Journal of Experimental Botany
doi:10.1093/jxb/erx148
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

RESEARCH PAPER

Photosynthetic limitations in two Antarctic vascular plants: importance of leaf anatomical traits and Rubisco kinetic parameters

Patricia L. Sáez[1,*], León A. Bravo[2], Lohengrin A. Cavieres[3], Valentina Vallejos[1], Carolina Sanhueza[3], Marcel Font-Carrascosa[4], Eustaquio Gil-Pelegrín[5], José Javier Peguero-Pina[5] and Jeroni Galmés[4]

[1] Laboratorio Cultivo de Tejidos Vegetales, Centro de Biotecnología, Departamento de Silvicultura, Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile
[2] Laboratorio de Fisiología y Biología Molecular Vegetal, Instituto de Agroindustria, Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agropecuarias y Forestales, Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile
[3] Laboratorio de ECOBIOSIS, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Concepción, Chile
[4] Research Group on Plant Biology under Mediterranean Conditions, Universitat de les Illes Balears-INAGEA, Balearic Islands, Spain
[5] Unidad de Recursos Forestales, Centro de Investigación y Tecnología Agroalimentaria, Gobierno de Aragón, 50059 Zaragoza, Spain

* Correspondence: patrisaez@udec.cl

Received 28 December 2016; Editorial decision 7 April 2017; Accepted 7 April 2017

Editor: Robert Sharwood, Australian National University

Abstract

Particular physiological traits allow the vascular plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. to inhabit Antarctica. The photosynthetic performance of these species was evaluated *in situ*, focusing on diffusive and biochemical constraints to CO_2 assimilation. Leaf gas exchange, Chl *a* fluorescence, leaf ultrastructure, and Rubisco catalytic properties were examined in plants growing on King George and Lagotellerie islands. In spite of the species- and population-specific effects of the measurement temperature on the main photosynthetic parameters, CO_2 assimilation was highly limited by CO_2 diffusion. In particular, the mesophyll conductance (g_m)—estimated from both gas exchange and leaf chlorophyll fluorescence and modeled from leaf anatomy—was remarkably low, restricting CO_2 diffusion and imposing the strongest constraint to CO_2 acquisition. Rubisco presented a high specificity for CO_2 as determined *in vitro*, suggesting a tight co-ordination between CO_2 diffusion and leaf biochemistry that may be critical ultimately to optimize carbon balance in these species. Interestingly, both anatomical and biochemical traits resembled those described in plants from arid environments, providing a new insight into plant functional acclimation to extreme conditions. Understanding what actually limits photosynthesis in these species is important to anticipate their responses to the ongoing and predicted rapid warming in the Antarctic Peninsula.

Key words: Antarctic plants, leaf traits, mesophyll conductance, photosynthesis, Rubisco, temperature.

© The Author 2017. Published by Oxford University Press on behalf of the Society for Experimental Biology.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Antarctica is the coldest, driest, and windiest continent on Earth, with mean summer air temperatures <0 °C in continental Antarctica and between 0 and 2 °C in Maritime Antarctica (Convey, 1996). The study of how organisms behave in this hostile habitat is of particular interest to unravel functional adaptations to extreme conditions (see Alberdi *et al.*, 2002; Cavieres *et al.*, 2016).

Deschampsia antarctica Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) are the only two vascular plants naturally colonizing these harsh conditions, being distributed along the west coast of the Antarctic Peninsula and its associated islands (Maritime Antarctica) down to Lazarev Bay on the north-west coast of Alexander Island (69°22.0'S, 71°50.7'W) (Convey *et al.*, 2011). Although several differences have already been described between these two species in dealing with the Antarctic conditions (Pérez-Torres *et al.*, 2007; Cavieres *et al.*, 2016), the performance of both Antarctic vascular species relies on a robust CO_2 assimilation machinery, including a high activation state of Rubisco and stromal fructose-1,6-biphosphatase (Pérez-Torres *et al.*, 2006). *Deschampsia antarctica* is more abundant and widely distributed along the Antarctic Peninsula than *C. quitensis* (Smith, 2003), colonizing different habitats ranging from mineral to organic soils, and from dry to waterlogged areas. In contrast, *C. quitensis* seems to be less tolerant to extreme conditions, preferring sparsely vegetated and sheltered sites with moist, but well-drained mineral soils (Smith, 2003). Although both species exhibit CO_2 assimilation rates similar to those of other herbaceous species from low-temperature environments such as alpine or arctic habitats (Chapin and Shaver, 1985; Körner, 2003), *D. antarctica* has a higher photosynthetic capacity than *C. quitensis* (Montiel *et al.*, 1999; Xiong *et al.*, 1999). Under laboratory conditions, *D. antarctica* and *C. quitensis* are able to maintain ~30% of their maximum photosynthetic rates at 0 °C, but both species display maximal CO_2 assimilation rates at 13 °C and 19 °C, respectively (Edwards and Smith, 1988). These findings led Xiong *et al.* (1999) to suggest that current and future warming would be beneficial for the carbon assimilation of these two Antarctic plants.

Although some information has been accrued on the responses of the Antarctic plants to the combination of cold and high light, and to the effect of temperature on their photosynthetic performance, most of these data come from laboratory-grown plants. Field assessments on the mechanisms involved in the photosynthetic regulation, on the anatomical and biochemical limitations to photosynthesis, and whether these limitations change among populations exposed to different environmental conditions are fundamental to understand the functional adaptations that these species have evolved to withstand the Antarctic conditions. This knowledge will equally provide powerful insights to anticipate how these species might respond to climate change.

Leaf mesophyll conductance of CO_2 (g_m) and Rubisco kinetic parameters are two key players in carbon assimilation needed for a proper understanding of the photosynthetic performance under field conditions. The kinetic traits describing Rubisco functioning differ among species (e.g. Delgado *et al.*, 1995; Kubien and Sage, 2008; Hermida-Carrera *et al.*, 2016; Orr *et al.*, 2016), with habitat-dependent variations in some parameters, mostly related to contrasting thermal environment, CO_2 concentration, and water availability (Galmés *et al.*, 2005, 2014*a*, 2016). Regarding g_m, several studies have quantified the importance of leaf anatomical traits in determining g_m and, consequently, the photosynthetic capacity of different plant species (Terashima *et al.*, 2001; Tomás *et al.*, 2013; Flexas *et al.*, 2016), or within the same species but growing under different environmental conditions (Peguero-Pina *et al.*, 2015*a*). Contrasting environmental conditions can induce changes in several leaf traits that affect g_m (Evans *et al.*, 1994; Kogami *et al.*, 2001; Terashima *et al.*, 2001). Differences in leaf anatomical traits and chloroplast ultrastructure have been reported for *D. antarctica* growing along the Maritime Antarctica (Jellings *et al.*, 1983; Gielwanowska and Szczuka, 2005). Regarding *C. quitensis*, plants growing at higher latitudes have smaller and thicker leaves than those grown at lower latitudes, evidenced by a smaller leaf cross-section, with higher mesophyll thickness, narrower adaxial surface, and reduced epidermis thickness (Cavieres *et al.*, 2016). Although these changes have been related to plastic responses to the Antarctic climate (Romero *et al.*, 1999), it is not clear to what extent they may affect the photosynthetic performance of these plants. More specifically, do ultrastructural mesophyll traits induce changes in g_m and other photosynthetic parameters affecting the carbon assimilation capacity in these Antarctic plant species?

The main objective of the present study was to evaluate *in situ* the photosynthetic performance of two natural populations of *D. antarctica* and *C. quitensis* located at different latitudes within the Maritime Antarctica. In particular, we analyzed the response of photosynthesis to varying measurement temperatures and the diffusive and biochemical constraints to CO_2 assimilation. To the best of our knowledge, this is the first field study thoroughly analyzing the responses and limitations of photosynthesis on these species.

Materials and methods

Study area and plant material

Two study sites were selected (Fig. 1): one located in King George Island (KGI), near to Henryk Arctowski Polish Antarctic Station (62°09'S, 58°28'W), and the other in Lagotellerie Island (LAG), in Marguerite Bay (67°53^20″S, 67°25′30″W). These sites were selected based on the visual similarity of the vegetation, exposure, and elevation, as well as their distance from the coast. The sites are characterized by wide open and relatively flat areas (KGI) or terraces (LAG) covered by vegetation. In both sites, individuals of *D. antarctica* and *C. quitensis* (see Supplementary Fig. S1 at *JXB* online) were randomly selected, within an area of ~300 m^2, for photosynthesis measurements and sampling of leaves during six sunny days in late January 2015 in LAG and during February 2015 in KGI.

Climate

Precipitation in both sites falls mainly as snow. Annual precipitation, which occurs mostly during summer, is higher in the northernmost

population (~1249 mm), while in Marguerite Bay it is ~360 mm (Turner *et al.*, 2002). During summer, the day length in KGI is ~15 h, and the mean air temperature is 0.7 °C, with average maximum and minimum air temperature of 2.9 °C and –4.1 °C, respectively. In LAG, summer day length is ~17 h, with mean air temperature of –3.5 °C and average maximum and minimum of 1 °C and –10.9 °C, respectively (Utah State University Database; https://climate.usurf.usu.edu).

Daily microclimatic records of air temperature at 10 cm above ground level, leaf temperature, and photosynthetically active radiation (PAR) were taken during 6 d in January 2015 (Fig. 2). According to these data, the daily integral of photosynthetic solar radiation in KGI was 30% greater than in LAG (Fig. 2A). Air temperature at KGI tended to be higher than at LAG during most of the daylight period (Fig. 2B). At both locations, leaf temperature of *D. antarctica* and *C. quitensis* was at least 2 °C above ambient temperature, reaching a maximum of ~10 °C (Fig. 2). Differences in leaf temperature between locations were evident during the night, where individuals of both species growing in LAG showed higher leaf temperature (~2 °C) compared with individuals from KGI (Fig. 2C, D). In contrast, during the day, there was a trend for higher leaf temperatures in KGI compared with LAG.

