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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Carbohydrate reserves in the facilitator cushion plant *Laretia acaulis* suggest carbon limitation at high elevation and no negative effects of beneficiary plants

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Abstract The elevational range of the alpine cushion plant *Laretia acaulis* (Apiaceae) comprises a cold upper extreme and a dry lower extreme. For this species, we predict reduced growth and increased non-structural carbohydrate (NSC) concentrations (i.e. carbon sink limitation) at both elevational extremes. In a facilitative interaction, these cushions harbor other plant species (beneficiaries). Such interactions appear to reduce reproduction in other cushion species, but not in *L. acaulis*. However, vegetative effects may be more important in this long-lived species and may be stronger under marginal conditions. We studied growth and NSC concentrations in leaves and stems of *L. acaulis* collected from cushions along its full elevational range in the Andes of Central Chile. NSC concentrations were lowest and cushions were smaller and much less abundant at the highest elevation. At the lowest elevation, NSC concentrations and cushion sizes were similar to those of intermediate elevations but cushions were somewhat less abundant. NSC concentrations and growth did not change with beneficiary cover at any elevation. Lower NSC concentrations at the upper extreme contradict the sink-limitation hypothesis and may indicate that a lack of warmth is not limiting growth at high-elevation. At the lower extreme, carbon gain and growth do not appear more limiting than at intermediate elevations. The lower population density at both extremes suggests that the regeneration niche exerts important limitations to this species' distribution. The lack of an effect of beneficiaries on reproduction and vegetative performance suggests that the interaction between *L. acaulis* and its beneficiaries is probably commensalistic.

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Introduction

The steep elevational gradients in mountain regions result in substantial environmental changes within a few kilometers of distance, with higher elevations experiencing lower temperatures, shorter growing seasons, higher solar radiation and higher wind speeds (Billings and Mooney 1968; Körner 2003). Generally, at the extremes of the elevational distribution of a plant species establishment and/or growth vigor tend to decline. The mechanisms by which plant growth is limited under such circumstances are still under discussion, a prime example being alpine treelines, which represent the elevational limit of an elsewhere dominant

life form (Körner 2012). A likely explanation for treelines focusses on the atmospheric coupling of trees due to their tall stature, preventing sufficient tissue warming to allow growth.

In trees, reduced growth at treeline appears to be due to direct low-temperature restrictions on biosynthesis rather than on carbon gain (the growth-limitation, or sink-limitation hypothesis, e.g. Hoch and Körner 2003; Piper et al. 2006). Under other stress conditions, e.g. drought, carbon gain likewise does not appear to limit tree growth, as indicated by stable or even increased levels of non-structural carbohydrates (NSC) observed in trees in stressful habitats (Hoch 2015). However, these observations are not universal and the dynamics of NSC in plants, including trees at the alpine treeline, are still a topic of hot debate (e.g. Wiley and Helliker 2012; Fajardo and Piper 2014).

Cushion plants are low stature plants with tightly knit canopies that privilege horizontal growth (Aubert et al. 2014). This growth form maximizes decoupling from the atmosphere and allows cushion plants to be well adapted to the stressful conditions found in the alpine zone (Billings and Mooney 1968; Körner 2003). As their tissues are much less coupled to the atmosphere than in trees, cushion plants may warm up far above the free-air temperature when growing in sunny microsites (Körner 2003). Nonetheless, the upper elevational species limit may still be related to low temperature, although it is unclear whether low-temperature extremes or a lack of warmth constitute the main problem. Conversely, in some mountains lower elevations are associated with drought conditions (Cavieres et al. 2006), which may limit photosynthesis and/or growth (Piper et al. 2016). If, at either stressful end of the distributional range, biosynthesis is more limited than carbon gain, NSC concentrations are predicted to increase with stress while growth decreases (Fajardo et al. 2012; Sala et al. 2012; Klein et al. 2014; Hoch 2015). In contrast, if photosynthesis limits growth under stressful conditions, this should decrease NSC concentrations and/or growth.

The relatively stable temperature and moisture conditions inside cushions compared to the surrounding bare soil (Hauri 1913; Gibson and Kirkpatrick 1985; Hager and Faggi 1990) may extend the habitat of other species that otherwise would not be able to survive (Cavieres et al. 2014). Hence, cushion plants often act as facilitators and can play an important role in plant community composition and biodiversity at the landscape level (Cavieres et al. 2016). However, the effect of beneficiaries on the facilitating cushion may also affect cushion fitness, which would indicate that the facilitation comprises a parasitic relationship. If being a facilitator negatively affects cushion fitness (e.g. Michalet et al. 2011; Schöb et al. 2014b), this can have consequences for the stability of the interaction and for the whole plant community.

