570.18 ± 3.41*	208.02 ± 18.11	343.8 ± 12.87*
g_s (mol H_2O m^{-2} s^{-1})	0.11 ± 0.02	0.11 ± 0.01	0.17 ± 0.01	0.34 ± 0.03*
l_s %	28.83 ± 2.87	21.80 ± 3.34	17.37 ± 1.22	11.42 ± 1.97*
l_m %	47.00 ± 3.51	59.80 ± 5.51	58.72 ± 2.57	62.40 ± 3.40
l_b %	24.50 ± 3.37	8.40 ± 3.04	23.95 ± 1.74	26.18 ± 1.67

Morphological and anatomical leaf traits

In *D. antarctica*, the leaf mass area (LMA), leaf density (LD), and leaf thickness (LT) were higher in plants growing inside OTC, with a significant positive relationship between LMA and LD (Table 5 and Fig. S3a).

Table 5. Leaf anatomical traits for *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas (OAs) and inside open top chambers (OTCs): Leaf mass area (LMA), leaf density (LD), and leaf thickness (LT), leaf mesophyll conductance modeled with anatomical parameters ($g_{m\text{-modeled}}$), mesophyll thickness (T_{mes}), cell wall thickness (T_{cw}), chloroplast thickness (T_{chl}), chloroplast length (L_{chl}), average distance between the chloroplasts and the cell wall (ΔL_{cyt}), mesophyll (S_m/S) and chloroplast (S_c/S) surface area facing intercellular air spaces per leaf area, fraction of intercellular air space (f_{ias}), conductivity gas-phase (g_{ias}) and liquid phase (g_{liq}). Values are means ± S.E. (n = 6-9). * indicate significant differences between OA$_S$ and OTCs for each species according to Sudent's t-test ($P < 0.05$).

Parameters	*D. antarctica*		*C. quitensis*	
	OAs	OTCs	OAs	OTCs
LMA (g m^{-2})	77.48 ± 1.65	94.99 ± 3.98*	121.75 ± 4.34	104.38 ± 7.91
LD (g cm^{-3})	0.43 ± 0.009	0.48 ± 0.02*	0.27 ± 0.005	0.24 ± 0.005*
LT (µm)	182.40 ± 3.76	201.99 ± 88.19*	457.27 ± 7.81	457.17 ± 9.58
$g_{m\text{-modeled}}$ (mol CO_2 kg^{-1} s^{-1})	0.018 ± 0.002	0.018 ± 0.001	0.15 ± 0.01	0.21 ± 0.01*
T_{mes} (µm)	148.43 ± 6.22	162.47 ± 8.61	418.96 ± 9.66	412.46 ± 12.01
T_{cw} (µm)	0.40 ± 0.01	0.34 ± 0.02*	0.45 ± 0.01	0.32 ± 0.02*
T_{chl} (µm)	2.86 ± 0.09	2.14 ± 0.11*	2.44 ± 0.2	2.04 ± 0.11
L_{chl} (µm)	5.0 ± 0.08	4.05 ± 0.11*	5.47 ± 0.02	5.57 ± 0.10
ΔL_{cyt} (µm)	0.4 ± 0.02	1.52 ± 0.11*	0.50 ± 0.06	0.46 ± 0.05
S_m/S (m^2 m^{-2})	7.22 ± 5.28	5.28 ± 0.61*	6.78 ± 0.72	5.84 ± 0.23
S_c/S (m^2 m^{-2})	0.60 ± 0.07	0.18 ± 0.04*	1.36 ± 0.26	1.37 ± 0.13
f_{ias}	0.27 ± 0.02	0.16 ± 0.04*	0.35 ± 0.02	0.49 ± 0.05*
g_{ias} (m s^{-1})	0.028 ± 0.005	0.030 ± 0.003	0.014 ± 0.001	0.020 ± 0.002*
g_{liq} (x10^{-4} m s^{-1})	4.2 ± 0.40	4.4 ± 0.3	4.4 ± 0.39	5.3 ± 0.32

Furthermore, several ultrastructural parameters underwent significant changes. At the chloroplast level, including cell wall thickness (T_{cw}), chloroplast thickness (T_{chl}), and chloroplast length (L_{chl}), where lower inside OTCs, while only the distance between the chloroplast and the cell wall (ΔL_{cyt}) was higher. Parameters such as mesophyll (S_m/S) and chloroplast (S_c/S) surface area facing intercellular air spaces per leaf area, as well as the fraction of intercellular air space (f_{ias}), also were significantly lowers (Table 5). In contrast, in *C. quitensis*, only LD decreased statistically inside OTCs (Table 5). However, like *D. antarctica*, a significant relationship was also observed between LMA and LD (Fig. S3b). Regarding ultrastructural traits, the leaf mesophyll conductance modeled through anatomical traits ($g_{m\text{-}modeled}$), T_{cw}, f_{ias}, and gas-phase conductivity (g_{ias}) was significantly higher within OTCs, while measurements associated with chloroplasts remained unaltered (Table 5). On the other hand, correlations between elasticity modulus (ε_{max}) and cell wall thickness (T_{cw}) were found only in *C. quitensis* (Fig. 3).

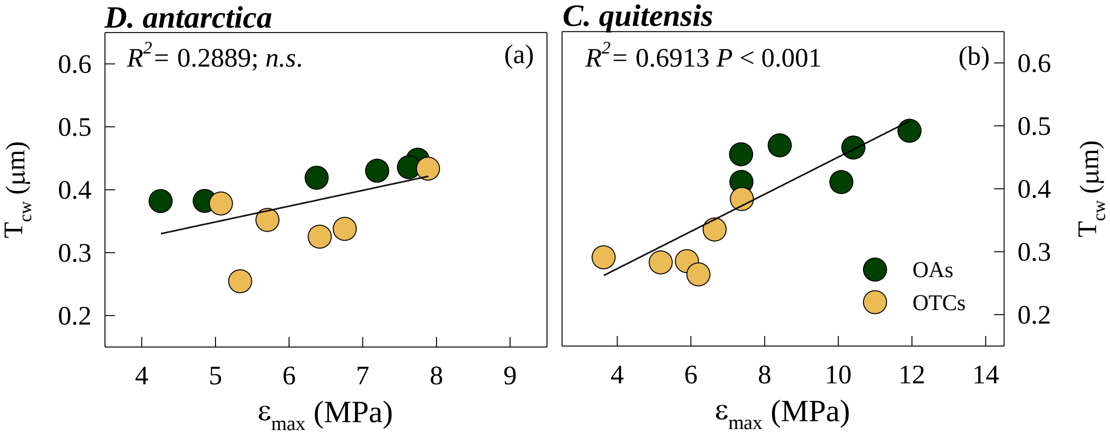
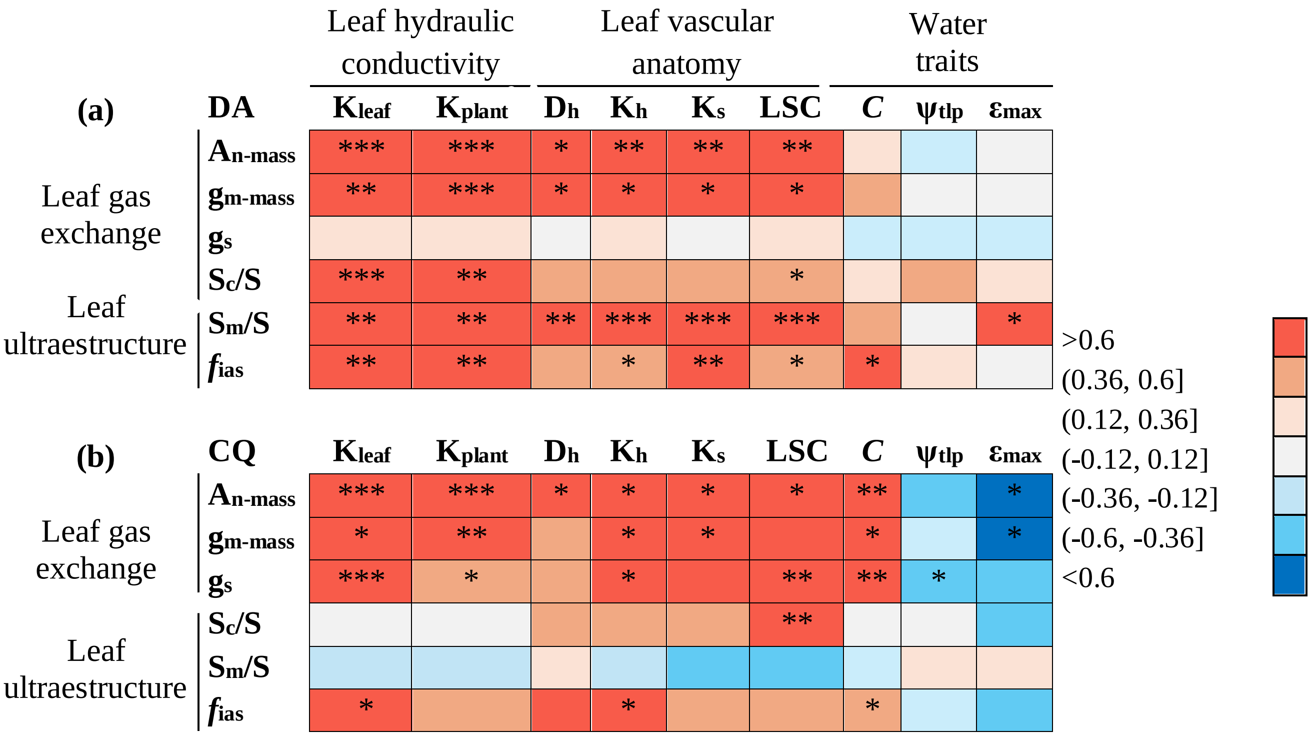


Figure 3. Relationships between cell wall thickness (T_{cw}) with maximum bulk modulus of elasticity (ε_{max}) in Deschampsia antarctica and Colobanthus quitensis growing in open

areas (OAs) and inside open top chambers (OTCs). The regression coefficient and the significance of the relationship are shown for each species, considering both growing conditions together

Coordination between leaf gas exchange and hydraulics

In both species, significant correlations between photosynthesis and hydraulic performance were established. These correlations extend across the functional, ultrastructural, and anatomical leaf traits, and some are species-specific (Fig. 4). With few exceptions, both species showed positive correlations between leaf gas exchange (i.e., A_N, g_s, and g_m) with hydraulic conductivity (K_{leaf} and K_{plant}), vascular anatomy, and theoretical conductivities (i.e., D_h, K_h, LSC, K_s). However, upon analyzing leaf ultrastructure (S_c/S, S_m/S, and f_{ias}), it was primarily observed that *D. antarctica* displayed correlations with hydraulic conductivity and vascular anatomy (Fig. 4a). Conversely, correlations between leaf gas exchange and water traits (C, Ψ_{tlp}, and ε_{max}) were found only in *C. quitensis* (Fig. 4b).



(a)

	DA	K_{leaf}	K_{plant}	D_h	K_h	K_s	LSC	C	ψ_{tlp}	ε_{max}
		Leaf hydraulic conductivity		Leaf vascular anatomy				Water traits		
Leaf gas exchange	$A_{n\text{-}mass}$	***	***	*	**	**	**			
	$g_{m\text{-}mass}$	**	***	*	*	*	*			
	g_s									
Leaf ultraestructure	S_c/S	***	**				*			
	S_m/S	**	**	**	***	***	***			*
	f_{ias}	**	**		*	**	*	*		

(b)

	CQ	K_{leaf}	K_{plant}	D_h	K_h	K_s	LSC	C	ψ_{tlp}	ε_{max}
Leaf gas exchange	$A_{n\text{-}mass}$	***	***	*	*	*	*	**		*
	$g_{m\text{-}mass}$	*	**		*	*		*		*
	g_s	***	*		*		**	**	*	
Leaf ultraestructure	S_c/S						**			
	S_m/S									
	f_{ias}	*			*			*		

Figure 4. Pearson´s correlations coefficients (*r*) and the significance of the relationship between photosynthesis performance (leaf gas exchange and leaf ultrastructure) and hydraulic performance (leaf hydraulic efficiency, leaf vascular anatomy, and water traits) for *Deschampsia antarctica* (a) and *Colobanthus quitensis* (b), considering both growing conditions together (OAs and OTCs) for each species. The *r* values are shown using different colours. Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Discussion

The microclimatic conditions under *in situ* experimental manipulation

Open top chambers (OTCs) have been widely used to simulate *in situ* warmer conditions and evaluate their effects on terrestrial biota. Contrary to these expectations, we observed that during our study period (season 2019), the daily air temperatures (i.e., mean, and minimum) were slightly higher outside than inside OTCs (Table 1). Interestingly, these findings align with other passive warming experiments conducted in the Alpine tundra where it has been argued that OTCs could provide minor warming compared to year-on-year variability (Hudson and Henry, 2010; Keuper *et al.*, 2011) because nighttime cooling could mask daily warming (Pieper *et al.*, 2011). Other studies suggested that simulated climate-change experiments have artifacts and are not perfect substitutes for ambient climate change (Kennedy, 1995; Marion *et al.*, 1997; Bokhorst *et al.*, 2013). However, when analysing OTCs temperatures during season 2019, they exhibited consistent patterns over the years (since 2014), maintaining an average air temperature of around 4.16 °C (Table S1). Furthermore, during the first seasons (2014 to 2017), OTCs temperatures were consistently higher than OAs (~ +1.6 for mean and +3 °C for maximum, Table S1). This increase aligns well with other studies utilizing similar systems, demonstrating a successful increase of mean daily temperatures by approximately 1.5-3 °C (Marion *et al.*, 1997; Hollister and Webber, 2000; Casanova-Katny *et al.*, 2016; Prather *et al.*, 2019). Additionally, the growing degree days (GDD_5) and temperatures above 10 °C were notably higher inside OTCs (Table 1) than OAs. These results align with Sierra-Almeida *et al.* (2018) and Sáez *et al.* (2018a) in the same experimental setup, but in the short-term.

Therefore, the system did have in the long-term a warming effect on plants, notwithstanding, the two last growing seasons (2018-2019) were unusually warm in open areas (Table S1) and in the Antarctic Peninsula in general (Turner *et al.*, 2021; Wille *et al.*, 2024), so OTCs results be surely more stressful from the point of view of the accumulation of hours with temperatures below zero. Overall, the changes exerted by OTCs and the notably warmer OAs, support, in part, the differences found in the long-term at the same experimental set-up reported by Sáez *et al.* (2018a) and Sierra-Almeida *et al.* (2018) in the short-term, and represented a challenge for the plant performance, which was reflected in the particular and contrasting responses displayed by both Antarctic species.