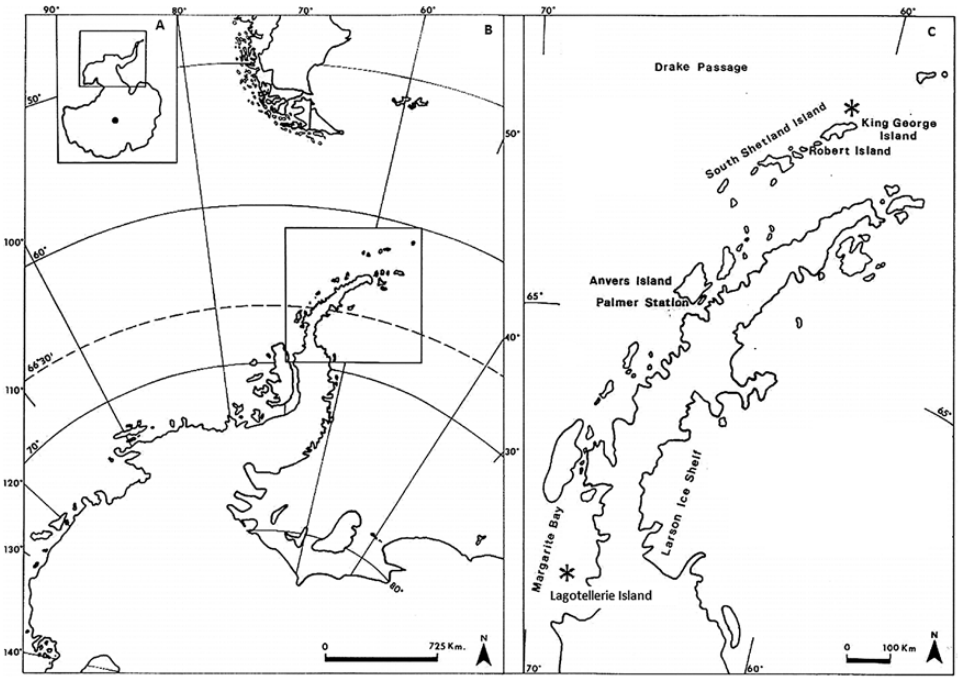

Fig. 1. The Antarctic continent (A), Antarctic Peninsula (B, inset from A), South Shetland Islands, and area of the Antarctic Peninsula (C, inset from B). Vascular plants from 62° to 67° South latitude, including the two study areas represented by an asterisk (*): King George Island (62°09'S, 58°28′W) and Lagotellerie Island (67°53′20″S, 67°25′30″W), Marguerite Bay (modified from Alberdi *et al.*, 2002).

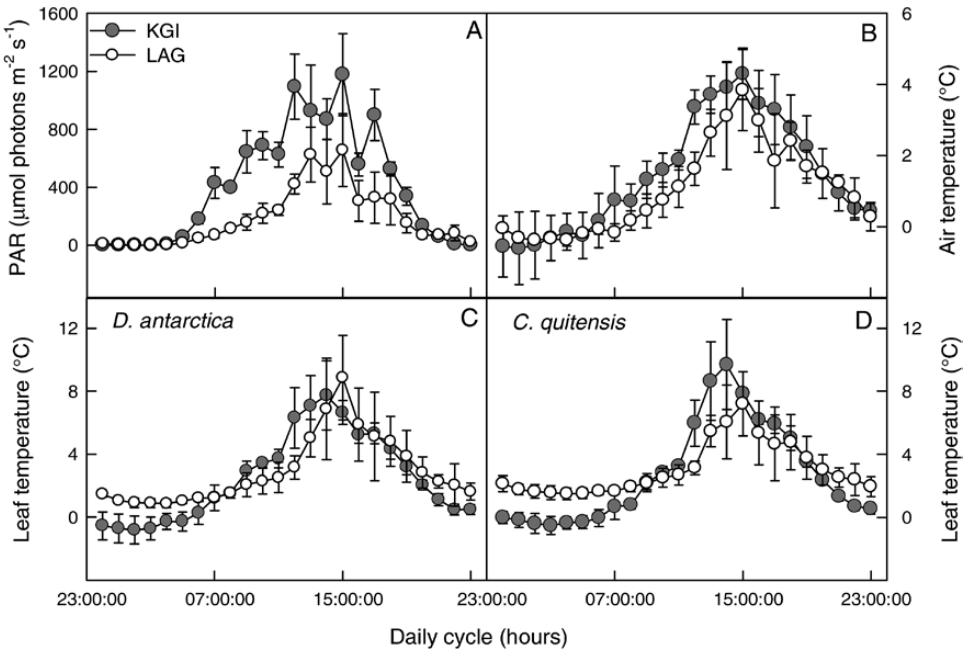

Fig. 2. Diurnal course of photosynthetically active radiation (A), air temperature (B), and leaf temperature in *D. antarctica* (C) and *C. quitensis* (D) in King George (KGI) and Lagotellerie Island (LAG) between 17 and 22 January 17 2015. Values are means ± SE

Leaf gas exchange and chlorophyll fluorescence

Instantaneous gas exchange and Chl *a* fluorescence measurements (Li-6400XT, Li-6400-40 leaf chamber, LI-COR Inc., Lincoln, NE, USA) were performed on a group of leaves (from a branch of *C. quitensis* or a tiller of *D. antarctica*), trying to cover all the IRGA's chamber area and avoiding leaf overlap. When the leaf area was lower than the chamber area, a correction by actual leaf area inside the chamber was performed.

The response of net photosynthesis CO_2 uptake (A_N) to varying substomatal CO_2 concentration (C_i) was studied with the so-called A_N–C_i curves. All measurements were made at 1000 µmol photons m^{-2} s^{-1}, air relative humidity of 40–50%, and at two leaf temperatures: 10 ºC and 15 ºC. These measurement temperatures corresponded to the maximal leaf temperatures recorded in the field (Fig. 2), and the average of optimal temperature for photosynthesis determined for these species in both populations, respectively (data not shown).

The A_N–C_i curves were initiated by allowing the leaf to reach steady state (typically 20–30 min after clamping the leaf). Thereafter, A_N–C_i curves were obtained at 11 different ambient CO_2 concentrations (C_as) from 0 to 2000 µmol CO_2 mol^{-1}. Leaves were left to equilibrate at least 5 min at each CO_2 concentration. Dark mitochondrial leaf respiration (R_{dark}) was obtained at pre-dawn at a C_a of 400 µmol CO_2 mol^{-1} and the two measuring temperatures (10 ºC and 15 ºC). Leaf temperature was measured with a fine-wire thermocouple touching the abaxial surface for the group of leaves. Corrections for the leakage of CO_2 into and out of the leaf chamber of the Li-6400 were applied to all gas exchange data, as described by Flexas *et al.* (2007).

The quantum efficiency of PSII-driven electron transport was determined using the equation: $\varphi_{PSII}=(F'_m-F_s)/F'_m$ where F_s is the steady-state fluorescence in the light (PPFD 1000 µmol quanta m^{-2} s^{-1}) and F'_m the maximum fluorescence obtained with a light-saturating pulse (8000 µmol quanta m^{-2} s^{-1}). As φ_{PSII} represents the number of electrons transferred per photon absorbed by PSII, the electron transport rate (ETR) can be calculated as: $ETR=\varphi_{PSII}\times PPFD\ \alpha\beta$, where PPFD is the photosynthetic photon flux density, α is the leaf absorptance, and β is the distribution of absorbed energy between the two photosystems, assumed to be 0.5. The leaf absorptance was directly measured in field-collected plants using a chlorophyll fluorescence system Imaging mini-PAM (Walz, Effeltrich, Germany). Successive illumination of the samples with red (R) and near infrared (NIR) light and the capture of each remission image allowed the calculation of leaf absorptance as follows: Abs=1–R/NIR. Leaf absorptance values were not significantly different between populations, with average values of 0.729 ± 0.02 for *D. antarctica* and 0.738 ± 0.02 for *C. quitensis* (mean ± SE, n=50).

The ratio of the electron transport rate and gross photosynthesis (ETR/A_G) was calculated at a C_a of 400 µmol CO_2 mol^{-1} air. Gross photosynthesis (A_G) was calculated from the sum of the net CO_2 assimilation rate (A_N) and half of the mitochondrial respiration in the dark (R_{dark}).

Estimation of g_m and C_c

Mesophyll conductance for CO_2 (g_m) was calculated from both combined gas-exchange and Chl *a* fluorescence measurements, and anatomical modeling. From the combined gas-exchange and Chl *a* fluorescence measurements, g_m was calculated as in Harley *et al.* (1992): $g_m=A_N/<C_i-\{\Gamma^*[ETR+8\ (A_N+R_L)]/[ETR-4\ (A_N+R_L)]\}>$, where A_N and C_i were obtained from gas exchange measurements at saturating light. The non-photorespiratory CO_2 evolution rate in the light (R_L) was assumed to be half of R_{dark}, and the chloroplast CO_2 compensation point (Γ*) was calculated according to Brooks and Farquhar (1985) from the Rubisco specificity factor ($S_{c/o}$) measured *in vitro*. Determination of g_m was used to calculate the chloroplast CO_2 concentration (C_c), converting A_N–C_i curves into A_N–C_c curves, as $C_c=C_i-(A_N/g_m)$.

The maximum velocity of carboxylation (V_{cmax}) was derived from A_N–C_c curves according to Farquhar *et al.* (1980) and using the kinetic constants for Rubisco determined for these species at the measurement temperatures (see below).

The approach of Tomás *et al.* (2013) was used for anatomical modeling of g_m. In the field, the central portions of leaves were fixed in formaldehyde, acetic acid, and ethanol, and 4% glutaraldehyde for optical and transmission electron microscopy (JEM1200 EXII, Japan), respectively. Six to 10 micrographs were randomly selected to measure the mesophyll thickness; the mesophyll area exposed to the intercellular air space (S_m) to total leaf surface (S) area ratio (S_m/S); the chloroplast-exposed surface area to total surface area ratio (S_c/S); the chloroplast length (L_{chl}); the chloroplast thickness (T_{chl}); and the cell wall thickness (T_{cw}). All images were analyzed with image analysis software (ImageJ; Wayne Rasband/NIH, Bethesda, MD, USA). The one-dimensional gas diffusion model of Niinemets and Reichstein (2003) was employed to estimate the different leaf anatomical characteristics determining g_m. The determinants of g_m were divided between gas-phase conductance and the different components of the cellular phase conductances: the cell wall (l_{cw}), the plasmalemma (l_{pl}), and inside the cells through the cytosolic path ($l_{cel,tot}$).