Several studies suggest that beneficiaries have negative effects on cushion species (e.g. Michalet et al. 2011; Cranston et al. 2012; Schöb et al. 2014a). Such reciprocal effects of facilitation may be related to resource (water, nutrients and space) competition (Holzapfel and Mahall 1999) or alterations in the cushion's microclimate. These negative effects may not be the same along the elevational distribution of the species. At dry distributional limits, competition for water may cause stronger negative effects on the vegetative performance of cushion plants (Michalet et al. 2016). Also, the beneficiary plants protruding from the smooth cushion surface disturb the natural boundary layer, which can increase transpiration and can cause cooling of the cushion surface. These changes in microclimate caused by the beneficiaries may also cause stronger negative effects at both the dry and the cold distributional limits.

Laretia acaulis, a cushion plant growing in the High Andes of Central Chile, is an example of a strong facilitator (Cavieres et al. 2006). In its natural habitat, high mountains with a Mediterranean-type rainfall seasonality, this species faces increasing drought conditions at lower elevations and increasingly low temperatures at higher elevations (Cavieres et al. 2006). In a recent study of facilitation by *L. acaulis* we found that along its elevational range there are no sustained effects of beneficiaries on the cushions' reproductive output (García et al. 2016), although such an effect has been suggested for other cushion species (e.g. Cranston et al. 2012; Al Hayek et al. 2014; Schöb et al. 2014a; Sortibrán et al. 2014). However, since *L. acaulis* is a very slow-growing and long-lived species, growth-effects may be more important for the fitness of this cushion than annual seed production. Unfortunately, to our knowledge nothing is known about the effects of beneficiaries on vegetative growth and survival in cushion plants.

In this study we aimed at quantifying NSC concentrations and growth, as two indicators for vegetative performance, in *L. acaulis* along its whole elevational distribution and as a function of beneficiary cover. We expected that NSC concentrations should increase towards the extreme ends (lower and upper) of the gradient, while growth should be reduced at both ends. Additionally, we expected an effect of beneficiaries on these two vegetative-performance parameters and changes in these effects along the elevational gradient. We hypothesized that there would be stronger negative effects at the extreme ends of the gradient, due to higher competition for water at the lower end and stronger disadvantages from disturbance of the boundary layer at both ends.

Methods

Study site

Our study was carried out in the High Andes of Central Chile at La Parva ca. 50 km NE of Santiago de Chile. We selected four sites of northwestern exposure and comparable slope along the entire elevational gradient of the known distribution of *L. acaulis* (2600, 2800, 3000, 3150 m a.s.l.). The sites experience a high-alpine climate with strong influence of the Mediterranean-type climate of lower elevations (with winter precipitation, mainly as snow, and a progressive drought in summer; Di Castri and Hajek 1976). Continuous snow cover lasts from June to September, but varies according to slope exposition and elevation (Rozzi et al. 1989). Snowmelt at the lowest site starts three to four weeks earlier than that at highest elevation. The start of the growing season thus differs between elevations, but the phenological lag between elevations becomes reduced during the season. We observed a difference of 2 weeks in the duration of the reproductive phase between elevations. Leaf senescence started in April/May. In the summer season of 1995–1996, mean air temperature of the warmest months (December to March) was 10.5 °C at 2600 m a.s.l. and 6.5 °C at 3600 m a.s.l. (Cavieres and Arroyo 1999). Maximum daily temperatures were registered in January and February, and mean air temperature decreased gradually with elevation and towards the end of the season (Cavieres et al. 2007).

Study species

Laretia acaulis (Apiaceae) is a perennial, compact cushion plant with a deep tap-root system. The entire cushion is made up by numerous rosettes (a circular arrangement of five to six lanceolate leaves of 15–25 mm) (Martinez 2003). Leaves senesce at the end of each growing season and stems are covered with old leaf remains, leading to an accumulation of dead organic matter below the cushion surface. Adventitious roots may access this resource (pers. obs.). Inflorescences are umbels with the number of flowers differing according to sex.

The growing season for *L. acaulis* and for most of the associated species starts after snowmelt at the end of October and finishes in April (Arroyo et al. 1981; Cavieres et al. 2007). Reproduction in *L. acaulis* begins at the end of December with floral bud emergence (phenological time lag is 2–3 weeks between 2600 and 3150 m a.s.l.) and finishes with detachment of fruits at the end of April.

Laretia acaulis cushions can reach up to 10 m² in size, with an increasing proportion of larger cushions

(>1 m²) at 3000 m a.s.l. At higher elevations the species is gradually replaced by *Azorella monantha* (Armesto et al. 1980). Growth rates in *L. acaulis* are unknown, but data from other cushion species with similar growth forms suggest very slow annual growth, at the order of a few millimeters of horizontal or radial increment (Ralph 1978; Halloy 2002; Kleier and Rundel 2004).