The hydraulics traits of Antarctic species in the field and their responses to changes in environmental conditions

In situ environmental experiments conducted on Antarctic vascular species have mainly focused on assessing their freezing resistance, growth, and photosynthetic responses (Day *et al.*, 1999, 2008; Sierra-Almeida *et al.*, 2018; Sáez *et al.*, 2018a). However, there remains a substantial gap in our understanding of their hydraulic performance. Our study addresses this gap, revealing that, on the one hand, both *D. antarctica* and *C. quitensis* growing in Antarctica (OAs) exhibit values of the leaf (K_{leaf}) and whole plant (K_{plant}) hydraulic conductivity within the range reported for herbaceous plants between 2.7 and 28 mmol m^{-2} s^{-1} MPa^{-1} (Sack and Holbrook, 2006; Bartlet *et al.*, 2012; Ocheltre *et al.*, 2016; Nolf *et al.*, 2016; Huang *et al.*, 2024). Additionally, conductivities and vascular anatomy (i.e., mean vessel diameter of the midrib) align closely with those reported in recent laboratory experiments on Antarctic plants under similar temperature conditions (5-8°C; Sáez *et al.*, 2024). However, there are some distinctions between natural and laboratory growing conditions that are crucial to recognize. Antarctic plants are subjected to various stressors such as wind, solar irradiation, freeze events, and temperature changes. Considering this, field observations reveal that plants displayed heightened water transport capacity, as evidenced by higher values of K_{leaf} and K_{plant} with less risky leaf vascular anatomy (i.e., lower number, size, and range of diameter vessel class) than the observed in the laboratory. In addition, field plants exhibited higher values of leaf capacitance (*C*), alongside slightly less negative osmotic potential at full turgor (π_o) and leaf water potential at the turgor loss point (Ψ_{tlp}) than those observed in the laboratory. These findings collectively suggest that field conditions offer a less stressful environment for water mobilization at both the leaf and whole plant levels (Bartlet *et al.*, 2012; Xiong and Nadal, 2020).

When plants are subjected to *in situ* manipulative experiments exerted by OTCs, both species exhibit notable differences in terms of hydraulic responses. *D. antarctica* showed lower K_{leaf} and K_{plant} when growing inside OTCs (Fig. 1a), while parameters derived by P-V curves were unchanged (Table 2). In contrast, *C. quitensis*, showed K_{leaf} and K_{plant} values significantly higher when growing inside OTCs (Fig. 1b), aligned with several adjustments in parameters derived by P-V curves (Table 2). For instance, plants within OTCs exhibited a more negative Ψ_{tlp} than plants growing in OAs, suggesting a greater capacity to maintain open stomata, which aligns with the enhanced photosynthetic performance observed in *C. quitensis* (Table 4). Additionally, a lower modulus of elasticity (ε_{max}), combined with higher apoplastic water (a_f) and *C*, helps maintain cell turgor and mitigate the impacts of lower water potentials, thereby preventing damaging cavitation and embolism events that could compromise hydraulic efficiency (Kozlowski *et al.,* 1990; Tyree and Jarvis, 1982., Clifford *et al.*, 1998; Barlett *et al.*, 2012; Xiong and Nadal 2020).

It is well-established that vessel size significantly influences a plant's capacity to transport water and directly affects its hydraulic conductivity (Tyree and Zimmermann, 2002; Schreiber *et al.,* 2015). Our results corroborate these assertions, showing several vascular adjustments in both species that support their contrasting responses within the OTCs. In both species, the variability of leaf vessel sizes, rather than the number, triggered changes in mean hydraulic diameter (D_h) and theoretical conductivities (K_h, K_s, and LSC). For instance, *D. antarctica* inside OTCs showed lower values of these traits, while *C. quitensis* showed higher values (Table 3). Notably, regardless of the direction of their responses, both species displayed positive correlations between *in vivo* conductivities (K_{leaf} and K_{plant}) and leaf vascular anatomy (Table S2). These findings imply that hydraulic responses exerted under the growing conditions are driven by modifications in the leaf vascular anatomy, confirming our first hypothesis. Additionally, the contrasting responses observed within OTCs between the species prompt new questions. Typically, the decline in hydraulic conductivity is attributed to hydraulic failure due to embolism, caused by bubbles formed during freezing that expand upon thawing, as well as drought stress (Tyree and Sperry, 1989; Hacke amd Sperry, 2001; Sevanto *et al.*, 2012; Hacke *et al.*, 2017). This raises a critical question, particularly for *D. antarctica*: Does hydraulic failure occur in this species? It is widely recognized that small vessels prevent hydraulic failure when plants encounter freezing conditions (Davis *et al.,* 1999; Pittermann and Sperry, 2003). *D. antarctica* exhibited small vessel sizes ranging from 1 to 10 µm (Fig. 2), with values among the lowest reported across different species (Ni *et al.,* 2022). Given this, the observed decline in hydraulic conductivity is unlikely to result from hydraulic failure due to cavitation during freeze-thaw cycles within the OTCs. However, since our study did not directly measure this phenomenon, we cannot be certain. On the other hand, recent hydraulic studies have suggested that anatomical traits outside the xylem as biochemical compounds can regulate change in K_{leaf} (Nardini *et al.*, 2005; Sack *et al*., 2005; Scoffoni *et al.,* 2016, 2017, 2018). For instance, features such as the xylem parenchyma, bundle sheath, and leaf mesophyll (Buckley *et al.,* 2015; Scoffoni *et al.,* 2016), as well as biochemical factors like aquaporins (Martre *et al.,* 2002; Prado *et al.,* 2013; Pou *et al*., 2013; Sade *et al.,* 2014; Rodríguez-Gamir *et al.,* 2019). Considering these studies, a new question arises: Are pathways outside the xylem contributing to the decline observed in *D. antarctica*? Thus, further investigation is needed to determine which components of the plant system (i.e., roots and stems) contribute to heightened resistance.

Another aspect that emerged from this study is the question of why only *D. antarctica* was negatively affected within OTCs despite both species experiencing the same freeze events. It is plausible to consider that *C. quitensis* showed a better hydraulic performance due to a higher capacity to take advantage of higher temperatures (over 10 °C) recorded inside OTCs (Table 1). These findings are consistent with previous studies, which showed that higher temperatures promote hydraulic performance in this species (Sáez *et al.*, 2024). This opportunist capacity, combined with accumulating the positive effects inside OTCs over time (leaf and vascular anatomy), may have prepared *C. quitensis* to deal with freeze events, unlike *D. antarctica*.

Photosynthetic responses

As observed in the hydraulic performance, photosynthetic responses also showed opposite responses between Antarctic species. *D. antarctica* displayed lower carbon assimilation inside OTCs, characterized mainly by lower photosynthetic rates (A_N) and mesophyll conductance (g_m) (Table 4). This response is attributed to the prevalence of sclerophyll leaves (e.g., higher LMA and LD), along with reductions in chloroplast features (e.g., T_{ch}, S_c/S, and S_m/S), as well as a decrease in intercellular air space (f_{ias}) (Table 5). These adjustments collectively increase the diffusion resistance of CO_2 into the leaves inside OTCs. In contrast, *C. quitensis* showed higher photosynthetic A_N and g_s when growth inside OTCs (Table 4). Leaves within OTCs were thinner (e.g., lower LD and T_{cw}) and the ultrastructural traits associated with gas-phase (e.g., gas-phase diffusion conductance, g_{ias} and f_{ias}) were also higher. This adjustment triggered a higher leaf mesophyll conductance modeled through anatomical traits ($g_{m\text{-modeled}}$), although *in vivo* g_m did not statistically change. (Tables 4 and 5). Despite this, mesophyll conductance remains the main photosynthetic limitation in both species, correlating strongly with A_N (Table 4, Fig. S2). In *C. quitensis*, these adjustments agree well with the previously reported by Sáez *et al.* (2018a) in the short-term (3 yrs.) persisting over time (7 yrs.). On the contrary, the observed response in the long term by *D. antarctica* is completely different from those observed at the short-term (where not changes were exerted by the OTCs at any of the evaluated parameters, see Saáez *et al.*, 2018a). Clemente-Moreno *et al.* (2020) highlighted that *D. antarctica* in the Antarctica Peninsula and under laboratory conditions growing at low temperature (5 °C) show a mix of green and yellow leaves, as a senescence symptom. These authors proposed that *D. antarctica* initiates a senescence program to dismantle key cell structures, enabling the transfer of nitrogen and carbon from source to sink tissues, particularly to meristematic areas in the stem. This process allows the plant to generate new leaves when environmental conditions improve (Clemente-Moreno *et al.*, 2020). In our study, *D. antarctica* also exhibited signs of senescence, likely as a response to the freeze events inside the OTCs. These stress signals probably led to a decline in hydraulic and photosynthetic performance, as well as a strategic shift towards recycling nutrients to ensure survival under challenging conditions. In the case of *C. quitensis*, the cushion shape of this species is likely advantageous to conserving higher temperatures inside the plants, which, in summary, could converge in higher protection against a freeze (Cavieres *et al.*, 2016). Thus, is likely that in this species, more stressful conditions are needed to affect photosynthetic and hydraulic performance.

Coordination between hydraulic and photosynthetic processes

Both species displayed numerous correlations between leaf hydraulic (e.g., hydraulic efficiency, vascular anatomy, and water traits) and photosynthetic (e.g., gas exchange and leaf anatomy) performance (Fig. 4). Many of these correlations have been previously reported across several species and growing conditions (Brodribb *et al.*, 2007). Interestingly, species-specific correlations were identified, which can aid in understanding the differing responses observed within OTCs between Antarctic species. For example, *D. antarctica* exhibited notable correlations between leaf ultrastructure (e.g., S_c/S, S_m/S, and f_{ias}) and hydraulic traits (conductivity and leaf anatomy), which were mostly absent in *C. quitensis*. In contrast, *C. quitensis* exhibits correlations between leaf gas exchange and water traits, a pattern not observed in *D. antarctica*. Thus, these contrasting patterns observed in the correlations provide potential explanations for the divergent responses exhibited between the two Antarctic species.

Leaf hydraulic conductivity has long been recognized as pivotal in determining plant hydraulic capacity and influencing shifts in g_s and A_N (Brodribb *et al.*, 2007; Sack and Holbrook, 2006; Scoffoni *et al.*, 2017; Huang *et al.*, 2022). More recently, g_m emerged as another key linked with K_{leaf}, as both partially share pathways for CO_2 diffusion and water transport within leaves (Flexas *et al.*, 2013; Buckley *et al.*, 2015; Xiong *et al.*, 2015; Xiong *et al.*, 2018). K_{leaf} includes pathways both inside (K_x) and outside (K_{ox}) the xylem, with the latter being crucial for understanding the coordination between K_{leaf} and g_m (Xiong & Nadal., 2020). This coordination is supported by anatomical traits such f_{ias}, S_m/S, and S_c/S, which widely influence g_m, which is also linked to K_{leaf} by modifying K_{ox} (Buckley *et al.*, 2015; Xiong *et al.*, 2017; Lu *et al.*, 2019; Sonawane *et al.*, 2021). Our results provide the first empirical evidence that external pathways significantly influence hydraulic and photosynthetic processes, particularly in *D. antarctica* (Fig. 4a, Table 5). Consequently, it is reasonable to suggest that the downregulation observed in *D. antarctica* within OTCs may be mediated by these anatomical adjustments, likely in response to freeze events. Similar responses have been documented under other adverse conditions, such as potassium deficiency (Lu *et al.*, 2020) and drought (Bouche *et al.*, 2016; Trifilò *et al.*, 2016; Scoffoni *et al.*, 2017; Corso *et al.*, 2020). Conversely, in *C.*

quitensis, only f_{ias} upregulated the correlation with K_{leaf} (Fig. 6b, Table 5), as in Hu *et al.* (2022). It's important to mention that the correlation between outside anatomy and K_{leaf} is still being debated; however, it has been anticipated that gas-phase transport may play an important role in outside-xylem water transport (Rockwell *et al.*, 2014; Buckley *et al.*, 2015; Xiong *et al.*, 2017).

Another novel finding is the correlation between water traits and leaf gas exchange, which is mainly observed in *C. quitensis*. In this species, *C* is higher inside OTCs and positively correlates with leaf gas exchange parameters (A_N, g_s, and g_m). This water trait emerges as a subsidiary aspect related to K_{leaf} and A_N, which buffers sudden fluctuations in Ψ_{leaf} (Xiong and Nadal, 2020). This implies that *C* can prevent damaging cavitation and embolism events that hinder hydraulic efficiency (Scholz *et al.*, 2011). Additionally, a novel trade-off has been proposed between the modulus of elasticity (ε_{max}) and leaf gas exchange parameters (Nadal *et al.* 2018). According to our results, we observed an inverse correlation between ε_{max} with A_N and g_m (Fig. 4b). The mechanistic basis for this trade-off remains unclear. However, cell wall thickness could be an important key that supports these correlations (Nadal *et al.*, 2018). In this line, it is well documented that T_{cw} strongly influences g_m (Evans *et al.*, 2009; Terashima *et al.*, 2011; Onoda *et al.*, 2017), and lately, it has also been related to ε_{max} (Peguero-Pina *et al.*, 2017). Cell wall thickness is one of the determinants of cell elasticity, where thinner cell walls result in lower ε_{max} (Zimmermann, 1978; Tyree and Jarvis, 1982). Indeed, a positive relationship arises when plotting the mean ε_{max} versus mean T_{cw} (Fig. 3b). Therefore, our study shows evidence that *C* and ε_{max} emerged as key traits that supported change in photosynthetic performance under growing conditions. The robust coordination observed between hydraulic and photosynthetic performance in both species supports our second hypothesis. This synchronization occurs across multiple physiological levels, facilitating the maintenance of homeostasis between carbon gain and water loss under diverse environmental conditions.

Concluding remarks

Our field study reveals that Antarctic species display more efficient hydraulic performance under their natural environment than in laboratory conditions, highlighting remarkable adaptability to the harsh Antarctic climate. On the other hand, unexpected freeze events occurred within OTCs than OAs, likely triggering divergent responses between the species. In *D. antarctica,* hydraulic and photosynthetic performance was negatively affected, while *C. quitensis* showed positive responses within OTCs. These responses were supported by adjustments in leaf and vascular anatomy, as well as water traits that influence the co-variation between hydraulic and photosynthetic performance. Notably, novel findings suggest that in *D. antarctica*, hydraulic and photosynthetic downregulation may be linked to specific leaf anatomical traits (e.g., f_{ias}, S_m/S, and S_c/S), whereas in *C. quitensis*, water traits (e.g., C and ε_{max}) have emerged as key factors which upregulated hydraulic and photosynthetic performance. After seven years of using OTCs, *C. quitensis* consistently demonstrated improved photosynthetic performance, as it occurred in the three years, while *D. antarctica*, initially showing no change, unexpectedly responded negatively over time. This highlights that each species adopts distinct survival pathways based on their sensitivity to environmental stresses, such as freeze events. It is plausible that *D. antarctica* activates a senescence program to recycle nutrients and use them to generate new leaves when environmental conditions improve. Conversely, the rounded shape of *C. quitensis* and its enhanced performance over time in OTCs contribute to its favorable hydraulic and photosynthetic efficiency.