Rubisco kinetic characterization at varying temperature

The Rubisco Michaelis–Menten constant for CO_2 under 21% O_2 (K_c^{air}) was determined in crude extracts obtained as detailed in Galmés et al. (2014*a*). Replicate measurements (n=3) were made using independent protein preparations from different individuals. To obtain the Rubisco carboxylase specific activity (k_{cat}^c), the maximum rate of carboxylation was extrapolated from the Michaelis–Menten fitted curve and divided by the number of Rubisco active sites in solution, quantified by [^{14}C]CABP (2′-carboxyarabinitol-1, 5-bisphosphate) binding (Yokota and Canvin, 1985) as described in Galmés *et al.* (2013). The carboxylase catalytic efficiency was obtained as the ratio k_{cat}^c/K_c^{air}.

Determination of the Rubisco specificity for CO_2/O_2 ($S_{c/o}$) at varying temperature

The Rubisco CO_2/O_2 specificity ($S_{c/o}$) was measured on purified extracts as in Gago *et al.* (2013), except that values were not normalized to those of wheat Rubisco. Measurements were performed at 5, 15, and 25 ºC, with 3–6 replicates per species and per assayed temperature. For comparative purposes, all Rubisco kinetic traits, including $S_{c/o}$, were also determined in wheat (*Triticum aestivum* 'Cajerne') at 25 ºC.

For all Rubisco assays, the pH of the assay buffers was accurately adjusted at each measurement temperature. The concentration of CO_2 in solution in equilibrium with HCO_3^- was calculated assuming a pK_a for carbonic acid of 6.31, 6.19, and 6.11 at 5, 15, and 25 ºC, respectively. The concentration of O_2 in solution was assumed to be 400.5, 316.4, and 258.9 nmol ml^{-1} at 5, 15, and 25 ºC, respectively (Truesdale and Downing, 1954).

Temperature response of the Rubisco kinetic constants

The temperature response of the Rubisco kinetic parameters was fitted for each individual temperature response data set by an Arrhenius-type temperature response function:

$$\text{Parameter} = \exp(c - \Delta H_a / RT) \quad (1)$$

where c is the scaling constant for the parameter, ΔH_a (J mol^{-1}) is the activation energy, R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T (K) is the temperature. Equation 1 was fitted to the data by iteratively minimizing the sum of squares between the measured and predicted values of each kinetic parameter using the Microsoft Excel Solver function.

Statistical analyses

Fully factorial two-way ANOVAs were performed on each species to assess differences between populations and measurement temperatures. Differences between means were assessed by *a posteriori* Tukey test (P<0.05). These analyses were performed with the SPSS statistics 19.0 software package (IBM-software, New York, USA). A Pearson correlation analysis was performed to assess the relationship between the anatomical derived g_m and studied anatomical traits. Goodness of fit to saturation curves was assessed in: A_N/g_{tot}, A_N/C_c, and A_N/g_m. All these analyses were done in Statistica 7.0 (Stat Soft Inc. Tulsa, OK, USA).

Results

Leaf carbon exchange in Antarctic plants at different measurement temperatures

Plants from LAG exhibited higher net CO_2 assimilation rates (A_N) at ambient CO_2 concentration than those from KGI, with no differences between measurement temperatures in the studied populations, although in both species the interaction between these two factors was significant (Supplementary Table S1; Supplementary Fig. S2). In *D. antarctica*, A_N was higher in LAG at 15 °C (15.11 ± 0.51 µmol CO_2 m^{-2} s^{-1}) compared with KGI (5.93 ± 0.60 µmol CO_2 m^{-2} s^{-1}; Fig. 3A), but A_N was similar across the two sites at 10 °C (~12 µmol CO_2 m^{-2} s^{-1}). In *C. quitensis*, the highest A_N value was observed in LAG at 10 °C (9.88 ± 1.71 µmol CO_2 m^{-2} s^{-1}) and the lowest in KGI at 10 °C (2.53 ± 0.37 µmol CO_2 m^{-2} s^{-1}; Fig. 3B). There were no differences in the dark respiration rate (R_{dark}) between populations or measurement temperatures (Fig. 3C, D; Supplementary Table S1). This indicates that the observed differences in A_N were not due to differences in R_{dark}, but to differences in the photosynthetic process and its determinants.

Diffusive limitations to photosynthesis

Stomatal (g_s) and leaf mesophyll (g_m) conductances for both species showed similar trends to those described for A_N (Fig. 4; Supplementary Table S1). Values of g_s for *D. antarctica* ranged between 0.08 ± 0.01 mol H_2O m^{-2} s^{-1} at KGI at 15 °C and 0.35 ± 0.03 mol H_2O m^{-2} s^{-1} in LAG at 15 °C. For *C. quitensis*, g_s varied from 0.14 ± 0.02 mol H_2O m^{-2} s^{-1} in LAG at 15 °C to 0.23 ± 0.03 mol H_2O m^{-2} s^{-1} in KGI at 15 °C (Fig. 4A, B). Estimated g_m values were lower than those of g_s, with the minimum value estimated in *C. quitensis* from KGI at 10 °C (0.01 ± 0.01 mol CO_2 m^{-2} s^{-1}), and the highest in *D. antarctica* from LAG at 15 °C (0.13 ± 0.02 mol CO_2 m^{-2} s^{-1}). The low g_m determined low total leaf conductance to CO_2 (g_{tot}), which significantly correlated with A_N (Fig. 5A). This indicates that the photosynthetic rates in the Antarctic vascular plants under field conditions were limited by diffusional components in general, and low g_m in particular (Fig. 5B). The good correspondence between A_N and the chloroplastic CO_2 concentration (C_c) further confirms that carbon fixation in these species was constrained by low availability of CO_2 at the carboxylation sites (Fig. 5C). In general, diffusion limitations to photosynthesis were more evident in *C. quitensis* (Figs 4, 5), which is consistent with the lowest CO_2 assimilation rates found in this species (Fig. 3B).

Mesophyll conductance modeled from leaf anatomy

The studied populations of *D. antarctica* showed similar leaf anatomy, with the exception of the mesophyll thickness (T_{mes}), which was higher in plants from LAG, and the chloroplast surface area facing intercellular air spaces per leaf area (S_c/S), which was larger in plants from KGI (Table 1). No differences between populations were found in ultrastructural leaf characteristics (Supplementary Fig. S3) such as cell wall thickness (T_{cw}), average distance between chloroplasts (ΔL_{cyt}), and their size (L_{chl} and T_{chl}). In addition, large numbers of organelles around chloroplasts were observed in both plant species (Supplementary Figs S3, S4). For *C. quitensis*, there were differences between populations

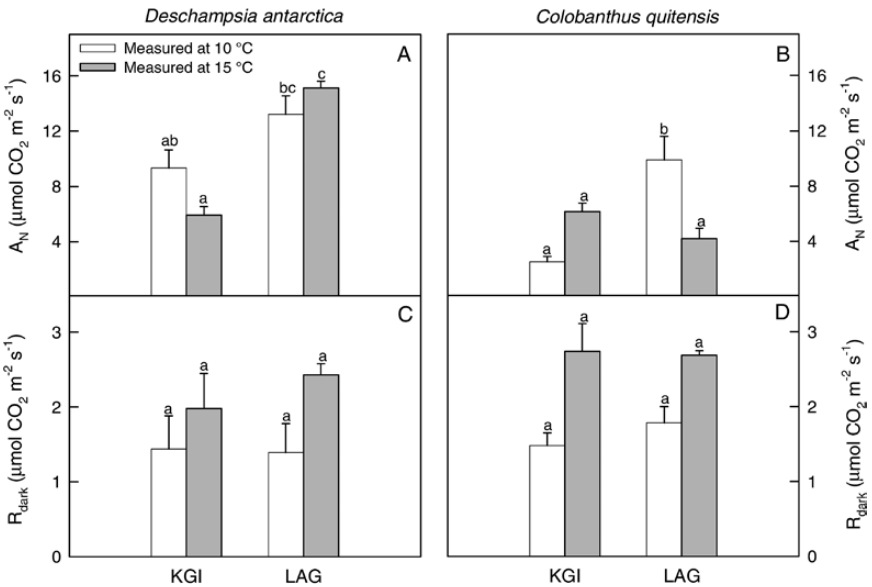

Fig. 3. The net photosynthetic CO_2 assimilation rate (A_N) and dark respiration (R_{dark}) of *D. antarctica* (A, C) and *C. quitensis* (B, D) in King George (KGI) and Lagotellerie Island (LAG), measured at 10 °C (white bars) or 15 °C (gray bars). Values are means ± SE (n=5–7). Different letters indicate statistically significant differences for each species between populations and measurement temperature according to Tukey (P<0.05).

