



Unexpected mechanisms sustain the stress gradient hypothesis in a tropical alpine environment

Fabien Anthelme, Belen Buendia, Charlotte Mazoyer & Olivier Dangles

Keywords

Andes; *Azorella aretioides*; Biogenic habitat; Competition; Cushion; Ecuador; Facilitation; RII; Species-specific effects

Abbreviations

RII = relative interaction index; SGH = stress gradient hypothesis

Nomenclature

Luteyn (1999)

Received 1 February 2011

Accepted 15 July 2011

Co-ordinating Editor: Rasmus Ejrnæs

Anthelme, F. (corresponding author, fabien.anthelme@ird.fr) & **Mazoyer, C.:** Institut de Recherche pour le Développement (IRD), UMR DIADE/AMAP, 911 avenue Agropolis BP 64501, 34394 Montpellier Cedex 5, France
Buendia, B. (guaguabelen@gmail.com) & **Dangles, O.** (Olivier.Dangles@legs.cnrs-gif.fr): Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Roca, Quito, Ecuador
Dangles, O. (Olivier.Dangles@legs.cnrs-gif.fr): Institut de Recherche pour le Développement (IRD), UR 072, LEGS, UPR 9034, CNRS 91198 Gif-sur-Yvette Cedex, France; Université Paris-Sud 11, 91405 Orsay Cedex, France

Introduction

Since the seminal paper of Bertness & Callaway (1994), the stress gradient hypothesis (SGH) has imposed itself as a major concept in understanding the effects of environmental stress on plant community distribution in a wide variety of environments (Callaway 2007; Brooker et al. 2008; Lortie 2010). As recently proposed by Maestre et al. (2009), it is crucial to determine the relative importance of resource (e.g. nutrients, light, water) vs. non-resource stress (e.g. temperature, wind, salinity) in order to achieve a better understanding of the high variability in the outcome of plant–plant interactions at the extremities of stress gradi-

Abstract

Questions: Does facilitation among plants increase with elevation in a humid tropical alpine system in which climatic and ecological conditions differ from other alpine environments? What mechanisms are involved in the interactions?

Location: Volcano Antisana, Eastern Cordillera of the Ecuadorian Andes (00°28'S, 78°09'W).

Methods: We selected the cushion-forming *Azorella aretioides* as a potential nurse plant along an altitudinal gradient (4400, 4550 and 4700 m) in the high tropical Andes. We quantified its effects on other plants at species and community levels by comparing the product vegetation cover \times number of individuals of every vascular species found inside and outside 265 cushions, using the relative interaction index. We inferred potential mechanisms behind the interactions through analysis of microclimate, soil moisture and soil nutrient measurements inside and outside cushions.

Results: Predictions of the stress gradient hypothesis (SGH) were corroborated at community level, with transition from competitive or neutral effects of *A. aretioides* at 4400 and 4550 m to facilitative effects at 4700 m. Strong species-specific effects were observed along the altitudinal gradient, with a substantial effect of local habitat disturbance on the outcome of plant–plant interactions. Surprisingly, cushions lowered air and soil temperatures and air humidity, which reduced at higher elevations. Facilitation appeared to be caused by higher soil moisture and nutrient content beneath cushions.

Conclusions: Our data extend the framework of the SGH by corroborating it for the first time in a tropical alpine system. However, the mechanisms underlying plant–plant interactions differed from those generally reported from alpine environments, with facilitation varying according to resource-mediated stress (nutrients). It remains to be tested whether this is specific to tropical alpine systems.

ents (Michalet 2007; Brooker et al. 2008; Maestre et al. 2009; le Roux & McGeoch 2010). This issue has been particularly addressed in alpine environments where plant–plant interactions have been suggested to be mainly driven by non-resource stress (Maestre et al. 2009), thereby explaining why facilitation processes are generally intense at the end of stress gradients in these ecosystems.

