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SINGULARIDAD ECOFISIOLÓGICA DE LOS RASGOS FOTOSINTÉTICOS E HIDRÁULICOS DE LAS PLANTAS VASCULARES ANTÁRTICAS

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II. RESUMEN

Deschampsia antarctica y *Colobanthus quitensis* son las únicas especies de plantas vasculares que han logrado colonizar naturalmente las condiciones climáticas extremas de la Antártida. Además de estar ampliamente distribuidas en casi toda la Antártida Marítima, desde los 62°S hasta los 68°S, *D. antarctica* se ha encontrado creciendo fuera de la Antártica, desde latitudes menores, alrededor de los 33°S, hasta la Patagonia, mientras que *C. quitensis* tiene una distribución más amplia a lo largo de toda la cordillera de los Andes (desde aproximadamente los 10°N hasta los 55°S). Bajo las condiciones antárticas, se ha reportado que la asimilación de carbono (A_N) en ambas especies está limitada por la conductancia del CO_2 a través del mesófilo foliar (g_m), lo que está relacionado con características fuertemente xerofíticas como una alta masa foliar por área (LMA) y densidad foliar (LD), esenciales para sobrevivir en el severo clima antártico. Para sobrellevar esta restricción en la difusión del CO_2 hacia los sitios de carboxilación en los cloroplastos, las propiedades cinéticas de la Rubisco de las especies antárticas muestran una alta especificidad por el CO_2 ($S_{c/o}$) y altas tasas de recambio catalítico (k_{catc}). A pesar del conocimiento extenso sobre los rasgos fisiológicos de las plantas vasculares antárticas, persisten vacíos respecto a la singularidad de estos rasgos. Específicamente, se desconoce si estos rasgos están determinados exclusivamente por el clima antártico, haciéndolos únicos en las especies que crecen en la Antártica, o si también están presentes en poblaciones subantárticas de estas especies, así como en otras especies filogenéticamente relacionadas o que se consideran potenciales invasoras de la Antártica. El objetivo de esta tesis fue analizar las características fotosintéticas e hidráulicas de *D. antarctica* y *C. quitensis* creciendo en ambientes subantárticos y antárticos, así como las características de especies filogenéticamente relacionadas que crecen en climas extremos, incluyendo dos potenciales especies invasoras en la Antártica. Las evaluaciones incluyeron características anatómicas, rasgos funcionales fotosintéticos (g_s, g_m, v_{cmax}) y características hidráulicas (K_{leaf}, ε_{max}, π_o) en plantas cultivadas bajo diferentes regímenes térmicos. Nuestros resultados subrayan algunas singularidades en la adaptación de las plantas vasculares antárticas en respuesta a la temperatura, destacando su eficiencia hidráulica y capacidad fotosintética relacionada a ajustes anatómicos foliares.

III. ABSTRACT

Deschampsia antarctica and *Colobanthus quitensis* are the only vascular plant species that have naturally colonized the extreme climatic conditions of Antarctica. Besides being widely distributed across almost all of Maritime Antarctica, from 62°S to 68°S, *D. antarctica* has been found growing outside Antarctica, as far north as 33°S, including Patagonia, while *C. quitensis* has a broader distribution along the Andes Mountain range (approximately between 10°N to 55°S). Under Antarctic conditions, carbon assimilation (A_N) in both species is reported to be limited by CO_2 conductance through the leaf mesophyll (g_m), which is related to strongly xerophytic characteristics such as high leaf mass per area (LMA) and leaf density (LD), essential for surviving the severe Antarctic climate. To overcome this restriction in CO_2 diffusion to the carboxylation sites in the chloroplasts, the kinetic properties of Rubisco in Antarctic species show high specificity for CO_2 ($S_{c/o}$) and high catalytic turnover rates (k_{catc}). Despite extensive knowledge about the physiological traits of Antarctic vascular plants, there are still gaps regarding the uniqueness of these traits. Specifically, it is unknown whether these traits are determined exclusively by the Antarctic climate, making them unique to species growing in Antarctica, or if they are also present in sub-Antarctic populations of these species, as well as in other phylogenetically related species or those considered potential invaders of Antarctica. The aim of this thesis was to analyze the photosynthetic and hydraulic characteristics of *D. antarctica* and *C. quitensis* growing in sub-Antarctic and Antarctic environments, as well as the characteristics of phylogenetically related species growing in extreme climates, including two potential invasive species in Antarctica. The evaluations included anatomical characteristics, photosynthetic functional traits (g_s, g_m, V_{cmax}), and hydraulic traits (K_{leaf}, ε_{max}, π_o) in plants grown under different thermal regimes. Our results highlight some singularities in the adaptation of Antarctic vascular plants in response to temperature, emphasizing their hydraulic efficiency and photosynthetic capacity related to adjustment in some anatomical leaf traits.

INTRODUCCION GENERAL

Deschampsia antarctica y *Colobanthus quitensis*, son las únicas dos especies de plantas con flores que han colonizado naturalmente la Antártida. Debido a su notable capacidad para adaptarse a uno de los entornos más extremos del planeta, estas especies han sido sujeto de diversos estudios. Si bien, *D. antarctica* y *C. quitensis* comparten algunos mecanismos de adaptación con otras especies de climas fríos hostiles (Alberdi et al., 2002; Bravo et al., 2009; Parkinoza et al., 2011; Cavieres et al., 2016), es al parecer la combinación única de características morfológicas y fisiológicas, incluyendo rasgos xerofíticos, alta resistencia a la congelación, capacidad de mantener la fotosíntesis a bajas temperaturas, y alta eficiencia en el manejo hidráulico (Alberdi et al. 2002; Bravo et al., 2009; Cavieres et al., 2016; Sáez et al.,2017;2018;2024), lo que les permiten prosperar en el hostil ambiente antártico (Bravo et al., 2009; Cavieres et al., 2016; Ramírez et al., 2024). Así, estas especies ocupan un papel determinante en la investigación científica y frecuentemente se utilizan como modelos para estudiar adaptaciones particulares a condiciones ambientales extremas, tanto en la Antártida como en regiones subantárticas (Giełwanowska 2005; Piotrowicz-Cieślak et al. 2005; Kellmann-Sopyła et al. 2015; Kellmann-Sopyłay y Giełwanowska 2015; Cavieres et al., 2016; Koc et al. 2018; Dulska et al. 2019).

Las características anatómicas xerofíticas de *D. antarctica* y *C. quitensis* se traducen en bajos valores de conductancia del CO_2 a través del mesófilo (g_m) lo que impone la mayor restricción en la adquisición de este gas para la fotosíntesis. A nivel ultraestructural, rasgos como el tamaño y disposición de los cloroplastos, así como el grosor de la pared celular y los componentes citoplasmáticos y del estroma son determinantes en la resistencia al movimiento del CO_2 en condiciones antárticas (Sáez et al., 2017; 2024). Estos rasgos se complementan con mecanismos compensatorios para mantener un funcionamiento adecuado de la capacidad fotosintética a bajas temperaturas (Ramírez et al., 2024), estando estrechamente vinculados a las propiedades hidráulicas foliares, asegurando la eficiencia en el transporte de agua y minimizando el riesgo de colapso frente a temperaturas de congelación (Sáez et al., 2024). A pesar de los avances en la comprensión de cómo estas plantas regulan el intercambio de agua y CO_2 a través de sus hojas, así como la eficiencia de la asimilación de carbono mediante Rubisco (Sáez et al., 2017, 2018b, 2024), aún queda mucho por conocer respecto a su singularidad biológica, por ejemplo, los factores que influyen en su distribución, la base genética de su resistencia al estrés, y rasgos bioquímicos asociados a la proporción de ácidos grasos y la distribución de los carbohidratos no estructurales (Ramírez et al., 2024).

En otros ambientes de gran elevación, como en Cordillera de los Andes, es posible encontrar condiciones climáticas tan extremas como en la Antártida (esto es, temperaturas extremadamente bajas, disponibilidad variable de agua líquida, alta radiación, suelos poco desarrollados, etc.), ofreciendo una oportunidad para estudios comparativos que podrían arrojar luz sobre la singularidad de los rasgos ecofisiológicos observados en condiciones antárticas. Los estudios de gradientes latitudinales son esenciales para dilucidar las respuestas de las plantas a una gama más amplia de condiciones ambientales tanto abióticas como bióticas, y podrían utilizarse como indicadores del impacto del cambio climático. En este sentido, se han reportado a *C. quitensis* creciendo en varios sectores de los Andes, hasta la Península Antártica , así como a *D. antarctica* creciendo desde los 33°S de latitud hasta la Antártida. Sin embargo, los estudios ecofisiológicos son aún escasos para *C. quitensis* fuera de la Antártida y completamente inexistentes para *D. antarctica*. Esta brecha limita nuestra comprensión de las adaptaciones ecofisiológicas necesarias para la supervivencia en condiciones antárticas y subraya la falta de estudios comparativos entre las especies de alta montaña y las de la Antártida. Desentrañar los rasgos ecofisiológicos que permiten a estas especies resistir el duro entorno antártico, así como en otros ambientes con condiciones estresantes similares, puede ayudar a explicar su excepcional distribución geográfica. Además, considerando el rápido calentamiento regional documentado en la Península Antártica (Turner et al., 2021; Chown et al., 2022), es crucial investigar aspectos biológicos aún no completamente estudiados en estas especies vegetales. El cambio climático no solo añade urgencia a estas investigaciones, sino que también anticipa un impacto significativo en los procesos morfológicos y fisiológicos de las especies antárticas. El aumento de temperatura podría facilitar la llegada de especies invasoras que se beneficiarían de las condiciones más cálidas, representando una amenaza adicional para el equilibrio del ecosistema Antártico. Para comprender mejor estos impactos, se requieren estudios adicionales en diversas poblaciones y especies, incluyendo gradientes latitudinales que funcionen como laboratorios naturales. Estos puntos establecen la base para explorar en los capítulos siguientes los rasgos fisiológicos relacionados con la fotosíntesis y su coordinación con las propiedades hidráulicas bajo diferentes regímenes térmicos en las procedencias antárticas y subantárticas de *D. antarctica* y *C. quitensis*, así como en especies filogenéticamente relacionadas, incluidas especies reportadas como potenciales invasores de la Antártida. Este análisis no solo contribuirá a revelar la singularidad ecofisiológica de los rasgos presentes en las plantas antárticas, sino que también permitirá prever sus respuestas futuras a los cambios ambientales y entender mejor el potencial impacto de las especies invasoras en este frágil ecosistema.

CAPÍTULO 1: UNIQUENESS OF PHOTOSYNTHETIC AND HYDRAULIC TRAITS OF ANTARCTIC VASCULAR PLANTS: A COMPARISON WITH CLOSELY RELATED SPECIES

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ABSTRACT

The survival of *Deschampsia antarctica* and *Colobanthus quitensis* in Antarctica has sparked scientific interest due to their unique adaptations. While traits like cold tolerance, photosynthetic capacity, and hydraulic management are key for their success, it remains unclear if these traits are unique to Antarctica or present in Subantarctic populations or in related species. We study leaf anatomy, photosynthetic gas exchange, hydraulic traits, and vascular anatomy in *D. antarctica* and *C. quitensis* from Antarctic and Subantarctic provenances, as well as in related species (*Deschampsia caespitosa*, *Avenella flexuosa*, and *Colobanthus apetalus*) growing at 5°C, 15°C, and 23°C. We hypothesized that the traits related to carbon assimilation and hydraulics and its coordinated response to increase temperature correspond to a unique adaptation to the Antarctic climate and is not present in Subantarctic provenances or related species. *D. antarctica* showed exceptional adaptability to temperature variations, with effective coordination between hydraulic and photosynthetic traits in both Antarctic and Subantarctic provenances, exhibiting a high hydraulic efficiency with low K_{leaf}, associated with narrow xylem vessel diameters. Additionally, *D. antarctica* showed higher net photosynthesis (A_N) and diffusive and biochemical determinant (g_m, V_{cmax}) and a dynamic structural trait adjustment (leaf mass area, LMA; leaf density, LD and elasticity, ε_{max}) in response to temperature, compared to phylogenetically related species. In contrast, *C. quitensis* displayed specific adaptations based on its origin, with Antarctic populations showing integrated coordination between photosynthetic and hydraulic traits, suggesting a specialized water conservation strategy. In contrast, Subantarctic populations had higher photosynthetic and K_{leaf} rates with less trait coordination, possibly due to ecotypic differentiation. *C. apetalus* showed thicker leaves and lower g_s at lower temperatures, which limited photosynthesis. However, it adjusted g_s, LMA, and V_{cmax} to enhance photosynthetic efficiency at higher temperatures, achieving A_N values comparable to those of *C. quitensis* from Subantarctic regions, despite having a lower K_{leaf-} These results underscore the uniqueness and adaptive significance of the physiological traits in *D. antarctica* and *C. quitensis*, highlighting their crucial role in thriving under extreme Antarctic conditions.

INTRODUCTION

It has been widely recognized that plants that inhabit extreme environments, especially those associated with the cryosphere, would be the most affected by the accelerated climate change (Lee et al., 2017; IPCC, 2019). *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) are two extremophile plant species inhabiting highly stressful environmental conditions. *D. antarctica* is found from approximately 33°S, while *C. quitensis* is distributed throughout the Andes Mountain range, with both species extending to the southernmost region of Patagonia (Convey, 1996; Van de Wouw et al., 2008; Moore, 1970; Hoffmann et al., 1998; Smith, 2003). Despite other related species also thrive in stressful conditions and some share this extensive gradient, *D. antarctica* and *C. quitensis* stand out as the only two flowering plants that naturally colonized the Antarctica (Barcikowski et al., 2001). These species have been the subject of several studies, describing morphological and biochemical features related to freezing resistance, high photosynthetic capacity at low temperature and other stressful environmental factors such as low nutrient availability, and high radiation (Bravo et al., 2009 and Ramirez et al., 2024). In both Antarctic species, xerophytic anatomical traits, such as high leaf mass area (LMA) and leaf density (LD). The photosynthetic performance of Antarctic plants is determined by diffusional components mainly associated with g_m, as well as biochemical determinants linked to Rubisco kinetic traits (Sáez et al., 2017). It has been reported, that g_s in the Antarctic vascular species remain relatively high, probably as a compensatory response to the limitation imposed by the significantly low g_m, which is at the same time, compensated by a high Rubisco specificity ($S_{c/o}$) (Sáez et al., 2017). In recent years, it has been reported that the photosynthesis of Antarctic plants benefits from warmer conditions through specific leaf anatomical adjustments (decreased LMA, LD, and cell wall thickness), that facilitate CO_2 diffusion to chloroplasts (Sáez et al., 2018a, 2018b, 2024). These adjustments are associated with modifications in leaf vascular traits, increasing K_{leaf} and whole-plant hydraulic conductivity (g_{plant}) in response to temperature increase, resulting in a tight coordination between photosynthetic and hydraulic processes (Sáez et al., 2024). The relationship between K_{leaf} and g_m is crucial in this context, as both are interrelated through a shared diffusion pathway in the mesophyll apoplast, ensuring efficient regulation of water and CO_2 under varying environmental conditions (Flexas et al., 2013; Xiong et al., 2017). This co-regulation of leaf hydraulics and gas exchange traits, such as photosynthesis, g_s, and g_m, is well documented in general (Brodribb et al., 2007; Flexas et al., 2013; Xiong et al., 2018), and is essential for the optimal functioning of vascular plants (Sáez et al., 2024). Although considerable knowledge has been accrued on *D. antarctica* and *C. quitensis* growing in Antarctica, it remains unclear whether their physiological traits are determined by the Antarctic climate and, therefore, are unique, or whether they are also present in populations of these species growing outside Antarctica, as well as in other phylogenetically related species that inhabit hostile environments.

The geographical isolation of Antarctica, resulting from the Gondwana fragmentation, led to the formation of an extreme and unique environment (Cantrill & Poole, 2012; Convey et al., 2008). As Antarctica progressively separated from Australia and South America, the continent experienced gradual cooling, crucial for the development of the Antarctic Circumpolar Current, which triggered global cooling and established the severe climatic conditions characteristic of Antarctica (Powell et al., 1981; Clifford & Simon, 1981; Gili et al., 2016; Evangelinos et al., 2024). It is proposed that the successful colonization of *D. antarctica* and *C. quitensis* in Antarctica likely occurred during a more favorable climate, before the Antarctic Circumpolar Current formation (Parnikoza et al., 2007; 2011). As the Antarctic environment evolved, these species developed specialized adaptations allowing them to thrive under the harsh conditions, including low temperatures, intense winds, freeze-thaw cycles, varying water and nutrient availability, and high radiation levels (Xiong & Day, 2001; Alberdi et al., 2002; Robinson et al., 2003; Mosyakin et al., 2007; Convey et al., 2011; Parnikoza et al., 2011; Cavieres et al., 2016). These adaptations have driven the unique physiological traits of *D. antarctica* and *C. quitensis*, enabling their remarkable stability in such an inhospitable environment However, the specific traits that make *D. antarctica* and *C. quitensis* uniquely suited to the Antarctic environment remain unclear, especially when compared to related species in nearby Subantarctic habitats. Understanding these adaptations is crucial, given the close phylogenetic relationship with species in geographically adjacent regions (Sáez et al., 2019; González et al., 2021; Albán et al., 2022).

Although summer temperatures in the Andean-Subantarctic are higher compared to Antarctica, other factors like water availability, nutrients, light, and winds exhibit certain similarities as ecosystems associated with the cryosphere (Pisano,1974; Torres and Campodonico, 2022). In the Andes of Argentina and Chile south of 30°S, there is a high diversity of *Deschampsia* species, with 15 species and two monospecific genera segregated from *Deschampsia*, *Avenella* and *Vahlodea,* growing typically in wetlands and humid environments (Chiapella and Zuloaga, 2010). Among these species, *D. caespitosa* (L.) P. Beauv is a frost-resistant species thriving in permafrost conditions and is the most widespread and successful species of this genus (Lawrence, 1945; Tieszen and Bonde, 1967; Chiapella, 2000; Nuzhyna et al., 2019). It has been reported that *D. caespitosa* has a close genetic affinity with *D. antarctica* (Chiapella, 2007). Similarly, *Avenella flexuosa* (L.) Drejer (formerly *Deschampsia flexuosa* (L.) Trin) is a perennial, widely distributed species that grows in Subarctic regions in Patagonia (Scurfield, 1954; Gargaglione et al., 2014). Morphologically, it shares similarities with *D. caespitosa* and has highly repetitive DNA sequences related to *D. antarctica* and *D. caespitosa*, suggesting a common ancestor (Amosova et al., 2017; Ishchenko et al., 2020). On the other hand, the genus *Colobanthus* includes 25 species distributed across South America, sub-Antarctic islands, and various islands in the Indian and Pacific Oceans, such as New Zealand and Tasmania (Moore, 1970; Correa, 1984; Hoffmann et al., 1998; Atlas of Living Australia, 2019). In particular, *Colobanthus apetalus* (Labill.) Druce has been recorded in New Zealand and southeastern Australia, including Tasmania (Allan, 1961). This species is also adapted to cold and stressful environments and shares highly similar chloroplast genomes with *C. quitensis* and forms a closely related clade within the Caryophyllaceae family, also showing similarity in genes involved in photosynthesis within this clade (Androsiuk et al., 2018; 2020).