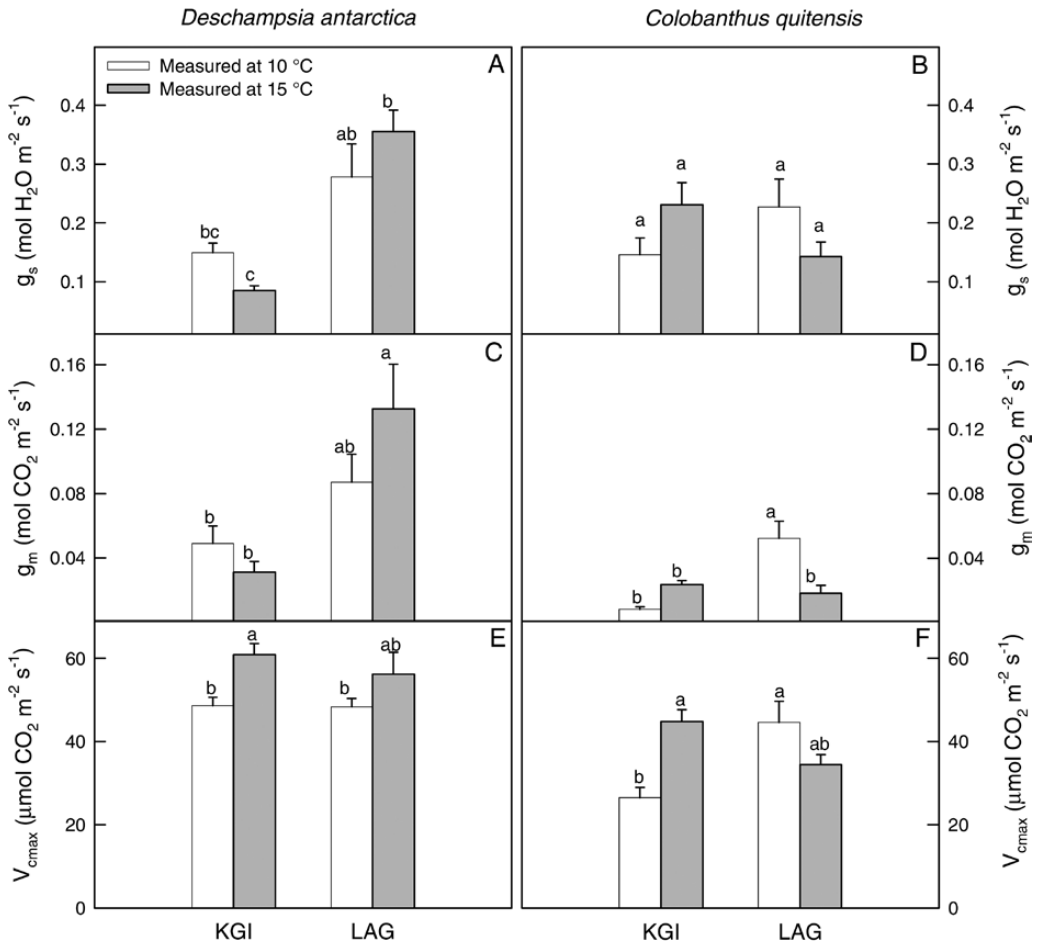

Fig. 4. The stomatal (A, B) and the leaf mesophyll (C, D) conductances, and the maximum rate of Rubisco carboxylation (E, F) of *D. antarctica* (left) and *C. quitensis* (right) in King George (KGI) and Lagotellerie Island (LAG), measured at 10 °C (white bars) or 15 °C (gray bars). Values are means ± SE (n=5–7). Different letters indicate statistically significant differences between populations for each species and measurement temperature according to Tukey (P<0.05).

in most of the anatomical parameters evaluated, except in T_{mes} and the chloroplast length (L_{chl}) (Table 1). In particular, *C. quitensis* plants from KGI exhibited higher T_{cw}, ΔL_{cyt}, and chloroplast thickness (T_{chl}) than plants from LAG (Table 1). The contrasting anatomical traits between both populations of *C. quitensis* determined differences in g_m modeled from leaf anatomy (0.013 ± 0.001 mol CO_2 m^{-2} s^{-1} in KGI versus 0.039 ± 0.004 mol CO_2 m^{-2} s^{-1} in LAG).

In general, anatomy-based g_m values were lower than those estimated *in vivo* by the method of Harley *et al.* (1992) (Table 1). It should be noted that anatomical measurements do not consider the different leaf temperatures recorded between populations (Fig. 2); therefore, the comparison with the 'Harley' g_m should be treated with caution. Despite this limitation, some of the described trends for g_m estimated by the method of Harley *et al.* (1992) were also found with the anatomy-based g_m (compare Fig. 4D with Table 1). For example, *C. quitensis* from LAG at 10 ºC showed higher values compared with the KGI. In contrast, the higher 'Harley' g_m found in *D. antarctica* from LAG compared with KGI (Fig. 4C) was not supported by the anatomical modeling of g_m (Table 1).

In *C. quitensis*, the mesophyll (S_m/S) and chloroplast (S_c/S) surface areas facing the intercellular air spaces per leaf area were significantly higher in LAG compared with KGI (Table 1), and the values of S_m/S and S_c/S correlated positively with g_m modeled from leaf anatomy (Fig. 6). In *D. antarctica*, S_c/S but not S_m/S differed between populations, and only S_m/S but not S_c/S correlated with g_m (Fig. 6).

In both species, the CO_2 transfer conductance across the liquid phase (g_{liq}) was lower than that across the intercellular air space (g_{ias}) (Table 1), which yielded a low quantitative g_m limitation by the intercellular air spaces (l_{ias}) (Table 2). Regarding the components of g_{liq}: the cell wall (l_{cw}), but mainly the cytoplasm and stroma ($l_{cet,tot}$) limitations determined the low internal diffusion of CO_2 observed in the Antarctic vascular plants.

Biochemical determinants of photosynthesis

The maximum rate of Rubisco carboxylation (V_{cmax}) increased with the measurement temperature in both plant species from KGI, but non-significant differences between measurement temperatures were found in plants from LAG (Fig. 4E, F). For all the range of A_N–C_c curves, A_N was linearly correlated to C_c (Supplementary Fig. S5), which impeded the estimation of the maximum rate of electron transport (J_{max}).

The ratio between the electron transport rate and gross CO_2 assimilation rate (ETR/A_G) which is used as a proxy of the amount of reducing power per unit of fixed CO_2 (Flexas *et al.*,

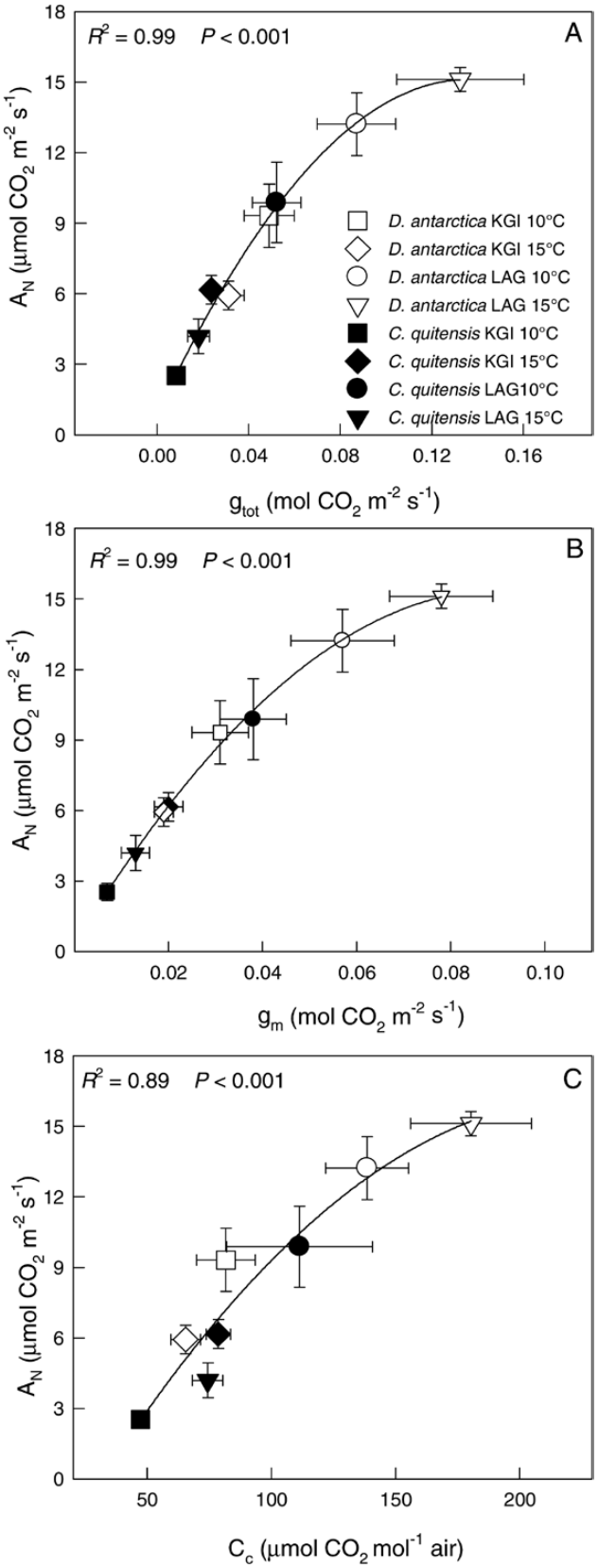

Fig. 5. The relationship of (A) the total leaf conductance (g_{tot}), (B) the leaf mesophyll conductance (g_m), and (C) the chloroplast CO_2 concentration (C_c) to the net photosynthetic CO_2 assimilation rate (A_N) of *D. antarctica* (open circles) and *C. quitensis* (filled circles) in King George (KGI) and Lagotellerie Island (LAG). Goodness of fit with a saturation model is shown for both species and considering all populations and measurement temperatures together. Values are means ± SE (n=5–7).

2002), varied between measurement temperatures and populations in both species (Fig. 7). In plants from KGI, the increase in the measurement temperature induced an increase in ETR/A_G in *D. antarctica* but a decrease in *C. quitensis*. In plants from LAG, there were no temperature-driven changes in ETR/A_G in either of the two species. The relationship between ETR/A_G and C_c was highly significant and negative (Supplementary Fig. S6), indicative of enhanced photorespiration rates under low CO_2 availability. Except for KGI at 15 ºC, *C. quitensis* exhibited higher values for ETR/A_G—consistent with lower values for C_c.

Rubisco in vitro *kinetic parameters and their temperature dependence*

The affinity of Rubisco for CO_2 measured as K_c^{air} at 25 ºC was 18.7 ± 0.4 µM and 23.4 ± 1.8 µM for *C. quitensis* and *D. antarctica*, respectively (Table 3). The maximum rate of the carboxylase catalytic turnover (k_{cat}^c) was 3.76 ± 0.29 s^{-1} in *C. quitensis* and 4.10 ± 0.75 s^{-1} in *D. antarctica*. The carboxylase catalytic efficiency assessed by the k_{cat}^c/K_c^{air} ratio ranged between 0.18 s^{-1} μM^{-1} and 0.20 s^{-1} μM^{-1} in both Antarctic species. At 25 ºC, the values of the Rubisco specificity factor ($S_{c/o}$) were 99.5 ± 4.8 mol mol^{-1} in *D. antarctica* and 97.1 ± 2.4 mol mol^{-1} in *C. quitensis* (Table 3).

Differences were observed in the temperature response of Rubisco between *D. antarctica* and *C. quitensis* (Table 3). For instance, *D. antarctica* presented higher values for K_c^{air} than *C. quitensis* at 5 °C and 15 °C. Differences in k_{cat}^c between *D. antarctica* and *C. quitensis* were found only at 5 °C. In consequence, the Rubisco carboxylase catalytic efficiency did not differ between species at any of the assayed temperatures. Regarding $S_{c/o}$, differences between the two Antarctic plant species were observed only at 15 ºC, where *D. antarctica* presented higher values than *C. quitensis* (P<0.05).