Most (if not all) of what we know about SGH predictions in alpine systems has been learned from mountains in temperate and sub-polar latitudes. A world map view of locations of the many SGH studies performed so far in alpine environments shows that tropical region virtually remains a 'SGH *terra incognita*' (Fig. 1; but see Cavieres & Badano

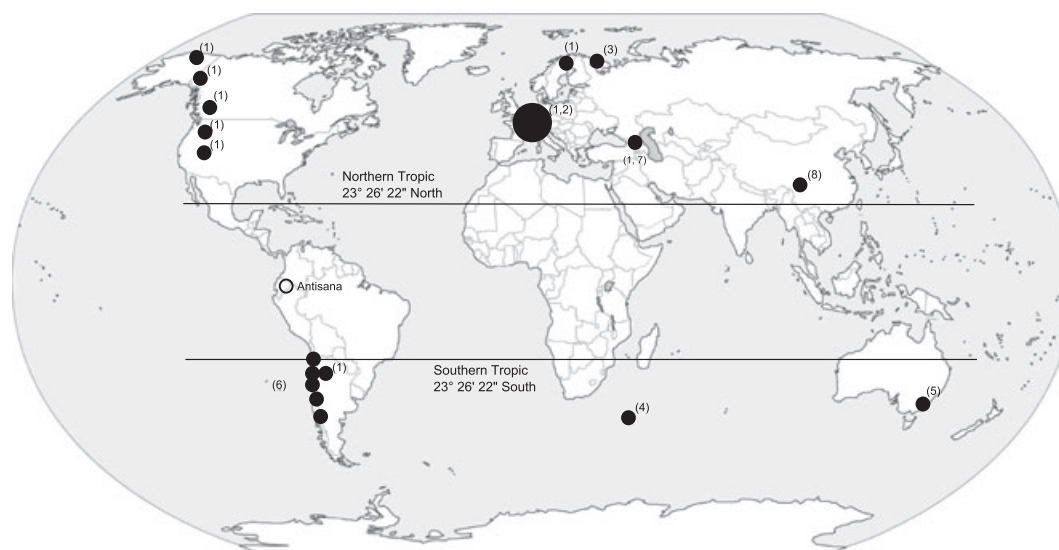


Fig. 1. The stress gradient hypothesis (SGH) as explicitly studied in alpine environments (black circles). In Europe, many studies were conducted in various mountain ranges (large black circle). Within the tropics, our study is the first to test the SGH (empty black circle). (1) Choler et al. (2001), Callaway et al. (2002), Kikvidze et al. 2005; (2) Dullinger et al. (2007); (3) Eränen & Kozlov (2008); (4) le Roux & McGeoch (2008, 2010); (5) Venn et al. (2009); (6) Cavieres & Badano (2009) (see also Chilean references cited in our paper); (7) Onipchenko et al. (2009); (8) Yang et al. (2010).

2009 at the southern limit). The most integrative study on plant–plant interactions in alpine environments (Callaway et al. 2002) spanned a wide range of biogeographical regions but failed to take into account tropical alpine sites, whether humid or dry. The only available report on this (Sklenář 2009) did not consider predictions of the SGH. The overall lack of ecological studies (Körner 2003; Stocks et al. 2008), and especially the absence of data on the SGH in tropical alpine systems, may result from their classification as ‘alpine environments’, implicitly suggesting that basic knowledge collected from temperate latitudes also applies to the tropics.

Why would alpine systems behave differently depending on their latitudinal position? Despite common features of alpine systems (e.g. reduction in air temperature, pressure and relative humidity, and increasing solar radiation; Körner 2003), tropical alpine environments are characterized by unique environmental characteristics (especially in their most humid part) resulting from biogeographical and climatic specificities (Malhi et al. 2010). Among these are aseasonality, the absence of prolonged dry periods and marked day-to-day climatic variation (Rundel et al. 1994; Körner 2003; Buytaert et al. 2011). Consequently, tropical mountains have evolved singular plant communities characterized by a continuous vegetative period and exceptional taxonomic and functional diversity (resulting from both physiological and morphological adaptations) even within a given plant growth form (e.g. cushion plants; Ramsay & Oxley 1997; Luteyn 1999; Sklenář & Balslev

2005). These singularities of tropical alpine systems may have potential impacts on the SGH predictions, especially on the commonness of facilitation processes and the role of species-specific interactions (due to the high functional diversity of tropical communities), and on the underlying mechanisms of facilitation (due to the specific abiotic and biotic characteristics of tropical mountains).