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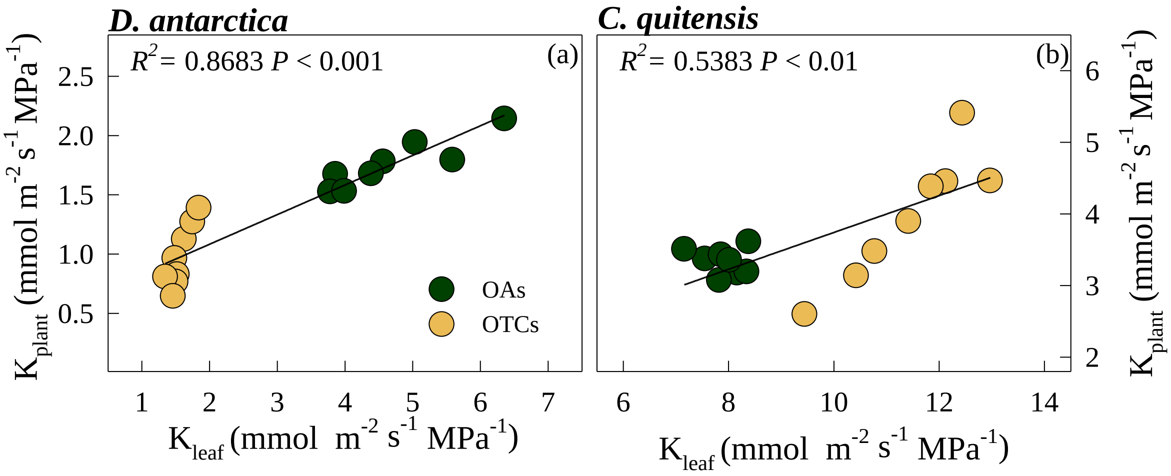

Figure S1. Relationship between the whole plant hydraulic conductivity (K_{plant}) and the leaf hydraulic conductivity (K_{leaf}) in *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas (OAs) and inside chambers (OTCs). The regression coefficient and the significance of the relationship are shown for each species, considering both growing conditions together.

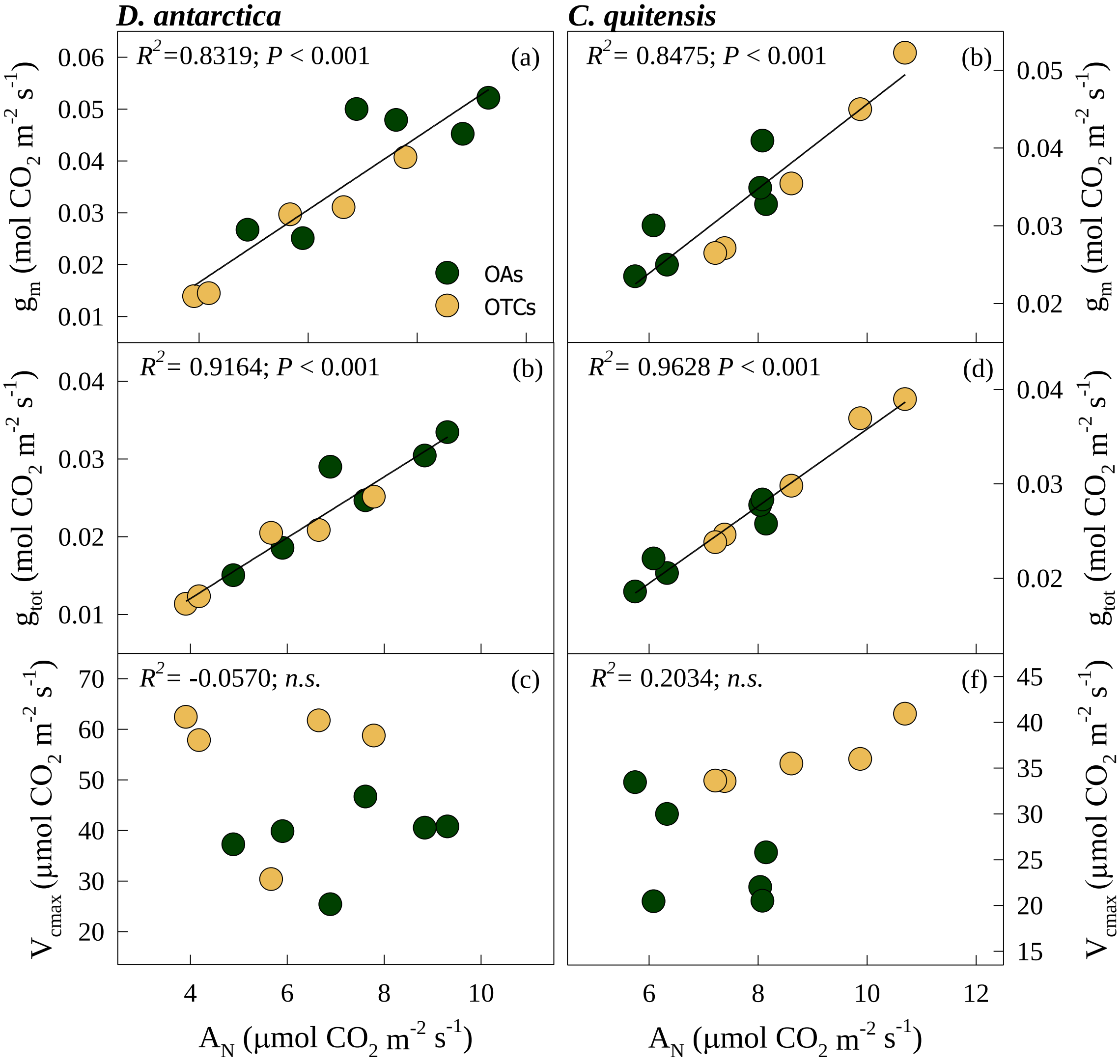

Figure S2. The relationship between the leaf mesophyll CO_2 conductance (a, b), total leaf conductance (c, d), and maximum Rubisco carboxylation rate (e, f) with the net photosynthetic CO_2 assimilation rate (A_N) for *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas (OAs) and inside open top chambers (OTCs). The regression coefficient and the significance of the relationship are shown for each species, considering both growing conditions together. *n.s.* not significant.

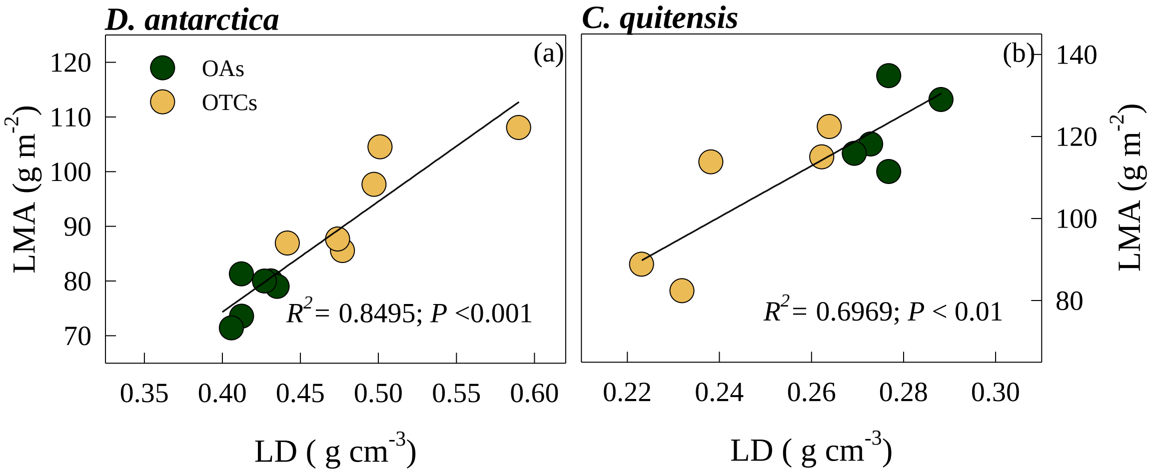

Figure S3. Relationship between leaf mass area and leaf density for *Deschampsia antarctica* and *Colobanthus quitensis* growing in open areas (OAs) and inside open top chambers (OTCs). The regression coefficient and the significance of the relationship are shown for each species, considering both growing conditions together.

Table S1. Daily air temperature parameters for open areas (OAs) and open top chambers (OTCs) during six growing seasons, from December 2013 until February 2019.

Season	Date	Variable	*n*	OAs	OTCs
2014	11 Dec 2013-8 Mar 2014	Mean air temperature (T_{mean}, °C)	88	1.68 ± 0.23a	3.13 ± 0.27a*
		Maximum air temperature (T_{max}, °C)		3.95 ± 0.30a	6.58 ± 0.44a*
		Minimum air temperature (T_{min}, °C)		-0.60 ± 0.20a	-0.02 ± 0.17a*
		Time below 0 °C (h)		893	682
2015	10 Jan 2015 -5 Mar 2015	Mean air temperature (T_{mean}, °C)	55	2.71 ± 0.31a	4.09 ± 0.34ab*
		Maximum air temperature (T_{max}, °C)		4.83 ± 0.40a	7.81 ± 0.50ab*
		Minimum air temperature (T_{min}, °C)		0.71 ± 0.29b	0.92 ± 0.30b
		Time below 0 °C (h)		251	246
2016	18 Dec 2015-12 Feb 2016	Mean air temperature (T_{mean}, °C)	57	2.60 ± 0.28a	4.41 ± 0.34b*
		Maximum air temperature (T_{max}, °C)		4.79 ± 0.35a	8.57 ± 0.52ab*
		Minimum air temperature ($T_{min,}$ °C)		0.43 ± 0.23ab	0.76 ± 0.24ab
		Time below 0 °C (h)		357	324
2017	1 Dec 2016-8 Mar 2017	Mean air temperature (T_{mean}, °C)	98	2.52 ± 0.25a	4.13 ± 0.31ab*
		Maximum air temperature (T_{max}, °C)		4.74 ± 0.32a	8.35 ± 0.51ab*
		Minimum air temperature (T_{min}, °C)		0.43 ± 0.22ab	0.66 ± 0.216ab
		Time below 0 °C (h)		704	664
2018	1 Dec 2017-8 Mar 2018	Mean air temperature (T_{mean}, °C)	84	6.68 ± 0.34c	4.89 ± 0.31b*
		Maximum air temperature (T_{max}, °C)		9.07 ± 0.35c	9.27 ± 0.46b
		Minimum air temperature (T_{min}, °C)		4.46 ± 0.38d	1.07 ± 0.23b*
		Time below 0 °C (h)		120	336
2019	1 Dec 2018-15 Feb 2019	Mean air temperature (T_{mean}, °C)	77	5.12 ± 0.22b	4.30 ± 0.26ab*
		Maximum air temperature (T_{max}, °C)		7.49 ± 0.30b	8.45 ± 0.43ab
		Minimum air temperature (T_{min}, °C)		2.95 ± 0.18c	0.92 ± 0.16b*
		Time below 0 °C (h)		32	309

n: total days of each growing season. Different letters indicate significant differences for each parameter among the six-season within each growing condition (i.e., OAs or OTCs), according to Tukey's test ($P < 0.05$). The asterisk (*) indicates statistically significant differences between OAs and OTCs for each season according to Student's *t*-test ($P < 0.05$).

Table S2. Pearson´s correlations coefficients and the significance of the relationship mean hydraulic diameter (D_h), theoretical hydraulic conductivity (K_h), specific hydraulic conductivity (K_s), and leaf-specific hydraulic conductivity (LSC) with the leaf (K_{leaf}) and whole plant (K_{plant}) hydraulic conductivity for *Deschampsia antarctica* and *Colobanthus quitensis* considering both growing conditions together (OAs and OTCs) for each specie.

Parameters	*D. antarctica*		*C. quitensis*	
	K_{leaf}	K_{plant}	K_{leaf}	K_{plant}
D_h	0.663**	0.684**	0.755*	0.685*
K_h	0.696**	0.736**	0.886**	0.716*
K_s	0.699**	0.789***	0.754*	0.710*
LSC	0.790***	0.764***	0.917***	0.839**

Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

CAPÍTULO III: Hydraulic and photosynthetic performance of antarctic plants under successive freeze-thaw cycles

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Summary

- Climate change projections anticipate a warming trend and heightened weather variability in polar regions, potentially altering freeze-thaw patterns. Nonetheless, it is still unclear how rising temperatures and more frequent freeze-thaw events could affect the water and CO_2 management of Antarctic plants.
- Laboratory evaluations determined how growth temperature (5 and 15 ºC) and successive freeze-thaw cycles affected the hydraulic and photosynthetic performance of *Deschampsia antarctica* and *Colobanthus quitensis*.
- We found that: (i) warmer conditions enhanced the hydraulic and photosynthetic performance in both Antarctic vascular species, driven by anatomical adjustments in leaf xylem vessels; (ii) plants exposed to successive freeze-thaw cycles exhibited a coordinated decline in their whole plant hydraulic conductivity and leaf gas exchange, regardless of the growth temperature; (iii) the magnitude of change (%) in photosynthetic traits after successive freeze-thaw cycles varied among species. *D. antarctica* exhibited similar changes at both growth temperatures, whereas changes in *C. quitensis* were more noticeable at the lower temperature.
- Overall, Antarctic plants exhibited higher photosynthesis at warmer temperatures, maintaining or enhancing their ability to withstand freeze-thaw cycles. Nevertheless, the cumulative effects of freeze-thaw cycles in natural environments could disrupt the hydraulic balance and irreversibly limit photosynthesis.

Keywords: Antarctic vascular plants, climate change, *Colobanthus quitensis*, *Deschampsia antarctica*, freeze-thaw cycles, hydraulic conductivity, photosynthetic performance.

Introduction

The extension of the growing season in temperate or polar ecosystems due to climate change is associated with higher risks of frost occurrence (Liu *et al*., 2018). This is particularly important for polar regions because most climate change scenarios predict a general warming trend and increased variability in weather conditions (IPCC, 2019; IPCC, 2022), including alterations in precipitations and thawing patterns (Liang *et al*., 2022). The transition between frozen and thawed states coupled with periods of drought could impair plant physiological performance in several ways (McCulloh *et al.*, 2023). For instance, freeze-thaw cycles can hinder the photosynthetic capacity of leaves and lead to an excess of absorbed light energy, causing photoinhibitory damage (Demmig-Adams & Adams, 2006). In stems, freeze-thaw events can produce severe hydraulic dysfunction via embolisms caused by bubbles formed during a freeze, which then expand upon thaw (Tyree & Sperry, 1989; Hacke & Sperry, 2001; Sevanto *et al*., 2012; Hacke *et al*., 2017). This hinders the transport of water and nutrients, thus increasing the risk of mortality (Sperry, 2000; Zwieniecki *et al.*, 2015; Venturas *et al*., 2017; Qaderi *et al*., 2019).

The thermal fluctuations caused by freeze-thaw cycles can be more detrimental than prolonged exposure to cold temperatures alone (Kennedy, 1993; Guy, 2003). In cold climates, plant species deploy specific strategies to mitigate freeze damage. For example, at the biochemical and molecular levels, plants can synthesize and accumulate cryoprotectant molecules, such as soluble sugars (e.g., sucrose, glucose, and raffinose) and specific amino acids (e.g., proline and glycine) (Zinta e*t al.*, 2022). Additionally, cold climate plants can upregulate their production of antifreeze proteins and dehydrins, which help protect cellular structures from freeze-induced damage (Pearce, 2001; Zinta *et al.*, 2022; Jahed *et al.*, 2023). Regarding morphological and anatomical strategies, plants from cold climates can adjust their height and leaf size, minimizing the surface area exposed to cold temperatures (Körner, 2016). They may also increase their epidermal thickness to provide additional insulation and reduce the size of xylem vessels to prevent cavitation and embolism (Davis *et al.*, 1999; Zinta *et al.*, 2022).