The differences in the temperature response of Rubisco resulted in a trend for higher absolute values of the energy of activation (ΔH_a) of the different kinetic parameters in *C. quitensis*, although significant differences between both species were only observed in ΔH_a of k_{cat}^c (78.8 ± 1.5 kJ mol^{-1} in *D. antarctica* versus 98.3 ± 6.6 kJ mol^{-1} in *C. quitensis*).

Discussion

The leaf anatomical and biochemical traits described here for the Antarctic vascular plants growing at different latitudes within Antarctica and their implications on the photosynthetic performance of these species constitute new insights into the plant functional responses to cold conditions. While ultrastructural traits of the leaf mesophyll restricted CO_2 transfer and limited the photosynthetic capacity of the two Antarctic vascular plants, the kinetic traits of Rubisco, characterized by high affinity for CO_2 and relative high values for k_{cat}^c, seem crucial to optimize carbon assimilation despite the restrictions of CO_2 transport inside the leaf. The former suggests an important functional adaptation that, together with other traits (Cavieres *et al.*, 2016), allows *D. antarctica* and *C. quitensis* to survive and grow in the harsh climate conditions of the Antarctica. As these two Antarctic species differ in their habitat requirements,

Table 1. *The mesophyll thickness between the two epidermal layers (T_{mes}), the cell wall thickness (T_{cw}), the average distance between chloroplasts (ΔL_{cyt}), the chloroplast thickness (T_{chl}), the chloroplast length (L_{chl}), the CO_2 transfer conductances across the intercellular air space (g_{ias}), the liquid phase (g_{liq}), and the mesophyll conductance for CO_2 (g_m) calculated from leaf anatomical measurements, the mesophyll (S_m/S) and chloroplast (S_c/S) surface area facing intercellular air spaces per leaf area, for* D. antarctica *and* C. quitensis *from King George (KGI) and Lagotellerie Island (LAG)*

	D. antarctica		***C. quitensis***	
	KGI	**LAG**	**KGI**	**LAG**
T_{mes} (µm)	101.03 ± 5.43 a	136.27 ± 5.14 b	322.29 ± 13.42 a	290.77 ± 18.91 a
T_{cw} (µm)	0.26 ± 0.01 a	0.22 ± 0.03 a	0.35 ± 0.06 b	0.21 ± 0.02 a
ΔL_{cyt} (µm)	0.68 ± 0.18 a	0.3 ± 0.16 a	0.53 ± 0.06 b	0.05 ± 0.03 a
T_{chl} (µm)	2.96 ± 0.24 a	3.58 ± 0.52 a	5.12 ± 0.66 b	1.64 ± 0.12 a
L_{chl} (µm)	4.40 ± 0.36 a	5.48 ± 0.56 a	5.32 ± 0.28 a	5.92 ± 0.36 a
g_{ias} (m s^{-1})	0.046 ± 0.006 a	0.031 ± 0.004 a	0.013 ± 0.001 a	0.019 ± 0.001 b
g_{liq} (m s^{-1})	0.0005 ± 0.0001 a	0.0005 ± 0.0001 a	0.0003 ± 0.0000 a	0.0001 ± 0.0001 b
g_m (mol m^{-2} s^{-1})	0.022 ± 0.002 a	0.023 ± 0.004 a	0.013 ± 0.001 a	0.039 ± 0.004 b
S_m/S (m^2 m^{-2})	6.40 ± 0.75 a	6.28 ± 0.51 a	4.33 ± 0.48 a	8.56 ± 0.53 b
S_c/S (m^2 m^{-2})	2.24 ± 0.48 b	1.16 ± 0.08 a	2.06 ± 0.32 a	4.42 ± 0.63 b

Values are means ± SE (n=6–10).
Different letters indicate statistically significant differences between populations for each species (P<0.05).

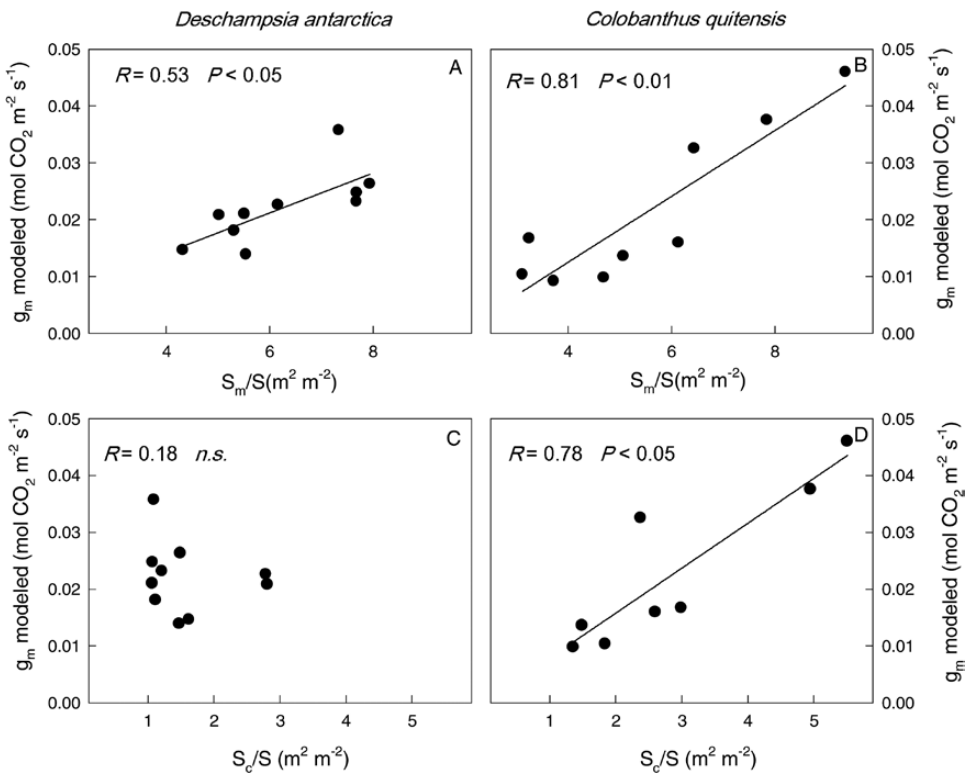

Fig. 6. Correlations of the leaf mesophyll conductance modeled with anatomical parameters (g_m modeled) with the mesophyll (S_m/S) and chloroplast (S_c/S) surface area facing intercellular air spaces per leaf area in *D. antarctica* (left) and *C. quitensis* (right). The Pearson correlation coefficient and the significance of the relationship are shown for each species considering both populations together. Values are means ± SE (n=3–5).

plant morphology, leaf anatomy, and photosynthetic optimum temperature (Cavieres *et al.*, 2016), an interspecific comparison was not considered here, except for Rubisco kinetic parameters.

Leaf mesophyll conductance to CO_2 limits carbon assimilation in Antarctic plants

This is the first study assessing g_m in Antarctic vascular plants, and how changes in the ultrastructure of the mesophyll affect g_m, and hence the carbon acquisition in these species. According to our results, the range of g_m values and the g_m/g_s ratio of these plant species are among the lowest reported so far for higher plant species (De Lucía et al., 2003; Flexas *et al.*, 2009, 2014; Tomás *et al.*, 2013; Peguero-Pina *et al.*, 2015*a*). Under certain conditions, g_m can be the most significant photosynthetic limitation (Flexas *et al.*, 2012; Tomás *et al.*, 2013; Galmés *et al.*, 2014*a*; Niinemets and Keenan, 2014; Carriquí *et al.*, 2014; Peguero-Pina *et al.*, 2015*a*, *b*). As

the photosynthetic rates of the Antarctic vascular plants were highly correlated with g_m (Fig. 5B), this factor seems to be the main constraint for the photosynthetic process in these plant species in the field. Positive correlations between A_N and g_m have been previously reported (e.g. Evans and Loreto, 2000; Warren, 2008), and recent meta-analyses suggest that g_m is also associated with the structure of leaves, determining the diffusion limitations of photosynthesis (Niinemets and Sack, 2006; Warren, 2008). Thus, our results highlight that morphoanatomical leaf characteristics regulating g_m are key determinants of the photosynthetic functioning in the two Antarctic vascular plant species.

Large differences in g_m have been shown both between and within species with different leaf forms and habits (e.g. Flexas *et al.*, 2008; Warren, 2008). The low g_m found in the Antarctic vascular plants in the field seems to be related to leaf anatomical traits that affect CO_2 diffusion across the intercellular air space (Table 1), especially with the limitations associated with cell walls, cytoplasm, and stroma (Table 2). The leaf mesophyll diffusion limitations were especially evident in *C. quitensis*, which is consistent with the low photosynthetic rate of this species and with the high values of ETR/A_G (Fig. 7), indicative of enhanced photorespiration rates. Higher leaf mesophyll thickness is commonly associated with a greater number of leaf mesophyll cell layers (Niinemets *et al.*, 2009). In *C. quitensis*, the highest leaf mesophyll thickness was accompanied by the largest area of leaf mesophyll exposed to intercellular air space (S_m), and thus the greatest S_m to total leaf surface area ratio (S_m/S) (Table 1). Provided that the numbers of chloroplasts are similar in mesophyll cells, a larger S_m/S also implies a greater ratio between the chloroplast-exposed surface area and the total surface area (S_c/S) (Terashima *et al.*, 2005, 2006). As a larger S_c/S implies more parallel pathways for CO_2 liquid-phase diffusion, g_m correlates positively with S_c/S (Fig. 6). Actually, higher leaf density has been associated with reduced gas-phase volume, and smaller and more densely packed leaf mesophyll cells with thicker cell walls (Niinemets, 1999; Niinemets and Sack, 2006). Such modifications could reduce the liquid-phase diffusion conductance and g_m (Terashima *et al.*, 2005; Evans *et al.*, 2009). In addition, the observed differences in g_m between both populations of *C. quitensis* can be partially attributed to the variation in other leaf anatomical traits such as T_{cw}, ΔL_{cyt}, and T_{chl} (Table 1). Specifically, the cell wall and the resistances imposed by the cytoplasm and stroma exerted the highest limitations in the g_m (Table 2). Further, as was noted above, S_m/S and S_c/S are two of the most important anatomical traits influencing g_m (Tosens *et al.*, 2012; Peguero-Pina *et al.*, 2012; Carriquí *et al.*, 2014).