The tropical Andes represent an interesting region to study the outcome of the SGH in tropical alpine environments, as they cover 94% of all tropical lands above 3000 m a.s.l. (Jacobsen 2008). Among the particular growth forms developed in this region, large cushion-forming plants are outstandingly diverse and abundant (Ramsay & Oxley 1997; Cavieres et al. 2007) and are, at high altitudes, among the best facilitators of the development of other plants through the creation of biogenic habitats (Badano & Marquet 2009). In this study, we selected the cushion-forming species *Azorella aretioides* (Apiaceae) as a potential nurse plant within the humid tropical alpine communities found at the highest altitudes in the Oriental Cordillera of the Ecuadorian Andes (4700 m a.s.l.). Various species of the genus *Azorella* are known for their key positive effects on vegetation (Arroyo et al. 2003; Cavieres et al. 2005, 2006; Badano & Marquet 2009; Sklenář 2009; le Roux & McGeoch 2010) and other components of biodiversity (Hugo et al. 2004).

Along a 300-m altitudinal gradient, we observed variations in the effects of the nurse on the fine-scale distribution of plant species. Our objective was to test the validity of the SGH, described in temperate alpine ecosystems, at

the level of a whole tropical plant community. We aimed to specifically explore: (1) species–species effects in light of the SGH, and (2) mechanisms explaining the variability in plant–plant interaction outcomes along the altitudinal gradient.

Methods

Study area and sites

The study was carried out in the superpáramo of the Antisana Ecological Reserve, in the Eastern Cordillera of the Ecuadorian Andes (00°28'S; 78°09'W). In the humid tropical Andes of Colombia, Ecuador and Venezuela, the superpáramos represent the highest belt of vegetation, between ca. 4300 and 4800 m a.s.l. (Luteyn 1999). At 4850 m, the annual rainfall is ca. 1000 mm, with a mean relative humidity of 80% and a mean temperature of 1.15 °C, with marked daily variations (Wagnon et al. 2009). Cushions, tussock grasses and small rosettes are the most common growth forms, with *Azorella* spp., *Xenophyllum rigidum*, *Calamagrostis* spp., *Lucilia kunthiana* and *Baccharis caespitosa* being the dominant species.

We tested the SGH along an altitudinal gradient mainly related to decreasing temperature. Study sites were located at three elevations (4400, 4550 and 4700 m) between Laguna Santa Lucia and the 15α Glacier, on the northwest side of the Antisana volcano. This gradient, although relatively shallow, corresponds to the whole altitudinal range of *A. aretioides* locally, and is correlated with a significant decrease in the minimum daily temperature (4400 m: $-0.31\text{ °C} \pm 0.15\text{ SE}$; 4550 m: $-0.74\text{ °C} \pm 0.22\text{ SE}$; 4700 m: $-1.21\text{ °C} \pm 0.22\text{ SE}$; F. Anthelme, unpubl. data) with higher frequency of frost events at higher sites. The density of herbivores (llamas, cows, wild horses, rabbits) was similar among sites (F. Anthelme pers. observ.) while abiotic factors such as snow duration and the presence of permafrost was negligible at all sites (B. Francou, pers. comm.). At the lowest site (hereafter 4400), the presence of sporadic water streams generated habitat heterogeneity, with continuous vegetation alternating with channels of eroded bare soil. A study of the proportions of vegetated area vs. bare soil at each site (measured with a decametre in ten 20-m long vegetation transects) showed that vegetation was discontinuous at 4400 m ($44.26\% \pm 5.25\text{ SE}$ of total soil cover), almost continuous at 4550 m ($89.88\% \pm 1.67\text{ SE}$) and scattered at 4700 m ($24.92\% \pm 1.57\text{ SE}$).

Nurse and beneficiary species

We selected the species *A. aretioides* (hereafter *Azorella*; Appendix S1), which is a semi-hemispherical giant cushion found on the NW slopes of the Antisana volcano, from

4350 to 4750 m (F. Anthelme, unpubl. data). Its surface is composed of compact leaves structured in a multitude of small rosettes. The relative cover (RC) of *Azorella* reaches a peak at the intermediate site 4550 ($10.51\% \pm 1.77\text{ SE}$), but decreases to $6.21\% \pm 1.61\text{ SE}$ at site 4400 and $5.06\% \pm 1.08\text{ SE}$ at site 4700 (data collected from the vegetation transects described above).

We took into account vascular plant species found inside or in the vicinity of *Azorella*. Because of difficulties with identification of seedlings of various species of grass (*Calamagrostis intermedia*, *Festuca andicola*, *Agrostis foliata*, *Calamagrostis fibrovaginata*, and *Agrostis breviculmis*), we grouped them into a single category, Poaceae. All bryophyte species were assigned to a single group. All identifications were carried out at the herbarium of the Pontifical Catholic University of Ecuador, with the help of the generic flora of the Páramos (Sklenář et al. 2005).