In Antarctica, freeze-thaw cycles occur at different frequencies, from monthly and weekly to even daily (Batista *et al.*, 2022). Since the 1950s, the Antarctic Peninsula has experienced one of the most pronounced warmings on Earth (Gonzalez *et al.*, 2018; Carrasco *et al.*, 2021; Turner *et al.*, 2021). Climate projections indicate that the annual mean surface air temperature over Antarctica is expected to increase between 0.5 °C and 3.6 °C by 2081-2100, depending on the CO_2 emission scenario (Chown *et al.*, 2022). This warming has already proven to improve the environmental conditions for plant colonization, including longer growing seasons, higher temperatures, and increased rainfall (Fox & Cooper, 1998; Cook *et al.*, 2005). Tus, the only two native Antarctic vascular plant species, *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. have exhibited significant expansions in their local range and population numbers at several sites in Maritime Antarctica (Torres-Mellado *et al.*, 2011; Cannone *et al.*, 2016; 2021; 2022). This expansion can be attributed to specific physiological adjustments that have enhanced their performance under warmer conditions. For instance, Sáez *et al.* (2018a) reported that the increased growth rate of *C. quitensis*, grown under *in situ* warming conditions, was associated with an increase in carbon assimilation, mediated by specific adjustments in leaf anatomical traits (i.e., reduced fibber content, leaf mass per area and leaf density), resulting in an increase in the leaf mesophyll conductance (g_m). In contrast, no significant responses were found in *D. antarctica* grown under *in situ* warming conditions (Sáez *et al.*, 2018a). However, subsequent laboratory experiments at higher temperatures (10 °C and 16 °C) revealed that *D. antarctica* could respond similarly to *C. quitensis*, increasing g_m and, consequently, its net photosynthetic CO_2 fixation (Sáez *et al.*, 2018b). Recently, Sáez *et al.* (2024) examined the leaf hydraulic properties and their response to varying growth temperatures in both Antarctic vascular species. This study revealed that Antarctic vascular plants adjusted some leaf xylem anatomical traits with increasing growth temperatures, enabling coordinated increases in leaf hydraulic conductivity (K_{leaf}) and photosynthetic capacity (A_N).

Overall, the evidence available thus far has shown that the increase in temperature is beneficial for Antarctic vascular species, favouring traits that ultimately result in greater growth. The assumption here is that the increase in CO_2 assimilation under warmer conditions increases the sugars and amino acids for growth, as well as for metabolism maintenance and defence (Unterholzner *et al.*, 2022; Zepeda *et al.*, 2022). Soluble sugars serve as important regulators for Antarctic vascular species' physiological adjustment to freezing stress (see Ramírez *et al.*, 2024). Moreover, while warmer conditions may enhance productivity, it is noteworthy that Antarctic vascular plants have maintained their stress tolerance capacities. For instance, in 2024 Sáez *et al.* found that despite the increase in K_{leaf} under warmer conditions, hydraulic traits, such as leaf vessel size, did not change, with values amongst the lowest reported in the literature, while the osmotic potential at full turgor showed some of the highest values ever reported. Similarly, *in situ* warming experiments on Antarctic vascular plants have shown that warmer temperatures did not generate a reduction in their freezing tolerance (Sierra-Almeida *et al.*, 2018); on the contrary, they maintained the lowest values for these parameters reported thus far for vascular plants. Nonetheless, it remains unclear how their enhanced performance under warmer conditions could support their ability to cope with extreme events, such as freeze-thaw cycles.

In the present study, we hypothesized that the hydraulic and photosynthetic performance of Antarctic vascular plants would be less affected by repeated freeze-thaw cycles when grown at higher temperatures as compared to lower temperatures. To test this hypothesis, we evaluated the effects of growth temperature on the hydraulic and photosynthetic performance of Antarctic vascular plants facing successive freeze-thaw cycles. There is a notable knowledge gap regarding how these cycles impact vascular plants, particularly their hydraulic and photosynthetic processes, even though these traits largely determine plant growth, productivity, and survival. A clear understanding of these physiological aspects is essential to improve our comprehension regarding the expansion of Antarctic vascular species related to global warming under the predicted weather conditions.

Materials and Methods

Plant material and growth conditions

Deschampsia antarctica and *Colobanthus quitensis* were collected from an Antarctic population located on King George Island (KGI), near the H. Arctowski Polish Antarctic Station (62° 09′S, 58° 28′ W). Plants were then transferred to the laboratory and cultivated in 500 mL pots in a substrate of sterile organic soil, vermiculite, and peat (3:1:1 v/v) in a growth chamber at 4 °C (Pi-Technology Inc. Santiago, Chile) at 80 ± 5% RH, with a light intensity of 150 μmol photons m^{-2} s^{-1} and an 18 h day length. Plants were fertilized with 0.02 g L^{-1} Phostrogen (Distribudora Yates Ltda., Chile) every two weeks. Subsequently, plants were randomly assigned to two different day/night temperature regimes (T_g): 5 °C/2 °C and 15 °C/2 °C because 5 °C corresponds to the highest average diurnal air temperature recorded during the Antarctic summer, while 15 °C represents the previously determined optimal photosynthetic temperature for *D. antarctica* and *C. quitensis* (Sáez *et al.*, 2018a,b). After one month at each thermoperiod, plants were exposed to successive freeze-thaw cycles to determine how these affected the hydraulic and photosynthetic performance.

Freeze-thaw experiment

Both Antarctic species were exposed to an experimental period of ten successive freeze-thaw cycles (C_n; Fig. 1a) applied in a programmable growth chamber with controlled temperature and light conditions. At each growth temperature (T_g, 5 °C or 15 °C), each C_n (Fig. 1b) started at 1:00 h; then, the air temperature gradually decreased for three hours until the target temperature of -4 °C was reached and maintained for seven hours until 8:00 h (Fig. 1b). This target temperature was chosen to mimic the temperature experienced by soil and air in the natural summer conditions of the Antarctic Peninsula (see Sierra-Almeida *et al.*, 2018). During the next three-hour period (from 8.00 to 11:00 h), the temperature was gradually increased to the target growth temperature (5 °C or 15 °C), which was maintained for the next 11 hours until 22:00 h, daytime for the next C_n to start. Light conditions (yellow period, Fig. 1b) were also controlled to mimic the Antarctic photoperiod. To prevent photodamage, plants were gradually exposed to increasing light intensities, initially using fluorescent tubes. At 4:00 h, plants from both growth temperatures were exposed to a light intensity of 50 µmol photons m^{-2} s^{-1} (on, Fig. 1b), which was gradually increased by 50 µmol photons m^{-2} s^{-1} per hour until 10:00 h, reaching a light intensity of 300 µmol photons m^{-2} s^{-1}. From 10:00 to 16:00 (purple dotted line in Fig. 1b), the plants were exposed to higher light intensity, reaching a level of 1000 µmol photons m^{-2} s^{-1}, facilitated by a supplementary LED lighting system (GP-180W, Innova-Led, Santiago, Chile). The plants remained under this light intensity for one hour prior to all measurements. From 13:00 to 16:00 h, physiological measurements were conducted before the freeze treatment (C_0, control), after the 4th (C_4), and the10th cycle (C_{10}) (M inside the green circle in Fig. 1b). All measurements were carried out during maximum transpiration rates (see below) with a 30-min interval between plants, to ensure uniform light exposure. From 16:00 to 22:00 h, the LED panel was turned off, and the light was maintained with the basic system. Finally, the light was turned off from 22:00 to 4:00 h (off, Fig. 1b) to initiate a new thaw event. Throughout the experiment, air and soil temperatures were recorded using a weather station (HOBO®-U-30, Onset Computer Co, Bourne, MA, USA). The freeze-thaw cycles were applied to the same plants throughout the experiment.

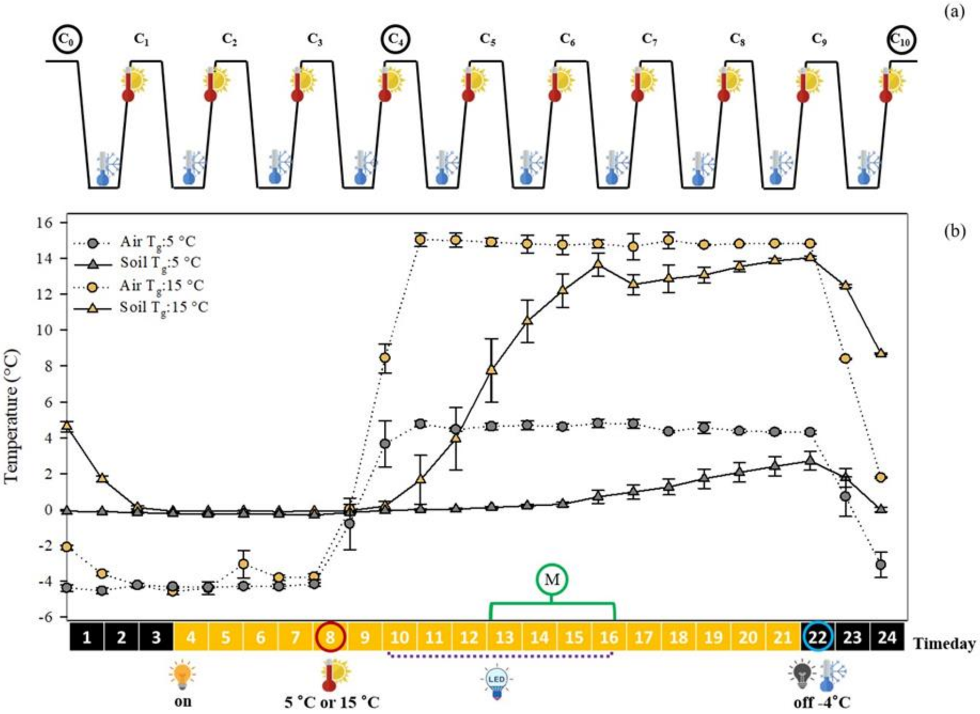

Figure 1. a) Graphic representation of ten successive freeze-thaw cycles (C_n), and b) the soil and air temperature for every freeze-thaw cycle, together with the light-dark photoperiod. The red circle indicates the time at which the temperature in the growth chamber was changed to thaw (growth temperature, T_g: 5 °C or 15 °C), and the blue circle the time at which the temperature in the growth chamber was changed to freezing (-4 °C). Black circles in A) indicate when experimental measurements were performed: before the application of a freeze-thaw cycle (C_0) and after the fourth (C_4) and tenth (C_{10}) cycles. The green circle in B) indicates the period when physiological measurements (M) were

performed. The purple dotted line indicates the period using a LED supplementary light system.

Whole plant hydraulic conductivity

Whole plant hydraulic conductivity (K_{plant}, mmol m^{-2} s^{-1} MPa^{-1}) was determined according to Brodribb and Cochard (2009) for plants growing at 5 °C and 15 °C before (C_0) and after the 4th and 10th cycles (C_4 and C_{10}, respectively). First, leaf transpiration rate (E, mmol m^{-2} s^{-1}) and leaf water potential (Ψ_{leaf}, MPa) were simultaneously measured using a portable photosynthesis system (Li-6400; LI-COR Inc., Lincoln, NE, USA) and Scholander pressure chamber, respectively. Then, K_{plant} was calculated as:

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

where Ψ_{soil} is the soil water potential. Considering that the soil was well irrigated throughout the experiment and the temperature had virtually no effect on Ψ_{soil}, it was assumed that $\Psi_{soil} = 0$ MPa (Cochard *et al.*, 2000). K_{plant} was obtained for 6-7 plants per species and growing condition (T_g). K_{plant} was then corrected for the effects of temperature on water viscosity by standardizing it to 20 °C (Sack *et al*., 2002; Brodribb *et al.*, 2007). Additionally, the leaf relative water content (% RWC) was calculated as: (fresh weight - dry weight)/(turgid weight - dry weight)*100 for fresh leaves collected from each plant. The turgid weight was determined from the weight of leaves immersed in distilled water at 4 °C for 48 h in the dark, and the dry weight was obtained after drying the leaves in an oven at 65°C for 72 h.

Leaf xylem anatomy

After one month of acclimation to each thermoperiod (5 °C and 15 °C), and before subjecting the plants to freeze-thaw treatments, central portions of the leaves were collected and fixed in formaldehyde, acetic acid, and ethanol, then stored at 4 °C. Following standard methods, tissues were processed for embedding in paraffin (Ruzin, 1999). In brief, leaf tissues were dehydrated in a graded series of ethyl alcohols, cleared in xylene, and embedded in paraffin. Paraffin sections were cut at 10 μm thickness using a rotary microtome mounted on a glass slide. Cross-sections were deparaffinized in xylene and rehydrated in a graded series of ethyl alcohols and distilled water (ddH$_2$O). To study leaf xylem anatomy, leaf cross-sections of each species were prepared and stained using a 0.5% toluidine blue solution. The samples were observed using an optical microscope (CX31, Olympus, Japan), and micrographs were captured using a digital PC-attached camera USB-2.0 (Cmex-5, Euromex, Holland). For each species and growth temperature, four micrographs were randomly selected to measure the leaf xylem vessel lumen diameter (d) and the number of vessels in the main vein. All the images were analysed using ImageJ (ImageJ; Wayne Rasband/NIH, Bethesda, MD, USA). The mean hydraulic diameter (D_h, μm) was calculated according to Corcuera *et al.* (2012) as $D_h = \Sigma d^5/\Sigma d^4$.

Gas exchange and mesophyll conductance estimation

Leaf gas exchange parameters were measured using a portable photosynthesis system (LI-6400; LI-COR, Inc., Nebraska, USA) with an infrared gas analyser coupled with a 2 cm^2 leaf fluorescence chamber (LI-6400-40 leaf chamber fluorometer; LI-COR, Inc.). All measurements were performed on the same plants that were selected for whole plant hydraulic conductivity (K_{plant}) measurements. Leaves were carefully clamped into the leaf-cuvette, avoiding overlaps, and ensuring contact with the leaf thermocouple. The leaf temperature was fixed at 5 °C or 15 °C (according to growth temperature), with an air flow rate of 300 µmol s^{-1} and a relative humidity of 50-70%. Leaf steady-state conditions were induced at an ambient CO_2 concentration (C_a) of 400 µmol mol^{-1} air and saturating photosynthetic photon flux density of 1000 µmol photons m^{-2} s^{-1} (PPFD). Once steady-state conditions were reached, instantaneous measurements of light-saturated net CO_2 assimilation (A_N), stomatal conductance to CO_2 diffusion (g_s), and the substomatal CO_2 concentration (C_i) were registered. Also, values of steady-state fluorescence (F_s) were registered immediately after the steady-state conditions for gas exchange were achieved. Then, a saturating white light flash of approximately 8000 µmol m^{-2} sec^{-1} was applied to determine the maximum fluorescence ($F_{m'}$). The electron transport rate (ETR) was estimated according to Genty *et al.* (1989) as $ETR = PPFD \times \Phi PSII \times \alpha \times \beta$, where ΦPSII is the efficiency of photosystem II, α the leaf absorbance and β the electrons partitioning between photosystems I and II. ΦPSII was estimated as $\Phi PSII = (F_{m'} - F_s)/F_{m'}$ (Genty *et al.*, 1989). The $\alpha \times \beta$ parameter was estimated following Valentini *et al.* (1995). Light response curves under non-photorespiratory conditions in a low O_2 atmosphere (< 2%) were used to establish the relationship between ΦPSII and ΦCO_2 under non-photorespiratory conditions (with $\Phi CO_2 = (A + R_d)/PPFD$), then considering $\alpha \times \beta = 4/b$ where b is the slope of the $\Phi PSII \sim \Phi CO_2$ relationship.