Anatomical characteristics could not be the only explanation for the observed differences in g_m, as those were also found between measurement temperatures in plants growing in LAG (Fig. 4C, D). It seems likely that some (not yet fully understood) biochemical components of g_m could be involved in the regulation of g_m. Among them, carbonic anhydrase (CAs) (Fabre *et al.*, 2007) and aquaporins (AQPs) (Terashima and Ono, 2002) have been shown to modify g_m *in vivo* in response to varying measurement temperatures. The discrepancy between g_m estimates based on anatomical measurements and those based on conventional gas-exchange methods suggests the existence of these facilitating mechanisms for CO_2 diffusion. However, to the best of our knowledge, there are no reports on the activity and abundance of these proteins in Antarctic vascular plants, and thus we cannot draw any general conclusion on this issue.

The anatomical features of the Antarctic species have been regarded as adaptive responses to the harsh climate conditions of Antarctica (Cavieres *et al.*, 2016). Among them, the large number of organelles (mitochondria or peroxisomes) around the chloroplasts in both Antarctic species (Supplementary

Table 2. *Quantitative limitation analysis of the leaf mesophyll conductance to CO_2 (g_m) for* D. antarctica *and* C. quitensis *from King George (KGI) and Lagotellerie Island (LAG) due to different anatomical components of the diffusion pathway: intercellular spaces (l_{ias}), cell wall (l_{cw}), plasmalemma (l_{pl}), and inside the cell ($l_{cel, tot}$)*

	D. antarctica		*C. quitensis*	
	KGI	**LAG**	**KGI**	**LAG**
l_{ias} (%)	1.1 ± 0.1 a	1.7 ± 0.2 a	2.5 ± 0.4 a	4.6 ± 0.1 b
l_{cw} (%)	34.4 ± 1.3 a	27.4 ± 2.1 b	35.4 ± 5.4 a	49.5 ± 6.2 a
l_{pl} (%)	2.3 ± 0.1 a	2.3 ± 0.2 a	2.0 ± 0.3 a	3.3 ± 0.1 b
$l_{cel, tot}$ (%)	62.3 ± 1.5 a	68.4 ± 2.1 a	59.8 ± 5.4 a	42.5 ± 6.1 b

Values are means ± SE (n=4–10).
Different letters indicate statistically significant differences between populations for each species (P<0.05).

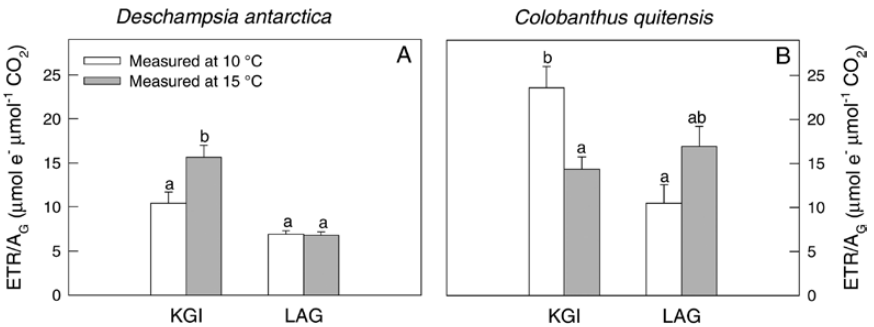

Fig. 7. The ratio of the electron transport rate and gross photosynthesis (ETR/A_G) for *D. antarctica* (A) and *C. quitensis* (B) in King George (KGI) and Lagotellerie Island (LAG), measured at 10 °C (white bars) or 15 °C (gray bars). Values are means ± SE (n=5–7). Different letters indicate statistically significant differences for each species between populations and measurement temperatures according to Tukey (P<0.05).

Table 3. In vitro *Rubisco kinetic parameters and their temperature response in* D. antarctica *and* C. quitensis

The Rubisco Michaelis–Menten constant affinity for CO_2 under 21% O_2 (K_c^{air}), the maximum carboxylase catalytic turnover rate (k_{cat}^c), the carboxylase catalytic efficiency (k_{cat}^c/K_c^{air}), and the specificity factor ($S_{c/o}$). For each parameter, the scaling constant (*c*) and the activation energy (ΔH_a) are shown.

Assay temperature	*D. antarctica*				*C. quitensis*			
	K_c^{air} (μM)	k_{cat}^c (s^{-1})	k_{cat}^c/K_c^{air} (s^{-1} μM^{-1})	$S_{c/o}$ (mol mol^{-1})	K_c^{air} (μM)	k_{cat}^c (s^{-1})	k_{cat}^c/K_c^{air} (s^{-1} μM^{-1})	$S_{c/o}$ (mol mol^{-1})
5 ºC	10.9 ± 1.2 a*	0.4 ± 0.1 a*	0.04 ± 0.00 a	167.5 ± 2.9 c	6.9 ± 0.4 a*	0.2 ± 0.1 a*	0.03 ± 0.00 a	172.4 ± 2.0 c
15 ºC	14.7 ± 0.8 a*	1.4 ± 0.2 a	0.09 ± 0.01 ab	151.3 ± 4.5 b*	11.1 ± 0.6 b*	1.1 ± 0.1 b	0.10 ± 0.01 b	129.8 ± 3.7 b*
25 ºC	23.4 ± 1.8 b	4.1 ± 0.8b	0.18 ± 0.04 b	99.5 ± 4.8 a	18.7 ± 0.4 c	3.8 ± 0.3 c	0.20 ± 0.01 c	97.1 ± 2.4 a
c	14.5 ± 1.8	33.2 ± 0.8	18.4 ± 2.5	–1.7 ± 0.6	17.1 ± 0.7	41.0 ± 2.7	20.9 ± 0.2	–3.2 ± 0.5
ΔH_a (kJ mol^{-1})	28.2 ± 4.4	78.8 ± 1.5*	49.9 ± 5.6	–15.9 ± 1.4	35.2 ± 1.6	98.3 ± 6.6*	55.6 ± 0.5	–19.3 ± 1.2

Different letters indicate statistically significant differences among assay temperatures in each species, and an asterisk denotes statistically significant differences between *D. antarctica* and *C. quitensis* for each assay temperature (P<0.05).
Values are means ± SE (n=3–6).
For comparative purposes, we also measured the kinetic parameters at 25 ºC in Rubisco from wheat (*Triticum aesticum* 'Cajerne'). The values were as follows: K_c^{air}=16.0 μM; k_{cat}^c=2.2 s^{-1}; k_{cat}^c/K_c^{air}=0.14 s^{-1} μM^{-1}; $S_{c/o}$=101.1 mol mol^{-1}.

Figs S3, S4) have been suggested as facilitators for the CO_2 exchange between respiration and photorespiration processes (Gielwanoeska and Szczuka, 2005). In addition, both species have xeromorphic leaf characteristics (Nobel, 1980; Vieira and Mantovani, 1995; Romero *et al.*, 1999), which are related to the water limitations due to the low temperature and strong winds that characterize the Antarctic climate (Smith, 1993). Some features, such as the presence of two bundle sheaths in leaf vascular bundles (mestome), of *D. antarctica* have been associated with an adaptation to high radiation in order to optimize photosynthesis and water use efficiency (WUE) (Pyykkö, 1966). Evert *et al.* (1985) suggested that the mestome functions as a leaf endodermis, limiting apoplastic movement of water across the mesophyll. This trait, along with a high stomatal density and leaf mass area, confer a high capacity to control water loss on *D. antarctica* (Vieira and Mantovani, 1995; Xiong *et al.*, 2000; Alberdi *et al.*, 2002). Decreased water movement across the leaf mesophyll could be advantageous to avoid heat loss in cold environments, being an important adaptation for these habitats (Sage, 2001). These characteristics are consistent with the low g_m values determined in the present study using both gas-exchange/fluorescence and anatomical methods.

The low g_m values resulted in low g_{tot} values, and therefore low C_c. According to our results, A_N was highly correlated with g_m, g_{tot}, and C_c (Fig. 5), confirming the predominant role of leaf CO_2 diffusion in the photosynthetic performance of Antarctic plant species. In this sense, reduced photosynthesis, due to low g_m, seems to be the penalty of structurally robust leaves that the Antarctic plant species have to pay to survive in extremely stressful conditions.

Rubisco performance alleviates the low mesophyll conductance

The Rubisco kinetic parameters, and their temperature response, have been related to species differences in the photosynthetic performance under varying conditions (Galmés *et al.*, 2014*a*, 2015, 2016; Sharwood *et al.*, 2016). The temperature dependence of Rubisco kinetics revealed some differences between the two Antarctic angiosperm species. In particular, the energy of activation (ΔH_a) for k_{cat}^c was higher in *C. quitensis* compared with *D. antarctica*, indicative of higher thermal sensitivity (Table 3). The values for ΔH_a of k_{cat}^c in the two Antarctic species are among the highest in Streptophyta, and do not support the reported trend (see compilation by Galmés *et al.*, 2015) that the Rubisco enzymes of C_3 species adapted to cool habitats have a lower plastic response to temperature changes compared with Rubisco of C_3 species from warm environments. The molecular and biochemical causes of this apparent discrepancy, already observed in other species from cool habitats such as *Atriplex glabriuscula* (Badger and Collatz, 1977), are unknown and should be explored in depth. In contrast, the high values for both $S_{c/o}$ and ΔH_a of k_{cat}^c observed in the Antarctic species are in accordance with the transition state theory of Tcherkez *et al.* (2006).

In fact, the values of $S_{c/o}$ measured at 25 ºC in both Antarctic plants (Table 3) are among the highest values reported so far for higher plant species (e.g. Galmés *et al.*, 2005; Orr *et al.*, 2016). We note that for comparative purposes between data sets, we also measured Rubisco kinetic parameters in wheat at 25 ºC (shown in the footnotes of Table 3) and the obtained values were similar to previous data (e.g. Galmés *et al.*, 2005; Prins *et al.*, 2016; Orr *et al.*, 2016). Regarding K_c^{air} at 25 ºC, both Antarctic plants, but in particular *D. antarctica*, presented values comparable with those reported for species adapted to xeric environments (Galmés *et al.*, 2014*a*). Notably, despite their high affinity for CO_2, Rubisco from the Antarctic plant species retained high k_{cat}^c, similar to species closely related to *D. antarctica* such as *Poa arctica* and *Poa pratensis* (Sage, 2002). The high k_{cat}^c leads to values for the carboxylase catalytic efficiency (k_{cat}^c/K_c^{air}) higher than those reported for species from xeric habitats (Galmés *et al.*, 2014*a*). Further, at 15 ºC (a temperature similar to the maximal diurnal leaf temperature recorded in the field; Fig. 2), Rubisco from both Antarctic plants increased the specificity for CO_2 to levels similar to that reported for xeromorphic species (Galmés *et al.*, 2005).