Sampling design

Biotic measurements

At each altitude, we selected between 85 and 90 cushions of *Azorella* that were separated by at least 3 m. The diameters of selected cushions all exceeded 12 cm (mean area: $1076.7 \pm 44.2\text{ cm}^2$), to avoid ontogenetic effects of the potential nurse. We observed no particular microtopographical patterns in the recruitment of young *Azorella*, suggesting the absence of pre-established environmental patterns for the species. In addition, the biogenic habitat developed by the semi-hemispherical, giant cushions is generally poorly connected to pre-existing soil/substrate, making this plant an interesting model to assess nurse effects using observational methods. For each cushion, we randomly selected an area of similar size to that of the cushion and located ca. 1 m from its border in order to compare plant performance inside and outside cushions. Within each area (inside and outside the cushion), we counted the number of individuals (NI) of all vascular species and visually estimated their RC (%). An individual was considered 'inside a cushion' when its main stem at the soil surface was part of the cushion. Because of the high diversity of growth forms in our study area, we multiplied these two measurements to provide a synthetic index of establishment performance for each species. For example, for the specific case of species with strong lateral spread (e.g. *B. caespitosa*, *L. kunthiana*), the combination of plant abundance and cover allowed us to avoid underestimation (when considering solely the NI) or overestimation (when considering solely the cover). We took into account intra-specific associations, as we observed that *Azorella* beneficiaries were easily identifiable from *Azorella* nurses by developing rosettes with a distinctive structure.

Abiotic measurements

Air temperature and relative humidity were measured 10 cm above ground level (both inside and outside cushions, paired samples) using HOBO-Pro RH/Temp Data Loggers (Onset Computer Corp., Pocasset, MA, USA; Appendix S2). Soil temperature was measured 5 cm below the soil surface (both inside and outside cushions, paired samples) using TidbiT v2 Temp Data Loggers and probes connected to HOBO microstations (Onset Computer Corp.). All data were acquired every 15 min over at least 16 d at 11 (soil temperature) and 7 (air temperature and humidity) locations separated by at least 10 m at each elevation. We focused our analyses on daily minimum values, which are expected to reflect more precisely the stress undergone by plants (Körner 2003). Because of the aseasonality of our study area (e.g. Jacobsen et al. 2010), evidenced from climatic data in our study site at 4800 m a.s.l. (Appendix S3), we assumed that our spatial repetitions associated with temporal measurements every 15 min provide consistent microclimate data.

A total of 60 soil samples (ca. 500 g each) were extracted on a sunny day during a relatively dry period in Mar 2009 (ten inside and ten outside cushions, at each elevation). Soil moisture (g H₂O/g fresh soil) was estimated by calculating the difference between fresh and oven-dried weight (96 h, 60 °C). Weights were measured to the nearest 0.1 mg using a TU-OI balance (FA-2104, Mhand, Fuzhou, China). Soil samples were then sealed in plastic bags and sent to the Instituto Nacional Autonomo de Investigaciones Agropecuarias (INIAP, Quito, Ecuador) for laboratory analysis of available soil nutrients (NH₄, P, S, Zn, Cu, Fe, Mn and B in ppm; K, Ca and Mg in meq·100 ml⁻¹) and soil organic matter (SOM, %). For both analyses, living material from cushions and other plants (roots, green leaves and stems) was removed from the samples.

Data analyses

The relative interaction index (RII; Armas et al. 2004) was used to quantify the outcome of the interactions between *Azorella* and the potential beneficiaries. RII ranges from pure competition (−1) to pure facilitation (+1), is symmetrical around 0, and can be scaled up and used to measure multi-species interactions at the community level. RII was calculated as follows:

$$RII = \frac{(P_{+N} - P_{-N})}{(P_{+N} + P_{-N})}, \quad (1)$$

where P_{+N} and P_{-N} represent the performance (NI × RC) of potential beneficiary species in the presence and absence of *Azorella*, respectively.

Because the intensity and importance of plant–plant interactions can respond differently to environmental gradients (Brooker et al. 2005), we also calculated an index of importance (I_{imp} , Seifan et al. 2010) to diminish the risk of result misinterpretation (Brooker & Kikvidze 2008). As both indices yielded similar results, we focused solely on the RII in the Results section, and present data for I_{imp} in Appendix S4.