Considering the presence of both *D. antarctica* and *C. quitensis* populations outside Antarctica, particularly in the Patagonian and Subantarctic regions, as well as the existence of phylogenetically related species that tolerate similar stressful conditions, we inquire whether the traits exhibited by *D. antarctica* and *C. quitensis* growing in Antarctica are unique or they also present in Subantarctic populations and in phylogenetically related species. We hypothesize that the physiological traits related to carbon assimilation and hydraulics observed in *D. antarctica* and *C. quitensis* and its coordinated response to increase temperature are specific adaptations to the extreme Antarctic climate. Therefore, these traits are unique to the Antarctic environment and are not present in the Subantarctic provenances of these species or in phylogenetically related species that inhabit similarly stressful environment. Comparisons with Subantarctic populations of *D. antarctica* and *C. quitensis*, as well as with phylogenetically related species that grow in cold habitats outside of Antarctica, will contribute to assessing the uniqueness of Antarctic vascular plants and their physiological responses to extreme environmental conditions.

MATERIAL AND METHODS

Plant material and experimental conditions

Individuals of *D. antarctica* and *C. quitensis* were collected at the King George Island near the Henryk Arctowski Polish Antarctic Station (62°09'S, 58°28'W). In the Subantarctic region, *D. antarctica* was collected at Torres del Paine National Park (50°59'S, 73°05'W) and *C. quitensis*, at Sierra del Toro (51°0.5'S, 72°42'W). All plant collection was performed following the methodology described in Sáez et al. (2017). Plants were vegetatively propagated in plastic pots using a sterile soil mixture (soil/vermiculite/peat 3:1:1 v/v) and were kept under common garden conditions in growth chambers (Pi-Technology Inc., Santiago, Chile) at 15°C (Xiong et al. 1999, Sáez et al. 2017) with an irradiance of 300 μmol photons $m^{-2}s^{-1}$ and an 18/6 h photoperiod. Seeds of *D. caespitosa*, *A. flexuosa* and *C. apetalus* were germinated and then seedlings were maintained under controlled conditions at 15°C with an irradiance of 300 μmol photons $m^{-2}s^{-1}$ and 18/6 h photoperiod. All plants were fertilized with Ultrasol 0.4 gL^{-1} (Solaris, Buckinghamshire, United Kingdom) once a week and watered periodically. Subsequently, all plants were randomly divided into subgroups in different growth chambers for temperature treatments. The selected temperatures were determined based on the average maximum daily temperature of the Antarctic summer (5°C), the optimal photosynthetic temperature for *D. antarctica* and *C. quitensis* (15°C) (Sáez et al., 2018a), and the optimal photosynthetic temperature determined for the phylogenetically related species (23°C). For all treatments, a nighttime temperature of 5°C was considered. Plants were maintained at each temperature for at least 45 days for acclimation. Mature leaves were randomly chosen for all measurements.

Leaf morphological traits

Leaf mass area (LMA) was calculated as the ratio of dry mass to leaf area. Leaf area was determined in fresh leaves by analyzing photos with ImageJ (Wayne Rasband/NIH, Bethesda, MD, USA). The dry mass of these leaves, approximately 10 to 12 per plant from around 10 plants per species, was determined after drying at 65°C for 72 hours. Leaf density (LD) was determined by dividing LMA by leaf thickness.

Leaf thickness was obtained from cross-sectional leaf analysis with an optical microscope (BX51, Olympus, Tokyo, Japan).

Gas exchange

CO_2 response curves were performed using a chlorophyll fluorescence measurement system (Li-6400XT, LI-COR Inc., Lincoln, NE, USA). The net photosynthesis (A_N) response to the sub-stomatal CO_2 concentration was studied as reported in Sáez et al. (2017). Corrections for CO_2 leakage from the Li6400XT leaf chamber were applied to all gas exchange data as described in Flexas et al. (2007). Gas exchange measurements were performed at leaf temperatures of 5°C, 15°C, and 23°C, with six replicates per species for each treatment. The quantum efficiency of photosystem II (PSII)-driven electron transport (ϕPSII) was determined using the equation: $\phi PSII = (F'_m - F_s) / F'_m$, where F_s is the steady-state fluorescence in light (1000 μmol quanta m^{-2} s^{-1}) and F'_m is the maximum fluorescence obtained with a light saturation pulse (8000 μmol quanta m^{-2} s^{-1}). Since ϕPSII represents the number of electrons transferred per photon absorbed by PSII, the electron transport rate (ETR) can be calculated as: $ETR = \phi PSII \cdot PPFD\ \alpha\beta$, where PPFD is the photosynthetic photon flux density, α is leaf absorptance, and β is the energy distribution between the two photosystems, assumed to be 0.5. Leaf absorptance was measured as described by Sáez et al. (2017, Table S1).

Estimation of leaf mesophyll conductance

The mesophyll conductance to CO_2 (g_m) was calculated from the combination of gas exchange and chlorophyll fluorescence measurements according to Harley et al. (1992): $g_m = A_N / (C_i - (\Gamma^* (ETR + 8 (A_N + R_L)) / (ETR - 4 (A_N + R_L))))$. The rate of non-photorespiratory CO_2 evolution in light (R_L) was determined as half of R_d and 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). g_m values were used to calculate the chloroplast CO_2 concentration (C_c) by converting A_N -C_i curves to A_N -Cc curves, where $C_c = C_i - (A_N / g_m)$. The maximum Rubisco carboxylation rate (V_{cmax}) was obtained from A_N -C_c curves according to Farquhar et al. (1980). The *in vitro* kinetic constants of Rubisco reported in Sáez et al. (2017) for *D. antarctica* and *C. quitensis* were used for *Deschampsia* and *Colobanthus* species, respectively.

Vascular anatomy

Leaves, previously fixed in formaldehyde, acetic acid, and alcohol (FAA), were chemically cleaned with 0.5% NaOH and stained with toluidine blue. A minimum of 10 leaves from six plants were used for analysis. The leaves were visualized under an optical microscope (BX51, Olympus, Tokyo, Japan) to obtain micrographs and anatomical variables were measured from leaf cross-sections. For this, a 0.5% w/v toluidine blue stain was used to identify diameter (d) and the number of vascular bundles (n). With these measurements, the mean hydraulic diameter was calculated as $D_h = \Sigma d^5/\Sigma d^4$ (Corcuera et al., 2012), and the theoretical hydraulic conductivity (K_h, $m^{-2}\ s^{-1}\ MPa^{-1}$) was determined according to Hagen-Poiseuille's law as: $K_h = \Sigma\ ((d^4\pi\rho) / (128\eta_w))$, where d is the diameter of xylem vessels (mm), ρ is the density of water, and ηw is the viscosity of water at different growth temperatures. All these variables were measured using ImageJ software.

Pressure-volume curves

PV curves were constructed using the free transpiration method described in Corcuera et al. (2002) with modification reported by Sáez et al. (2024). Briefly, 20-30 leaves from the same plant were separated for P-V curve construction using a pressure pump (PMS-600). For each species and growth conditions, P-V curves were performed on six different plants. According to the methods proposed by Liu and Osborne (2015), P-V curves were drawn and analyzed to estimate the following parameters: osmotic potential at full turgor (π_o), water potential at the turgor loss point (Ψ_{tlp}), relative water content at the turgor loss point (RWC_{tlp}), and maximum wall elasticity modulus (ε_{max}).

Measurement of leaf hydraulic conductance

Leaf hydraulic conductivity (K_{leaf}) was measured using the rehydration kinetic method described by Brodribb and Holbrook (2003) and following the modification described in Sáez et al. (2024). For this analysis, 20-25 leaves from six different plants per species were used to ensure representative measurements and account for individual variation. K_{leaf} was calculated as: $C \ln [\Psi_0 / \Psi_f] / t$, where Ψ_0 is the water potential before rehydration, Ψ_f is the water potential after rehydration, t is the rehydration time, and C is the leaf capacitance for each species determined as the initial slope of the pressure-volume curves normalized by leaf area (Tyree and Hammel, 1972; Brodribb et al., 2005).

Statistical analyses

The influence of growth temperature (5, 15, and 23 °C) on photosynthetic, hydraulic, and anatomical leaf traits of the study species including *D. antarctica* and *C. quitensis* from two provenances (Antarctic and Subantarctic), *D. caespitosa*, *A. flexuosa*, and *C. apetalus*, was evaluated using linear models (LMs). In cases of non-normality of residuals, variables were log-transformed. For some variables and species, normality of residuals was not met in spite of data transformation. Due to this, generalized linear models (GLMs) were conducted. Post-hoc multiple comparisons (Tukey tests) were carried out using the emmeans package. Fisher's Exact Test was employed to evaluate the statistical differences in the distribution of xylem vessel diameters across temperature treatments and to assess differences between species. Pearson correlation analyses were performed to evaluate the relationships between response variables (A_N, g_m, K_{leaf}, ε_{max}, LMA, LD, g_s, and g_m). Additionally, a principal component analysis (PCA) was employed to explore parameters that explain the observed variability in the data and to examine how different species and provenances are distributed along this variability. Prior to conducting the PCA, data were normalized by the median and subsequently centered and scaled. The PCA, linear models (LMs) and generalized linear models (GLMs) were performed using R (v. 3.5.1; R Core Team 2018) with lattice (Deepayan, 2008) and pcamethods libraries (Stacklies et al., 2007).

RESULTS

Leaf anatomy and photosynthetic gas exchange

D. antarctica from both Antarctic and Subantarctic provenances exhibited higher values in leaf mass area (LMA) and leaf density (LD) at both 5°C and 15°C (Fig. 1A, B). In contrast, the related species *D. caespitosa*, and particularly *A. flexuosa*, presented lower LMA and LD. However, at 23°C, *D. caespitosa* exhibited LMA and LD values similar to those of *D. antarctica* from both provenances (Fig. 1A, B). As compared to related species, *D. antarctica* tend to display the highest values for the net CO_2 assimilation rate (A_N), the dark respiration (R_{dark}), the leaf mesophyll conductance to CO_2 (g_m), the maximum velocity of Rubisco carboxylation (V_{cmax}), and the intrinsic water use efficiency (WUE), increasing with growth temperature, with no differences between Antarctic and Subantarctic provenances of *D. antarctica* (Fig. 1). As for the stomatal conductance (g_s), this trend was less pronounced, and for some growth temperatures *D. antarctica* presented lower values than relative counterparts.

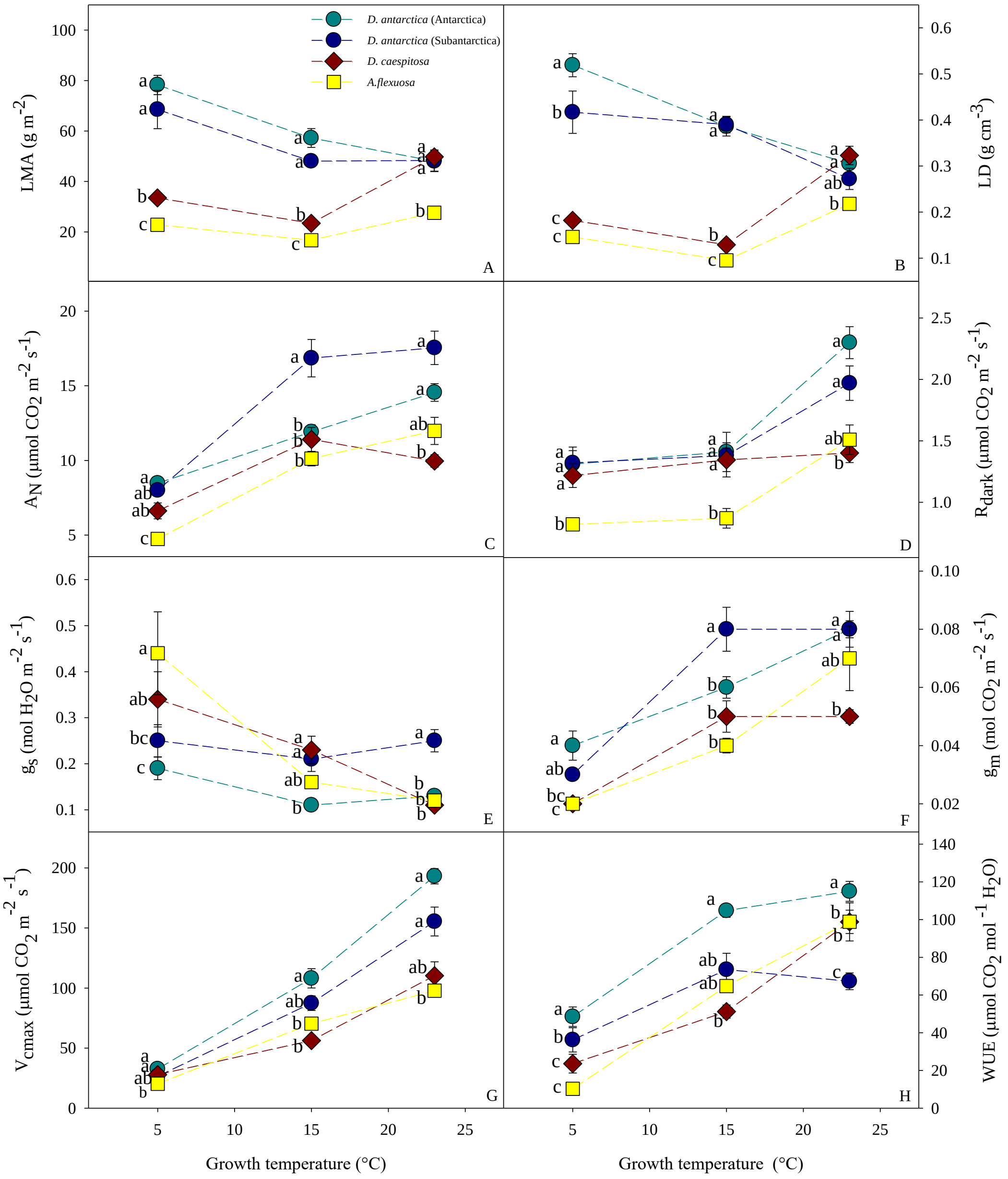

Figure 1. Leaf anatomy and gas exchange parameters for *D. antarctica* from Antarctic and Subantarctic provenances, and the closely related species D. caespitosa and *A. flexuosa* grown at 5 °C, 15 °C and 23 °C. Values are means ± S.E. (n= 10-12 for LMA and LD and n = 6–8 for gas exchange). Leaf mass area (LMA), leaf density (LD), net photosynthetic CO_2 assimilation rate (A_N), respiration rate (R_{dark}), stomatal conductance (g_s), mesophyll conductance (g_m), maximum Rubisco carboxylation rate (V_{cmax}) and water use efficiency (WUE). Different letters indicate statistically significant differences among species at different growth temperatures according to Tukey's test ($P < 0.05$).

The response of LMA and LD to growth temperature was dissimilar in Antarctic and Subantarctic *C. quitensis* and *C. apetalus*. In Antarctic *C. quitensis*, LMA and LD were lower at 15 ºC, while the opposite was observed in Subantarctic *C. quitensis*, and *C. apetalus* displaying the highest LMA and LD at 5 ºC (Fig. 2). As observed for *D. antarctica*, there was a trend for increased values in the main gas exchange parameters with increasing growth temperature for both *C. quitensis* and *C. apetalus* (Fig. 2). With a few exceptions, *C. quitensis* from Subantarctic provenance displayed higher values for the photosynthetic gas exchange parameters compared to *C. quitensis* from Antarctica across all three growth temperatures (Fig. 1). The comparison with *C. apetalus* is more complex, as this species shows the lowest values for given conditions (e.g., g_s at 5 ºC) and the highest values in others (e.g., g_s at 15 ºC).

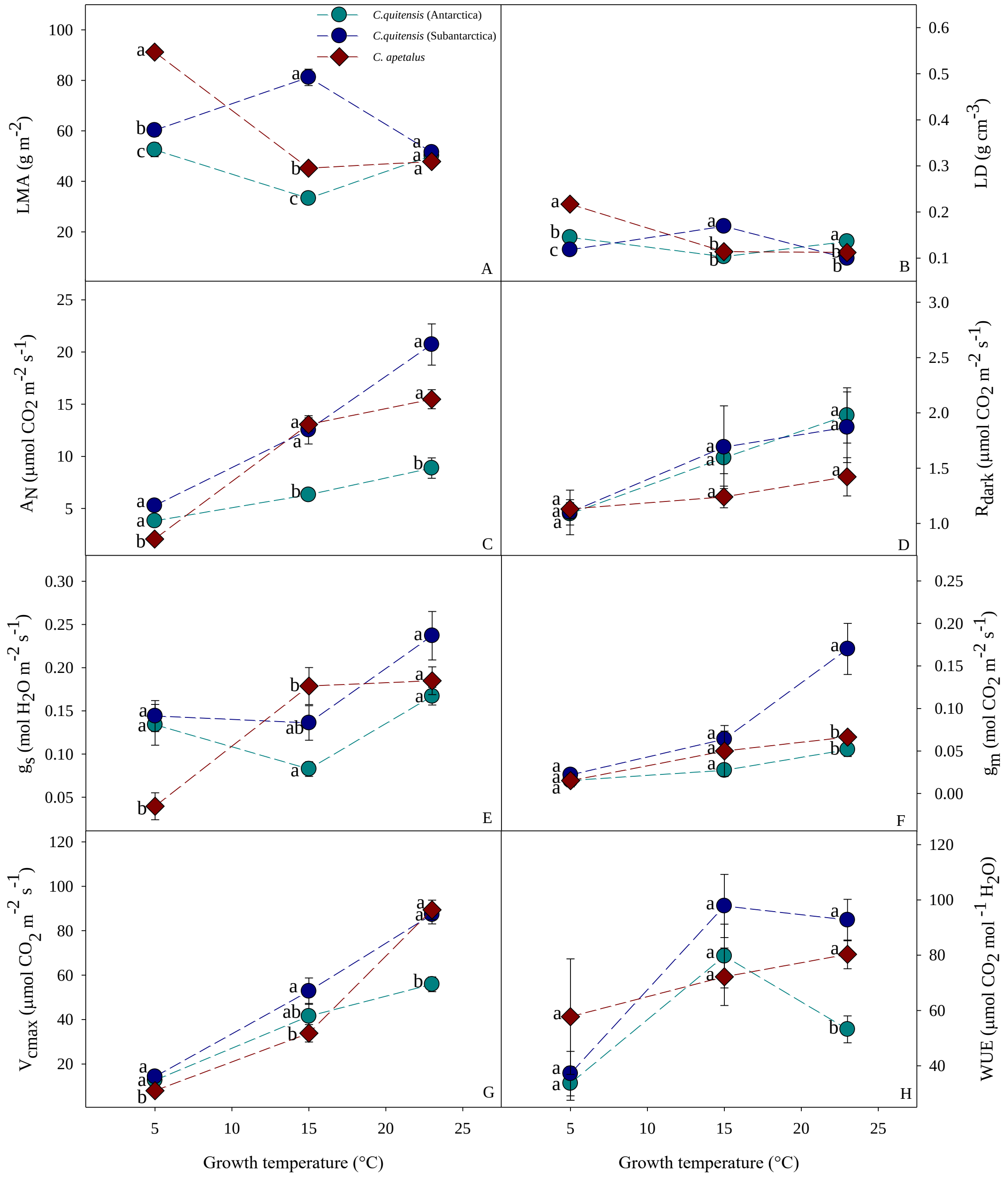

Figure 2. Leaf anatomy and gas exchange parameters for *C. quitensis* from Antarctic and Subantarctic provenances, and the closely related species *C. apetalus* grown at 5 °C, 15 °C and 23 °C. Values are means ± S.E. (n= 10-12 for LMA and LD and n = 6–8 for gas exchange). Leaf mass area (LMA), leaf density (LD), net photosynthetic CO_2 assimilation rate (A_N), respiration rate (R_{dark}), stomatal conductance (g_s), mesophyll conductance (g_m), maximum Rubisco carboxylation rate (V_{cmax}) and water use efficiency (WUE). Different letters indicate statistically significant differences among species at different growth temperatures according to Tukey's test ($P < 0.05$).