Although it has been suggested that the selective pressures for greater CO_2 affinity for Rubisco have been high in species adapted to high temperature and low soil water availability (Galmés *et al.*, 2005, 2014*a*), there is also evidence that in cold environments the selective pressures may favor Rubisco with higher k_{cat}^{c} (Sage, 2002; Yamori *et al.*, 2009). Interestingly, the Antarctic species have evolved under both dry and cold conditions, and here we found that their Rubiscos show a high affinity for CO_2 and retain high k_{cat}^{c}. The high $S_{c/o}$ in the Antarctic plants is additional evidence in favor of the hypothesis that CO_2 limitations shaped the evolution of their Rubisco kinetics (e.g. Raven, 2000; Young *et al.*, 2012; Galmés *et al.*, 2014*b*). In the case of the Antarctic plants, the low availability of CO_2 at the sites of carboxylation is driven by adaptive anatomical traits to resist the extreme climatic conditions of low temperature and strong winds. It has been demonstrated that under conditions that promote drought, higher $S_{c/o}$ reduces ribulose bisphosphate (RuBP) oxygenation and favors the carboxylase reaction (Galmés *et al.*, 2005). Thus, it seems likely that the anatomical features that determine a low CO_2 diffusion in these species are partially counterbalanced by a highly efficient Rubisco. The concentration of active Rubisco, calculated from $V_{cmax}=k_{cat}^{c}\times$[active Rubisco sites], was notably high in both Antarctic species at 15 ºC: ~44.57 ± 1.9 µmol m^{-2} and 42.31 ± 2.84 µmol m^{-2} in *D. antarctica* and *C. quitensis*, respectively. Although these data should be confirmed by direct measurements in leaf extracts, they suggest that the decrease in k_{cat}^{c} at low temperatures is compensated by increased amount of [active Rubisco sites], following trends already reported in other species (e.g. Yamori *et al.*, 2005, 2006).

The high $S_{c/o}$ and low K_c^{air}, as well as the constitutive low g_m of Antarctic plants, are not their unique traits, resembling those documented for drought-adapted species. The presence of bundle sheaths found in *D. antarctica* and also in *C. quitensis* (Vieira and Mantovani, 1995) is a characteristic of plants from xeric climates (Sage, 2001). In addition, Montiel *et al.* (1999) reported remarkably high values of instantaneous WUE in *D. antarctica*, more typical of C_4 and Crassulacean acid metabolism (CAM) species than of C_3 species. The high WUE of *D. antarctica* is likely to be related to the diffusive and biochemical determinants identified in the present study.

Concluding remarks

The present study provides new field data on the photosynthetic performance and diffusive and biochemical limitations of the two Antarctic plants. This work incorporates leaf anatomical traits related to CO_2 assimilation and increases the range of knowledge of the diversity of Rubisco kinetics parameters in two relevant species.

The ultrastructural traits of the leaf mesophyll in field-grown Antarctic plants ultimately restricted the CO_2 leaf transfer capacity and limited the photosynthetic capacity of these species. Under the cold and dry climate conditions of the Antarctica, the high Rubisco affinity for CO_2 and relatively high values for k_{cat}^{c} seem crucial to optimize carbon assimilation. Overall, these results constitute new insights regarding the functional adaptations to highly stressful conditions in plants and the properties that enable the distribution and abundance of vascular plants in Antarctica. Considering the strong climate changes experienced in the Antarctic Peninsula that include rapid warming during the last decades, and a pause of that warming during recent years (Turner *et al.*, 2016), the next challenge should be to assess the effect of different durations of long-term exposure to warmer temperatures on the photosynthetic performance of *D. antarctica* and *C. quitensis*.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. ANOVA of the effects of populations, measurement temperature, and their interaction.

Fig. S1. *Deschampsia antarctica* (left) and *Colobanthus quitensis* (right).

Fig. S2. The response of the net photosynthetic CO_2 assimilation rate (A_N) to varying internal CO_2 concentration (C_i).

Fig. S3. Transverse section of the mesophyll and mesophyll cells of *D. antarctica*.

Fig. S4. Transverse section of the mesophyll and mesophyll cells of *C. quitensis*.

Fig. S5. The response of the net photosynthetic CO_2 assimilation rate (A_N) to varying chloroplast CO_2 concentration (C_c).

Fig. S6. The relationship between electron transport rate and gross photosynthesis ratio (ETR/A_G) and CO_2 concentration at the site of carboxylation (C_c).

Acknowledgements

This research was funded by The National Fund for Scientific and Technological Development (FONDECYT 11130332), The Associative Research Program of CONICYT (CONICYT-PIA ART-1102), The Chilean Antarctic Institute (INACH AN-02-12 and FI_02-13), and the Spanish Ministry of Science and Innovation (AGL2009-07999 and AGL2013-42364R awarded to JG). LAC acknowledge funds from F ICM P05-02 and PFB-023 supporting the Institute of Ecology and Biodiversity (IEB). The authors thank Dr Charles L. Guy (University of Florida) for English editing and help with grammar. Permits for entrance and plant collection in the ASPA 128 and 115 were provided by INACH.

References

Alberdi M, Bravo LA, Gutiérrez A, Gidekel M, Corcuera LJ. 2002. Ecophysiology of Antarctic vascular plants. Physiologia Plantarum **115,** 479–486.

Badger MR, Collatz GJ. 1977. Studies on the kinetic mechanism of ribulose-1, 5-bisphosphate carboxylase and oxygenase reactions, with particular reference to the effect of temperature on kinetic parameters. Carnegie Institute of Washington Yearbook **76,** 355–361.

Brooks A, Farquhar GD. 1985. Effect of temperature on the CO_2/O_2 specificity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta **165,** 397–406.

Carriquí M, Cabrera HM, Conesa MÀ, *et al.* 2015. Diffusional limitations explain the lower photosynthetic capacity of ferns as compared with angiosperms in a common garden study. Plant, Cell and Environment **38,** 448–460.

Cavieres LA, Sáez P, Sanhueza C, Sierra-Almeida A, Rabert C, Corcuera LJ, Bravo LA. 2016. Ecophysiological traits of Antarctic vascular plants: their importance in the responses to climate change. Plant Ecology **217,** 343–358.

Chapin FS III, Shaver GR. 1985. Arctic. In: Chabot BF, Mooney HA, eds. Physiological ecology of North American plant communities. Dordrecht: Springer Netherlands, 16–40.

Convey P. 1996. The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. Biological Reviews **7,** 191–225.

Convey P, Hopkins D, Roberts S, Tyler A. 2011. Global southern limit of flowering plants and moss peat accumulation. Polar Research **30,** 8929.

De Lucia E, Whitehead D, Clearwater MJ. 2003. The relative limitation of photosynthesis by mesophyll conductance in cooccurring species in a temperate rainforest dominated by conifer *Dacrydium cuperessinum*. Functional.Plant Biology **30,** 1197–1204.

Delgado E, Medrano H, Keys AJ, Parry MAJ. 1995. Species variation in Rubisco specificity factor. Journal of Experimental Botany **46,** 1775–1777.

Edwards JA, Smith RL. 1988. Photosynthesis and respiration of *Colobanthus quitensis* and *Deschampsia antarctica* from the maritime Antarctica. British Antarctic Survey Bulletin **81,** 43–63.

Evans JR, von Caemmerer S, Setchell BA, Hudson GS. 1994. The relationship between CO_2 transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of Rubisco. Functional Plant Biology **21,** 475–495.

Evans JR, Loreto F. 2000. Acquisition and diffusion of CO_2 in higher plant leaves. In: Leegood RC, Sharkey TD, von Caemmerer S, eds. Photosynthesis: physiology and metabolism. Dordrecht: Springer Netherlands, 321–351.

Evans JR, Kaldenhoff R, Genty B, Terashima I. 2009. Resistances along the CO_2 diffusion pathway inside leaves. Journal of Experimental Botany **60,** 2235–2248.

Evert RF, Botha CEJ, Mierzwa RJ. 1985. Free-space marker studies on the leaf of *Zea mays* L. Protoplasma **126,** 62–73.

Fabre N, Reiter IM, Becuwe-Linka N, Genty B, Rumeau D. 2007. Characterization and expression analysis of genes encoding α and β carbonic anhydrases in Arabidopsis. Plant, Cell and Environment **30,** 617–629.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C 3 species. Planta **149,** 78–90.

Flexas J, Barbour MM, Brendel O, *et al*. 2012. Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. Plant Science **193,** 70–84.

Flexas J, Barón M, Bota J, *et al*. 2009. Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (*V. berlandieri*×*V. rupestris*). Journal of Experimental Botany **60,** 2361–2377.

Flexas J, Bota J, Escalona J, Sampol B, Medrano H. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Functional Plant Biology **29,** 461–471.

Flexas J, Diaz-Espejo A, Berry JA, *et al*. 2007. Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. Journal of Experimental Botany **58,** 1533–1543.

Flexas J, Díaz-Espejo A, Conesa MA, *et al*. 2016. Mesophyll conductance to CO_2 and Rubisco as targets for improving intrinsic water use efficiency in C3 plants. Plant, Cell and Environment **39,** 965–982.

Flexas J, Díaz-Espejo A, Gago J, Gallé A, Galmés J, Gulías J, Medrano H. 2014. Photosynthetic limitations in Mediterranean plants: a review. Environmental and Experimental Botany **103,** 12–23.

Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H. 2008. Mesophyll conductance to CO_2: current knowledge and future prospects. Plant, Cell and Environment **31,** 602–621.

Gago J, Coopman RE, Cabrera HM, *et al*. 2013. Photosynthesis limitations in three fern species. Physiologia Plantarum **149,** 599–611.

Galmés J, Andralojc PJ, Kapralov MV, *et al*. 2014*b*. Environmentally driven evolution of Rubisco and improved photosynthesis and growth within the C_3 genus *Limonium* (Plumbaginaceae). New Phytologist **203,** 989–999.

Galmés J, Flexas J, Keys AJ, *et al*. 2005. Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. Plant, Cell and Environment **28,** 571–579.