Sampling

Samples from 2012 and 2013 were used to evaluate growth and NSC concentrations, while samples of 2014 were used exclusively to test differences in NSC concentrations along the gradient and between the beginning and end of the growing season. In 2014, we collected samples twice: first, samples were taken at the beginning of the growing season (10-Dec), after full expansion of the leaves but before initiation of the flower buds, and second, at the end (20-Mar), after fruits had been dropped but before leaf senescence (same phenological stages as in 2012 and 2013). At the beginning of the growing season, the cushions at the higher elevations were less developed than those at the lower elevations so that these early-season data (2014) were not compared between elevations.

At each elevation, we selected ten similar-sized cushions (ca. 1 m²) with low beneficiary plant cover (<10% of cover), ten cushions with high cover (between 40 and 50% cover) and ten cushions without beneficiaries, as these were cut in the previous season and maintained free of plants during the rest of the growing season (see García et al. 2016). Samples were collected at 2800 m a.s.l. in 2012 (10-Mar) to test the method, while the sampling was completed for the other three elevations in 2013 (28-Mar). Sampling was repeated for ten cushions at each elevation in 2014 (see above) to study seasonal differences and the consistency of the elevational pattern between years.

In 2012 and 2013, samples of leaves and stems (1–3 cm below the rosette) of each cushion were taken from three 10 cm × 10 cm quadrats at increasing distance from beneficiary plants: directly adjoining (S1), at 20 cm (S2) and at 40 cm (S3) to evaluate local effects of beneficiaries on growth and NSC concentrations. On cushions without beneficiaries, we selected sub-quadrants randomly. For all sub-quadrants we counted and weighed the rosettes to determine dry foliar biomass density (g cm⁻²) and rosette weight (g rosette⁻¹). Subsequently, stems and leaves were used to measure NSC concentrations (see below). In 2014, we randomly collected ten rosettes and one stem portion (3 cm below the leaves) per cushion. All samples were collected during the morning to minimize the effect of diurnal fluctuations in NSC.

All tissue samples were rinsed with water to remove soil remains and micro-waved for 30 s as soon as we arrived

at the refuge site, on the day of collection, in order to stop enzymatic activity and breakdown of stored carbohydrates. Then, samples were dried at 80 °C and stored frozen at −4 °C. During transportation and during a refrigerator breakdown, samples were unfrozen for several weeks. However, as samples were completely dry, this is unlikely to have affected NSC concentrations (Fick and Nolte 1986).

We could not directly measure radial cushion growth because our markers were moved by periglacial soil movements. We did measure cushion sizes (length x width) and population density in a 50 m × 50 m area at each elevation.

For valid comparisons of NSC concentrations, plants should be sampled at the same phenological stage (e.g. just after bud-break, i.e. later at higher elevations) or sampling should take place after shoot growth and leaf maturation have finished and carbohydrate levels are more constant (i.e. late in the growing season, but before senescence starts, as this may start earlier at higher elevation: Hoch 2015). Samples to study the elevational gradient were, therefore, collected at the end of the growing season, but still when fully green.

Similarly important is the type of organs studied. Generally, NSC concentrations in trees vary much more in leaves and branches than in stems, and carbohydrates allocated to reproduction are those produced and temporarily stored in neighboring leaves and branches (Kozlowski and Pallardy 1997; Hoch and Körner 2003; Schädel et al. 2009). Consequently, we expect branch and leaf NSC concentrations to depend largely on local carbon dynamics. Even though leaves senesce at the end of the growing season and are thus no long-term storage organs, foliar NSC concentration indicates how much is being produced and available for translocation to the rest of the plant. In cushion plants, we consider stem sections just below leaves to be functionally equivalent to branches. Although roots may also serve as a NSC reserve organ, logistically it was not possible to collect root tissue because this would have caused serious damage to the plants. We collected samples of green leaves (produced during the current growing season) and stems.

NSC analysis

It has been recently shown that the results of NSC measurements can differ widely between methods and research labs (Quentin et al. 2015), so that cross-lab comparisons of absolute values are difficult without cross-calibration. Within labs, however, values should be comparable. We determined all NSC concentrations at the Functional Ecology group of Oldenburg University using the same protocol: About 20 mg of ground sample was mixed with distillated water and heated at 80 °C for 30 min. After three consecutive centrifugation and dilution steps, the supernatant was used for measuring soluble sugar concentrations

(fructose, glucose, maltose, sucrose) via HPLC (ICS-3000, Dionex Corporation, Sunnyvale, California). For starch, each residual from the first extract was mixed with amylase and amyloglucosidase, distilled water and acetate buffer to breakdown starch into glucose. Again, after three consecutive centrifugation and dilution steps, starch concentration was measured using HPLC. Concentrations (mg g^{-1}) of each sugar were determined via comparison with standards. Total NSC concentration is the sum of all carbohydrates.

Data analysis

All the analyses and figures were prepared in R 2.13.1 (R Core Team 2013).