Leaf hydraulic traits

No differences in leaf hydraulic conductivity (K_{leaf}) were observed between the Antarctic and Subantarctic provenances of *D. antarctica*, with values ranging between 2.3 and 4.3 mmol m^{-2} s^{-1} MPa^{-1}. *D. caespitosa* showed comparable values to those of *D. antarctica*, except at 5 °C where it displayed highest values (3.67 mmol m^{-2} s^{-1} MPa^{-1}), while *A. flexuosa* exhibited the highest K_{leaf} across all temperatures, ranging between 6.28 and 7.8 mmol m^{-2} s^{-1} MPa^{-1} (Fig. 3A). Maximum cell wall elasticity (ε_{max}) decreased with growth temperature in all species (Fig. 3B), with the highest values recorded in Antarctic *D. antarctica* at 5°C (12.4 MPa). There were no significant differences in e_{max} among species at 15°C and 23°C, with values ranging between 5-7 and 3-4 MPa, respectively (Fig. 3B).

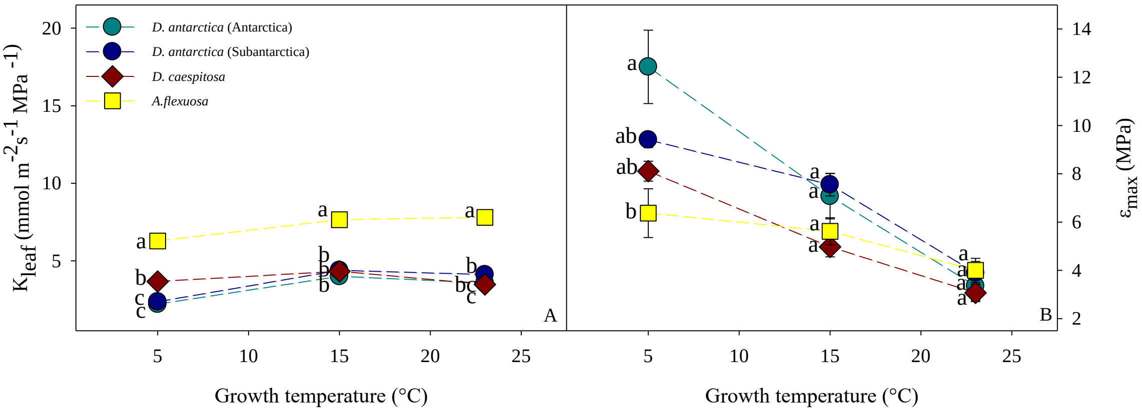

Figure 3. Leaf hydraulic conductivity (K_{leaf}), and cell wall elasticity (ε_{max}) for *D. antarctica* from Antarctic and Subantarctic provenances, and the closely relative species *D. caespitosa* and *A. flexuosa* grown at 5 °C, 15 °C and 23 °C. Different letters indicate statistically significant differences among species at different growth temperatures according to Tukey's test ($P < 0.05$).

Among *Colobanthus* species, K_{leaf} generally increased with temperature, with the lowest values observed at 5°C. Subantarctic *C. quitensis* exhibited the highest K_{leaf} values at 5 and 15°C (7.47 and 17.44 mmol m^{-2} s^{-1} MPa^{-1}, respectively). At 23°C, K_{leaf} values were approximately 13 mmol m^{-2} s^{-1} MPa^{-1} for *C. quitensis* provenances. In contrast, *C. apetalus* showed the lowest K_{leaf} values at 15 and 23°C (6.9 and 6.16 mmol m^{-2} s^{-1} MPa^{-1}, respectively). In all *Colobanthus* species, ε_{max} decreased with increasing growth temperature (Fig. 4B), with the maximum values recorded at 5 ºC for Antarctic *C. quitensis* (10.69 ± 0.73 MPa), while the minimum was measured in *C. apetalus* at 15°C (3.01 ± 0.22 MPa). At 23°C, no significant differences in ε_{max} were observed across species and provenances.

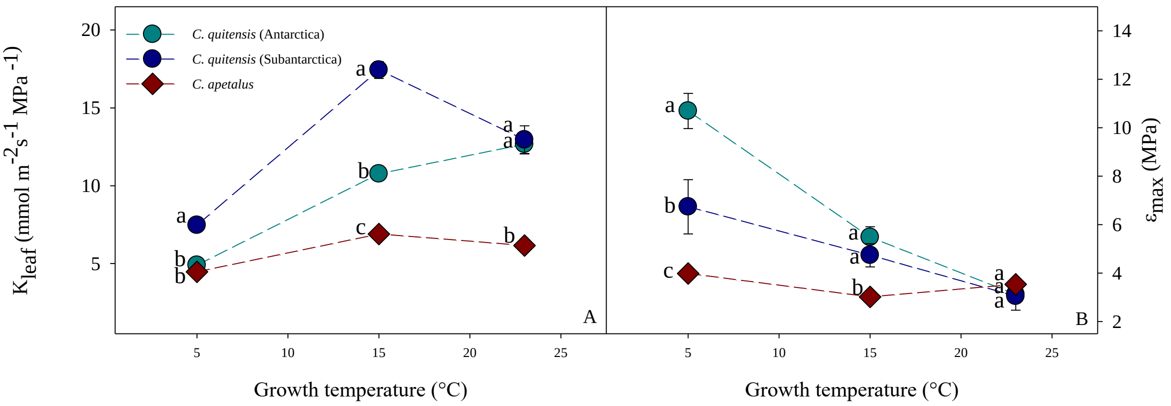

Figure 4. Leaf hydraulic conductivity (K_{leaf}), and cell wall elasticity (ε_{max}) for *C. quitensis* from Antarctic and Subantarctic provenances, and the closely relative species *C. apetalus* grown at 5 °C, 15 °C and 23 °C. Different letters indicate statistically significant differences among species at different growth temperatures according to Tukey's test ($P < 0.05$).

Leaf vascular anatomy

In *Deschampsia* species, the distribution of diameter classes of leaf xylem vessels was largely concentrated in the range of 3 to 6 µm at the three growth temperatures (Fig. 5). Noteworthy, *D. antarctica* from Antarctica presented a higher frequency of xylem vessel diameters at 5 ºC as compared to related species and the Subantarctic population. Another distinctive feature of *D. antarctica* from Antarctica is the narrower range of vessel diameters at the three growth temperatures (up to 15 µm at 5 ºC and up to 12 µm at 15 ºC and 23 ºC). By contrast, *D. antarctica* from the Subantarctic and *A. flexuosa* exhibited a broader range of diameters, reaching leaf xylem vessel diameters up to 18 µm (Fig. 5).

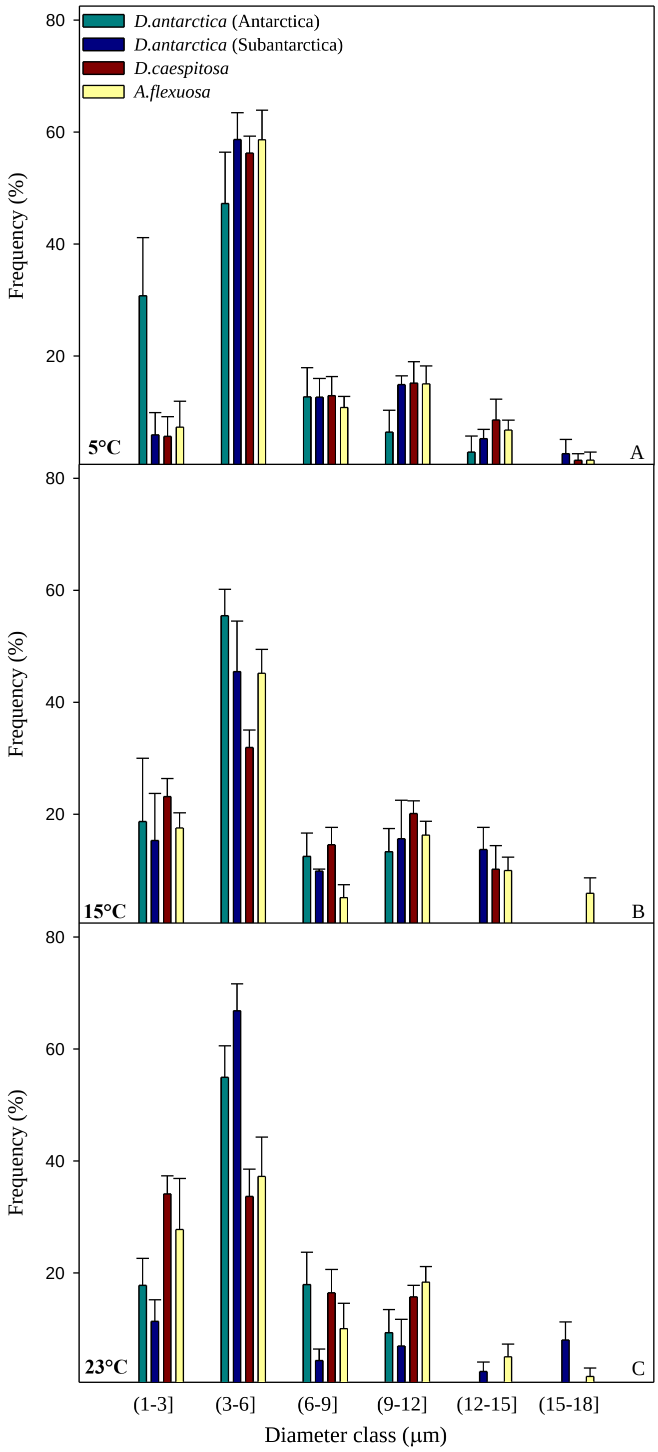

Figure 5. Frequency distribution of leaf xylem vessels diameters for *D. antarctica* from Antarctic and Subantarctic provenances and the closely related species *D. caespitosa* and *A. flexuosa* growing at 5 °C, 15 °C, and 23 °C. Statistically significant differences in distribution among species at different temperatures were analyzed according to the χ^2 test ($P<0.05$).

The distribution of leaf xylem vessel diameters largely differed among *Colobanthus* species and provenances (Fig. 6). *C. quitensis* from Antarctica presented, in general, the highest frequency of vessel diameters below 4 µm, irrespective of the growth temperature. On the contrary, *C. quitensis* from Subantarctica and *C. apetalus* presented a significant portion of vessels at diameters range above 5 µm (Fig. 6). There was not a clear effect of the growth temperature in the frequency distribution of vessel diameters in any of the three species and provenances.

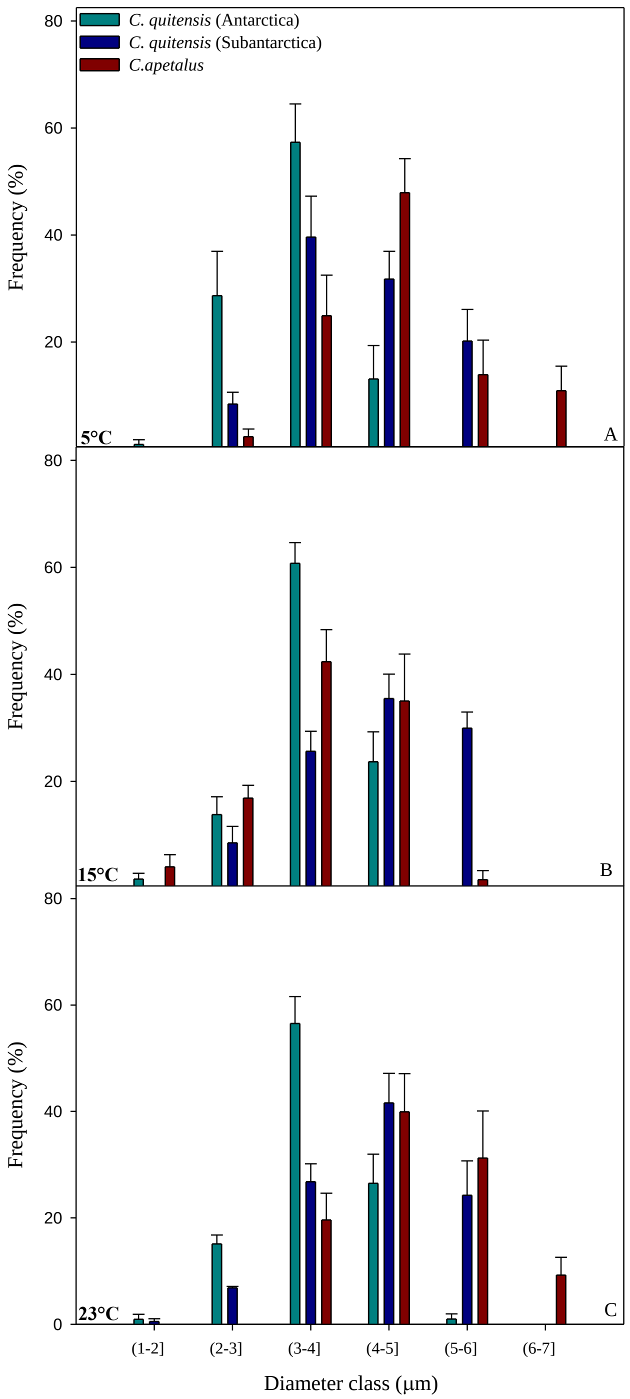

Figure 6. Frequency distribution of leaf xylem vessels diameters for *C. quitensis* from Antarctic and Subantarctic provenances and the closely related species *C. apetalus* growing at 5 °C, 15 °C, and 23 °C. Statistically significant differences in distribution among species at different temperatures were analyzed according to the χ2 test (P<0.05).

Coordination between Photosynthetic and Hydraulic traits

All Pearson correlations run between the main photosynthetic, hydraulic and anatomical parameters were statistically significant ($p < 0.05$) for both provenances of *D. antarctica* (Table 1). In this sense, positive correlations were observed in A_N *vs*. K_{leaf}, A_N *vs*. g_m, K_{leaf} *vs*. g_m, and ε_{max} *vs*. LMA, while the combinations A_N *vs*. LMA, K_{leaf} *vs*. LMA, K_{leaf} *vs*. g_s, and ε_{max} *vs*. K_{leaf} were negatively correlated (Table 1). In contrast, some of these trait-correlation became not statistically significant ($p > 0.05$) for the closely related species. In *D. caespitosa*, significant positive correlations were found between A_N and g_m, and negative correlations for K_{leaf} *vs*. LMA and ε_{max} *vs*. LMA. In *A. flexuosa*, significant positive correlations were observed for A_N *vs*. K_{leaf}, A_N *vs*. g_m, K_{leaf} *vs*. g_m, and negative correlations for K_{leaf} *vs*.g_s, and ε_{max} *vs*. K_{leaf} (Table 1).

Table 1. Pearson correlations between different photosynthetic and hydraulic traits for *D. antarctica* from Antarctic and Subantarctic provenances and closely related species: *D. caespitosa* and *A. flexuosa*. The correlations are presented for trait combinations between the following parameters: net CO_2 assimilation rate (A_N), leaf hydraulic conductivity (K_{leaf}), leaf mass per area (LMA), leaf mesophyll conductance to CO_2 (g_m), and maximum cell wall elasticity (ε_{max}). For each correlation, data measured at the different growth temperatures were combined. Pearson coefficients (*r*) and the significance (*p*) are shown. Ns means non-significant correlation ($p > 0.05$).

Traits	*D. antarctica*				Closely related species			
	Antarctic		Subantarctic		*D. caespitosa*		*A. flexuosa*	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
A_N -g_m	0.94	<0.001	0.99	<0.001	0.94	<0.001	0.92	<0.001
A_N-K_{leaf}	0.685	<0.002	0.66	<0.002	0.34	ns	0.91	<0.001
K_{leaf}-g_s	-0.81	<0.001	-0.47	<0.05	-0.42	ns	-0.76	<0.001
K_{leaf} -g_m	0.58	<0.02	0.66	<0.002	0.29	ns	0.73	<0.002
A_N -LMA	-0.73	<0.001	-0.50	<0.02	-0.12	ns	0.08	ns
K_{leaf} -LMA	-0.63	<0.01	-0.61	<0.01	-0.63	<0.002	-0.06	ns
ε_{max}-LMA	0.78	<0.001	0.43	<0.05	-0.45	<0.05	-0.26	ns
ε_{max} -K_{leaf}	-0.55	<0.02	-0.62	<0.002	-0.007	ns	-0.68	<0.01
ε_{max} - A_N	-0.81	<0.001	-0.66	<0.002	-0.61	<0.01	-0.76	<0.001

As for *Colobanthus* species, the sign (whether positive or negative) of the Peason correlation coefficient between the photosynthetic, hydraulic and anatomical traits was similar to that observed for *Deschampsia* species. Hence, significant positive correlations were found for A_N *vs.* K_{leaf}, and A_N *vs.* g_m, and negative correlation for ε_{max} *vs.* A_N for all *Colobanthus* species and provenances (Table 2). However, the number of significant trait-correlations was higher for *C. quitensis* from Antarctica and *C. apetalus* as compared to Subantarctic *C. quitensis*, for which five correlations were not significant (Table 2).

Table 2. Pearson correlations between different photosynthetic and hydraulic traits for *C. quitensis* from Antarctic and Subantarctic provenances and the closely related species *C. apetalus*. The correlations are presented for trait combinations between the following parameters: net CO_2 assimilation rate (A_N), leaf hydraulic conductivity (K_{leaf}), leaf mass per area (LMA), leaf mesophyll conductance to CO_2 (g_m), and maximum cell wall elasticity (ε_{max}). For each correlation, data measured at the different growth temperatures were combined. Pearson coefficients (*r*) and the significance (*p*) are shown. ns means non-significant correlation ($p > 0.05$).

Traits	*C. quitensis*				Closely related species	
	Antarctic		Subantarctic		*C. apetalus*	
	r	*P*	*r*	*p*	*r*	*p*
A_N -g_m	0.98	<0.001	0.95	<0.001	0.67	<0.002
A_N-K_{leaf}	0.76	<0.001	0.41	<0.05	0.73	<0.001
K_{leaf}-g_s	0.08	Ns	-0.12	ns	0.72	<0.001
K_{leaf} -g_m	0.69	<0.001	0.34	ns	0.41	ns
A_N -LMA	-0.12	Ns	-0.33	ns	-0.91	<0.001
K_{leaf} -LMA	-0.49	<0.05	0.29	ns	-0.81	<0.001
ε_{max}-LMA	0.46	<0.1	0.41	ns	0.58	<0.01
ε_{max} -K_{leaf}	-0.91	<0.001	-0.47	<0.05	-0.33	ns
ε_{max} -A_N	-0.71	<0.001	-0.67	<0.002	-0.61	<0.01

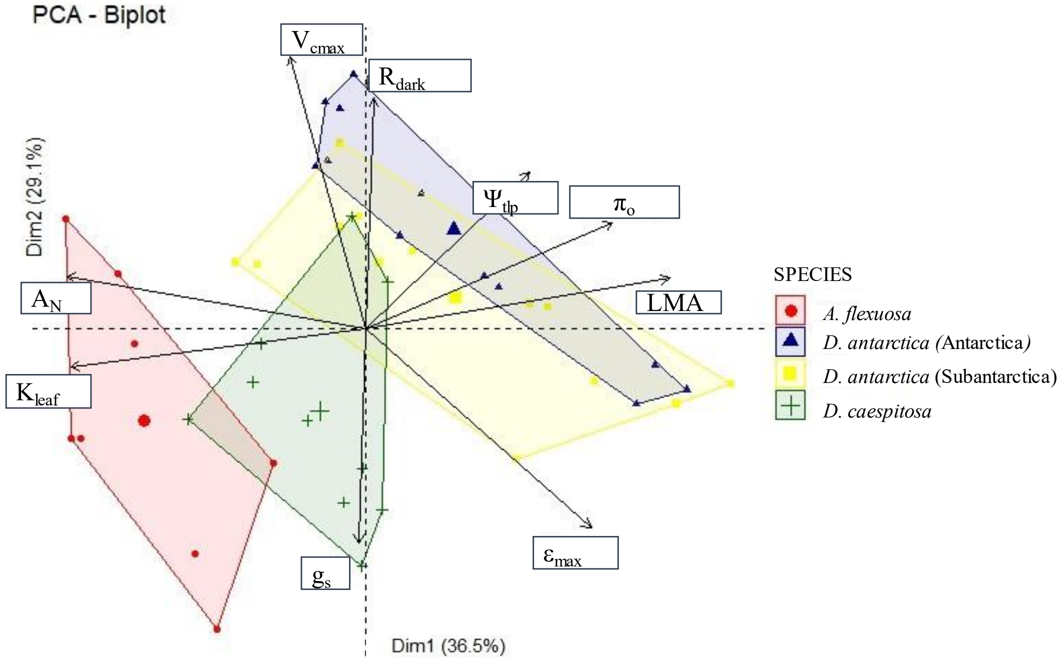

Figure 7. Principal component analysis (PCA) for *D. antarctica* from Antarctic and Subantarctic provenances, and the closely related species *D. caespitosa* and *A. flexuosa*. All data obtained at the three growth temperatures were considered, and included the following parameters: net CO_2 assimilation rate (A_N), stomatal conductance (g_s), dark respiration rate (R_{dark}), maximum Rubisco carboxylation rate (V_{cmax}), leaf hydraulic conductance (K_{leaf}), leaf mass per area (LMA), water potential at turgor loss point (Ψ_{tlp}), leaf capacitance (C), osmotic potential (π_o), and maximum bulk modulus of elasticity (ε_{max}).