We calculated RII values at community level by pooling individual values of all species. At 4400 m, we observed that locally distributed water run-offs reduced plant cover. Consequently, we calculated RII index using a subsample of the 30 subplots with highest vegetation cover outside cushions.

At each spatial repetition, we averaged all daily minimum values to ensure the consistency of our data. Variation in the micro-environment due to the presence of *Azorella* (paired differences between *Azorella* treatment and soil treatment) were tested with paired sample *t*-tests at each altitude, and the effect of altitude was tested with ANOVAs and *post hoc* Tukey tests. Statistical analyses were performed with Minitab 15 (State College, PA, USA).

Results

Effects of *Azorella* on the micro-environment

Cushions had an overall negative effect on soil and air temperature and air relative humidity, especially at 4400 and 4550 m (Fig. 2a–c; ANOVAs, $P < 0.05$). Air temperature and relative humidity inside cushions were similar among elevations (ANOVAs, $P > 0.05$) whereas they were significantly different outside cushions (ANOVAs, $P < 0.05$). Soil temperature inside and outside cushions varied significantly among elevations (ANOVAs, $P < 0.001$).

Both soil moisture and SOM increased significantly inside cushions of *Azorella aretioides* at all elevations (paired sample *t*-test, $P < 0.01$; Fig. 2d and e). The strongest increase in soil moisture by cushions was observed at 4700 m ($+51.79 \text{ g·g}^{-1} \pm 5.69 \text{ SE}$; Fig. 2d) which was also the elevation where soil moisture reached its lowest level outside cushions ($17.26 \text{ g·g}^{-1} \pm 4.20 \text{ SE}$; Fig. 2d). Increase in SOM beneath cushions was stronger at the intermediate elevation of 4550 m than at 4700 m ($53.6\% \pm 4.7 \text{ SE}$ and $21.1\% \pm 6.7 \text{ SE}$, respectively; Fig. 2e). SOM content was high at 4550 m ($31.6\% \pm 3.9 \text{ SE}$) while it was particularly low at 4400 and 4700 m ($7.5\% \pm 1.1 \text{ SE}$ and $2.2\% \pm 0.4 \text{ SE}$, respectively; Fig. 2e).

Azorella had an overall positive effect on the availability of many soil nutrients, including macronutrients K, Ca and Mg (Table 1). However, it did not modify the availability of NH₄ and P and had a negative effect on Cu and Fe (Table 1). Positive effects of *Azorella* were predominantly found at elevations of 4400 and 4700 m, while effects were