From the combined gas exchange and Chl*a* fluorescence measurements, *in vivo* mesophyll conductance to CO_2 (g_m) was calculated as in Harley *et al.* (1992):

$g_m = A_N/(C_i - (\Gamma^*(ETR + 8 (A_N + R_L))/(ETR - 4 (A_N + R_L))))$

where A_N and C_i were obtained previously from instantaneous measurements of gas exchange at saturating PPFD. The rate of non-photorespiratory CO_2 evolution in the light (R_L) was assumed to be half of R_{dark}, determined after exposing plants to darkness for at least 30-min (Niinemets *et al.*, 2005). The chloroplast CO_2 compensation point (Γ^*) was calculated according to Brooks & Farquhar (1985) from the Rubisco specificity factor ($S_{c/o}$) measured *in vitro* (Sáez *et al.*, 2017).

Statistical analysis

Repeated measured ANOVAs were conducted on several parameters, including whole plant hydraulic conductivity and photosynthetic performance, to examine the effects of growth temperature (Tg: 5 °C and 15 °C) and freeze-thaw cycles (C_0, C_4, and C_{10}), with cycles as the repeated factors. To assess the differences among means, post hoc Tukey tests were conducted at a significance level of $P < 0.05$. Additionally, we used a Student t-test to assess the effects of growth temperature on the mean hydraulic diameter. Chi-square (χ^2) tests were used to assess the statistical differences on the distribution of xylem vessel diameters among growth temperatures. Regression analyses were performed to examine the relationship between whole plant hydraulic conductivity and photosynthetic traits (A_N, g_s, and g_m). Statistical analyses were conducted using Statistica 8.0 (Statsoft, Tulsa, USA).

Results

The effect of freeze-thaw cycles on whole-plant hydraulic conductivity

The whole plant hydraulic conductivity (K_{plant}) was, in general, significantly lower in plants grown at the low temperature and significantly reduced by repeated freeze-thaw cycles (Fig. 2). In *D. antarctica*, before the freeze treatment (C_0), mean K_{plant} values were 0.71 ± 0.12 and 1.69 ± 0.10 mmol m^{-2} s^{-1} MPa^{-1} in plants grown at 5 °C and 15 °C, respectively. These values decreased by around 50% and 36% after the 10th cycle (C_{10}) in plants grown at 5 °C and 15 °C, respectively (Fig. 2a, Table 1). A similar trend was observed in *C. quitensis*, with K_{plant} in C_0 of 2.59 ± 0.08 and 3.29 ± 0.13 mmol m^{-2} s^{-1} MPa^{-1} at 5 °C and 15 °C, respectively, decreasing around 78% after the C_{10} for both growth temperatures (Fig. 2b; Table 1).

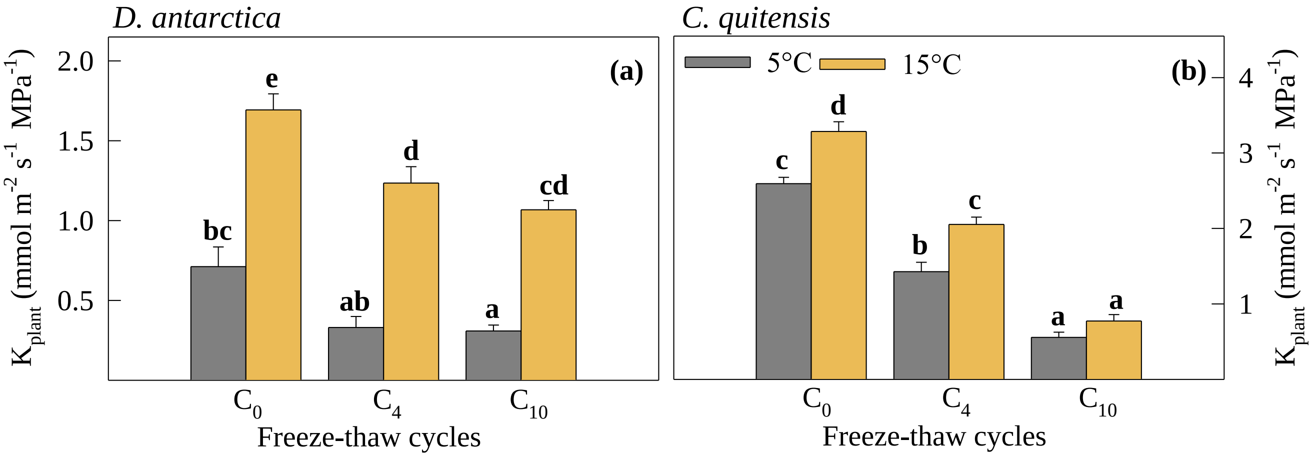

Figure 2. Effects of freeze-thaw cycles on the whole plant hydraulic conductivity (K_{plant}) of *Deschampsia antarctica* (a) and *Colobanthus quitensis* (b) grown at 5 °C and 15 °C. Measurements were performed before the application of a freeze-thaw cycle (C_0) and after

the fourth (C_4) and tenth (C_{10}) cycles. For each species, different letters indicate statistical differences between growth temperatures and freeze-thaw cycles together (Tukey´s HSD test, $P < 0.05$).

Table 1. The magnitude of change (%) between cycles (C_0-C_4 and C_0-C_{10}) at both growth temperatures (5 °C and 15 °C) for hydraulic and photosynthetic performance. Whole plant hydraulic conductivity (K_{plant}), net photosynthetic CO_2 assimilation rate (A_N), stomatal conductance (gs), and mesophyll conductance to CO2 (g_m) for *Deschampsia antarctica* (DA) and *Colobanthus quitensis* (CQ) grown at 5 °C and 15 °C. The percentage of decrease was calculated considering C_0 as the 100%. Values are means ± SE (n = 6-8).

Species	Cycles	K_{plant} %	A_N %	g_s %	g_m %
$DA\_{5°C}$	C_0-C_4	47.2 ± 10.9	52.2 ± 3.9	32.3 ± 10.7	57.5 ± 3.6
	C_0-C_{10}	45.7 ± 8.6	55.8 ± 5.3	27.5 ± 4.9	64.7 ± 4.3
$DA\_{15°C}$	C_0-C_4	24.5 ± 8.2	23.0 ± 5.6	41.0 ± 4.3	16.7 ± 3.7
	C_0-C_{10}	35.8 ± 4.7	49.0 ± 5.3	40.2 ± 5.6	56.6 ± 5.4
$CQ\_{5°C}$	C_0-C_4	44.9 ± 4.9	43.4 ± 6.6	26.3 ± 5.6	49.3 ± 4.2
	C_0-C_{10}	78.4 ± 2.2	66.2 ± 2.1	65.8 ± 2.6	66.0 ± 2.0
$CQ\_{15°C}$	C_0-C_4	36.5 ± 4.4	21.2 ± 3.0	34.7 ± 7.3	30.7 ± 5.7
	C_0-C_{10}	76.4 ± 2.7	39.8 ± 2.4	72.7 ± 1.9	35.7 ± 5.0

The effects of growth temperature on leaf xylem anatomy and hydraulic traits

Regarding the number of leaf xylem vessels in the main vein and the mean hydraulic diameter (D_h), both species showed significant differences between growth temperatures (T_g). In *D. antarctica*, the number of leaf xylem vessels was higher in plants grown at 15 °C (18 ± 0.84) than those grown at 5 °C (11 ± 0.41, inset graph Fig. 3a). In contrast, in *C. quitensis*, the number of vessels (inset graph Fig. 3b) was lower in plants grown at 15 °C (16 ± 0.80) than those grown at 5 °C (19 ± 0.64). In both species, D_h was higher in plants grown at 15 °C than those grown at 5 °C (Fig. 3).

Regarding the distribution of leaf xylem vessels, the Antarctic species exhibited contrasting patterns. *D. antarctica* showed a wider range of vessel diameters, extending up to the range of 10-11 µm, and a higher frequency of xylem vessels in narrow diameter classes, with the highest frequency being within the 3-4 µm range, regardless of T_g (Fig. 3a). On the other hand, *C. quitensis* displayed a constrained range of diameter classes, up to 3-4 µm in plants grown at 5 °C, and up to 5-6 µm in plants grown at 15 °C (Fig. 3b). Chi-square analyses revealed a significant effect of T_g on the distribution of diameter classes in both species (Fig. 3).

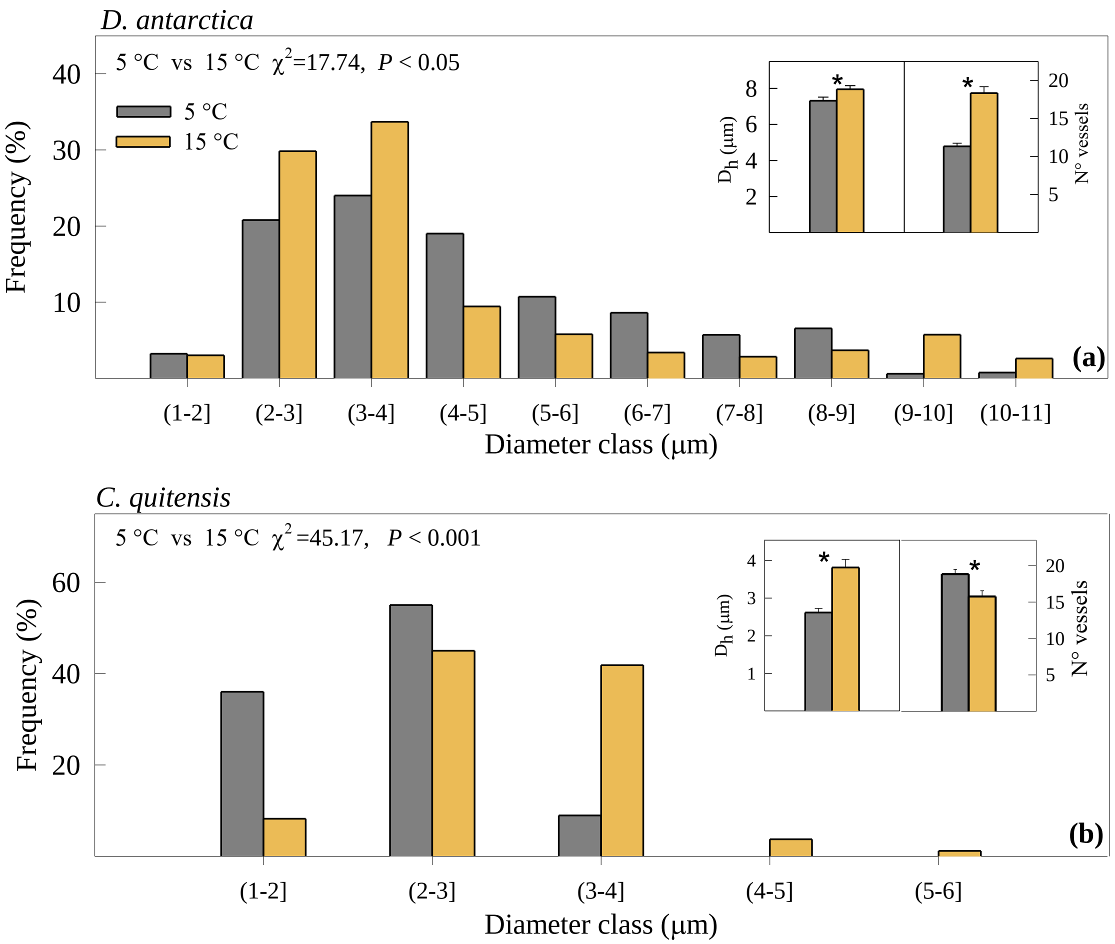

Figure 3. Frequency distribution of leaf xylem vessel classes of *Deschampsia antarctica* (a) and *Colobanthus quitensis* (b) grown at 5 °C and 15 °C. The effects of growth temperatures on vessel frequency distribution were evaluated within each species using the Chi-square (χ^2) homogeneity test. The inset graphs show the mean hydraulic diameter (D_h) and the mean number of leaf xylem vessels of the main vein (N° vessels). The asterisk indicates statistical differences between growth temperatures, according to the Student-t test ($P < 0.05$).

The effects of freeze-thaw cycles on leaf gas exchange

In both species, the net CO_2 assimilation rate (A_N), the stomatal conductance (g_s), and the mesophyll conductance (g_m) were significantly higher in plants grown at 15 °C (Fig. 4). When comparing the two growth temperatures across freeze-thaw cycles, plants grown at 15 °C showed a progressive decline in leaf gas exchange parameters after being exposed to freeze-thaw cycles (Fig. 4). The magnitude of this decline was more pronounced in *C. quitensis* than in *D. antarctica*, particularly when considering the percentage of change from C_0 to C_{10} between growth temperatures (Table 1). In addition, for *C. quitensis*, g_s was more sensitive to freeze-thaw cycles than g_m, while the contrary was found for *D. antarctica*. This could explain the contrasting pattern observed between the two species for intrinsic water use efficiency (Fig. 4).