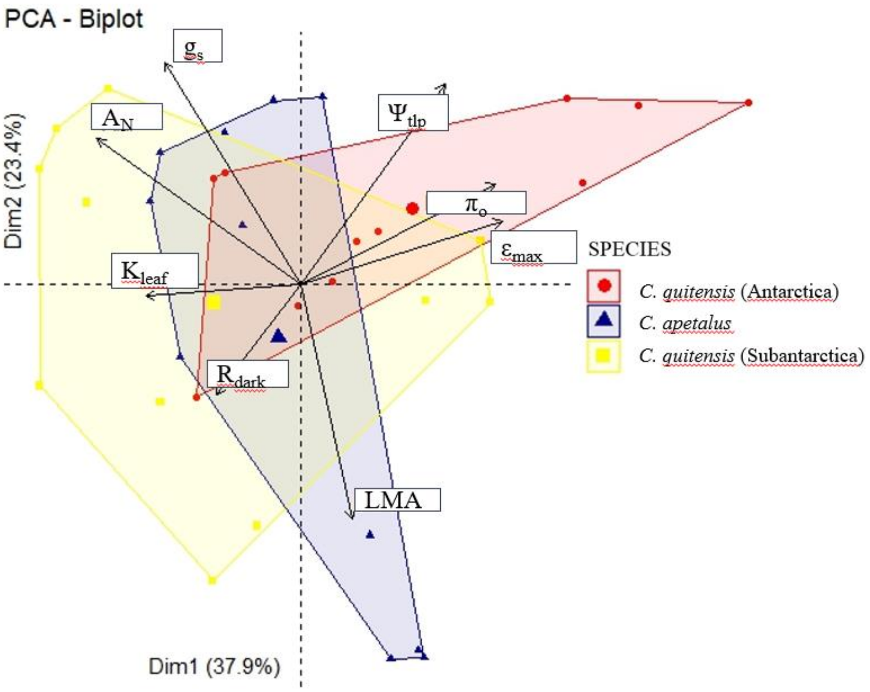

Figure 8. Principal component analysis (PCA) for *C. quitensis* from Antarctic and Subantarctic provenances, and the closely related species *C. apetalus*. All data obtained at the three growth temperatures were considered, and included the following parameters: net CO_2 assimilation rate (A_N), stomatal conductance (g_s), dark respiration rate (R_{dark}), maximum Rubisco carboxylation rate (V_{cmax}), leaf hydraulic conductance (K_{leaf}), leaf mass per area (LMA), water potential at turgor loss point (Ψ_{tlp}), leaf capacitance (C), osmotic potential (π_o), and maximum bulk modulus of elasticity (ε_{max}).

DISCUSSION

Antarctic vascular plants and related species show divergent patterns in hydraulic conductance and photosynthesis

The relationship between K_{leaf} and A_N highlight how an efficient hydraulic system can enhance the plant photosynthetic capacity (Brodribb et al., 2007; Scoffoni et al., 2016b; Xiong et al., 2017). An efficient K_{leaf} ensures adequate water supply and facilitates stomatal opening, facilitating transpiration while allowing CO_2 to enter the leaves. This gas exchange is essential for photosynthesis and growth, particularly in stressful environments where the ability to optimize resource use becomes crucial for plant survival.

Principal component analyses (PCA) were run to investigate how the different species and provenances clustered within the observed variability. For *Deschampsia* species, the two dimensions of the PCA accounted for up to 65 % of the total variability (Fig. 7). Dimension 1 was primarily driven by traits related to water conservation, such as K_{leaf}, Ψ_{tlp}, and LMA, along with A_N, while dimension 2 mainly correlated with some gas-exchange parameters, particularly V_{cmax}, R_{dark} and g_s. Interestingly, the different species clustered in a continuous trend in which the extremes were occupied by *D. antarctica* from Antarctica and *A. flexuousa*, with a high degree of overlapping between the two *D. antarctica* provenances. *D. caespitosa* situated in between *A. flexuosa* and Subantarctic *D. antarctica*, indicating intermediate traits in terms of both hydraulic efficiency and photosynthetic capacity. Antarctic and Subantarctic provenances of *D. antarctica* exhibit similar physiological traits, including efficient hydraulic properties and strong photosynthetic capacity. The significant correlation between K_{leaf} and A_N (Table 1) demonstrates that effective water transport enhances CO_2 diffusion and photosynthesis. Genetic analysis suggests that the observed similarity in physiological traits of *D. antarctica* could be attributed to a reduction in genetic variability. Antarctic populations may have originated from a limited number of Patagonian genotypes (Fasanella et al., 2017), which has reduced genetic diversity. This reduction has led to greater uniformity in physiological traits, reflecting a strong fixation of adaptive characteristics through natural selection that optimizes survival in extreme conditions. Regarding related species, *D. caespitosa* showed a lack of a critical correlation between A_N and K_{leaf}, suggesting a decoupling of water transport efficiency and carbon assimilation. Instead, it prioritizes other physiological traits such as g_s and ε_{max}, which become more influential depending on the temperature, likely due to anatomical differences (Ocheltree et al., 2016). *A. flexuosa*, on other hand, prioritizes photosynthetic efficiency, exhibiting a strong positive correlation between K_{leaf} and A_N, but a weaker relationship with drought tolerance which is explained by the lesser influence of LMA and ε_{max}. This suggests a trade-off between rapid growth and drought resistance in this species. These differences highlight the diverse adaptive strategies of plants and underscore the uniqueness of *D. antarctica*, which maintains specific hydraulic traits while preserving a co-variation between hydraulic and photosynthetic traits.

For *Colobanthus*, the two dimensions accounted for ca. 60% of the observed variability (Fig. 8). Dimension 1 was mainly associated with water conservation traits

(Ψ_{tlp}, π_o, ε_{max}, K_{leaf}) and some photosynthetic parameters (A_N and g_s). Dimension 2 correlated also correlated with A_N, g_s and Ψ_{tlp}, as well as with LMA. Contrarily to *Deschampsia* species, the Subantarctic provenance of *C. quitensis* was not situated in the transition between the Antarctic population and the related species, though a higher degree of overlapping between species and provenances was observed in the case of *Colobanthus*. Antarctic *C. quitensis* is distinguished by its high ε_{max} and low g_s, Ψ_{tlp}, and K_{leaf}, indicating adaptations for water conservation (Fig. 8). The strong coordination between A_N, K_{leaf}, and g_m (Table 2) suggests an effective adaptive strategy for optimizing photosynthesis and water transport under the harsh conditions of Antarctica. In contrast, subantarctic *C. quitensis* exhibits higher A_N and K_{leaf} but with less pronounced correlations, suggesting a different adaptive approach. These physiological differences underscore the uniqueness of Antarctic *C. quitensis*, whose exceptional adaptations to the severe conditions of Antarctica distinguish it from its Subantarctic counterpart (Gianoli et al., 2004; Acuña-Rodríguez et al., 2017). Ecotypes of *C. quitensis*, such as Andean and Antarctic, have been defined, showing notable morphological and genetic variability, likely due to selection processes in response to habitat conditions (Acuña-Rodríguez et al., 2017; Cuba et al., 2017; Androsiuk et al., 2015; Koc et al., 2018). On the other hand, *C. apetalus* prioritizes structural resistance, adapting differently to cold conditions. The strong correlation between K_{leaf} and A_N suggests a close relationship between hydraulic capacity and photosynthesis. Additionally, the greater influence of LMA in *C. apetalus* is associated with lower K_{leaf} response, which suggests an adaptive strategy focused on foliar structure to withstand adverse conditions rather than maximizing hydraulic conductance. This highlights a different adaptive strategy compared to *C. quitensis*.

Anatomy and photosynthesis facing increased growth temperature

The photosynthetic performance of both Antarctic and Subantarctic provenances of *D. antarctica* and *C. quitensis*, alongside their phylogenetically related species *D. caespitosa*, *A. flexuosa*, and *C. apetalus*, exhibits a general trend towards enhanced CO_2 assimilation rate (A_N) with increasing growth temperatures (Figs. 1 and 2). This improvement was associated with larger values for the leaf mesophyll conductance to CO_2 (g_m) and the maximum rate of Rubisco carboxylation (V_{cmax}).

Among *Deschampsia* species, *D. antarctica* exhibited the highest photosynthetic capacity throughout the range of evaluated growth temperatures. This fact confirms that, though it is adapted to low temperatures, the maximum CO_2 assimilatory potential of *D. antarctica* is obtained at temperatures above 15 ºC (see Ramírez et al. 2024). Despite the geographic separation, *D. antarctica* from Antarctica and Subantarctic showed notable similarities in the main photosynthetic traits (Fig. 1). This consistency suggests comparable adaptations to temperature variations and a high degree of uniformity in its functional traits for extreme conditions. While previous studies did not explore these physiological traits beyond Antarctica, Edwards and Smith (1988) also noted similarities in the photosynthetic response curves among different Antarctic maritime locations, along with variations in leaf morphoanatomy and chloroplast traits (Moore et al., 1970; Jellings et al., 1983; Giełwanowska et al., 2005). The higher photosynthetic capacity of *D. antarctica*, as compared to the related species, was accompanied by a distinctive response pattern of leaf structure to the different growth temperatures. In *D. antarctica*, both the leaf dry matter per area (LMA) and leaf density (LD) tend to decrease at higher growth temperatures (Fig. 1 A, B). In this sense, the reported increase in A_N correlated with a decrease in LMA and LD across the range of growth temperatures (Table 1), in line with previous observations (Sáez et al.2017, 2018, 2024). LMA inversely correlated with g_m, allowing a higher CO_2 transfer capacity along the leaf mesophyll, and ultimately, a higher A_N in *D. antarctica.* On the contrary, no significant correlations were found between leaf structure and A_N in the related species (Table 1). However, a positive correlation was identified between A_N and g_m, suggesting a complex relationship between leaf anatomy and physiological function (Table 1). Traits such as cell wall thickness (T_{cw}) and the surface area of mesophyll cells (S_m) and/or chloroplasts (S_c) exposed to intercellular air spaces per leaf area (Tomàs et al., 2013; Peguero-Piña et al., 2017b; Sáez et al., 2017), along with the activity of transporters and enzymes like aquaporins (Terashima and Ono, 2002) and carbonic anhydrases (Fabre et al., 2007), could also have also influence g_m and, consequently, photosynthetic capacity in *Deschampsia* species.

In *Colobanthus* species, both Antarctic and Subantarctic *C. quitensis* exhibited similar rates of A_N, g_s, g_m, V_{cmax}, and WUE at 5°C (Fig. 2). However, at higher growth temperature, Subantarctic *C. quitensis* displayed higher A_N than the Antarctic provenance, driven by enhanced g_m and/or V_{cmax}. Contrarily to what was observed for *Deschampsia*, no correlation was found between A_N and LMA in either provenance. Instead, significant correlations between A_N and g_m were observed, underscoring the importance of efficient internal CO_2 diffusion for photosynthesis in this species regardless of the provenance (Sáez et al., 2017). Recent studies have revealed differences between Antarctic and Subantarctic populations of *C. quitensis* in g_m and A_N, related to anatomical variations and the accumulation of calcium oxalate (CaOx) crystals (Gómez et al., 2021, 2024). In Antarctic populations, lower values of A_N and g_m were correlated with higher LMA and LD, and lower abundance of CaOx. The breakdown of CaOx crystals appears to activate a stress-response photosynthesis mechanism, providing internal CO_2 under stressful conditions. This relationship highlights the differential adaptation of *C. quitensis* provenances and supports ecotypic differentiation in response to extreme conditions. Conversely, significant correlations were found between A_N and both LMA and g_m in *C. apetalus*. At 5°C, high LMA and significantly low g_s resulted in reduced A_N compared to both provenances of *C. quitensis*. These traits restricted gas exchange and negatively affect photosynthesis (Wright et al., 2005; Medrano et al., 2009; Poorter et al., 2009; Flexas et al., 2014). However, the higher A_N observed in *C. apetalus* compared to Antarctic *C. quitensis* at 15 and 23°C was associated with lower LMA, higher g_s, and increased values in WUE and V_{cmax}.

Hydraulic responses of Antarctic and Subantarctic *D. antarctica* and *C. quitensis*, and their closely species

D. antarctica, from both provenances, exhibited consistently low K_{leaf}, even considering increases at higher temperatures (Sáez et al., 2024) (Fig. 3). This trade-off between water transport efficiency and embolism resistance suggests that species with low K_{leaf} are more resistant to hydraulic failure (Ocheltree et al., 2016; Wright et al., 2004; Onoda et al., 2017; Xiong & Flexas, 2018). In comparison, *D. caespitosa* showed similar low K_{leaf} values to those of *D. antarctica*, while *A. flexuosa* exhibited the highest K_{leaf} across all treatments, indicating better water transport due to higher capacitance (Table S3). The narrow vessel diameters and high cell wall rigidity in *Deschampsia* species, especially in *D. antarctica*, are key adaptations for survival in cold environments. These traits minimize the risk of embolism, ensuring efficient water transport (Sakai and Larcher, 1987; Day et al., 1999; Medek, 2010), and enhance tolerance to turgor loss, allowing plants to maintain higher relative water content before reaching the critical turgor loss point where stomata close (Sáez et al., 2024). High ε_{max} values at low temperatures indicate adaptations to freeze dehydration and thermal fluctuations, helping to prevent intracellular freezing and manage ice spread, possibly involving thicker cell walls and specific gene regulation (Solecka et al., 2008; Panter et al., 2020). The lower ε_{max} in all species at higher temperatures suggests a trade-off between the rigidity needed for cold tolerance and the flexibility required for efficient gas exchange during photosynthesis (Blackman et al., 2010; Nadal et al., 2018). Interestingly, both provenances of *D. antarctica* maintain low osmotic potential (π_o), enhancing water conservation in cold environments, and a more negative leaf turgor loss point (Ψ_{tlp}), extending the range of leaf water potential (Ψleaf) over which the leaf maintains turgor and function (Sack et al., 2003; Lenz et al., 2006) (see Table S3). This underscores the exceptional water conservation ability of *D. antarctica* compared to closely related species.

Hydraulic trait differences highlight the distinct adaptations of Antarctic versus Subantarctic *C. quitensis*. Antarctic *C. quitensis* seems to prioritize xylem resistance, likely due to increased exposure to freezing-thawing cycles in its environment (Pittermann & Sperry, 2006; Mayr & Sperry, 2010; Sáez et al., 2024). This is indicated by lower K_{leaf} values at 5°C and 15°C, as well as smaller xylem vessels and higher ε_{max}, suggesting adaptations for improved water retention and reduced dehydration in freezing conditions. These adaptations, along with more negative values of π_o and Ψ_{tlp} support greater water conservation, optimizing physiological processes like g_s, K_{leaf}, and A_N (Farrell et al., 2017; Trueba et al., 2019; Sorek et al., 2021) under constant cold conditions. In contrast, Subantarctic *C. quitensis* feature larger and more numerous leaf xylem vessels, deploying a higher mean hydraulic diameter (D_h, Table S3), which can enhance the xylem hydraulic capacity at the cost of increased hydraulic resistance (Pittermann et al., 2006). Their higher specific conductivity (K_s) and theoretical hydraulic conductivity (K_h) (Table S3) facilitate efficient water transport, signifying an enhanced xylem capacity to transport water under a hydraulic pressure gradient (Tyree and Ewers, 1991). This capacity is reflected in the higher K_{leaf} values observed at 5°C and 15°C. However, at 23°C, Subantarctic *C. quitensis* exhibited lower leaf capacitance, which is associated with a reduced ability to store and release water, despite maintaining similar K_{leaf} values compared to the Antarctic population.

The similar K_{leaf} values between *C. apetalus* and *C. quitensis* from Antarctica at 5°C were associated with the small diameter of xylem vessels and high LMA. However, *C. apetalus* exhibited lower ε_{max} and higher π_o and Ψ_{tlp} values (Table S2), which explained the reduction in g_s and A_N at low temperatures. The lower leaf capacitance in *C. apetalus* at higher temperatures (15°C and 23°C) resulted in a lower K_{leaf} compared to *C. quitensis*. However, *C. apetalus* exhibited higher A_N, suggesting that it had developed structural and functional adaptations to optimize gas exchange at warmer temperatures. Notably, *C. apetalus* showed a strong correlation between K_{leaf} and both LMA and g_s, a relationship that was not observed in *C. quitensis*. These findings indicated that *C. apetalus* might have efficient stomatal regulation and greater water use efficiency, along with internal mechanisms that enhance CO_2 capture, allowing effective photosynthesis despite a lower K_{leaf}.

CONCLUDING REMARKS AND PROSPECTS

Our results partially confirm the initial hypothesis, showing that the physiological traits of Antarctic populations of *D. antarctica* and *C. quitensis*, where photosynthetic and hydraulic traits are coordinated in response to temperature, are also evident in their sub-Antarctic counterparts, though differently. *D. antarctica*, from both Antarctic and sub-Antarctic provenances, exhibited similar physiological traits, with notable coordination in water conservation and high photosynthetic capacity, suggesting a conservative strategy that allows it to maintain stable physiological performance despite environmental variations between the regions. In contrast, *C. quitensis* exhibited distinct responses between its Antarctic and Subantarctic populations. The Antarctic populations showed a closer integration between photosynthesis and hydraulic traits, reflecting a specialized strategy for water conservation under Antarctic conditions. Conversely, the Subantarctic populations demonstrated less coordination between these traits but had higher values in individual characteristics, suggesting divergent physiological strategies. These differences highlight how *C. quitensis* has adjusted its traits according to the prevailing conditions in each region, efficiently adapting to its specific environment. Additionally, when analyzing the responses of phylogenetically related species, it was observed that they did not exhibit the same responses and displayed divergent strategies in hydraulic and photosynthetic coordination at different temperatures, emphasizing the uniqueness of adaptations in Antarctic plants. These findings underscore the adaptability of *D. antarctica* and *C. quitensis* to their respective environments, reflecting key differences in their physiological strategies in response to temperature, and highlight the need for future research to assess how these adaptations might respond to projected climate changes, including rising temperatures and shifts in water availability.