Comparison of NSC concentrations in leaves and stems of *L. acaulis* between the beginning and end of the season and along the elevational gradient were analyzed using a nested ANOVA with the data of 2014. Response variables were fructose, glucose, maltose, sucrose, starch, total soluble sugars or total NSC concentrations in leaves and stems or foliar biomass density (g cm^{-2}). These variables were log normalized when it was necessary. We used a mixed model (l mm in R) with cushion id as the random factor to deal with temporal replication and pseudo-replication from using several plots per cushion.

Kruskal–Wallis and Dunn post hoc tests were used to compare cushion size and abundance between sites (employing the kruskalmac function from the R package pgirmess; Giraudoux 2011), as cushion size and abundance per site were not normally distributed (Fig. 2 and Table S3).

The relationship between foliar biomass density (expressing vegetative growth) and NSC concentrations (each sugar, starch and total) in leaves and stems of *L. acaulis* was evaluated with analyses of covariance (ANCOVA). Explanatory variables were log normalized when it was necessary (Table 2). Further explanatory variables were elevation, beneficiary cover and their interactions.

The effect of beneficiary plant cover (high, low and missing) and distance from beneficiaries (sub-quadrants: S1, S2 and S3) on NSC concentrations and growth expressed as foliar biomass density were evaluated using an ANOVA with the data of 2012 and 2013. The analyses were done separately for the site at 2800 m a.s.l. (samples from 2012). For samples from 2013 (three sites: 2600, 3000 and 3150 m a.s.l.), the models also included elevation and its interactions with beneficiary cover and distance from beneficiaries. Explanatory variables were square-root normalized when it was necessary. We used linear mixed models (lmm, from R package lme4: Bates et al. 2014) and cushion id was used as random factor to deal with pseudo-replication. We analyzed 28 models, i.e. for each carbohydrate fraction, for leaves and stems and for two sets of years (2012–2013). Therefore, we applied a Bonferroni

correction by setting the upper α to 0.05/28 = 0.002, i.e. at p values <0.002, effects were considered significant (Tables S5 and S6).

All response variables were normalized where necessary. In all analyses we selected the most parsimonious models by deleting first non-significant interactions and then successively those variables explaining the least variation and comparing the original and simplified models. To obtain p-values for model differences, we used a type II ANOVA with the function Anova from the R package Car (Fox et al. 2011), with an F or Chisq test depending on the model. These test-results are reported, whereby F and Chisq statistics refer to the difference between the model with and without this parameter, after removal of all less-significant effects (Tables 1, 2, S1, S2, S4, S5 and S6). For models with significant differences we did an a posteriori evaluation using lsmean (Lenth and Hervé 2014).

Results

Spatial and temporal variation of NSC in *L. acaulis*

NSC concentrations were quite dynamic temporally, both between and within years (Fig. 1, S2). In general, leaves had significantly higher total soluble sugar and starch concentrations at the beginning of the season than at the end, while this pattern was mostly reversed in both years for stems (Fig. 1, S1 and Table S1). As a result, NSC concentrations were higher in stems than in leaves in autumn. For leaves, this seasonal pattern was consistent among the measured carbohydrates (Fig. S2). For stems, the higher soluble sugar concentrations in autumn were caused by higher sucrose levels, while the other sugars did not differ or, for maltose, showed lower concentrations than in spring.

Table 1 Means of foliar biomass parameters: biomass per area (g cm^{-2}), biomass per rosette (g rosette^{-1}) and number of rosettes per area (rosettes cm^{-2}), of *Laretia acaulis* along an elevational gradient (2600, 3000, 3150 m a.s.l.) in the Andes of Central Chile

	Elevation (m a.s.l.)		
	2600	3000	3150
Foliar biomass (g cm^{-2})	0.06a	0.05b	0.06a
Foliar biomass (g rosette^{-1})	0.11a	0.10b	0.10b
Rosettes cm^{-2}	0.66b	0.68b	0.75a

Data were taken at the end of the growing season in 2013. Data were analyzed with a linear mixed model (l mm) and cushion id was used as random factor. Letters indicate significant differences between elevations according to a Tukey test α < 0.05. Number of cushions per elevation = 30, with three samples per cushion. Further tested co-variables were beneficiary cover and distance from beneficiaries (directly adjoining, at 20 cm and at 40 cm), but theses co-variables were not retained in the final most parsimonious model

Because carbohydrate levels can fluctuate strongly with phenology early in the season, we compared elevational patterns only for the end-of-season data. At the end of the season in 2014, cushions from the highest elevation had the lowest starch and soluble sugars levels in both leaves and stems (Fig. 1). This is consistent with the pattern found in 2013 (Fig. S1). In 2014, stem NSC differed little between the other elevations, while in 2013 no data were gathered at 2800 m (data for 2800 m in Fig. S1 is from 2012) so that these cannot be compared between all elevations. Leaf NSC was highest at the intermediate elevation of 3000 m in both years (excluding the 2800 m a.s.l.; Figs. 1 and 4). At the beginning of the season, leaf sucrose (and hence soluble sugar) concentrations were highest at the highest elevation (3150 m a.s.l.), while in stems they were lowest (Fig. 1). At the same time, leaf starch concentrations in leaves were very low at this elevation. This is probably due to the fact that leaf development started a few weeks later than at lower sites.