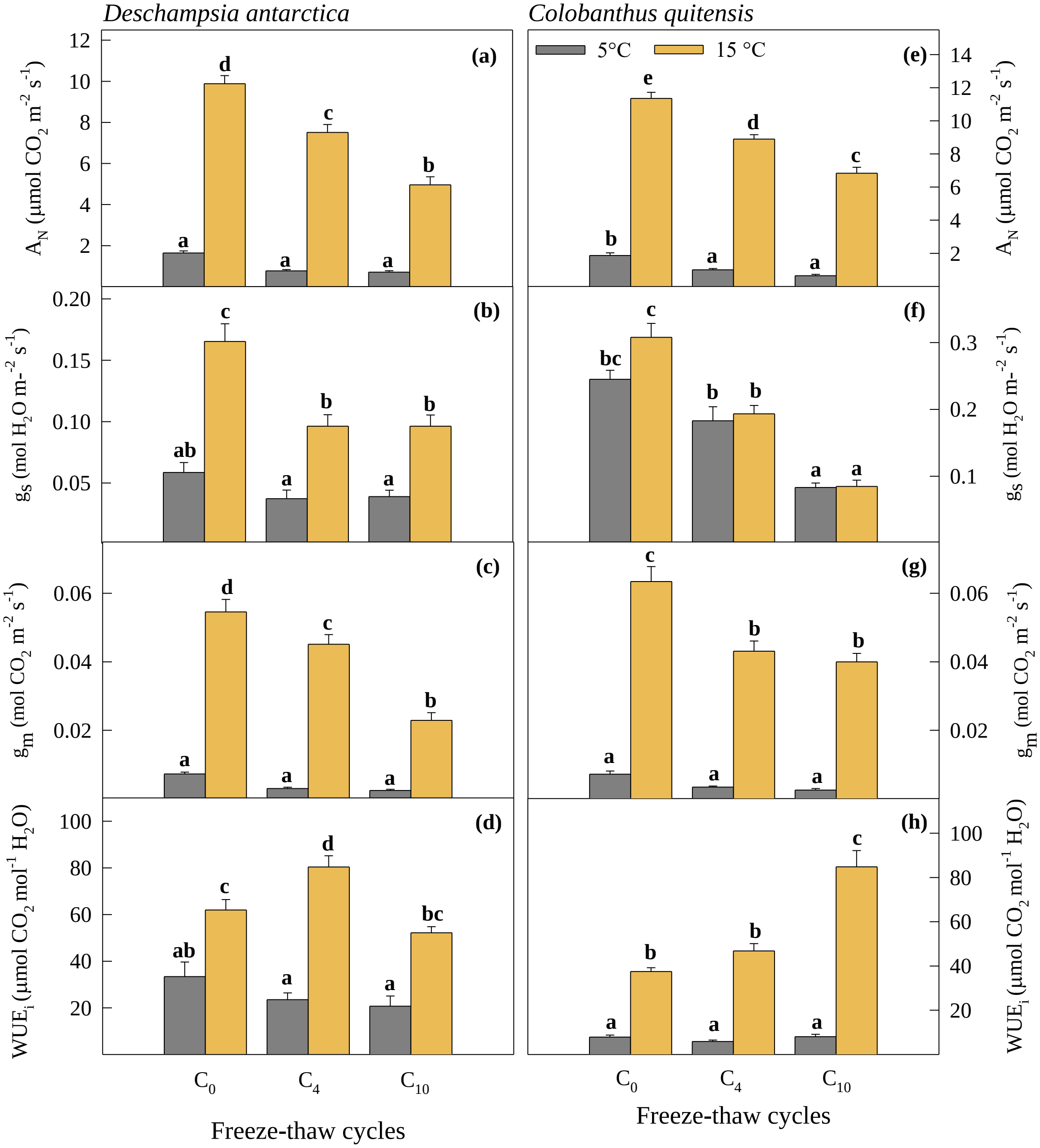

Figure 4. Effects of freeze-thaw cycles on the gas exchange of *Deschampsia antarctica* (a-d) and *Colobanthus quitensis* (e-h) grown at 5 °C and 15 °C. Measurements of net CO_2 assimilation rate (A_N), stomatal conductance (g_s), mesophyll conductance (g_m), and intrinsic water use efficiency (WUE_i) were performed before the application of a freeze-

thaw cycle (C_0) and after the fourth (C_4) and tenth (C_{10}) cycles. For each species, different letters indicate statistical differences between growth temperatures and freeze-thaw cycles together, according to Tukey´s HSD test ($P < 0.05$). The data were fitted by linear regressions.

The coordination of hydraulic and gas exchange traits

Both Antarctic species showed a significant relationship between hydraulic and gas exchange parameters, where the co-variation between K_{plant} and the gas exchange parameters depended on T_g (Fig. **5**). The slopes of the relationships were higher for plants grown at 15 °C, indicating a higher sensitivity of gas exchange parameters to any change in K_{leaf} compared to plants grown at 5 °C.

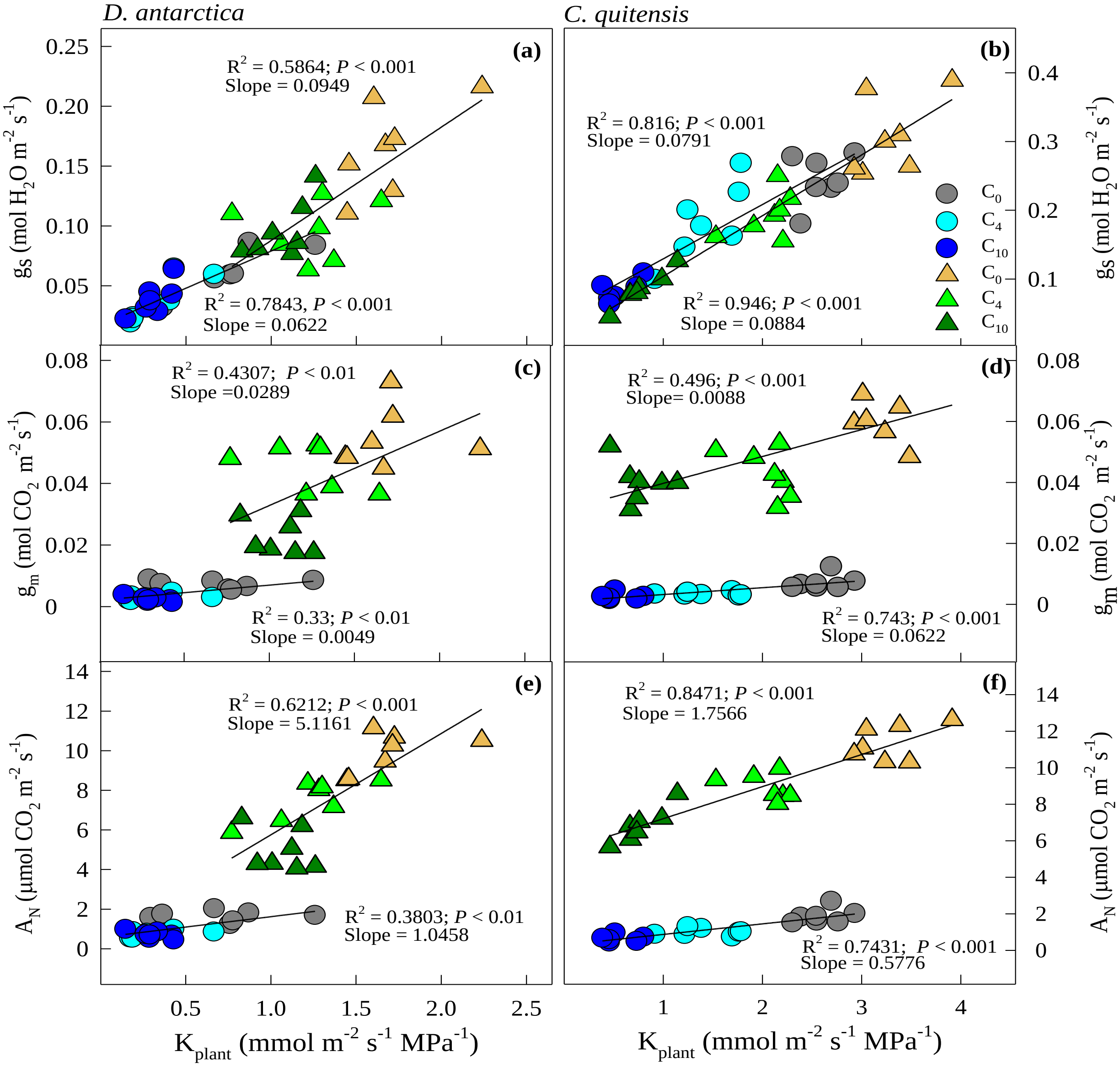

Figure 5. Relationships between whole plant hydraulic conductivity (K_{plant}) and stomatal conductance (g_s; a, b), mesophyll conductance (g_m; c, d), and net CO_2 assimilation rate (A_N; e, f) for Deschampsia antarctica and Colobanthus quitensis grown at 5 °C (circles) and 15 °C (triangles) before the application of a freeze-thaw cycle (C_0, grey and yellow) after the fourth (C_4; light blue and light green) and tenth (C_{10}; dark blue and dark green) cycles. The data were fitted by linear regressions.

Discussion

One of the predicted effects of climate change is the increased frequency of freeze-thaw events, especially at polar latitudes (IPCC, 2019; Liang *et al.*, 2022). Although cold climate species are adapted to the occurrence of these events, increased exposure to warmer conditions could alter some physiological processes. As previously reported, warmer conditions have proven to positively influence the hydraulic and photosynthetic performance of Antarctic vascular plants. Nevertheless, a crucial question arises from these findings: what potential trade-offs or costs might these adjustments imply in the face of successive freeze-thaw events?

The hydraulic performance of Antarctic vascular plants facing successive freeze-thaw events

Several studies have indicated that plants can adjust their xylem conduits (e.g., number, distribution, and clustering in vessels or tracheid) in response to environmental conditions to enhance their hydraulic efficiency (Hacke *et al.*, 2000; Fisher *et al.*, 2007; Martínez-Vilalta *et al.*, 2009; Choat *et al.*, 2011; Plavcová and Hacke, 2012; Schreiber *et al.*, 2015; Li *et al.*, 2023). In the present study, both Antarctic species presented lower K_{plant} under colder growing conditions (Fig. 2), most likely due to adjustments in leaf xylem vessels, including variations in the diameter and the vessel frequency (Fig. 3). When grown at the lower temperature, *D. antarctica* exhibited a reduced number of leaf xylem vessels and a lower mean hydraulic diameter (D_h), displaying a lower frequency of vessels in smaller diameter classes compared to those observed at 15 °C (Fig. 3a and inside graph).

Conversely, although *C. quitensis* also showed lower D_h at the lower temperature, the number of leaf vessels increased (inside graph Fig. 3b). Notably, under warmer conditions, this species presented a higher frequency of vessels in larger diameter classes (Fig. 3b). This result agrees with Sáez *et al.* (2024), where Antarctic species also displayed different behaviour, adjusting some leaf anatomical traits, and enabling coordinated increases in leaf (K_{leaf}) and whole (K_{plant}) hydraulic conductivity with the increase in growth temperature.

A lower K_{plant} has been associated with increased resilience to the embolism induced by freeze-thaw cycles (Choat *et al.*, 2011). In this sense, plants grown at 5 °C could be more resilient than those grown at 15 °C. The loss of hydraulic function due to freeze-thaw-induced embolism is well-documented in conifers and woody angiosperms (Pittermann and Sperry, 2006; Mayr *et al.*, 2007; Charrier *et al.*, 2014; Dai *et al.*, 2020; Charra-Vasckou *et al.*, 2023), while limited data is available for herbs due to technical limitations (Stiller and Sperry, 2002; Li *et al.*, 2009; Saha *et al.*, 2009; Lens *et al.*, 2016; Bourbia *et al.*, 2021). According to our results, regardless of the growth temperature, both Antarctic species progressively reduced K_{plant} in response to successive freeze-thaw cycles (Fig. 2), which could be evidence of embolism. However, both species showed notably smaller mean leaf vessel diameters for the midrib sizes compared to several shrub, tree, and herb species (Fig. S2), even when plants were grown at 15 °C, a temperature at which higher K_{plant} and D_h were observed. Embolism resulting from freeze-thaw cycles has always been associated with the size of xylem vessels. For instance, Davis *et al.* (1990) established that in stems conduits greater than or equal to 44 µm would be expected to cavitate, while all less than this diameter would remain functional. Later, Cavender-Bares *et al.* (2005) reduced this limit, showing that a higher loss of hydraulic conductivity occurred in diameters between 20-25 µm in oak species. Lütz *et al.* (2010) suggested a tracheid diameter of 30 µm to resist embolism in plants growing in cold environments. According to our results, Antarctic plants, which have among the smallest leaf xylem diameters reported (around 4-8 µm), showed embolism symptoms. Considering the range of vessel sizes that prevent freeze-thaw embolism, our study prompts a reconsideration of these thresholds, especially for small herbaceous species. Based on the methodology of the present study, we cannot guarantee that the decrease in K_{plant} is due to embolism. Furthermore, we cannot be certain as to which part of the soil-plant-atmosphere continuum (i.e., root, stem, or leaf) embolism occurred. Several studies have suggested that anatomical and biochemical adjustments play a role in hydraulic regulation. At the leaf level, changes in the xylem parenchyma, bundle sheath, and leaf mesophyll have been proposed (Buckley *et al.*, 2015; Scoffoni *et al.*, 2017; Xiong *et al.*, 2017). In roots, the presence of abscisic acid is a key factor (Bhattacharya, 2022). Additionally, aquaporins in both leaf and root tissues may impact the efficiency of water movement within plants (Martre *et al.*, 2002; Prado *et al.*, 2013; Pou *et al.*, 2013; Sade *et al.*, 2014; Rodríguez-Gamir *et al.*, 2019). Consequently, several questions remain unanswered and require further investigation.

It is noteworthy that freeze-thaw events are common in Antarctica (Batista *et al.*, 2022). Nevertheless, the effects of these events on hydraulic performance have never been evaluated, specifically under conditions mimicking the Antarctic summer in combination with warming scenarios. Both species showed a clear decrease in K_{plant} at consecutive cycles at each growth temperature (Fig. 2). However, the magnitude of change in K_{plant} was very similar between both growth temperatures from C_0-C_{10} (Table 1).

At warmer conditions, *D. antarctica* exhibited higher rates of K_{plant} than at lower temperatures. This could be attributed to the increased occurrence of small diameter vessel classes in *D. antarctica*, serving as a buffer against the detrimental effects of freeze-thaw cycles on K_{plant} (Fig. 3a). Water traits such as the leaf relative water content (RWC) and the leaf water potential (ψ_{leaf}) did not differ for the two evaluated growth temperatures from C_0 to C_{10} (Table S1). Therefore, *D. antarctica* appeared to exhibit a greater resistance to the adverse effects of freeze-thaw cycles. In *C. quitensis,* RWC and ψ_{leaf} significantly decreased between C_0 and $C_{10,}$ regardless of the growth temperature (Table S1), but as in *D. antarctica*, no differences were observed when comparing the two evaluated growth temperatures from C_0 to C_{10}.

RWC and ψ_{leaf} are important indicators of the plant's hydration status; however, the values of ψ_{leaf} were not close to the leaf water potential at the turgor-loss point (ψ_{tlp}) previously informed for this species, mainly at 15 °C (Sáez *et al.*, 2024). This suggests that they may have adopted a riskier strategy to maintain other physiological processes (e.g., photosynthesis), while K_{plant} dropped drastically at both growth temperatures (around 78%) (Fig. 2, Table 1).