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ANEXOS

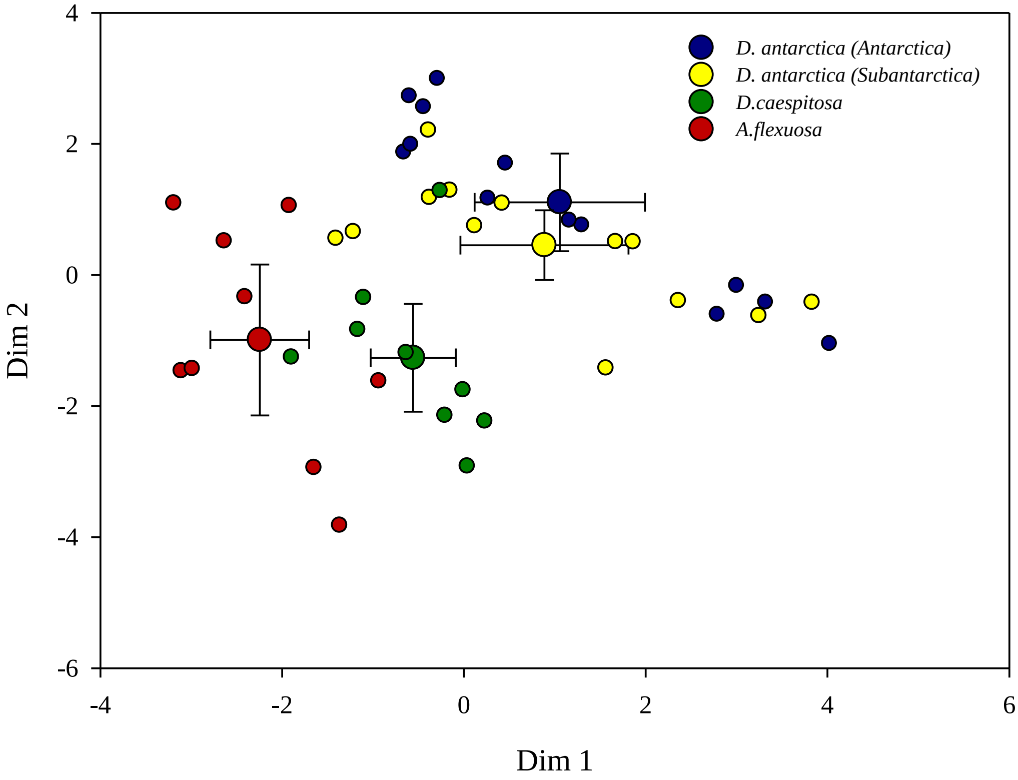

Figure S1. Scores of observations obtained from principal component analysis (PCA) of photosynthesis (A_N), stomatal conductance (g_s), leaf hydraulic conductance (K_{leaf}), leaf mass per area (LMA), water potential at turgor loss point (Ψ_{tlp}), leaf capacitance (C), osmotic potential (π_o), dark respiration rate (R_{dark}), maximum Rubisco carboxylation rate (V_{cmax}), and maximum bulk modulus of elasticity (ε_{max}) for *D. antarctica* from Antarctic and Subantarctic provenances, and the closely related species *D. caespitosa* and *A. flexuosa* grown at 5 °C, 15 °C and 23 °C.

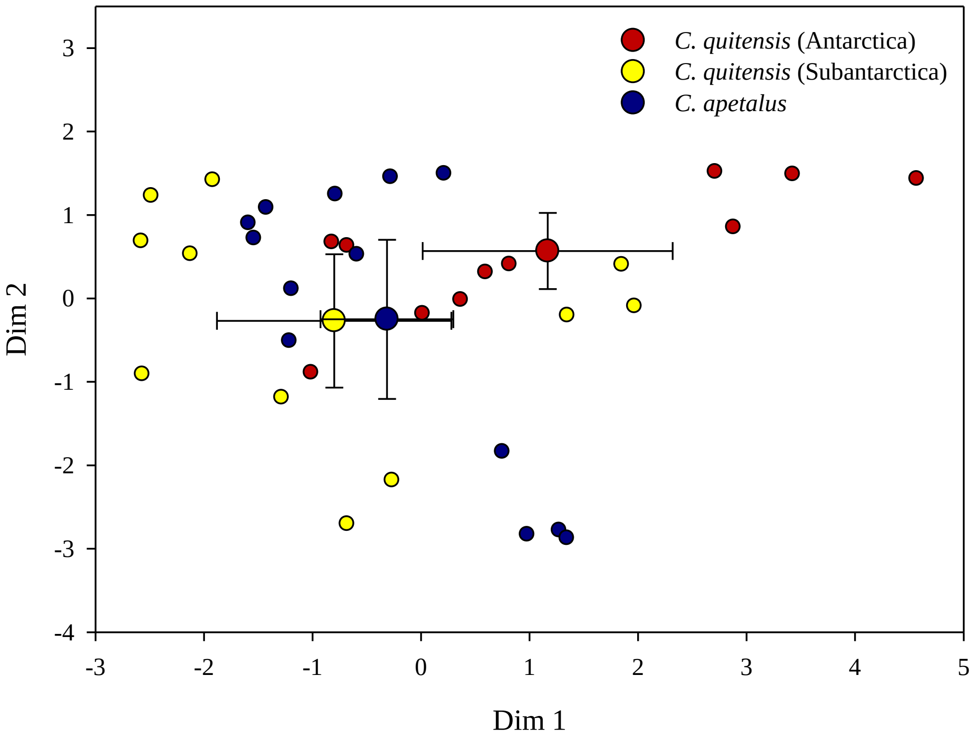

Figure S2. Scores of observations obtained from principal component analysis (PCA)of photosynthesis (A_N), stomatal conductance (g_s), leaf hydraulic conductance (K_{leaf}), leaf mass per area (LMA), water potential at turgor loss point (Ψ_{tlp}), leaf capacitance (C), osmotic potential (π_o), dark respiration rate (R_{dark}), maximum Rubisco carboxylation rate (V_{cmax}), and maximum bulk modulus of elasticity (ε_{max}) for *C. quitensis* from Antarctic and Subantarctic provenances, and the closely related species *C. apetalus* grown at 5 °C, 15 °C and 23 °C.

Table S1. Leaf absorbance for *D. antarctica* and *C. quitensis* from Antarctic and Subantarctic provenances and their respective closely species: *D. caespitosa, A. flexuosa* and *C. apetalus* growing at 5 °C, 15 °C and 23 °C. Values are means ± S.E. (*n*=6). Different letters for *D. antarctica* and *C. quitensis* indicate statistically significant differences among species at different growth temperatures according to Tukey's test (P < 0.05).

Absorbance	5°C	15°C	23°C
D. antarctica (Antarctica)	0.86±0.005a	0.85 ± 0.01a	0.81±0.01b
D. antarctica (Subantarctica)	0.85±0.01a	0.77± 0.01b	0.76±0.01c
D. caespitosa	0.87±0.01a	0.78±0.01b	0.86±0.01[a]
A. flexuosa	0 .85±0.01a	0.86±0.004a	0.86±0.01a
C. quitensis (Antarctica*)*	0.89±0.006b	0.89±0.006b	0.8±0.005b
C. quitensis (Subantarctica)	0.92±0.009a	0.94±0.006a	0.88±0.007a
C. apetalus	0.8±0.005c	0.80±0.008c	0.89±0.003a

Table S2. Parameters derived from the pressure-volume curves for *D. antarctica* and *C. quitensis* from Antarctic and Subantarctic provenances and their respective closely related species: *D. caespitosa*, *A. flexuosa* and *C. apetalus* growing at 5 °C, 15 °C and 23 °C: leaf capacitance (C), 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$_{tlp}$) Values are means ± S.E. (*n*=6). Different letters indicate statistically significant differences among species at different growth temperatures according to Tukey's test ($P < 0.05$).

	5 °C	15 °C	23 °C	5 °C	15 °C	23 °C
	Deschampsia antarctica (Antarctica)			*Colobanthus quitensis* (Antarctica)		
C (mol m^{-2} MPa^{-1})	0.35± 0.01b	0.67± 0.03b	0.73±0.04b	1.08± 0.013b	2.7± 0.079a	3.353±0.098a
π_o (MPa)	-1.26± 0.02a	-1.11± 0.03a	-0.92±0.05a	-0.946 ± 0.071b	-0.763± 0.034a	-0.684±0.047a
RWC$_{tlp}$ (%)	95± 0.01a	93± 0.01a	90 ±0.01b	91.4± 0.008a	84.8± 0.007b	85.2 ±0.025b
Ψ_{tlp} (MPa)	-1.06± 0.03a	-1.25± 0.05a	-0.98 ±0.08a	-1.18± 0.05b	-0.9± 0.018a	-0.976 ±0.102a
	Deschampsia antarctica (Subantarctica)			*Colobanthus quitensis* (Subantarctica)		
C (mol MPa^{-1} m^{-2})	0.36±0.04b	0.54±0.01b	0.83±0.06b	1.53±0.11a	3.157±0.103a	2.368±0.319b
π_o (MPa)	-1. 01±0.11a	-1.14±0.04a	-0.86 ±0.06a	-0.794-±0.039ab	-0.704±0.045a	-0.675 ±0.036a
RWC$_{tlp}$	95±0.01a	94±0.01a	91±0.01ab	92.1±0.008a	92.7±0.007a	91.6±0.009a
Ψ_{tlp} (MPa)	-1.02±0.02a	-1.22±0.06a	-0.94±0.09a	-0.988-±0.042a	-0.802±0.035a	-0.926±0.059a
	Deschampsia caespitosa			*Colobanthus apetalus*		
C (mol m^{-2} MPa^{-1})	0.45± 0.02b	0.74± 0.08b	0.69±0.11b	1.099± 0.055b	1.72± 0.109b	1.634±0.104c
π_o (MPa)	-0.6± 0.0b	-0.66± 0.04b	-0.77± 0.03a	-0.667± 0.024a	-0.638± 0.034a	-0.743± 0.064a
RWC$_{tlp}$ (%)	94± 0.0a	93±0.01a	94±0.01a	94.5± 0.007a	93.8±0.008a	91.8±0.008a
Ψ_{tlp} (MPa)	-0.79± 0.02b	-0.84±0.04b	-1.07± 0.03a	-0.832± 0.057a	-0.804±0.037a	-1.058± 0.04a
	Avenella flexuosa					
C (mol m^{-2} MPa^{-1})	0.8± 0.05a	1.18± 0.02a	1.29±0.12a			
π_o (MPa)	-0.7-±0.07b	-0.63±0.07b	-0.73±0.05a			
RWC$_{tlp}$ (%)	95±0.00a	94±0.01a	94± 0.01a			
Ψ_{tlp} (MPa)	-0.67±0.05b	-0.9±0.05b	-1.03±0.04a			

Table S3. The mean hydraulic diameter (D_h), theoretical hydraulic conductivity (K_h), and specific conductivity (K_s) for *D. antarctica* and *C. quitensis* from Antarctic and Subantarctic provenances, and their respective closely related species *D. caespitosa*, *A.flexuosa* and *C. apetalus* grown at 5°C, 15°C, and 23°C. Values are means ± S.E. (n=4-8). Different letters indicate statistically significant differences among species at different growth temperatures according to Tukey's test ($P < 0.05$).

	5 °C	15 °C	23 °C	5 °C	15 °C	23 °C
	Deschampsia antarctica (Antarctica)			*Colobanthus quitensis* (Antarctica)		
D_h (µm)	9.46± 0.84a	8.78± 1.00b	9.09±0.58b	3.65±0.13b	3.86± 0.068b	3.93± 0.108b
K_h (x 10^{-10} kg m s^{-1} MPa^{-1})	4.46±1.51b	5.32±1.31b	5.89±1.1b	0.53±0.079c	0.76± 0.065b	0.92±0.093b
K_s (kg m^{-1} s^{-1} MPa^{-1})	1.46±0.24a	1.71± 0.33b	2.08± 0.21b	0.26±0.018b	0.39± 0.015b	0.47± 0.025b
	Deschampsia antarctica (Subantarctica)			*Colobanthus quitensis* (Subantarctica)		
D_h (µm)	11.97± 0.73a	12.89± 0.26a	15.36± 0.86a	5.01± 0.096a	5.13± 0.085a	5.17± 0.17a
K_h (x 10^{-10} kg m s^{-1} MPa^{-1})	12.4±2.6a	18±1.61a	38.5±5.7a	2.81±0.42a	2.97±0.28a	5.06±3.7a
K_s (kg m^{-1} s^{-1} MPa^{-1})	2.28± 0.30a	3.52± 0.13a	6.04± 0.68a	0.47± 0.018a	0.658± 0.021a	0.80± 0.05a
	Deschampsia caespitosa			*Colobanthus apetalus*		
D_h (µm)	11.57± 0.73a	12.34± 0.73a	10.54± 0.46b	5.2±0.14a	4.11±0.16b	5.3±0.17a
K_h (x 10^{-10} kg m s^{-1} MPa^{-1})	9.8±2ab	14±3.3a	9.4±1.4b	1.44 ±0.21b	0.94±0.17b	3.95± 0.53a
K_s (kg m^{-1} s^{-1} MPa^{-1})	2.26± 0.27a	3.45± 0.48a	2.97± 0.29b	0.49±0.025a	0.43±0.035b	0.89±0.055a
	Avenella flexuosa					
D_h (µm)	11.21± 0.64a	13.41± 0.76a	10.96± 0.64b			
K_h (x 10^{-10} kg m s^{-1} MPa^{-1})	9.2 ± 2ab	24±5.5a	12 ±3.1b			
K_s (kg m^{-1} s^{-1} MPa^{-1})	2.03± 0.23a	4.21± 0.49a	3.29± 0.33b			

Table S4. Statistical differences in the frequency distribution of leaf xylem vessel diameters among *Deschampsia antarctica* (Antarctic and Subantarctic provenances), *D. caespitosa*, and *A. flexuosa* at 5 °C, 15 °C, and 23 °C, according to Fisher's exact test (P<0.05).

Growth temperature (°C)	Comparison (Fischer's test)	p-value
5 °C	*D. antarctica* (Antarctica) vs *D. antarctica* (Subantarctica)	< 0.05
	D. antarctica (Antarctica) vs *D. caespitosa*	< 0.05
	D. antarctica (Antarctica) vs *A. flexuosa*	< 0.05
	D. antarctica (Subantarctica) vs *D. caespitosa*	ns
	D. antarctica (Subantarctica) vs *A. flexuosa*	ns
	D. caespitosa vs A. flexuosa	ns
15	*D. antarctica* (Antarctica) vs *D. antarctica* (Subantarctica)	< 0.05
	D. antarctica (Antarctica) vs *D. caespitosa*	< 0.05
	D. antarctica (Antarctica) vs *A. flexuosa*	< 0.05
	D. antarctica (Subantarctica) vs *D. caespitosa*	ns
	D. antarctica (Subantarctica) vs *A. flexuosa*	ns
	D. caespitosa vs *A. flexuosa*	ns
	D. antarctica (Antarctica) vs *D. antarctica* (Subantarctica)	ns
23	*D. antarctica* (Antarctica) vs *D. caespitosa*	< 0.05
	D. antarctica (Antarctica) vs *A. flexuosa*	< 0.05
	D. antarctica (Subantarctica) vs *D. caespitosa*	< 0.05
	D. antarctica (Subantarctica) vs *A. flexuosa*	ns
	D. caespitosa vs *A. flexuosa*	< 0.05

Table S5. Statistical differences in the frequency distribution of leaf xylem vessel diameters among *Colobanthus quitensis* (Antarctic and Subantarctic provenances) and *C. apetalus* at 5 °C, 15 °C, and 23 °C, according to Fisher's exact test ($P<0.05$).

Growth temperature (°C)	Comparison (Fischer's test)	p-value
5	*C. quitensis* (Antarctica) vs *C. quitensis* (Subantarctica)	< 0.05
	C. quitensis (Antarctica) vs *C. apetalus*	< 0.05
	C. quitensis (Subantarctica) vs *C. apetalus*	< 0.05
15	*C. quitensis* (Antarctica) vs *C. quitensis* (Subantarctica)	< 0.05
	C. quitensis (Antarctica) vs *C. apetalus*	< 0.05
	C. quitensis (Subantarctica) vs *C. apetalus*	ns
23	*C. quitensis* (Antarctica) vs *C. quitensis* (Subantarctica)	< 0.05
	C. quitensis (Antarctica) vs *C. apetalus*	< 0.05
	C. quitensis (Subantarctica) vs *C. apetalus*	< 0.05

CAPÍTULO 2: ARE THE PHOTOSYNTHETIC AND HYDRAULIC TRAITS OF *Deschampsia antarctica* SIMILAR TO THOSE FOUND IN POTENTIALLY INVASIVE *Poa* SPECIES IN ANTARCTICA?

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ABSTRACT

One of the most significant effects of climate change is the potential arrival of non-native species, which is particularly important in sensitive ecosystems like Antarctica, where climate change is already evident. In this context, *Poa annua* and *Poa pratensis* have been suggested as potential invasive species. This study investigates the photosynthetic and hydraulic responses of these two species in comparison to the native Antarctic grass *Deschampsia antarctica* growing at different temperatures: the optimal photosynthetic temperature for *Poa* species (23°C), the optimal photosynthetic temperature for *D. antarctica* (15°C), and the average maximum Antarctic summer temperature (5°C). We focused on photosynthetic determinants (both diffusive and biochemical), hydraulic traits (P-V curves parameters and hydraulic conductivity), and leaf anatomical characteristics to understand how these plants manage photosynthesis and water conductivity across temperatures. Our findings reveal that all species maintain similar photosynthetic rates across temperatures, indicating robust photosynthetic machinery as at optimal as well as suboptimal temperatures. At low temperature, cell walls in all species become more rigid, limiting cell shrinkage and preserving high relative water content. Distinctly, *D. antarctica* coordinate net photosynthesis and water use efficiency with specific leaf traits, such as leaf density and leaf mass per area, which enhance its resilience to cold temperatures. In contrast, *P. annua* and *P. pratensis* exhibit greater hydraulic efficiency in warmer conditions without significant changes in leaf structure, suggesting potential shifts in competitive interactions under future climate scenarios... Despite having similar photosynthetic and hydraulic traits, further studies on additional factors are necessary to fully determine the invasive potential of *Poa* species.

INTRODUCTION

Climate change is rapidly reshaping ecosystems impacting the distribution and survival of several species (IPCC, 2019). As temperatures rise and climate patterns shift, habitats are undergoing significant transformations, challenging the adaptive capacities of native species (Root et al., 2003; Parmesan, 2006) and encouraging the proliferation of non-native species (Hellmann et al., 2008; Early & Sax, 2011), posing severe threats to biodiversity and natural habitats (Walther et al., 2009; Simberloff et al., 2013). For instance, in the South American Andes, climate change has facilitated the invasion of exotic species into high mountain ecosystems, displacing endemic flora (Cavieres et al., 2014; Pauchard et al., 2016). Similarly, in subantarctic islands, warming has enabled the establishment of invasive species, threatening the unique biodiversity of these isolated ecosystems (Frenot et al., 2005; Lebouvier et al., 2011). The impacts of climate change on these sensitive environments highlight the need to understand how climate and biological invasions interact (Wookey et al., 2009; McDougall et al., 2011). Cold regions, such as high-altitude environments and polar areas, play crucial role in the global cryosphere system, regulating climate and providing vital freshwater resources while harboring unique biodiversity adapted to extreme ecosystems (Körner, 1999; Convey et al., 2012). Recent IPCC reports (2019, 2022) reveal that these ecosystems are undergoing dramatic changes due to climate change, including declines in snow cover, glaciers, and permafrost, which contribute to habitat loss and disrupt hydrological and climatic patterns. In Antarctica, particularly in the Antarctic Peninsula, temperatures have increased more rapidly than in most other regions of the Southern Hemisphere (Mulvaney et al., 2012; Turner et al., 2016, Chown et al., 2022, Siegert et al. 2023). This rising temperature is profoundly altering the Antarctic landscape by lengthening growing seasons, retreating glaciers and ice shelves, and shifting precipitation patterns (Torres-Mellado et al., 2011; Canonne et al., 2016). As a result, the expansion of the only two native vascular plant species, *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae), has been facilitated (Smith, 1994; Gerighausen et al., 2003; Torres-Mellado et al., 2011, Cannone et al., 2016;2022).