Growth patterns along the elevational gradient

Median cushion size differed along the elevational gradient (Kruskal–Wallis, $\chi^2 = 35.7$, df = 3, $p < 0.001$). Cushion area was significantly smaller at the highest elevation (Kruskal–Wallis, post hoc testing, $p < 0.05$, Fig. 2 and Table S3). Cushion numbers were lowest at the highest site (212 cushion ha^{-1}), whereas they were around 1500 ha^{-1} at the intermediate sites and 1040 ha^{-1} at the lowest site, respectively.

The foliar biomass per cushion area (foliar density of green leaves, in g cm^{-2}) was highest at the extreme elevations (2600 and 3150 m; Table 1, not measured at 2800 m). This is due to a denser packing of small, thick-leaved rosettes, while at the lowest elevation this was due to larger rosettes.

There was a positive relationship between foliar biomass density (g cm^{-2}) and starch concentration in leaves and stems at each of the three elevations tested (Fig. 3 and Table S4). There was also a positive relationship between foliar biomass density and sucrose concentration in leaves, but not in the case of the other sugars (data not shown).

Effects of beneficiary cover

NSC concentrations at the end of the growing season differed little between cushions with high, low or no beneficiary cover, with most carbohydrates showing no differences (Figs. 4, S3, S4 and Tables S5, S6). During 2012, maltose concentrations in leaves at 2800 m a.s.l. were significantly lower in cushions without beneficiaries, while in 2013 they were highest in these cushions and lowest in cushions with high beneficiary cover, but
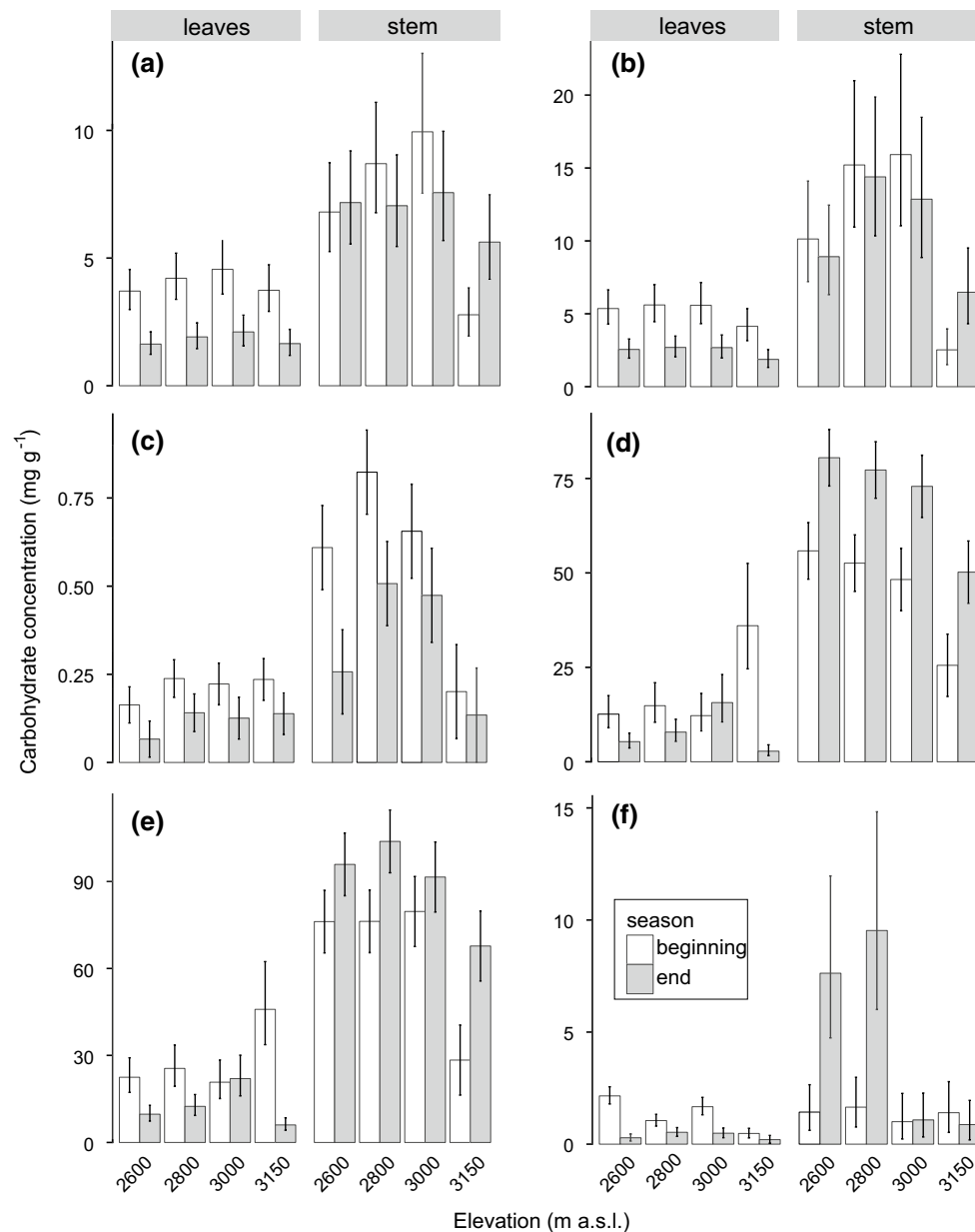
Fig. 1 NSC concentrations of **a** fructose, **b** glucose, **c** maltose, **d** sucrose, **e** soluble NSC and **f** starch in leaves and stems of *Laretia acaulis* at the beginning and end of the growing season and along an elevation gradient (2600–3150 m a.s.l.) in the Andes of Central Chile. Shown are means and 95% confidence intervals. Non-overlapping error bars indicate significant differences at $\alpha < 0.05$. Number of cushion pair samples was 31 for leaves and 35 for stems. Data correspond to the 2014 growing season