Antarctic vascular plants showed contrasting sensibility of photosynthesis to successive freeze-thaw events

As observed in the hydraulic conductivity, changes in net photosynthesis (A_N) and diffusive determinants were also observed as the freeze-thaw cycles progressed. The photosynthetic performance of the two evaluated species differed after freeze-thaw cycles when comparing the results of the two growth temperatures (Fig. 4 and Table 1). In *D. antarctica,* g_m was the most affected parameter, with a decrease in C_{10} with respect to C_0 of around 66% in plants grown at 5 °C and 55% in plants grown at 15 °C. In fact, the reduction in A_N was triggered mainly due to a decrease in g_m, resulting in a strong correlation between A_N and g_m (Fig. S1). Despite the sustained reduction of g_m, A_N maintained positive rate, decreasing by around 50% from C_0 to C_{10} at both growth temperatures, and showing lower changes in stomatal conductance (g_s), which explain the sustained water use efficiency (WUE_i) among cycles (Fig. 4). These results, along with those found in hydraulic traits, further confirmed that *D. antarctica* deployed a water conservation strategy when exposed to freeze-thaw cycles. This hypothesis is also supported by other traits deployed in this species: i) leaf curling, reported by Gielwanowska *et al*. (2005) and Romero *et al.* (1999), and ii) the senescence program reported by Clemente-Moreno *et al.* (2020). This species has been observed curling its leaves towards the adaxial leaf surface, enclosing most of the stomata, which restricts the transpiratory surface. Additionally, this species has proven to activate a senescence program to cope with environmental stressors, playing a role in water conservation (Zhao *et al.*, 2022). As the freeze-thaw cycles increased, the leaves of *D. antarctica* at both growth temperatures turned more yellow, accompanied by a decrease in the chlorophyll content (Table S1) at C_{10} for both growth temperatures, as has been reported in other species (Guo *et al.*, 2022; Zhao *et al.*, 2022). Therefore, apparently *D. antarctica* experiences a trade-off between degrading cell structures and sustaining leaf functionality (i.e., gas exchange), which could be an additional key strategy to deal with freeze-thaw events.

Regarding *C. quitensis*, decreases in gas exchange were also observed; however, in this species, g_s was the most affected parameter, showing a reduction of around 66% and 73% from C_0 to C_{10} in plants grown at 5 °C and 15 °C, respectively (Fig. 4, Table 1). On the other hand, A_N and g_m were also affected, but a higher drop in these parameters was found in plants grown at low temperatures (66% between C_0 and C_{10}, Table 1). At the higher temperature, the drops in both A_N and g_m did not exceed 40%. This lower drop in g_m could act as a buffer against the strong decrease in g_s, enabling a positive A_N with a higher WUE_i (Fig. 4), mainly in plants grown at the higher temperature. In fact, when comparing the magnitude of change of A_N and g_m from C_0 to C_{10} at both growth temperatures, plants at the lower temperature were more affected than those at the higher temperature (Table 1). These findings were partially unexpected, mainly in plants grown at the low temperature, due to several reports showing that Antarctic species display mechanisms to deal with the constant low temperatures of this climate (see Cavieres *et al.*, 2016; Ramírez *et al.*, 2024). This phenomenon could be attributed to the fact that plants growing at lower temperatures operate far from the optimal photosynthetic temperature. Additionally, this result may imply that the assimilated carbon serves primarily to maintain basal metabolism, potentially leaving insufficient resources to resist stresses such as successive freeze-thaw cycles. On the other hand, at the higher temperature, the significant increase in net photosynthesis could provide a greater source of carbohydrates, which could enable plants to have a more favourable response to successive freeze-thaw cycles.

Coordination between whole plant hydraulic conductivity and gas-exchange

Vascular plants require an efficient and safe water transport system to ensure their survival and maintain productivity. One of the first defences against abiotic stress is the regulation of the stomatal aperture in response to changes in water potential (Martínez-Vilalta and Garcia-Forner, 2017), with stomatal closure commonly coinciding with the beginning of hydraulic dysfunction (Nardini and Salleo, 2000; Skelton *et al.*, 2018; Tombesi *et al.*, 2015**).** Our results showed that g_s decreased after successive freeze-thaw cycles, aligning with a reduction in K_{plant} for both species (Fig. **5**). Similarly, g_m also decreased, suggesting a coordinated decline between g_m and K_{plant}. Recent studies have identified specific anatomical determinants related to the outside-xylem pathway, such as mesophyll (S_m) and chloroplast (S_c) surface areas that face intercellular air spaces per leaf area and the fraction of intercellular air space (f_{ias}), which intermediate the coordination between leaf hydraulic conductivity (K_{leaf}) and g_m (Buckley *et al.*, 2015; Scoffoni *et al.*, 2017; Xiong *et al.*, 2017). Provided that K_{leaf} is a component of K_{plant}, the anatomy outside the xylem most likely plays a key role in the observed response of Antarctic species, although further research is required.

In line with the above, A_N and K_{plant} showed a positive correlation (Fig. 5), which may be related to K_{leaf}, as reported by Sáez *et al.* (2024). Like the findings in this study, we found K_{plant} values to be relatively low, considering notably high values for A_N. It is likely that the high Rubisco specificity for CO_2 described for Antarctic species at 5 and 15 ºC (Sáez *et al.*, 2017) enabled a positive net photosynthesis, despite the low g_s and g_m triggered by the freeze-thaw cycles, and therefore, depending on less extent on K_{plant}.

Overall, our study evidences a significant co-variation between hydraulic and photosynthetic processes, consistent with findings from other studies. For instance, Gleason *et al.* (2017) showed a positive coordination between photosynthesis and hydraulics under drought stress. This coordination was also observed when plants experienced potassium deficiency (Lu *et al.*, 2019), varying light intensities (Xiong *et al.*, 2018), and different growth temperatures (Sonawane *et al.*, 2021; Sáez *et al.*, 2024). This underscores the robust interdependence of these physiological processes in Antarctic species, emphasizing the delicate equilibrium between carbon acquisition and water transport efficiency. However, different slopes in the K_{plant} and gas exchange (A_N, g_s, and g_m) relationship under successive freeze-thaw cycles were observed in plants grown at low and high temperatures (Fig. 5). At the lower temperature, plants showed relatively stable responses, suggesting some degree of resilience to cold stress. In contrast, at the higher temperature a more significant slope in the photosynthetic-hydraulic relationship could reflect a greater effect of freeze-thaw cycles on plants that have partially lost their ability to resist cold stress, although the higher photosynthetic rates achieved under warmer conditions helped to better resist these cycles. This raises the question of whether warmer temperatures could create greater challenges in the future, especially if temperatures continue to rise due to climate change.

Concluding remarks

Both Antarctic vascular species adjusted their leaf xylem anatomy at the higher growth temperature, revealing an acclimation capacity of the xylem and suggesting that the hydraulic system possesses an adaptive potential to confront the projected warming trends. When plants were exposed to successive freeze-thaw cycles, their hydraulics showed a coordinated decrease with leaf gas exchange, regardless of the growth temperature. Notably, Antarctic species have proven to maintain a highly conservative water management strategy and use, underpinning the fact that they are the only two species capable of naturally colonizing Antarctica. Regarding what potential trade-offs or costs these adjustments could imply in the face of successive freeze-thaw events, our results confirmed that though warmer conditions may favour productivity traits, this does not necessarily imply reductions in the stress tolerance of Antarctic species. However, it is likely that the accumulative effects of successive freeze-thaw cycles trigger other effects outside the xylem (i.e., K_{ox}) or biochemical (i.e., AQPs) factors not evaluated in this study, which may comprise the hydraulic response, constraining the photosynthetic gas exchange to a point of non-return. This is especially true in *C. quitensis*, which was more sensitive to freeze-thaw cycles than *D. antarctica*, in agreement with the greater response capacity that has already been described in this species when facing different climatic scenarios. Meanwhile, *D. antarctica* demonstrated a water-conservative strategy that mitigated or delayed the impact of freeze-thaw cycles on hydraulic conductivity and gas exchange.

This conservative approach most likely accounts for the more extensive colonization of *D. antarctica* throughout Maritime Antarctica, prioritizing coverage over rapid growth, in contrast with *C. quitensis,* which exhibits the opposite trend when exposed to warming.

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Anexos

Table S1. Water relation and biochemical traits for *Deschampsia antarctica* (DA) and *Colobanthus quitensis* (CQ) growing at 5 °C and 15 °C. Measurements were performed before the application of a freeze-thaw cycle (C_0) and after the fourth (C_4) and tenth (C_{10}) cycle. Ψ_{leaf}: leaf water potential; RWC: the relative water content; (Chl*a*+Chl*b*), total chlorophyll. Values are means ± SE (*n* = 6-8). For each species, different letters indicate statistical differences among growth temperatures and freeze-thaw cycles together (Tukey´s HSD test, $P < 0.05$).

Species	Cycles	ψ_{leaf} (MPa)	RWC (%)	Chl*a*+Chl*b* (μmol g^{-1} FW^{-1})
$DA\_{5°C}$	C_0	-0.69 ± 0.05a	94.31 ± 1.24b	2.43 ± 0.23b
	C_4	-0.87 ± 0.05ab	95.31 ± 1.26b	1.77 ± 0.26ab
	C_{10}	-0.91 ± 0.04b	86.39 ± 1.69a	1.50 ± 0.07a
$DA\_{15°C}$	C_0	-0.88 ± 0.03ab	94.14 ± 1.00b	3.25 ± 0.21c
	C_4	-0.82 ± 0.02ab	90.67 ± 1.52ab	2.43 ± 0.11b
	C_{10}	-0.96 ± 0.04b	89.04 ± 1.37ab	1.86 ± 0.11ab
$CQ\_{5°C}$	C_0	-0.65 ± 0.03a	83.81 ± 1.74c	0.52 ± 0.06a
	C_4	-0.91 ± 0.04b	82.49 ± 1.52bc	0.57 ± 0.04a
	C_{10}	-1.10 ± 0.03c	74.52 ± 4.5ab	0.58 ± 0.07a
$CQ\_{15°C}$	C_0	-0.86 ± 0.03a	83.70 ± 1.03c	0.88 ± 0.03b
	C_4	-0.92 ± 0.02b	72.94 ± 2.37ab	0.88 ± 0.07b
	C_{10}	-0.98 ± 0.02bc	71.23 ± 0.71a	0.89 ± 0.05b

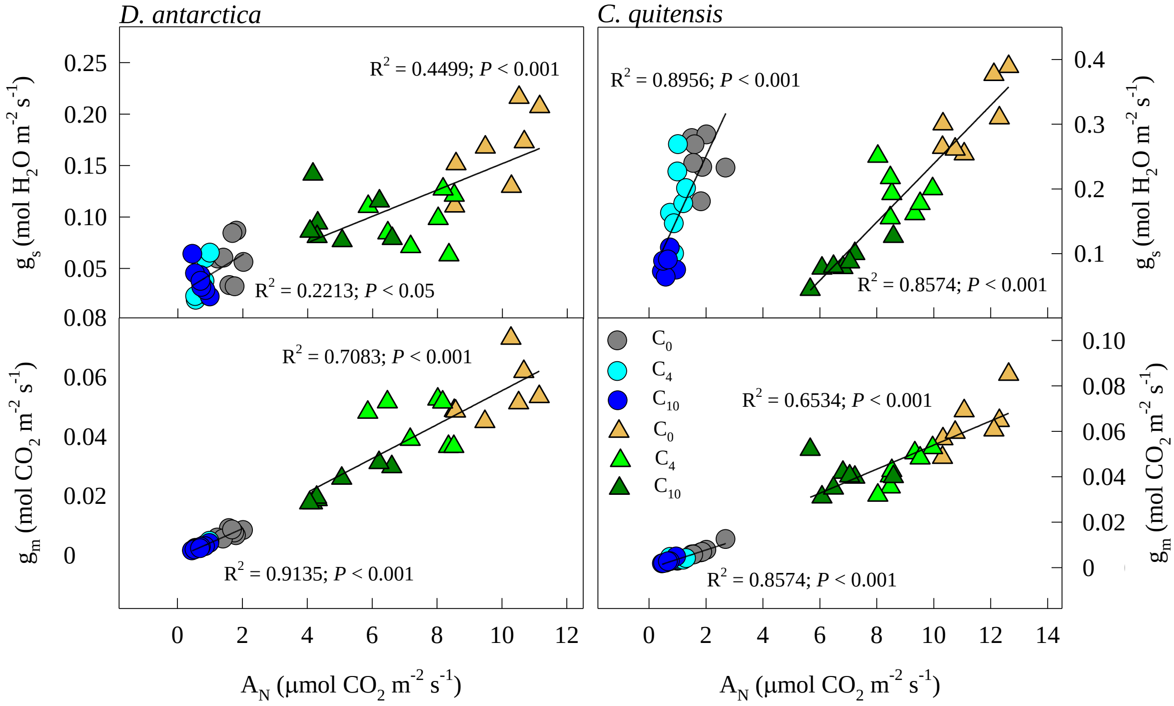

Figure S1. The relationship between the net CO_2 assimilation rate (A_N), the stomatal conductance (g_s), and the mesophyll conductance (g_m) for *Deschampsia antarctica* and *Colobanthus quitensis* growing at 5 °C (circles) and 15 °C (triangles). Measurements were performed before the application of a freeze-thaw cycle (C_0; green and yellow) and after the fourth (C_4; light blue and light green) and tenth (C_{10}; dark blue and dark green) cycle. The data were fitted by linear regressions.

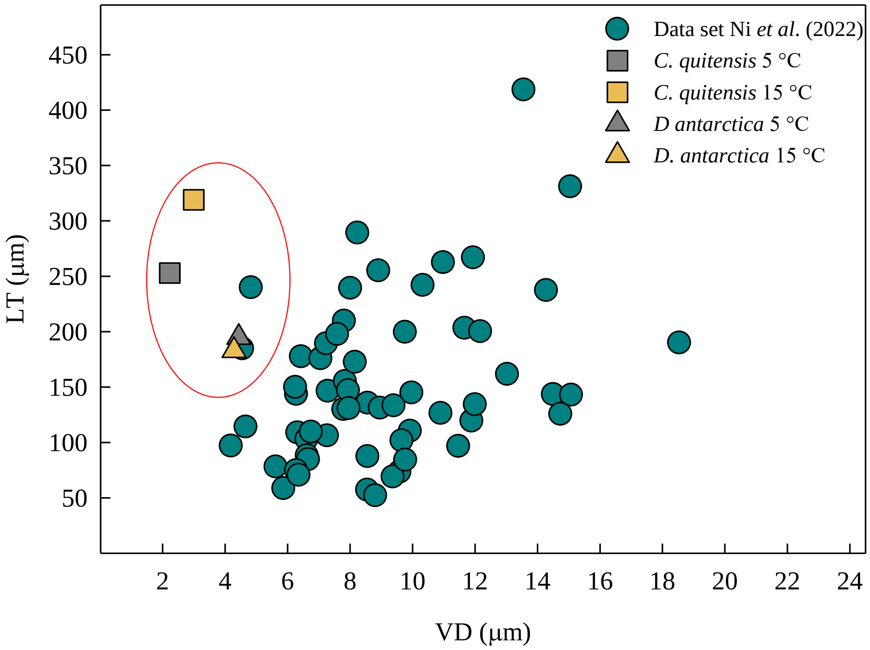

Figure S2. Leaf thickness (LT) *vs*. mean leaf vessel diameter of the midrib (VD) of Antarctic plants (*Colobanthus quitensis* and *Deschampsia antarctica*) compared to other angiosperms. Data for other angiosperms was compiled from Ni *et al*. (2022).