The impacts of climate change on Antarctic plants extend beyond temperature increases to alterations in water availability due to increased evapotranspiration rates and reduced precipitation (Robinson et al., 2003). This could potentially expose native plants to competitive pressures from invasive species (Cavieres et al., 2018). Although Antarctica has remained isolated over the past 10,000 years (Convey, 2006), studies suggest that plant propagules from various species, both native and non-native to subantarctic habitats, have reached Antarctica through natural ways or human assistance (Smith and Richardson, 2011; Chown et al., 2012). However, the capacity of these plant species to establish self-sustaining populations in natural environments without human intervention is extremely limited (Smith, 1996; Hughes and Convey, 2010; Chown et al., 2012). Over the past 50 years, increased anthropogenic pressure associated with scientific and tourist activities, along with the regional climate effects (primarily in the Antarctic Peninsula region) has altered this paradigm (Hughes et al. 2020). The introduction of plant species from other regions and the spread of propagules have significantly increased in the Maritime Antarctic, where climatic conditions are more suitable for plant growth compared to the continental Antarctic (Chown et al. 2012; Chwedorzewska et al. 2015; Molina-Montenegro et al. 2015; Fuentes-Lillo et al. 2017a; Bokhorst et al. 2021).

Poaceae species are among the most widely distributed and abundant vascular plants in the world, including the Antarctic and subantarctic regions (Pysek, 1998; Shaw et al., 2010). Two *Poaceae* species, *Poa annua* and *Poa pratensis*, are the only plant species from outside the Antarctic region that have persisted for extended periods on the Antarctic Peninsula (Molina-Montenegro et al., 2012; Pertierra et al., 2013). The ability of these grasses to withstand Antarctic conditions makes them excellent candidates for modeling the potential geographic distribution of non-native species in this region. *P. annua* is the most widespread exotic plant in the subantarctic islands (Frenot et al., 2005; Chwedorzewska et al., 2015), being considered invasive in many of them (e.g., Gremmen and Smith, 1999; Frenot et al., 2001, 2005; Williams et al., 2013). Similarly, *P. pratensis* has a global distribution, including several subantarctic islands (Frenot et al., 2005). Both grasses are cold adapted (Pertierra et al., 2017) and are predicted to become more aggressive invaders under a warming climate (Chown et al., 2012; Duffy et al., 2017; Hughes et al., 2020). Despite being introduced by human activities and not having undergone the same evolutionary processes as the two native Antarctic vascular plants, *P. annua* and *P. pratensis* exhibit functional adaptations for surviving extreme conditions, suggesting their potential to outcompete native species (Chown et al., 2012). The geographical isolation of Antarctica, coupled with its extreme conditions, minimizes competition among species and reduces the likelihood of successful colonization by new taxa (Kühn and Klotz, 2007). Unlike other cold-temperate regions, no other taxonomic group has managed to successfully colonize the Antarctic Peninsula (Fasanella et al., 2017). Despite this limited competition, the presence of relatively unoccupied niches in warmer environments increases the potential for non-native species to establish themselves in this unique ecosystem (Duffy and Lee, 2019). Interactions between native and non-native species can be influenced by a variety of factors, including growth temperatures (particularly the increase in the number of days with temperatures above 0°C and the length of the growing season; Cavieres et al., 2018), phylogenetic relatedness among species (Dóstal et al., 2011), functional traits (Cahill et al., 2008; Burns and Strauss, 2012), ontogeny (LeRoux et al., 2013), and phenotypic plasticity along with genetic differentiation (Alexander et al., 2016). Understanding these processes can enhance our comprehension of how biotic interactions, especially competition, might intensify due to climate change. Additionally, it could elucidate how non-native species could establish and disperse under the extreme climatic conditions currently prevailing in the Antarctic Peninsula (Brooker et al., 2008; Casanova-Katny and Cavieres, 2012; Hughes et al., 2020; Rew et al., 2021).

In the challenging Antarctic environment, characterized by intense winds, freeze-thaw cycles, and fluctuating water and nutrient availability (Robinson et al., 2003; Convey, 2006; Bramley-Alves et al., 2014), the Antarctic vascular species *D. antarctica* and *C. quitensis* have developed unique survival adaptations. Both species exhibit xerophytic anatomical characteristics, such as high leaf mass area (LMA) and a thick cuticle, which help minimize water loss and protect against harsh conditions (Romero et al.,1999; Vieira and Mantovani., 2000). At the ultrastructural level, features such as chloroplast size and arrangement, cell wall thickness, and cytoplasmic and stromal components confer resistance to CO_2 diffusion, thereby limiting photosynthesis (Sáez et al., 2017). Despite low internal CO_2 conductance (g_m), Antarctic plant species compensate with high stomatal conductance (g_s) and a Rubisco enzyme highly specific to CO_2 (Sáez et al., 2017). These photosynthetic adaptations are coupled with an efficient water transport system, minimizing xylem embolism risks (Sáez et al., 2024). Recent studies have associated regional warming with physiological and anatomical adjustments in Antarctic plants, promoting increased net photosynthesis, hydraulic conductivity and growth (see Ramirez et al., 2024). However, while warmer conditions may benefit Antarctic plant species in the short term, they could also have adverse long-term effects. Higher temperatures might shorten snow cover duration, crucial for protecting plants from wind and low winter temperatures in Antarctica. This could increase freeze-thaw events and frost exposure during the growing season, depleting water reserves (Easterling et al., 2000). Warmer conditions could also impact the ability of Antarctic plants to tolerate freezing temperatures, a critical adaptation for thriving in this hostile environment (Sierra-Almeida et al., 2018). These challenges, combined with the potential establishment of non-native species in Antarctica, raise the question of whether Antarctic vascular species can compete with potential invasive species. Laboratory evidence indicates that while Antarctic vascular plants can cope with competition from invasive species (Cavieres et al., 2018), under a climate change scenario, invasive plants have advantages (Molina-Montenegro et al., 2016; Cavieres et al., 2018). In the present study, we work with *D. antarctica*, the only monocotyledon to naturally colonize Antarctica (Barcikowski et al., 2001) and the potential invaders *P. annua* and *P. pratensis*, to elucidate how similar are the physiological traits of *D. antarctica* compared to potential *Poacea* invaders. In particular, we compared the photosynthetic and hydraulic responses of. *D antarctica* an *Poa* species growing at optimal photosynthetic temperatures for *Poa* species (23°C), optimal photosynthetic temperatures for *D. antarctica* (15°C), and maximum temperatures of the Antarctic growing season (5°C). Considering the adaptive mechanisms that *D. antarctica* has evolved to survive in the extreme Antarctic environment, we hypothesize that the physiological traits of *D. antarctica* related to carbon assimilation (photosynthesis) and water transport (hydraulics) will differ significantly from those of the potential

Poa invaders, *Poa annua* and *Poa pratensis*. These differences will highlight the distinct evolutionary pathways and adaptations of *D. antarctica* compared to species that are not yet naturally established in Antarctic environments.

MATERIAL AND METHODS

Plant material and experimental conditions

Individuals of *D. antarctica* were collected at King George Island, near to Henryk Arctowski Polish Antarctic Station (62°09'S, 58°28'W), following the methodology described in Sáez et al. (2019). *P. annua* seedlings were obtained from soil extracted from Antarctica, from the same place where *D. antarctica* was collected. Seeds of *P. pratensis* were obtained from the Servicio Agricola Ganadero (SAG) of Magallanes, Chile. *Poa* seedlings were cultured in plastic pots using a sterile soil mixture (soil/vermiculite/peat 3:1:1 v/v) and kept in a growth chambers (Pi-Technology Inc., Santiago, Chile) at 15°C (Xiong et al., 1999, Sáez et al., 2017) with an irradiance of 300 μmol photons m^{-2} s^{-1} and an 18/6 h photoperiod, along with de *D. antarctica.* Plants were fertilized with Phostrogen 0.4 g L^{-1} (Solaris, Buckinghamshire, United Kingdom) once a week and watered periodically. Subsequently, all plants were randomly divided into subgroups in different growth chambers for temperature treatments. The selected temperatures were determined based on the optimal photosynthetic temperature for *Poa* species (23°C), the optimal photosynthetic temperature for *D. antarctica* (15°C) (Sáez et al., 2018a), and the maximal average daily temperature of the Antarctic summer (5°C). For all treatments, a nighttime temperature of 5°C was considered. Plants were maintained at each growth temperature for at least 45 days for acclimation. Mature leaves were randomly chosen for all measurements.

Photosynthetic leaf gas exchange measurements

Leaf gas exchange with chlorophyll a fluorescence measurement was recorded using a Li-6400XT (LI-COR Inc., Lincoln, NE, USA). Six individuals from each growth temperature were randomly selected for the measurements. For each individual, gas exchange measurements were performed on a group of leaves (as described in Sáez et al., 2018a), trying to maximize the occupation of the leaf chamber but avoiding leaf overlap. The net photosynthetic response (A_N) to sub-stomatal CO_2 concentration (C_i) was studied as reported in Sáez et al. (2017). Corrections for CO_2 leakage from the Li6400XT leaf chamber were applied to all gas exchange data as described in Flexas et al. (2007). Gas exchange measurements were performed at leaf temperatures of 23°C, 15°C, and 5°C. The quantum efficiency of photosystem II (PSII)-driven electron transport (ϕPSII) was determined using the equation: $\phi PSII = (F'_m - F_s) / F'_m$, where F_s is the steady-state fluorescence in light (1000 µmol quanta m^{-2} s^{-1}) and F'_m is the maximum fluorescence obtained with a light saturation pulse (8000 µmol quanta m^{-2} s^{-1}). Since ϕPSII represents the number of electrons transferred per photon absorbed by PSII, the electron transport rate (ETR) was calculated as: $ETR = \phi PSII \bullet PPFD\ \alpha\beta$, where PPFD is the photosynthetic photon flux density, α is leaf absorptance, and β is the energy distribution between the two photosystems, assumed to be 0.5. Leaf absorptance was measured as described by Sáez et al. (2017, Table S1).

Leaf mesophyll conductance

Mesophyll conductance to CO_2 (g_m) was determined by combining gas exchange and chlorophyll fluorescence measurements. Following the method outlined by Harley et al. (1992), g_m was calculated as $g_m = A_N / (C_i - (\Gamma^* (ETR + 8 (A_N + R_L)) / (ETR - 4 (A_N + R_L))))$, where A_N and C_i were derived from gas exchange measurements conducted under saturating light. The rate of non-photorespiratory CO_2 evolution in light (R_L) was estimated as half of dark respiration (R_d), and the chloroplastic CO_2 compensation point (Γ^*) was calculated based on the Rubisco specificity factor ($S_{c/o}$) measured *in vitro* (Sáez et al. 2017). The g_m values were then used to derive the chloroplastic CO_2 concentration (C_c) by transforming A_N - C_i curves into A_N - C_c curves, where $C_c = C_i - (A_N / g_m)$. The maximum Rubisco carboxylation rate (V_{cmax}) was determined from the A_N - C_c curves following the approach of Farquhar et al. (1980) and using the *in vitro* Rubisco kinetic constants reported by Sáez et al. (2017) for *D. antarctica*.

Leaf structural traits

To determine leaf mass area (LMA), the ratio of dry mass to leaf area was calculated. For this, 10 individuals from each species and growth temperature were randomly selected and at least 10 leaves per individual were collected for measurements. Leaf area was determined with fresh leaves by analyzing photos with ImageJ (Wayne Rasband/NIH, Bethesda, MD, USA). Then, the dry mass of these leaves was measured after oven-drying for 72 h at 65 °C. Leaf density (LD) was determined by dividing LMA by leaf thickness. The leaf thickness was obtained from leaf cross-sections analyzed by optical microscopy.

Pressure-Volume curves and the leaf hydraulic conductivity

Pressure-volume (P-V) curves were constructed using the free transpiration method described by Corcuera et al. (2002) and modified by Sáez et al. (2024). For each species and growth condition, 20 to 30 leaves were collected from the same plant due to the fragility and small size of the plant species, and six P-V curves were performed on six different plants using a pressure chamber (PMS-600). Due to the morphology of *D. antarctica* and *Poa* species, leaves were placed in flexible silicone tubes (5 mm diameter) sealed with slightly moistened cotton wool to prevent gas leakage and dehydration. First, well-watered plants were covered with plastic bags overnight to ensure full hydration. The next day, 5-10 leaves or shoots per plant were collected in three to four series, weighed using an analytical balance (MS105U, accuracy ± 0.0001 g, Mettler-Toledo, Switzerland) to determine the fully saturated weight (W_{sat}), and allowed to dry slowly at room temperature under a dark plastic cover. During the dehydration process, leaf water potential (Ψ) and fresh weight (W_f) were recorded to complete the P-V curve. After the final measurement, leaves were oven-dried at 65°C for 72 hours to obtain dry weight (W_{dry}). Then, the relative water content (RWC) for each point was calculated as $(W_f - W_{dry})/(W_{sat} - W_{dry})$. P-V curves were then plotted and analyzed according to Liu and Osborne (2015) to estimate osmotic potential at full turgor (π_o), water potential at the turgor loss point (Ψ_{tlp}), relative water content at the turgor loss point (RWC_{tlp}), and maximum wall elasticity modulus (ε_{max}).

Leaf hydraulic conductivity (K_{leaf}) was measured using the rehydration kinetic method described by Brodribb and Holbrook (2003) and modified by Sáez et al.

(2024). Fully hydrated tillers were collected and enclosed in black plastic bags to allow slow drying at room temperature for about 1 hour, ensuring uniform water potential among all leaves. Water potential (Ψ) was measured in one or two leaves, with values around −1 MPa, assumed to be the water potential before rehydration (Ψ_0). A leaf was then cut under distilled, filtered, and degassed water, allowing rehydration for 120 seconds. Water potential after rehydration (Ψ_f) was measured, and the rehydration time (t) was selected to achieve approximately half of the initial water potential. K_{leaf} was calculated as: $C \ln [\Psi_0 / \Psi_f] / t$, where Ψ_0 is the water potential before rehydration, Ψ_f is the water potential after rehydration, t is the rehydration time, and C is the leaf capacitance for each species determined as the initial slope of the P-V curves normalized by leaf area (Tyree and Hammel, 1972; Brodribb et al., 2005).

Vascular anatomy

Leaf samples from the central portions of each species (n = 6) grown at the selected temperatures were collected and fixed in a solution of formaldehyde, acetic acid, and ethanol (FAA), then stored at 4 °C. The tissues were subsequently embedded in paraffin and sectioned into paraffin blocks. Leaf cross sections were then cut from these blocks using a rotary microtome and deparaffinized. Leaf cross-sections were stained with toluidine blue and analyzed by optical microscopy (Olympus; CX31, Japan). Micrographs were randomly selected to measure the number of leaf xylem vessels and leaf xylem vessel lumen diameter (d). All images were analyzed with image analysis software (ImageJ). The mean hydraulic diameter (D_h) was calculated as $D_h = \Sigma d^5 / \Sigma d^4$ (Corcuera et al., 2012). Additionally, from the leaf vessel number and lumen diameter, the theoretical hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}) was determined according to the Hagen–Poiseuille law (Tyree and Zimmermann, 2002; Eguchi et al., 2008): $K_h = \Sigma ((d^4 \pi \rho) / (128\eta))$, where d 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, 15 °C, and 23 °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.

Statistical Analysis

The influence of growth temperature (23°C, 15°C and 5 °C) on the photosynthetic, hydraulics, and leaf anatomical traits of *D. antarctica*, *P. annua* and *P. pratensis* was evaluated using linear models (LMs). In cases of non-normality of residuals, variables were log-transformed. For some variables and species, normality of residuals was not met despite data transformation. Due to this, generalized linear models (GLMs) were conducted. Post-hoc multiple comparisons (Tukey tests) were carried out using the emmeans package. All statistical analyses were performed in R software (v. 3.5.1; R Core Team 2018).

RESULTS

Leaf carbon exchange at different growth temperatures

In general, similar trends were observed in the photosynthetic gas exchange for *D. antarctica*, *P. annua*, and *P. pratensis* (Fig. 1). At 23°C, close to the optimal temperature for *Poa* species, *D. antarctica* and *P. pratensis* exhibited the highest net photosynthetic rates (A_N), with 14.55 ± 0.59 µmol CO_2 m^{-2} s^{-1} and 13.9 ± 0.48 µmol CO_2 m^{-2} s^{-1}, respectively, while *P. annua* showed a lower rate (10.47 ± 0.48 µmol CO_2 m^{-2} s^{-1}) (Fig. 1A). At 15°C, temperature closer to the optimal photosynthetic conditions for *D. antarctica*, as this species as *P. annua* showed lower A_N, while *P. pratensis* had the highest (18.29 µmol CO_2 m^{-2} s^{-1}). At 5°C, simulating the maximum summer temperature in Antarctica, A_N was similar among the species, ranging from 7 to 8 µmol CO_2 m^{-2} s^{-1}. Dark respiration (R_{dark}) gradually decreased with the decrease in the growth temperature, with no differences among species ranging from (2.3 to 1.4 mmol CO_2 m^{-2} s^{-1}) (Fig. 1B). Stomatal conductance (g_s) was lowest at 15°C for *D. antarctica* and *P. annua* with *P. pratensis* having higher g_s at 5°C (0.19±0.01 mol H_2O m^{-2} s^{-1}) (Fig. 1C). Mesophyll conductance (g_m) showed a similar trend to A_N with the highest values for *P. pratensis* growing at 15°C (0.1 mol CO_2 m^{-2} s^{-1}) and the lowest for *P. annua* and *P. pratensis* growing at 5 °C (0.03 mol CO_2 m^{-2} s^{-1}). Thus, a significant correlation between A_N and g_m was observed in all species (Table S2). The maximum Rubisco carboxylation rate (V_{cmax}) decreased with temperature in all species, with *D. antarctica* showing higher values at 23°C (193.13 mmol CO_2 m^{-2} s^{-1}) compared to *Poa* species (Fig. 1E). A significant correlation between A_N and V_{cmax} was also observed in all species (Table S2). *D. antarctica* had similar water use efficiency (WUE) compared to *P. annua* and *P. pratensis* across all temperatures (Fig. 1F), with the exception that at 23°C, *P. annua* had lower WUE.