only at 3000 m a.s.l. while the other elevations showed no clear pattern (Figs. 4, and S4). In both 2012 and 2013, starch concentrations in stems were lowest in cushions with high beneficiary cover. However, for 2012 this was not significant, while for 2013 it was significant only for the pooled data from all elevations and no differences were detected when considering each elevation separately (Fig. 4). NSC concentrations in leaves and stems were not affected by beneficiaries' distance, so this variable was excluded from all the models (Table S5 and S6).

Foliar biomass per area also did not differ with beneficiary cover or distance (Table S2).

Discussion

If growth under stressful conditions was limited by carbon gain, we would expect lower NSC concentrations at the extremes of a stress gradient. If, as has repeatedly been found for trees, NSC concentrations do not change or
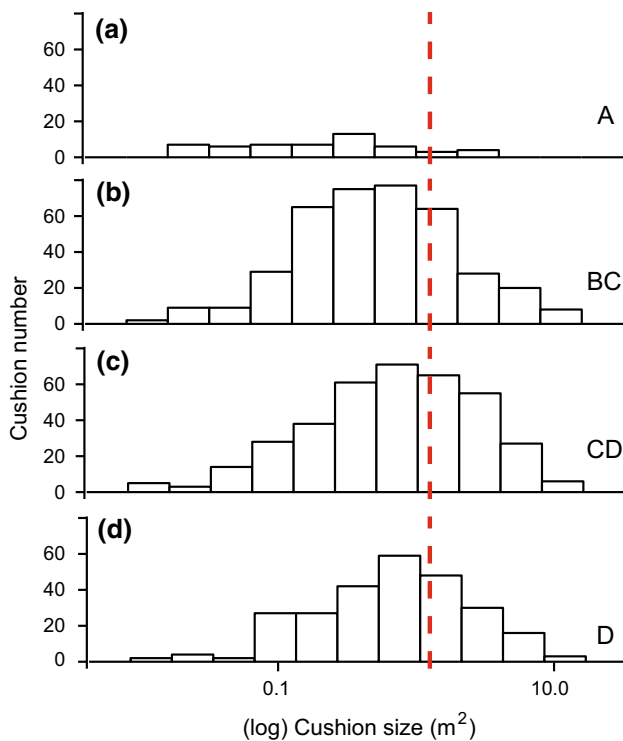
Fig. 2 Histograms of the size distribution of *Laretia acaulis* cushions in 50 m × 50 m plots at **a** 3150, **b** 3000, **c** 2800 and **d** 2600 m a.s.l. in the Andes of Central Chile. Capital letters *A*, *BC*, *CD* and *D* denote significant differences between sites according to Kruskal–Wallis post hoc testing, $p < 0.05$. The dashed red line represents the mean cushion size of all cushions. Number of cushions: $n = 260$ at 2600, $n = 373$ at 2800, $n = 386$ at 3000 and $n = 53$ at 3150 m a.s.l. Note the log-scale of the abscissa

Fig. 3 Relation of foliar biomass density per area and starch concentrations in **a** leaves and **b** stems of *Laretia acaulis* along an elevational gradient in the Andes of Central Chile. Different line styles indicate different elevations. See table S4 for model significances. Number of sampled cushions per elevation $n = 30$. Each symbol represents the mean of three 10 cm × 10 cm quadrants. Note the log-scale of the abscissa

even increase under adverse environmental conditions, it can be assumed that these conditions do not cause carbon-gain limitation (Fajardo et al. 2012; Hoch 2015). In *Laretia acaulis* cushions, NSC concentrations did not change towards the dry lower end of the elevational distribution, but they were lowest and cushions were smallest at the cold upper end. Beneficiaries did not affect carbohydrate stores or growth at any elevation.