DISCUSIÓN GENERAL

El calentamiento regional en la Península Antártica ha sido relacionado con la expansión de las plantas vasculares antárticas (Cannone *et al.*, 2016, 2022), lo que en parte se debe a una mejora en rendimiento fotosintético (Sáez *et al.*, 2018a; b ; 2019). Esta respuesta a nivel de asimilación de carbono se correlaciona con una serie de ajustes anatómicos específicos en la hoja que facilitan la transferencia de CO_2 hacia los sitios de carboxilación, incrementando la conductancia del mesófilo (g_m) y finalmente la asimilación fotosintética (A_N). Además de la importancia de la difusión de CO_2 dentro de las hojas, el transporte de agua es otro rasgo crucial que determina el éxito del rendimiento fotosintético. Por lo que la presente tesis abordó las propiedades hidráulicas de las especies antárticas y su relación con el rendimiento hidráulico a través de diferentes ensayos de laboratorio y campo.

De acuerdo con la primera la hipótesis planteada en el Capítulo I se comprobó que, en condiciones controladas de laboratorio, el aumento de la temperatura de crecimiento (5, 8 y 15 °C) incrementa significativamente la conductancia hidráulica de la hoja (K_{leaf}) y de la planta completa (K_{plant}), así como la tasa fotosintética. Estos resultados son consistentes con la literatura, donde una conductividad hidráulica mejorada asegura un suministro constante de agua a las células fotosintéticas, lo que permite una mayor tasa de intercambio gaseoso y una óptima fijación de carbono (Brodribb *et al.*, 2005, Yang *et al.*, 2020). Esta mejora se debe en parte a ajustes específicos en los vasos xilemáticos foliares. El diámetro de los vasos del xilema se considera uno de los rasgos más informativos en el campo de la hidráulica vegetal (Hacke and Sperry, 2001; Venturas *et al.*, 2017), pudiendo variar en respuesta a factores ambientales, incluso dentro de la misma planta (Davis *et al*., 1999; Pittermann and Sperry 2003, 2006, Medeck *et al*., 2010, Hacke *et al*., 2017). Específicamente, *C. quitensis* incrementó el diámetro hidráulico medio de los vasos (D_h), mientras que *D. antarctica* aumentó el número de vasos. Estos ajustes anatómicos diferenciales permiten a cada especie adaptar su sistema vascular foliar a las variaciones térmicas, manteniendo la integridad de la vía de transporte de agua desde la raíz hasta las hojas (Meinzer and McCulloh, 2013; McCulloh *et al*., 2019). Este capítulo confirma nuestra hipótesis inicial, y además destaca el éxito de las metodologías empleadas para determinar las propiedades hidráulicas en las especies vasculares. El uso de la cámara de Scholander presenta un desafío particular para especies pequeñas y con tejidos frágiles, como las especies antárticas. Para *C. quitensis*, la selección de un tamaño adecuado de roseta y la repetibilidad de las mediciones, junto adaptaciones mecánicas en *D. antarctica* para evitar el daño al obtener los potenciales hídricos, permitieron obtener valores precisos de los rasgos hidráulicos en estas especies. Estos ajustes metodológicos no solo validaron nuestros hallazgos, sino que nos permitieron avanzar en los siguientes capítulos que se abordaron en esta tesis.

Considerando que las especies antárticas respondieron de manera diferencial bajo condiciones *in situ* de calentamiento (Sáez *et al*., 2018a), y sabiendo que el incremento de las temperaturas en condiciones de laboratorio (Capítulo I) favorece tanto la respuesta hidráulica como la fotosintética, en el Capítulo II se evaluaron los rasgos fotosintéticos e hidráulicos de las plantas antárticas creciendo en condiciones de calentamiento *in situ* (Capítulo II). La simulación de calentamiento se realizó utilizando las cámaras de calentamiento pasivo tipo OTCs (open top chambers) establecidas por Sáez et al. (2018a), lo que permitió indirectamente evaluar respuestas de las plantas frente a calentamiento en el largo plazo (después de siete años de instaladas las OTC) en comparación con el corto plazo (tres años de calentamiento, Sáez *et al*., 2018). Si bien las OTC registraron una mayor cantidad de horas con temperaturas por encima de 10 °C, así como un mayor número de grados día de crecimiento (GDD_5) en comparación con las condiciones de crecimiento naturales de la Antártica (espacio abierto), también acumularon una cantidad considerable de horas con temperaturas bajo cero. Estas fluctuaciones térmicas condicionaron en parte las respuestas observadas entre las especies antárticas. *C. quitensis* mostró una notable capacidad para aprovechar las temperaturas más cálidas, probablemente gracias a su forma de cojín que seguramente les confiere más resistencia frente a los efectos negativos de los eventos de congelación (Cavieres *et al.*, 2016). Esta protección facilitó ajustes anatómicos a nivel vascular, como un mayor D_h, resultando en mayores valores de K_{leaf} y K_{plant} en comparación con el espacio abierto. Esta especie también ajustó algunos parámetros hidráulicos específicos que derivan de las curvas de presión-volumen, desplegando una mayor capacitancia (*C*) y un menor módulo de elasticidad (ε_{max}), ajustes que en su conjunto ayudaron a mantener el turgor celular, mantener los estomas abiertos y favorecer una alta tasa de fotosíntesis dentro de las OTC. En contraste, *D. antarctica* mostró una respuesta hidráulica más conservadora creciendo dentro de OTC. Esta especie redujo su D_h y presentó una mayor frecuencia de vasos xilemáticos foliares de diámetro pequeño, limitando su capacidad de transporte de agua y resultando en menores valores de K_{leaf} y K_{plant}. La embolia es una preocupación común en condiciones de congelación y descongelación. Sin embargo, ambas especies antárticas presentan diámetros de vasos xilemáticos foliares notablemente pequeños, los cuales de acuerdo con la literatura deberían ser menos susceptibles a la cavitación (Davis *et al.*, 1999; Ni *et al.*, 2022). Una explicación alternativa para las disminuciones de K_{leaf} antes de la cavitación es que las vías extravasculares podrían estar modulando en parte la respuesta hidráulica. La hidráulica de la hoja es un proceso complejo influenciado por el movimiento de agua a través del xilema (K_x) y por vías fuera del xilema (K_{ox}). Donde la vías extraxilemáticas incluyen cambios asociados al parénquima vascular, la vaina del haz y las células del mesófilo (Holbrook and Sack, 2006; Scoffoni *et al.*, 2016), así como cambios en la expresión de acuaporinas (Buckley *et al.*, 2015). Algunos estudios han indicado que las vías fuera del xilema son las principales responsables de la disminución de K_{leaf} durante sequía leve y moderada, en lugar de la embolia del xilema foliar, en diversas especies (Bouche *et al.*, 2016; Trifiló *et al.*, 2016; Scoffoni *et al.*, 2017, 2018; Corso *et al.*, 2020; Albuquerque *et al.*, 2020). Por tanto, es posible que, en lugar de sufrir un fallo hidráulico, *D. antarctica* esté realizando ajustes extraxilemáticos para manejar el estrés impuesto por las OTC (Buckley *et al.*, 2015; Scoffoni *et al.*, 2017). En este sentido, identificamos novedosas correlaciones que en parte pueden explicar las respuestas contrastantes entre especies. Los rasgos extraxilemáticos tales como fracción intercelular del área foliar (f_{ias}), la superficie del mesófilo ($S_{m/}S$) y del cloroplasto (S_c/S) frente a los espacios de aire intercelular por área foliar se posicionan como fuertes candidatos en la regulación negativa observada entre el rendimiento hidráulico y fotosintético. Mientras que en *C. quitensis*, los rasgos de hidráulicos específicos como C y el ε_{max} son rasgos claves que podrían facilitar la regulación positiva entre estos procesos. En términos generales, se confirma nuestra segunda hipótesis donde las respuestas hidráulicas de las plantas vasculares antárticas al calentamiento *in situ* depende de modificaciones en la anatomía del xilema foliar y, que además estas respuestas ocurren en coordinación con el intercambio de gases.

Este estudio también puso de manifiesto otros aspectos interesantes. Como se mencionó, permitió evaluar indirectamente los efectos del calentamiento *in situ* tanto a largo plazo (después de 7 años) como a corto plazo (después de 3 años, según Sáez *et al*., 2018a). Aunque este no es el foco principal de este Capítulo, son aspectos relevantes para considerar debido a las diferencias encontradas entre las especies. En este contexto, *C. quitensis* mostró mayores tasas fotosintéticas creciendo bajo condiciones de calentamiento *in situ* en comparación con las condiciones de espacio abierto en ambos estudios. Este incremento está condicionado por el desarrollo de hojas más delgadas, con menor densidad y grosor de la pared celular, lo cual facilita la transferencia de CO_2. Por otra parte, mientras que *D. antarctica* casi no respondió en el corto plazo, sí lo hizo a largo plazo, pero en la misma dirección que su capacidad hidráulica, es decir, con menores valores. Esto plantea nuevas incertidumbres, dado que ambas especies estuvieron bajo las mismas condiciones experimentales. Sin lugar a duda, es una cuestión que hay que seguir debatiendo respecto a la efectividad de este tipo de experimentos *in situ*. Sin embargo, hay algunas pistas que podrían ayudar a entender este comportamiento. Previamente se ha indicado que *D. antarctica,* tanto en campo como en condiciones de laboratorio (bajas temperaturas), desarrolla hojas amarillas. Esta coloración, se ha descrito como un signo de senescencia, un proceso que permite a la planta reciclar nutrientes y utilizarlos para generar nuevas hojas cuando las condiciones ambientales mejoran (Clemente-Moreno *et al.*, 2020). Esta estrategia de senescencia puede ser particularmente útil en entornos extremos como la Antártida, donde las condiciones climáticas pueden ser muy variables y estresante. La senescencia permite a *D. antarctica* conservar recursos y mantener su viabilidad a largo plazo, a pesar de las condiciones adversas. Además, la disminución de la capacidad hidráulica en *D. antarctica* puede estar relacionada con su estrategia de conservación de agua. Al reducir su capacidad de transporte de agua, la planta puede minimizar la pérdida de agua durante los períodos de estrés hídrico o cambios inusuales de temperatura. Este ajuste, aunque a primera vista puede parecer una desventaja, en realidad puede ayudar a la planta a sobrevivir en el difícil ambiente antártico, donde la disponibilidad de agua y las temperaturas favorables son limitadas. Los resultados de esta investigación subrayan la adaptabilidad diferencial entre *C. quitensis* y *D. antarctica* a las condiciones ambientales cambiantes, destacando la importancia de realizar estudios integrales que aborden diversas escalas temporales y condiciones ambientales para comprender de mejor manera los mecanismos de adaptación de estas especies frente al calentamiento regional.

Los eventos de congelación y descongelación son comunes en la Antártida (Batista *et al*., 2022). Sin embargo, por primera vez, tenemos indicios de que el aumento de la temperatura, junto con eventos repentinos de congelación y descongelación, podrían afectar negativamente el rendimiento hidráulico y fotosintético en las especies antárticas. Bajo esta premisa, en el Capítulo III evaluamos el efecto de la temperatura de crecimiento

(5 y 15 °C) en la hidráulica y el rendimiento fotosintético de las plantas vasculares antárticas frente a ciclos sucesivos de congelación y descongelación. Con este trabajo, confirmamos que ambas especies disminuyen los valores de conductividad hidráulica de la planta (K_{plant}) a medida que progresan los ciclos de congelación y descongelación. Sin embargo, debido a las limitaciones técnicas de las mediciones utilizadas, no podemos asegurar si realmente está ocurriendo una falla hidráulica por embolia. No obstante, de acuerdo con los resultados presentados en el Capítulo II, es plausible considerar que las vías extraxilemáticas están involucradas en la regulación hidráulica y fotosintética de estas especies. Esto cobra mayor sentido debido a las fuertes covariaciones encontradas entre los rasgos hidráulicos y fotosintéticos en todas las condiciones evaluadas (Capítulos I, II y III).

La evidencia sugiere que un aumento de temperatura por encima de los 20 °C puede afectar la eficiencia de carboxilación y la activación de Rubisco activasa (Xiong et *al.*, 1999; Salvucci and Craft-Brander, 2004), comprometiendo las tasas fotosintéticas, al menos en *D. antarctica*. Por otra parte, sabemos que los sucesivos ciclos de congelación y descongelación afectan el transporte de agua en ambas especies antárticas. Por lo tanto, si las temperaturas supraóptimas se vuelven más comunes, como lo registrado en los últimos años en la Península Antártica (Turner *et al.*, 2021; Gorodetskaya *et al.*, 2023), junto con eventos más frecuentes de congelación y descongelación durante la temporada de mayor actividad metabólica de las especies antárticas (Liang *et al.*, 2022), la asimilación de carbono y el transporte de agua en estas especies podrían verse negativamente afectados.

CONCLUSIONES GENERALES

El incremento de la temperatura de crecimiento induce una mayor conductividad hidráulica tanto a nivel de hoja como de planta completa. Estas mejoras en la eficiencia hidráulica están asociadas a cambios anatómicos vasculares en las hojas, específicos para cada especie. En *C. quitensis*, se reduce el número de vasos, pero son de mayor diámetro. En *D. antarctica*, se observa un aumento en el número de vasos sin modificaciones en su diámetro. Estos ajustes hidráulicos se alinean con las mayores tasas fotosintéticas bajo condiciones más cálidas.

Después de siete años bajo condiciones de calentamiento simulado *in situ*, las especies antárticas muestran respuestas hidráulicas y fotosintéticas divergentes. *D. antarctica* disminuye su eficiencia hidráulica y fotosintética, mientras que *C. quitensis* muestra un aumento en ambos procesos. Estos hallazgos sugieren que *C. quitensis* se beneficia de los efectos acumulativos de la OTCs mantenido una anatomía foliar (hoja delgada) y vascular (mayor diámetro hidráulico) más productiva. En contraste, *D. antarctica* adopta una estrategia conservadora para asegurar su supervivencia, reduciendo su capacidad hidráulica (menor diámetro hidráulico) y fotosintética (hoja gruesa) en respuesta a las fluctuaciones térmicas registradas dentro de las OTCs.

Los ciclos sucesivos de congelación y descongelación provocan una disminución coordinada entre la conductividad hidráulica a nivel de planta completa y el intercambio de gases, independientemente de la temperatura de crecimiento. *D. antarctica* muestra cambios similares en sus rasgos fotosintéticos a temperaturas bajas y altas, mientras que

C. quitensis exhibe una reducción más severa a baja temperatura. A pesar de que las temperaturas más cálidas mejoran la fotosíntesis, los efectos acumulativos de los ciclos de congelación y descongelación podrían alterar el equilibrio hidráulico y limitar irreversiblemente la fotosíntesis.

Estos resultados no solo amplían nuestro conocimiento sobre las respuestas fisiológicas de las plantas vasculares antárticas, sino que también subrayan la importancia de integrar diversos factores ambientales para anticipar de manera precisa la resiliencia de estos ecosistemas frente a los desafíos del cambio climático global.

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