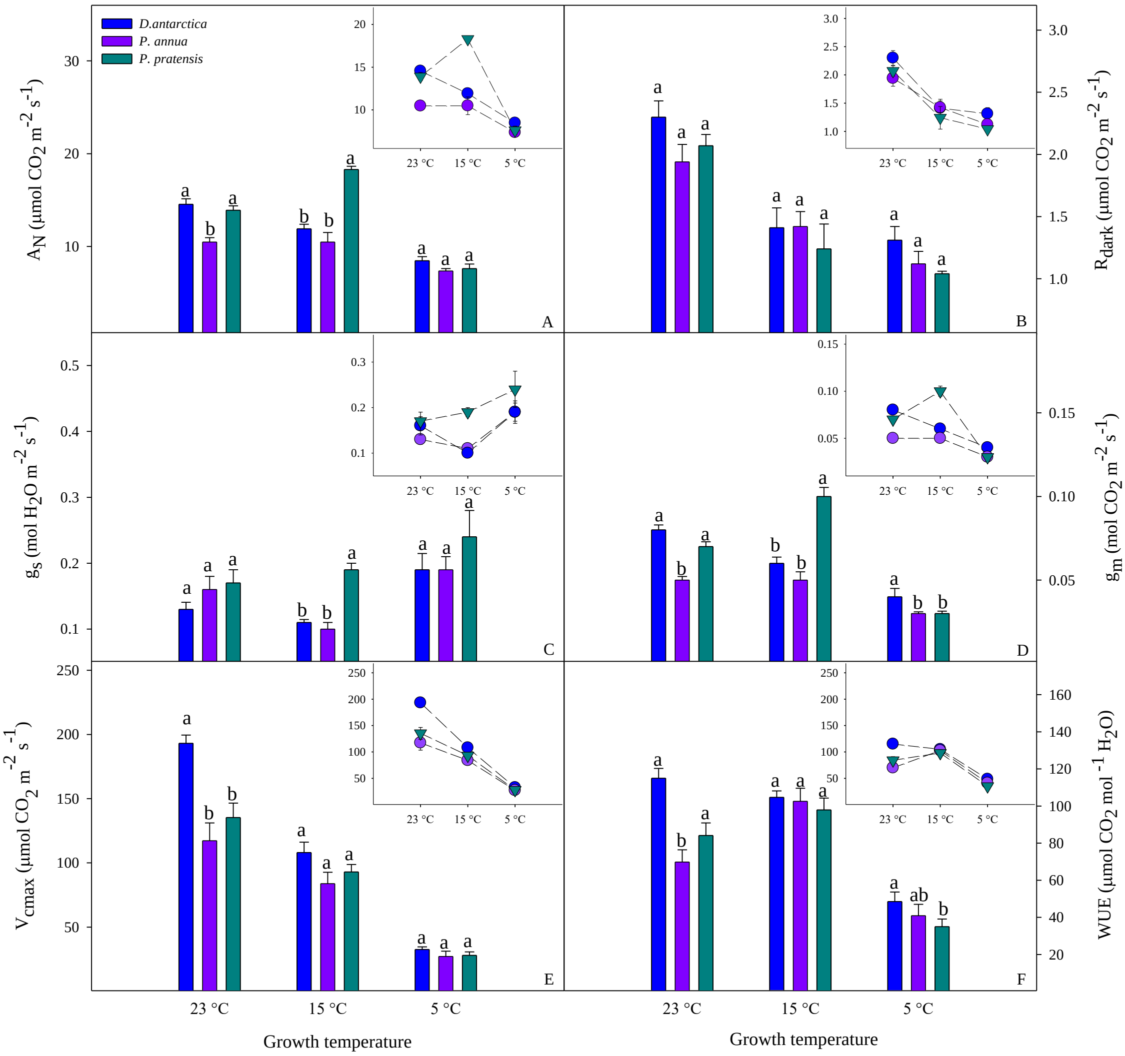

Figure 1. Leaf gas exchange parameters for *D. antarctica*, *P. annua* and *P. pratensis* grown at 23 °C, 15 °C and 5 °C. Values are means ± S.E. (n = 6–8). Net photosynthetic CO_2 assimilation rate (A_N), dark respiration rate (R_{dark}), stomatal conductance (g_s), mesophyll conductance (g_m), maximum Rubisco carboxylation rate (V_{cmax}) and water use efficiency (WUE). For each growth temperature, different letters indicate statistically significant differences among species, according to Tukey ($P < 0.05$). The inset graphs show the reaction norms for each parameter in response to decrease growth temperature for each species.

Leaf structural traits

At 23°C, *P. pratensis* exhibited the highest LMA (68.69 g m^{-2}), whereas *D. antarctica* and *P. annua* showed similar values (48.06 g m^{-2} and 42.96 g m^{-2}, respectively) (Fig. 2A). At 15°C, LMA was similar across all species, ranging from 50.53 to 57.23 g m^{-2}. However, at 5°C, *D. antarctica* deployed the highest LMA (78.25 g m^{-2}). Leaf density (LD) did not differ significantly among species (Fig.2 B). However, *D. antarctica* had a higher LD compared to *P. annua* and *P. pratensis*, when growth at 15°C and 5°C (0.386 g $cm^{-3}$and 0.519 g cm^{-3}, respectively, Fig. 2B). In addition, a significant negative correlation between LMA and A_N as temperature decreased was observed in *D. antarctica* and *P. annua* (Table S2).

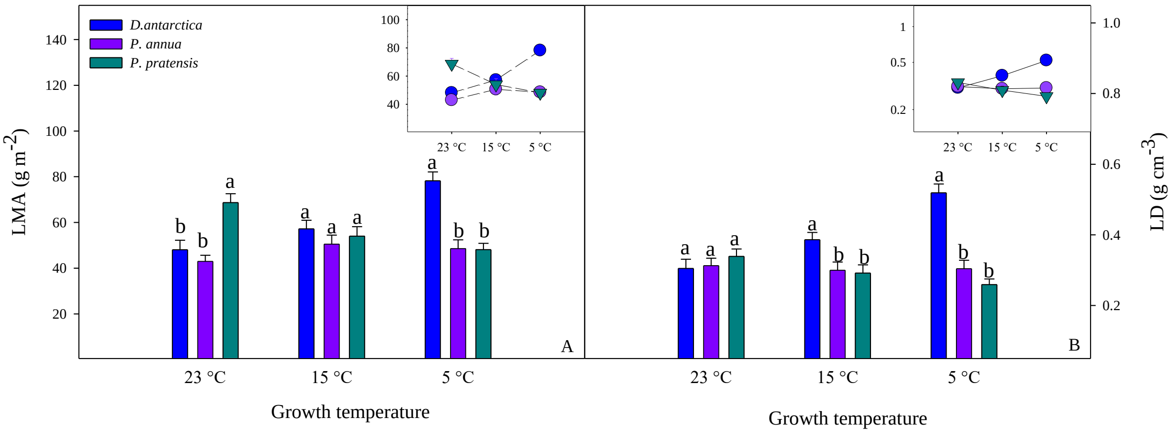

Figure 2. Leaf structural traits for *D. antarctica*, *P. annua* and *P. pratensis* grown at 23 °C, 15 °C and 5 °C. Values are means ± S.E. (n= 10-12). Leaf mass area (LMA) and leaf density (LD). For each growth temperature, different letters indicate statistically significant differences among species, according to Tukey (P < 0.05). The inset graphs show the reaction norms for each parameter in response to decrease growth temperature for each species.

Leaf hydraulic traits at different growth temperatures

In general, the leaf capacitance (C) was similar across all species, with the highest value for *P. pratensis* at 15°C (1.12 ± 0.06 (mol m^{-2} MPa^{-1}), respectively) and the lowest for *D. antarctica* at 5°C (0.35±0.01 mol m^{-2} MPa^{-1}). Consistently, this species displayed the lowest C at all growth temperatures (Fig. 3A). *D. antarctica* showed the most negative osmotic potential at full turgor (π_o) across all temperatures, while *P. annua* showed the least negative values at 15°C (Fig. 3B). The relative water content at the turgor loss point (RWC_{tlp}) was similar among species, however at 23°C, *D. antarctica* had the lowest RWC_{tlp} (90% ± 0.01) (Fig. 3C). The leaf water potential at the turgor loss point (Ψ_{tlp}), was similar at 23°C across all species. However, at 15°C and 5°C, *D. antarctica* consistently exhibited the most negative Ψ_{tlp} (-1.25 and -1.06 MPa, respectively) (Fig. 3D). Regarding the leaf hydraulic conductivity (K_{leaf}), the higher was found in *P. pratensis* growing at 23°C (6.65 ± 0.27 mmol m^{-2} s^{-1} MPa $^{-1}$) and the lowest in *D. antarctica* growing at 5°C (2.21 ± 0.03 mmol m^{-2} s^{-1} MPa $^{-1}$) (Fig. 3E). Maximum cell wall elasticity (ε_{max}) gradually increased with reduction in the growth temperature in all species, ranging between 3.35 to 12.4 MPa, with no significant differences observed among treatments (Fig. 3F). Distinctively, a strong correlation between ε_{max} and K_{leaf} was observed for *D. antarctica*, but not for the *Poa* species (Table S2).

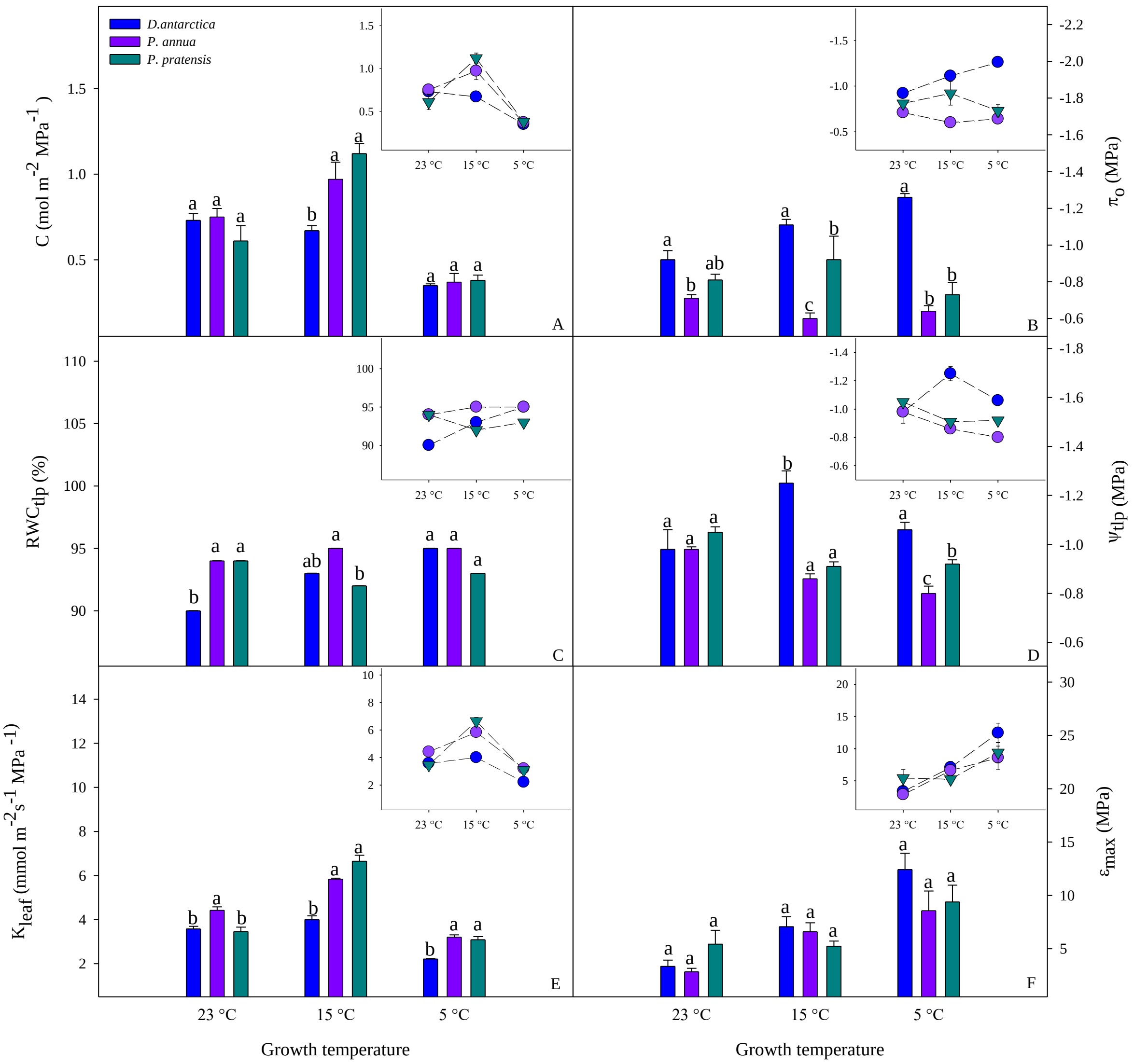

Figure 3. Leaf hydraulic traits for *D. Antarctica*, *P. annua* and *P. pratensis* growing at 23 °C, 15 °C and 5 °C Leaf hydraulic conductivity (K_{leaf}), cell wall elasticity (ε_{max}), capacitance (C), osmotic potential (π_o), relative water content at the turgor loss point (RWC_{tlp}) and water potential at the turgor loss point (Ψ_{tlp}). Values are means ± SE (n=4-8). For each growth temperature, different letters indicate statistically significant differences among species, according to Tukey ($P < 0.05$). The inset graphs show the reaction norms for each parameter in response to decrease growth temperature for each species.

Leaf vascular anatomy in response to temperature

Overall, the number of xylem vessels did not differ between species or growth temperatures (Fig. 4A), except at 23°C, where *P. pratensis* had the highest number of vessels (12.67), *D. antarctica* had intermediate values (11), and *P. annua* had the lowest number (8.2). *D. antarctica* consistently showed the lowest values for mean hydraulic diameter (D_h), theoretical hydraulic conductivity (K_h), and specific conductivity (K_s) across all temperatures. *P. pratensis* had the highest values for these parameters at 23°C and 5°C, while *P. annua* exhibited intermediate values at these temperatures but aligned more closely with *P. pratensis* at 15°C (Fig. 4B, C, D).

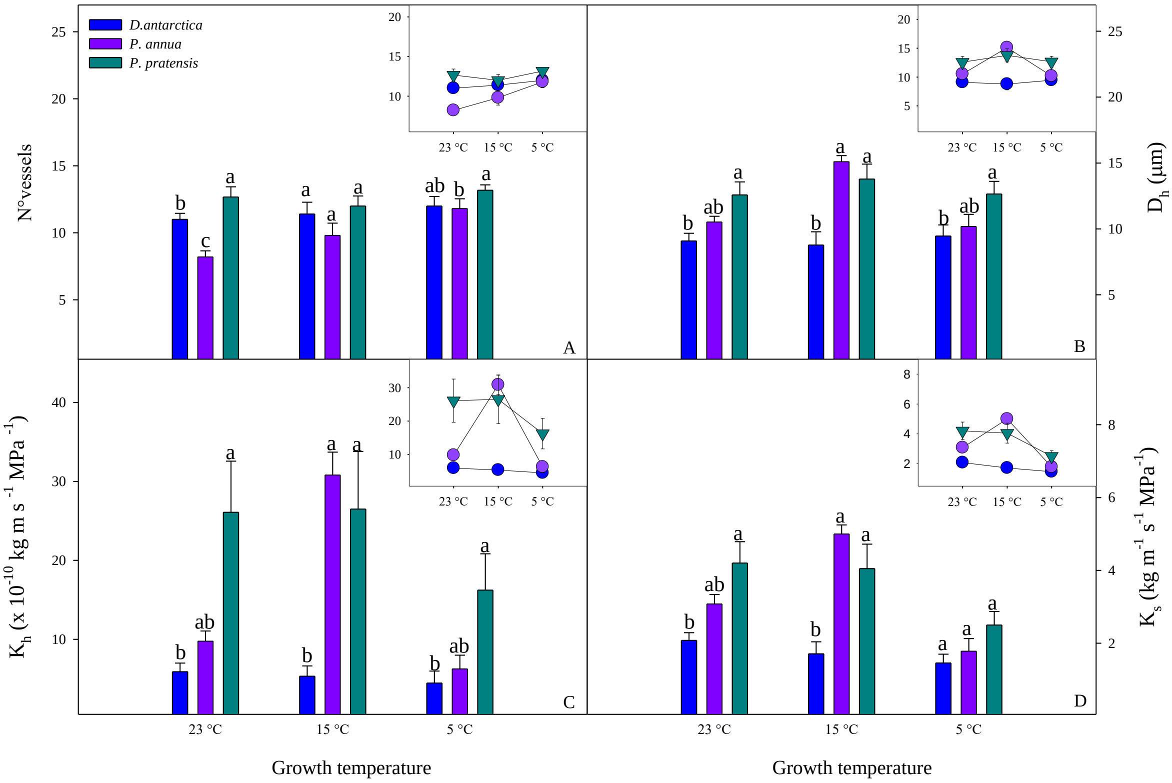

Figure 4. The number of vessels, mean hydraulic diameter (D_h), and the theoretical hydraulic constant: theoretical hydraulic conductivity (K_h), and specific conductivity (K_s) for *D. antarctica*, *P. annua* and *P. pratensis* grown at 23°C, 15°C, and 5°C. Values are means ± S.E. (n=4-8). For each growth temperature, different letters indicate statistically significant differences among species, according to Tukey ($P < 0.05$). The inset graphs show the reaction norms for each parameter in response to decrease growth temperature for each species.

DISCUSSION

Global warming is altering environmental conditions, affecting several physiological processes in plants (Dusenge et al., 2018). In Antarctica, warmer conditions have led to changes in the photosynthetic and hydraulic traits of Antarctic plants, which may influence their long-term survival (Sáez et al., 2018a, b; 2024). This study compares these traits in *D. antarctica* and the *Poa* species: *P. annua* and *P. pratensis*, allowing predictions about their behavior in the face of climate change and assessing their invasive potential and impacts on Antarctic flora.

The similar photosynthetic performance of *D. antarctica* and *Poa* species in response to temperature

Photosynthesis is crucial for plant survival, underpinning growth, reproduction, and overall adaptability to diverse environments (Larcher, 2003; Lambers et al., 2008; Taiz & Zeiger, 2002). In cold climates, plant species have developed unique adaptations that enhance their photosynthetic efficiency, enabling them to endure and thrive in suboptimal temperatures (Körner et al., 1999; Larcher et al., 2003). *D. antarctica* has been described as a species that, despite thriving in an inherently cold climate, exhibits compensatory traits that allow it to maintain high photosynthetic rates (see Ramirez et al., 2024). These traits include increased stomatal conductance (g_s) and a high activation of Rubisco which helps to sustain efficient gas exchange and photosynthesis even under climate conditions that reduce the CO_2 availability in the chloroplasts (Sáez et al., 2017). Interestingly, our results show that *D. antarctica* and *Poa* species show similar photosynthetic performance trends across different growth temperatures, including suboptimal temperatures (5°C), typical of Antarctic summer. *D. antarctica* and *Poa* species experience significant reductions in net photosynthesis (A_N) and its determinants, particularly mesophyll conductance (g_m), and the maximum Rubisco carboxylation rate (V_{cmax}), as the growth temperature is lower. This suggests a co-limitation of photosynthesis by both diffusive and biochemical determinants (see Table S2), indicating that these species may employ similar adaptive strategies to cope with low temperatures. The comparable A_N level between *D. antarctica* and *Poa* species suggests that these potential invaders may also have compensatory mechanisms that support similar performance under both optimal and supra-optimal conditions. In addition, all species showed a general trend of increased g_s at lower temperatures, resulting in reduced water use efficiency (WUE). The high g_s likely compensates for limited CO_2 diffusion (g_m) imposed by the low growth temperature, keeping stomata open to sustain photosynthesis rates (Atkin et al., 2006; von Caemmerer & Evans, 2014). These observations suggest effective adaptations of *Poa* species for the photosynthetic gas exchange in cold environments, including optimized stomatal control, which determine both WUE and hydraulic safety (Lawson & Blatt, 2014; Flexas et al., 2014).

At low temperatures, reductions in A_N and g_m are linked to higher LMA, a common trait among cold-climate species that enhances leaf reinforcement and minimizes water loss (Niinemets, 2001; Wright et al., 2002; Poorter et al., 2009; Onoda et al., 2017). *D. antarctica* and *P. annua* follow this pattern, with strong correlations between A_N and LMA, highlighting its adaptive strategy (Table S2). At higher temperatures, it reduces LMA and LD, effectively shifting from a conservative to an acquisitive strategy that facilitates enhanced gas exchange. This capacity for adjustment was not completely observed in the *Poa* species, which show less variability in leaf structure across temperature changes (Fig. 2). Specifically, the correlation between A_N and LMA, as well as the correlation between g_m and LMA, was absent in *P. pratensis* and weaker in *P. annua* compared to *D. antarctica*, potentially limiting their adaptability to Antarctic environments. Additionally, the strong correlation observed between A_N and g_m across all species (Table S2) suggests that additional traits such as cell wall thickness (T_{cw}), activation energy for CO_2 membrane permeability, diffusion path length to chloroplasts, and the roles of aquaporins and carbonic anhydrase, could be also crucial in determining g_m (von Caemmerer & Evans, 2015; Momayyezi et al., 2020).