Lower elevation limitations

Thus, at the lowest elevation, growth does not seem to be restricted as much or for the same reason as at the highest elevation, raising the question whether this lower species distribution limit is possibly set by biotic processes rather than physiological limitations. Competition may play a role, as shrubs are more abundant than cushions at this lower elevation and have higher growth rates. With their canopies and large root systems they may affect cushion performance (Armesto et al. 1980), although the lack of a beneficiary effect suggests that the cushions are relatively
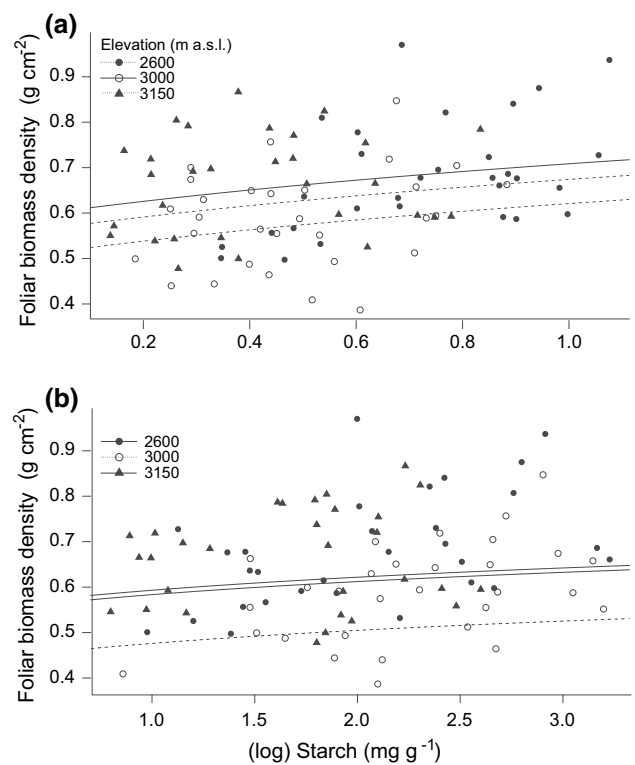
insensitive to competition. Interestingly, although NSC concentrations and growth were not reduced at the lowest elevation, population densities were lower at both extremes.

This suggests that apart from growth, or at the lowest elevation, instead of growth, establishment limitations may determine the elevational range of this species. A similar pattern was found for this area by Armesto et al. (1980) so that it appears that this decline towards the range edges is a general pattern. In the context of the reciprocity of facilitation, this population pattern also highlights the need to understand the effect of facilitation from other plant species or existing cushions on cushion-plant establishment chances and population dynamics.

Similar to our result for the lowest elevation, many studies have found increased NSC concentrations in plants exposed to mild or short drought conditions and concluded that the observed growth decrease was not caused by carbon limitation (e.g. Sala and Hoch 2009; Mitchell et al. 2013). Only under severe drought, NSC reserves are often depleted (though never completely) before the drought becomes lethal (Adams et al. 2013; Klein et al. 2014). However, drought-related changes in NSC concentrations
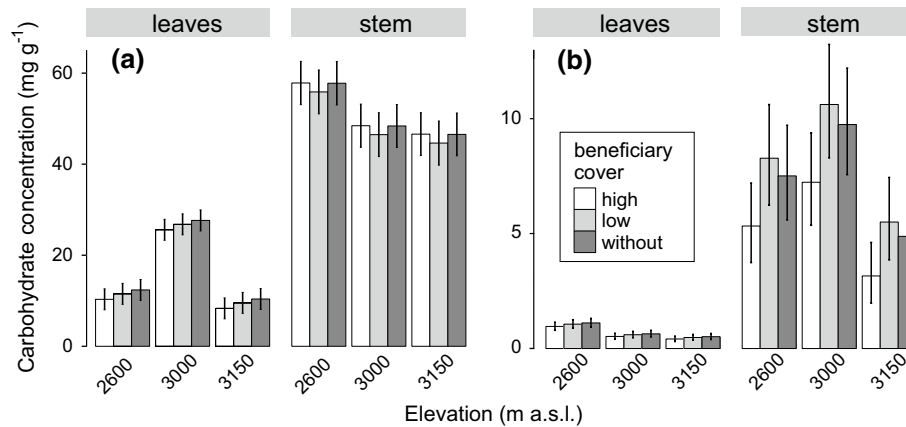
Fig. 4 Carbohydrate concentrations of **a** total soluble sugars and **b** starch in leaves and stems of *Laretia acaulis* with three levels of beneficiary cover: high, low and without (pruning) and along an elevation gradient (2600, 3000, 3150 m a.s.l.) in the Andes of Central Chile. Data correspond to the end of the growing season in 2013. Cushion per elevation = 30, replicates (distance from beneficiaries cover; leaves = 270, stems = 268). Note the different scales of the ordinates. Shown are means and 95% confidence intervals. Hence, non-overlapping error bars indicate significant differences at α < 0.05

may also be organ-specific (Galvez et al. 2013; Klein et al. 2014) or species-specific (Piper et al. 2006). From our study it appears that *L. acaulis* follows the common pattern of stable or increased NSC under drought.