Galmés J, Hermida-Carrera C, Laanisto L, Niinemets Ü. 2016. A compendium of temperature responses of Rubisco kinetic traits: variability among and within photosynthetic groups and impacts on photosynthesis modeling. Journal of Experimental Botany **67,** 5067–5091.

Galmés J, Kapralov MV, Andralojc PJ, Conesa MÀ, Keys AJ, Parry MA, Flexas J. 2014*a*. Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. Plant, Cell and Environment **37,** 1989–2001.

Galmés J, Kapralov MV, Copolovici LO, Hermida-Carrera C, Niinemets Ü. 2015. Temperature responses of the Rubisco maximum carboxylase activity across domains of life: phylogenetic signals, trade-offs, and importance for carbon gain. Photosynthesis Research **123,** 183–201.

Galmés J, Perdomo JA, Flexas J, Whitney SM. 2013. Photosynthetic characterization of Rubisco transplantomic lines reveals alterations on photochemistry and mesophyll conductance. Photosynthesis Research **115,** 153–166.

Gielwanowska I, Szczuka E. 2005. New ultrastructural features of organelles in leaf cells of *Deschampsia antarctica* Desv. Polar Biology **28,** 951–955.

Harley PC, Loreto F, Di Marco G, Sharkey TD. 1992. Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. Plant Physiology **98,** 1429–1436.

Hermida-Carrera C, Kapralov MV, Galmés J. 2016. Rubisco catalytic properties and temperature response in crops. Plant Physiology **171,** 2549–2561.

Jellings AJ, Usher MB, Leech RM. 1983. Variation in the chloroplast to cell area index in *Deschampsia antarctica* along a 16 degrees latitudinal gradient. British Antarctic Survey Bulletin **61,** 13–20.

Kogami H, Hanba YT, Kibe T, Terashima I, Masuzawa T. 2001. CO_2 transfer conductance, leaf structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and high altitudes. Plant, Cell and Environment **24,** 529–538.

Körner C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. 2nd edn. Berlin: Springer.

Kubien DS, Sage RF. 2008. The temperature response of photosynthesis in tobacco with reduced amounts of Rubisco. Plant, Cell and Environment **31,** 407–418.

Montiel P, Smith A, Keiller D. 1999. Photosynthetic responses of selected Antarctic plants to solar radiation in the southern maritime Antarctic. Polar Research **18,** 229–235.

Niinemets Ü. 1999. Research review. Components of leaf dry mass per area—thickness and density—alter leaf photosynthetic capacity in reverse directions in woody plants. New Phytologist **144,** 35–47.

Niinemets U, Díaz-Espejo A, Flexas J, Galmés J, Warren CR. 2009. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. Journal of Experimental Botany **60,** 2249–2270.

Niinemets Ü, Keenan TF. 2014. Photosynthetic responses to stress in Mediterranean evergreens: mechanisms and models. Environmental and Experimental Botany **103,** 24–41.

Niinemets Ü, Reichstein M. 2003. Controls on the emission of plant volatiles through stomata: a sensitivity analysis. Journal of Geophysical Research **108,** 4211.

Niinemets Ü, Sack L. 2006. Structural determinants of leaf light-harvesting capacity and photosynthetic potentials. In: Esser K, Lüttge U, Beyschlag W, Murata J, eds. Progress in botany. Berlin: Springer: 385–419.

Nobel PS. 1980. Leaf anatomy and water use efficiency. In: Turner NC, Kramer PJ. eds. Adaptation of plant to water and high temperature stress. New York: Wiley, 43–55.

Orr DJ, Alcântara A, Kapralov MV, Andralojc PJ, Carmo-Silva E, Parry MA. 2016. Surveying rubisco diversity and temperature response to improve crop photosynthetic efficiency. Plant Physiology **172,** 707–717.

Peguero-Pina JJ, Flexas J, Galmés J, *et al*. 2012. Leaf anatomical properties in relation to differences in mesophyll conductance to CO_2 and photosynthesis in two related Mediterranean *Abies* species. Plant, Cell and Environment **35,** 2121–2129.

Peguero-Pina JJ, Sancho-Knapik D, Flexas J, Galmés J, Niinemets Ü, Gil-Pelegrín E. 2015*a*. Light acclimation of photosynthesis in two closely related firs (*Abies pinsapo* Boiss. and *Abies alba* Mill.): the role of

leaf anatomy and mesophyll conductance to CO_2. Tree Physiology **36,** 300–310.

Peguero-Pina JJ, Sisó S, Fernández-Marín B, Flexas J, Galmés J, García-Plazaola JI, Niinemets Ü, Sancho-Knapik D, Gil-Pelegrín E. 2015*b*. Leaf functional plasticity decreases the water consumption without further consequences for carbon uptake in Quercus coccifera L. under Mediterranean conditions. Tree Physiology **36,** 356–367.

Pérez-Torres E, Bascuñán L, Sierra-Almeida A, Bravo LA, Corcuera LJ. 2006. Robustness of activity of Calvin cycle enzymes after high light and low temperature conditions in Antarctic vascular plants. Polar Biology **29,** 909–916.

Pérez-Torres E, Bravo LA, Corcuera LJ, Johnson GN. 2007. Is electron transport to oxygen an important mechanism in photoprotection? Contrasting responses from Antarctic vascular plants. Physiologia Plantarum **130,** 185–194.

Prins A, Orr D, Andralojc P, *et al*. 2016. Rubisco catalytic properties of wild and domesticated relatives provide scope for improving wheat photosynthesis. Journal of Experimental Botany **67,** 1827–1838.

Pyykkö M. 1966. The leaf anatomy of East Patagonian xeromorphic plants. Annales Botanici Fennici **3,** 453–622.

Raven JA. 2000. Land plant biochemistry. Philosophical Transactions of the Royal Society B: Biological Sciences **355,** 833–846.

Romero M, Casanova A, Iturra G, Reyes A, Montenegro G, Alberdi M. 1999. Leaf anatomy of *Deschampsia antarctica* (Poaceae) from the Maritime Antarctic and its plastic response to changes in the growth conditions. Revista Chilena de Historia Natural **72,** 411–425.

Sage RF. 2001. Environmental and evolutionary preconditions for the origin and diversification of the C_4 photosynthetic syndrome. Plant Biology **3,** 202–213.

Sage RF. 2002. Variation in the k_{cat} of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature. Journal of Experimental Botany **53,** 609–620.

Sharwood RE, Ghannoum O, Kapralov MV, Gunn LH, Whitney SM. 2016. Temperature responses of Rubisco from Paniceae grasses provide opportunities for improving C3 photosynthesis. Nature Plants **2,** 16186.

Smith RIL. 1993. Dry coastal ecosystems of Antarctica. In: van der Maarel E, ed. Ecosystems of the world. Amsterdam: Elsevier, 51–71.

Smith RIL. 2003. The enigma of *Colobanthus quitensis* and *Deschampsia antarctica* in Antarctica. In: Huiskes AHL, Gieskes WWC, Rozema J, Schorno RML, van der Vies SM, Wolff WJ, eds. Antarctic biology in a global context. Leiden: Backhuys Publishers, 234–239.

Tcherkez GG, Farquhar GD, Andrews TJ. 2006. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proceedings of the National Academy of Sciences, USA **103,** 7246–7251.

Terashima I, Araya T, Miyazawa S, Sone K, Yano S. 2005. Construction and maintenance of the optimal photosynthetic systems of the leaf, herbaceous plant and tree: an eco-developmental treatise. Annals of Botany **95,** 507–519.

Terashima I, Hanba YT, Tazoe Y, Vyas P, Yano S. 2006. Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. Journal of Experimental Botany **57,** 343–354.

Terashima I, Miyazawa SI, Hanba YT. 2001. Why are sun leaves thicker than shade leaves? Consideration based on analyses of CO_2 diffusion in the leaf. Journal of Plant Research **114,** 93–105.

Terashima I, Ono K. 2002. Effects of $HgCl_2$ on CO_2 dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO_2 diffusion across the plasma membrane. Plant and Cell Physiology **43,** 70–78.

Tomás M, Flexas J, Copolovici L, *et al*. 2013. Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: quantitative limitations and scaling up by models. Journal of Experimental Botany **64,** 2269–2281.

Tosens T, Niinemets U, Vislap V, Eichelmann H, Castro Díez P. 2012. Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in Populus tremula: how structure constrains function. Plant, Cell and Environment **35,** 839–856.

Truesdale GA, Downing AL. 1954. Solubility of oxygen in water. Nature **173,** 1236.

Turner T, Lachlan-Cope A, Marshall GJ, Morris EM, Mulvaney R, Winter W. 2002. Spatial variability of Antarctic Peninsula net surface mass balance. Journal of Geophysical Research **107,** 4173.

Turner J, Lu H, White I, *et al*. 2016. Absence of 21st century warming on Antarctic Peninsula consistent with natural variability. Nature **535,** 411–415.

Vieira RC, Mantovani A. 1995. Leaf anatomy of *Deschampsia antarctica* Desv. (Gramineae). Revista Brasileira de Botânica **18,** 207.

Warren CR. 2008. Does growth temperature affect the temperature responses of photosynthesis and internal conductance to CO_2? A test with Eucalyptus regnans. Tree Physiology **28,** 11–19.

Xiong FS, Ruhland CT, Day TA. 1999. Photosynthetic temperature response of the Antarctic vascular plants *Colobanthus quitensis* and *Deschampsia antarctica*. Physiologia Plantarum **106,** 276–286.

Yamori W, Noguchi K, Hanba YT, Terashima I. 2006. Effects of internal conductance on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. Plant and Cell Physiology **47,** 1069–1080.

Yamori W, Noguchi K, Hikosaka K, Terashima I. 2009. Cold-tolerant crop species have greater temperature homeostasis of leaf respiration and photosynthesis than cold-sensitive species. Plant and Cell Physiology **50,** 203–215.

Yamori W, Noguchi KO, Terashima I. 2005. Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant, Cell and Environment **28,** 536–547.

Yokota A, Canvin DT. 1985. Ribulose bisphosphate carboxylase/oxygenase content determined with [^{14}C]carboxypentitol bisphosphate in plants and algae. Plant Physiology **77,** 735–739.

Young JN, Rickaby RE, Kapralov MV, Filatov DA. 2012. Adaptive signals in algal Rubisco reveal a history of ancient atmospheric carbon dioxide. Philosophical Transactions of the Royal Society B: Biological Sciences **367,** 483–492.