Hydraulic similarities and key distinctive traits of *D. antarctica* and *Poa* species in response to temperature

Hydraulic conductance and water storage capacity are essential for maintaining physiological functions and managing water stress and temperature fluctuations (Tyree & Zimmermann, 2002; Yin et al., 2018; McCulloh et al., 2019). While hydraulic traits display some similar trends in *D. antarctica* and *Poa* species in response to temperature, some distinctive responses were found only in *D. antarctica* (Fig. 3). For instance, while at 23°C *D. antarctica* shows similar water potential at the turgor lost point (Ψ_{tlp}) than the *Poa* species, at their optimal temperature (15°C) and low temperature (5°C) *D. antarctica* display significant more negatives Ψ_{tlp}, indicating a stronger ability to maintain turgor at lower temperatures (Sack et al., 2003; Lenz et al., 2006; Blackman et al., 2010). This trait is associated with a more negative osmotic potential (π_o), likely associated to the high concentration of osmoprotective solutes reported for this species (Zuñiga-Feest et al., 2005; Bravo et al., 2009), which enhance cellular function and growth even under reduced water availability triggered for low temperature (Merchant et al., 2007; Bartlett et al., 2012).

Both *D. antarctica* and *Poa s*pecies show similar responses in adjusting cell rigidity (ε_{max}) to temperature variations. At warmer temperatures, lower cell rigidity facilitates CO_2 diffusion, enhancing photosynthesis and allowing resources to be allocated for growth and water transport. In contrast, at colder temperatures, increased cell rigidity improves resistance to water stress and freezing damage. This adaptive mechanism is present in both species, suggesting that adjusting cell rigidity in coordination with A_N and g_m (see correlations in Table S2), is a common strategy for coping with extreme conditions. For *Poa* species, this adaptation could be particularly beneficial for establishing in cold conditions (Joly & Zaerr, 1987; Chimenti & Hall, 1994; Martínez-Vilalta et al., 2012; Nadal et al., 2018). Recent research (Clemente-Moreno et al., 2019, 2020; Gago et al., 2023) suggests that *D. antarctica* can dynamically adjust ε_{max} to optimize metabolic processes under various stress conditions. In resource-scarce scenarios, the species prioritizes photoprotection and protein stabilization, whereas, in more favorable conditions, it focuses on accumulating osmoprotective amino acids and antioxidants. Distinctly, the increase in

ε_{max} with decrease temperature, aligns with higher LMA only in *D. antarctica* (Table S2), supporting its strategy of structural reinforcement in extreme environments.

In both *D. antarctica* and *Poa* species, trends in leaf capacitance and therefore, in leaf hydraulic conductivity (K_{leaf}) are similar, However, *Poa* species exhibit higher K_{leaf} at suboptimal temperatures (5 and 15°C) than *D. antarctica*. Thus, *D. antarctica* adopts a more conservative strategy with lower K_{leaf} across all temperature, and particularly at low temperature (Fig. 3). The differences observed in K_{leaf} are supported by differences in the leaf xylem anatomy (Fig. 4). Although in general there are no differences in the number of leaf vessels, *Poa* species have higher mean hydraulic diameters (D_h) across all temperature and notably higher specific conductivity (K_s) and theoretical hydraulic conductivity (K_h), which support the higher water transport efficiency at different growth temperatures. Larger vessel diameters facilitate faster water flow, benefiting situations with high water demand, but increasing the risk under low water conditions (Tyree & Zimmermann, 2002; Hacke et al., 2001; Medek et al., 2010). The lower values for these traits found in *D. antarctica* shed light on a strategy focused on avoiding cavitation (Tyree et al., 2003) which is key to survive under Antarctic conditions (Sáez et al., 2024).

The potential for non-native plants to establish in Antarctica depends on several factors

The similarity in photosynthetic performance and most hydraulic traits observed between *D. antarctica* and *Poa* species suggests that both *P. annua* and *P. pratensis* could have the potential to survive in Antarctica. However, the ability of a species to establish in extreme environments depends on overcoming several critical factors, such as dispersal capability (Nathan & Muller-Landau, 2000), tolerance to harsh climate conditions (Körner, 1999), competition with native species (Tilman, 1997), and efficient reproduction (Harper, 1977) among others. In Antarctica, non-native species face significant barriers to establishment. These include the short growing season, low summer temperatures, nutrient-poor soils, and the continent's isolation (Lee et al., 2017; Convey, 2019). The isolation, combined with the harsh environment, limits the arrival and establishment of non-native species. Furthermore, these adverse conditions restrict the life cycle of plants arriving through natural dispersal or human-assisted introduction (Frenot et al., 2005; Hughes et al., 2015). Although native Antarctic plants like *D. antarctica* exhibit improved reproductive performance under warmer conditions (Day et al., 1999), they continue to struggle under the extreme conditions of Antarctica (Convey, 1996, 2006). Thus, these barriers serve as a significant filter for colonization.

Climate change could reduce these barriers by lengthening the growing season and improving soil conditions (Convey, 1996). As the climate warms, these limitations may become less restrictive, facilitating not only the survival and reproduction of native species like *D. antarctica* but also allowing non-native species like *Poa annua* and *Poa pratensis* to establish and expand. The phylogenetic and functional similarity between *D. antarctica* and non-native species could intensify competition for resources (Cahill et al., 2008; Dostal et al., 2011; Burns & Strauss, 2012; Kunstler et al., 2012), challenging the photosynthetic and hydraulic strategies of *D. antarctica.* and potentially enabling invasive species to outcompete natives in a warmer environment. This shift towards a more favorable climate could significantly increase the growth and dispersal of non-native species, altering competition dynamics and enhancing vascular plant diversity in the Antarctic region (Duffy et al., 2017; Molina-Montenegro et al., 2012; 2016; 2019).

CONCLUDING REMARKS AND PROSPECTS

D. antarctica and *Poa* species show similar photosynthetic trends in response to temperature variations, including significant reductions in net photosynthesis (A_N), mesophyll conductance (g_m), and the maximum Rubisco carboxylation rate (V_{cmax}) at low temperature. However, *D. antarctica* exhibits a unique ability to adjust both its leaf mass per area (LMA) and leaf density (LD) in response to temperature changes, thereby optimizing its photosynthetic capacity, while *Poa* species maintain a more constant and less structural response. Regarding hydraulic functions, both *D. antarctica* and *Poa* species demonstrate notable management of water transport. *D. antarctica* employs a conservative strategy by reducing hydraulic conductance at cold temperatures to avoid cavitation through smaller vessel diameters. Conversely, *Poa* species, with larger xylem vessels and higher hydraulic conductivity, may have advantages in less extreme conditions but face additional challenges in the Antarctic environment, where cavitation risk and adaptation to extreme conditions are critical. These similarities and differences in photosynthetic and hydraulic strategies suggest that, although *Poa* species and *D. antarctica* have comparable capabilities overall, the specifics of their strategies could influence their potential expansion and competitive dynamics in the context of climate change. Understanding these mechanisms is crucial for anticipating how climate change could alter species distribution and the composition of Antarctic flora.

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ANEXOS

Table S1. Table S1. Leaf absorbance for D. antarctica and the potential invading species of Antarctica: Poa annua and Poa pratensis growing at 5 °C, 15 °C and 23 °C. Values are means ± S.E. (n=6). Different letters indicate statistically significant differences among species at different growth temperatures according to Tukey's test (P < 0.05).

Absorbance	5°C	15°C	23°C
D. antarctica	0.86±0.005a	0.85 ± 0.01a	0.81±0.01b
Poa annua	0.86±0.01a	0.83± 0.01a	0.82±0.01b
Poa pratensis	0.86±0.01a	0.84±0.01a	0.88±0.01a

Table S2. Pearson correlations between different photosynthetic and hydraulic traits for *Deschampsia antarctica*, *Poa annua* and *Poa pratensis*. The correlations are presented for trait combinations between the following parameters: net CO_2 assimilation rate (A_N), leaf hydraulic conductivity (K_{leaf}), leaf mass per area (LMA), leaf mesophyll conductance to CO_2 (g_m), and maximum cell wall elasticity (ε_{max}). For each correlation, data measured at the different growth temperatures were combined. Pearson coefficients (*r*) and the significance (*p*) are shown. ns means non-significant correlation (*p* > 0.05).

Traits	*D. antarctica*		*Poa annua*		*Poa pratensis*	
	r	*p*	*r*	*p*	*r*	*p*
A_N - g_m	0.94	<0.001	0.96	<0.001	0.98	<0.001
A_N-V_{cmax}	0.87	<0.001	0.85	<0.001	0.67	<0.002
A_N -LMA	-0.73	<0.001	-0.48	<0.05	0.36	ns
g_m - LMA	-0.73	<0.001	-0.51	<0.02	0.40	ns
ε_{max} -K_{leaf}	-0.55	<0.02	-0.31	ns	-0.32	ns
ε_{max}-LMA	0.78	<0.001	0.21	ns	-0.26	ns
ε_{max} - g_m	-0.87	<0.001	-0.57	<0.02	-0.54	<0.02
ε_{max} - A_N	-0.81	<0.001	0.53	<0.02	-0.48	<0.05

DISCUSION GENERAL

La capacidad única de *D. antarctica* y *C. quitensis* para sobrevivir en la Antártida ha generado un intenso debate sobre los mecanismos adaptativos que les permiten crecer bajo condiciones extremas. Factores como la variable disponibilidad de agua, las bajas temperaturas constantes, la corta temporada de crecimiento, la irradiancia, el viento y la abrasión influyen significativamente en el crecimiento y la morfología de las hojas en la Antártida (Körner, 1999; Larcher, 2003; Crawford, 2008; Convey et al., 2011). Así, las adaptaciones de las plantas antárticas a estos factores se describen como el resultado de procesos de aclimatación y respuestas adaptativas, reflejando una alta especialización a condiciones extremas (Parnikoza et al., 2007, 2011).

El calentamiento regional en la Península Antártica ha impulsado la expansión de las plantas vasculares, mejorando su rendimiento fotosintético (Cannone et al., 2016, 2022). El aumento en la asimilación de carbono se relaciona con ajustes anatómicos en las hojas que optimizan la conductancia del CO_2 en el mesófilo (g_m) (Sáez et al., 2018a; 2018b; 2019). La capacidad hidráulica, crucial para la fotosíntesis, se ve favorecida tambien con el aumento de la temperatura en las plantas vasculares antárticas (Sáez et al., 2024). La estrecha relación entre la conductancia hidráulica y la fotosíntesis resalta la importancia de integrar estos procesos para optimizar el transporte de agua y la eficiencia fotosintética (Brodribb et al., 2007; Scoffoni et al., 2016). Esta tesis explora la singularidad fisiológica de las propiedades hidráulicas y su relación con el rendimiento fotosintético en respuesta a la temperatura, comparando *D. antarctica* y *C. quitensis* procedentes de la Antártica, con procedencias subantárticas y otras especies filogenéticamente relacionadas que habitan ambientes similares y potenciales especies invasoras de la Antártica.

En el Capítulo I, se planteó como hipótesis que los rasgos relacionados con la asimilación de carbono y la hidráulica, así como su respuesta coordinada al aumento de temperatura, corresponden a una adaptación única al clima antártico, no presente en las procedencias subantárticas ni en especies relacionadas. Sin embargo, los resultados obtenidos revelan que las propiedades hidráulicas y fotosintéticas de

D. antarctica son sorprendentemente similares entre las procedencias antárticas y subantárticas de esta especie. *D. antarctica* mantiene una alta coordinación entre sus propiedades hidráulicas y fotosintéticas, independiente de su origen, debido a una combinación de determinantes difusivos (g_s, g_m) y bioquímicos (V_{cmax}), una alta capacidad de ajustes estructurales (LMA, LD, ε_{max}) así como la presencia vasos xilemáticos foliares con diámetros notablemente pequeños. Estas características permiten un transporte eficiente de agua, con bajo riesgo de cavitación y una fotosíntesis efectiva a temperaturas tanto supra como sub optimas. Aunque las especies filogenéticamente relacionadas, *D. caespitosa* y *A. flexuosa*, presentaron tendencias similares en respuesta a la temperatura, como habíamos sugerido, *D. antarctica* muestra una coordinación única de los rasgos fotosintéticos e hidráulicos, lo que podría ser considerada una característica clave y singular que le permite ser la única monocotiledónea capaz de establecerse exitosamente en la Antártica.

Por otro lado, *C. quitensis*, muestra respuestas fisiológicas diferenciales entre las procedencias antártica y subantártica. La procedencia antártica exhibe una alta coordinación entre rasgos fotosintéticos e hidráulicos, con menor conductancia hidráulica foliar debido a vasos xilemáticos más pequeños y mayor rigidez en las paredes celulares, lo que aumenta la resistencia a fallo hidráulico. En contraste, la procedencia subantártica presenta tasas fotosintéticas y K_{leaf} más altas, con vasos xilemáticos de mayor diámetro, especialmente en condiciones de crecimiento más cálidas, pero con una menor coordinación entre estos rasgos. Estas diferencias podrían ilustrar una diferenciación ecotípica, donde la adaptación al estrés de congelación ha dado lugar a poblaciones genéticamente distintas, tal como lo sugerido previamente Gianoli et al. (2004) y Acuña-Rodriguez et al. (2017) y reflejando la intensa presión selectiva de los ambientes fríos (Körner, 1999). Por su parte, *C. apetalus* mostró una alta coordinación entre fotosíntesis e hidráulica, con hojas más gruesas y menor g_s a bajas temperaturas, lo que limitó la fotosíntesis. Sin embargo, esta especie ajustó g_s, LMA y V_{cmax} para mejorar su capacidad fotosintética a temperaturas más altas, alcanzando valores de asimilación neta (A_N) similares a los de *C. quitensis* subantártica, a pesar de tener un K_{leaf} más bajo. Esto sugiere una estrategia conservadora del agua adaptada a ambientes más cálidos.

Las proyecciones climáticas sugieren una disminución en la cobertura de nieve y un aumento en la frecuencia de eventos de congelación durante la temporada de crecimiento en la Antártida (Chown et al., 2022). Estos cambios, junto con el incremento de eventos de temperatura supra óptima en la Península Antártica, podrían afectar el transporte de agua y la asimilación de carbono en las especies vasculares antárticas (Turner et al., 2021; Gorodetskaya et al., 2023; Liang et al., 2022; Sáez et al., 2024). Adicionalmente, estos cambios ambientales podrían también favorecer la invasión y establecimiento de nuevas especies en la región. En este contexto, el Capítulo II comparó los rasgos fotosintéticos e hidráulicos en respuesta a la temperatura en *D. antarctica* y en las dos especies de plantas vasculares no nativas que han mantenido poblaciones persistentes en la Antártida, *Poa annua* y *Poa pratensis*. En este capítulo se hipotetizó que los rasgos fisiológicos de *D. antarctica* relacionados con la asimilación de carbono (fotosíntesis) y el transporte de agua (hidráulica) diferirán significativamente de los de los posibles invasores del género *Poa*, *Poa annua* y *Poa pratensis*. Los resultados muestran que las potenciales invasoras, reducen el rendimiento fotosintético y la eficiencia hidráulica en respuesta a bajas en la temperatura de crecimiento, en tasas similares a las observadas *en D. antarctica*. Los niveles comparables de fotosíntesis neta sugieren que *P. annua* y *P. pratensis* podrían tener mecanismos compensatorios similares a los de *D. antarctica*, funcionando tanto en condiciones de temperatura óptima como subóptimas por lo que, desde el punto de vista de estos rasgos, podrían ser capaces de vivir en la Antártica.

En términos hidráulicos, tanto *D. antarctica* como las especies de *Poa* ajustan su ε_{max} en respuesta a la temperatura. *D. antarctica* destaca por su estructura foliar reforzada y estrategias conservadoras, con valores más negativos de Ψ_{tlp} y π_o que priorizan la seguridad hidráulica conjugado con vasos xilemáticos foliares más estrechos. En contraste, las especies de *Poa* presentan una mayor capacidad de transporte de agua gracias a diámetros de vasos más grandes, lo que se refleja en una mayor K_{leaf} Sin embargo, a bajas temperaturas estos rasgos podrían resultar más susceptibles a fallo hidráulico. Aunque *D. antarctica* está adaptada al frío y puede ajustarse a condiciones más cálidas, las especies invasoras podrían superar su rendimiento en escenarios más cálidos (Chwedorzewska et al., 2015; Cavieres et al., 2018). Esta ventaja competitiva de las especies invasoras podría intensificarse con el cambio climático y el aumento en la disponibilidad de recursos, otorgándoles una superioridad sobre las especies nativas (Cavieres et al., 2018; Molina-Montenegro et al., 2012; 2016). Sin embargo, para que una especie pueda establecerse exitosamente en un ambiente, debe ser capaz de superar ciertos filtros no evaluados en esta tesis (competencia con especies nativas, la capacidad reproductiva, y la eficiencia en el uso de recursos), por tanto, se sugieren más estudios en este sentido con el fin de dilucidar la vulnerabilidad del ecosistema antártico a la llegada de especies no nativas gatillada por el cambio climático.

CONCLUSION GENERAL

Nuestros resultados subrayan algunas singularidades de las plantas vasculares antárticas en su adaptación a condiciones antárticas. *D. antarctica* mostró una coordinación sobresaliente entre las propiedades hidráulicas y fotosintéticas en sus procedencias antárticas y subantárticas, superando a las especies filogenéticamente relacionadas en términos de eficiencia en el uso del agua y capacidad fotosintética. *C. quitensis* presenta diferencias según su origen, con procedencias antárticas mostrando una estrategia centrada en la conservación del agua, mientras que las subantárticas exhibieron una mayor capacidad fotosintética y conductancia hidráulica, pero con menor coordinación entre estos rasgos. *C. apetalus* mostró una alta coordinación entre fotosíntesis e hidráulica. La conductividad hidráulica se mantuvo baja en todas las temperaturas, mientras que la capacidad fotosintética fue más baja a temperaturas frías, pero aumentó en condiciones más cálidas.

Las potenciales especies invasoras, *P. annua* y *P. pratensis*, mostraron un rendimiento fotosintético comparable al de *D. antarctica*, relacionado con mecanismos compensatorios entre los determinantes difusivos y bioquímicos de la fotosíntesis. Ambas especies presentaron tendencias similares en sus respuestas hidráulicas, aunque exhibieron una mayor K_{leaf}, asociada a vasos xilemáticos más grandes en comparación con *D. antarctica*. Esto podría hacerlas más vulnerables en el ambiente antártico, pero le conferirle una ventaja adaptativa en escenarios de cambio climático en un entorno antártico menos extremo.

Estos hallazgos amplían nuestra comprensión de las respuestas fisiológicas de las plantas vasculares antárticas y destacan la necesidad de considerar múltiples factores ambientales, como el aumento de las temperaturas, los cambios en la disponibilidad hídrica y los cambios en la disponibilidad de nutrientes, para prever mejor la resiliencia de estos ecosistemas frente a los nuevos escenarios de cambio climático. Futuras investigaciones deben centrarse en comprender los mecanismos moleculares detrás de los rasgos adaptativos y en estudiar una mayor diversidad de poblaciones y especies, así como la vulnerabilidad de los ecosistemas antárticos a la llegada de especies no nativas.

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