Upper-elevation limitations

Although decreases in NSC concentrations have been observed in plants under extreme drought conditions, extreme low-temperature conditions do not appear to have the same effect (e.g. Piper et al. 2006; Hoch and Körner 2012). However, our cushion plants did show such a decrease under the coldest conditions although low temperatures have been shown to affect tissue formation (i.e. carbohydrate use) before it affects photosynthesis (e.g. Körner 2008). A plausible explanation could be the successful decoupling from the atmosphere by the low, compact and smooth growth form, particularly at the highest elevation. A cushion can warm up far above air temperature and could mean that a lack of warmth at high elevations is not the main stress factor for the cushions and that photosynthesis is limited by other factors. Alternatively, cushions at higher elevations may allocate more resources to other functions, such as fast reproduction in the short alpine growing season, instead of reserve accumulation (Monson et al. 2006). Although for the lower NSC in leaves phenological causes cannot be ruled out (e.g. export of reserves preceding senescence), even if our sampling took place in March, well before visible senescence which started in April/May, this explanation seems very unlikely for the stem reserves, which should, if anything, go up during this stage.

Besides having lower NSC concentrations, cushions were also smaller and less abundant at the highest

elevation (Fig. 2). Although this could theoretically also be due to a younger population age at this elevation, there are no signs of recent glaciation at our sites, making this explanation unlikely. Instead, the more compact growth form at high elevation suggests higher stress levels and less emphasis on lateral expansion. This would agree with the smaller cushion sizes, but without aging the cushions or following lateral growth through time it cannot be determined with certainty whether the smaller cushion sizes result from lower growth rates.

Our measure of growth, annual leaf biomass produced per area, showed the highest values at both altitudinal extremes, though the effect size was small. So, the smallest (=high-elevation) cushions had high 'growth' values. Here, this was due to higher density of rosettes (Table 2), while at the lowest elevation this was due to a higher biomass per rosette. This could indicate that biomass produced during one growing season in relation to existing biomass is lower at the highest elevation site, as indicated by a lower per-rosette biomass than at the lowest sites. A similar gradient from compact cushions at high to more open and soft cushions at low elevations was recently reported for *Silene acaulis*, where cushion form influenced the mitigating effects on microclimate and thereby the role of these cushions as facilitator (Michalet et al. 2011; Bonanomi et al. 2016). Thus, the two-way interaction between cushions and their beneficiaries can change along elevation in a complex interplay of direct environmental stress, relative costs and benefits, differences in amelioration strength due to cushion morphology, as well as the cover and identity of the beneficiaries (Michalet et al. 2011, 2016; Al Hayek et al. 2014). In our system, the net reciprocal effect of facilitation, however,

appears to be neutral along the entire gradient (García et al. 2016).

Beneficiary effects on cushion fitness

In terms of fitness, the most important measure of growth for these long-lived stress-tolerant plants would be the production of reproductive units (rosettes that can build inflorescences) and the maintenance of a form that assures long-term survival. Although the compact cushion forms appears well-suited to the high-elevation conditions, the lower tissue NSC concentrations at the highest elevation could compromise the cushions' long-term survival. Low NSC concentrations at the end of the season can affect the survival of plants during the dormant period (Wyka 1999; Landhäusser and Lieffers 2003) and may allow less growth during the next season, as indicated by the positive correlation between NSC and biomass within elevations (Fig. 3). Low NSC concentrations can also increase the susceptibility to disturbances (e.g. herbivores, Louda and Collinge 1992). Additional reductions in NSC could theoretically be brought about by beneficiary cover over the cushions. However, total soluble sugars and starch concentrations in leaves and stems of *L. acaulis* did not change with beneficiary cover. Only maltose concentrations differed significantly between cushions as a function of beneficiary cover, but this seems spurious as the nature of the difference was inconsistent between elevations. As the importance of this sugar is quantitatively negligible (ca. 1% of total NSC) (Fig. 1), we consider this statistical difference biologically irrelevant.

Similar to NSC concentrations, growth expressed as foliar biomass per area did not change with beneficiaries (neither cover nor distance). Thus, consistent with our previous finding of a neutral net effect of beneficiaries on the reproduction of *L. acaulis* (García et al. 2016), we found no evidence for an effect on vegetative performance. The lack of a detectable signal could result from compensation of both positive and negative effects, but the neutral net effect is decisive for the fitness of the cushions. It thus appears that *L. acaulis* performs its role as foundation species and facilitator without experiencing negative reciprocal effects from the presence of beneficiaries. This species interaction is thus likely to be evolutionary and ecologically stable.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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