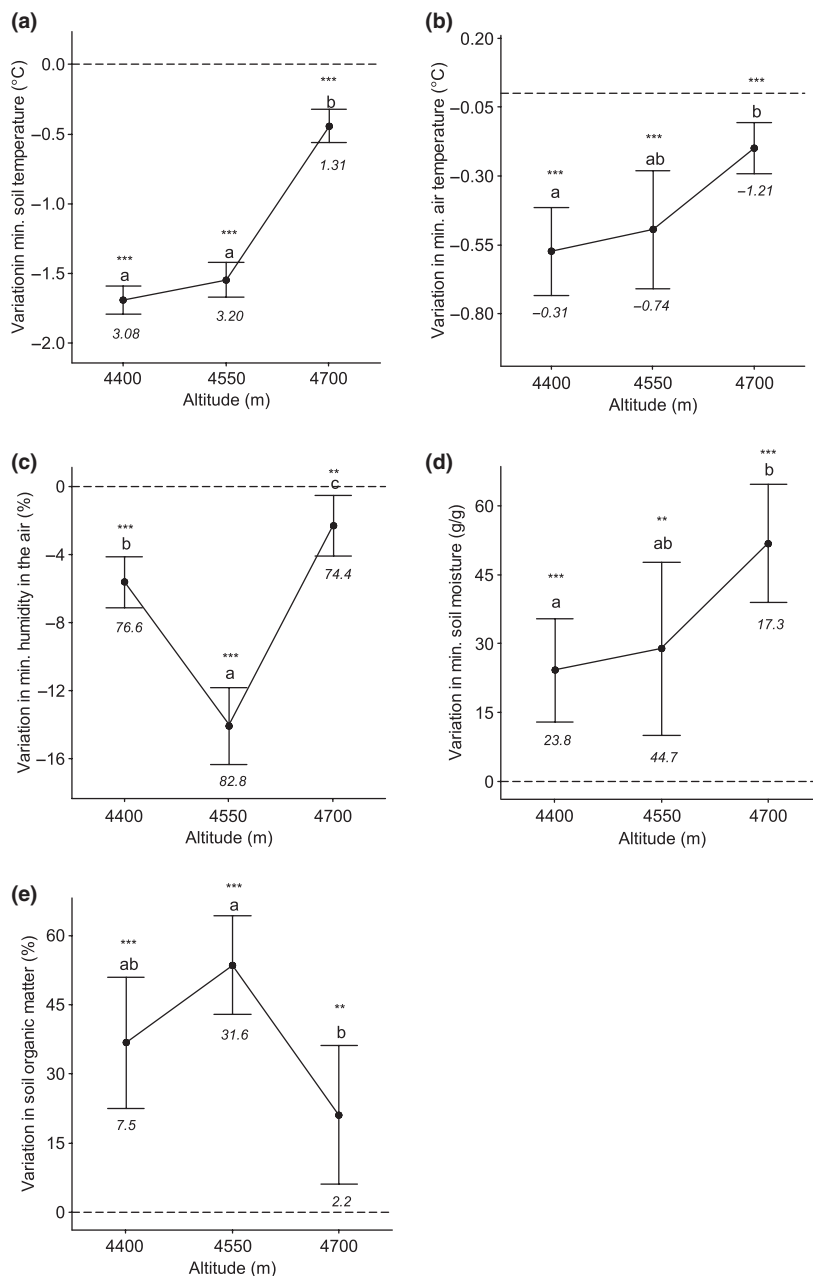


Fig. 2. Impact of the presence of *Azorella aretioides* on the micro-environment: (a) soil temperature 5 cm inside cushions/soil; (b) air temperature 10 cm above cushions/soil; (c) relative humidity 10 cm above cushions/soil; (d) soil moisture; (e) soil organic matter. Error bars represent 95% CI. Different letters: significant variation among sites (*post hoc* Tukey tests). Variation significantly greater or less than 0 (dashed lines) calculated with paired sample *t*-tests: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Mean values outside cushions at each site displayed in italic below error bars.

neutral at 4550 m. Nutrient gain was generally higher at 4400 m than at 4550 m.

Facilitation at community level

The RII at community level revealed that the effects of *Azorella* on neighbouring plants were neutral at site

4400, significantly negative at site 4550, and significantly positive at site 4700 (one-sample *t*-tests, $P < 0.05$; Fig. 3). However, when considering the subsample with highest values of vegetation cover at 4400 m, the RII became negative and not significantly different to the RII value at 4550 m (-0.20 ; *post hoc* Tukey test; $P > 0.05$).

Table 1. Availability of soil nutrients in the presence or absence of *Azorella*.

Elevation (m)	Treatment	NH ₄ ⁺	P	K	Mg	Ca	Cu	Fe	S	Zn	Mn	B
4400	<i>Azorella</i>	85.4	10.5	0.4	3.5	10.1	1.8	196.0	15.2	3.8	9.9	0.3
	Soil	58.7	3.6	0.2	1.3	2.0	3.1	169.8	11.4	1.2	7.2	0.1
	<i>Azorella</i>	+	+	+	+	+	—	n.s.	n.s.	+	n.s.	+
	Elevation	a	a	a	a	a	a	a	a	a	a	a
4550	<i>Azorella</i>	64.4	10.8	0.8	2.2	8.2	1.2	17.4	11.7	9.1	34.4	0.4
	Soil	101.3	14.3	0.5	2.2	9.2	1.5	303.4	14.8	8.9	11.2	0.2
	<i>Azorella</i>	—	n.s.	+	n.s.	n.s.	—	—	n.s.	n.s.	+	n.s.
	Elevation	b	b	a	b	b	a	b	b	a	b	a
4700	<i>Azorella</i>	51.7	7.4	0.5	3.3	5.4	1.9	102.6	6.9	2.3	11.0	0.6
	Soil	49.0	9.3	0.2	1.1	1.3	2.3	53.2	7.1	0.7	1.8	0.2
	<i>Azorella</i>	n.s.	n.s.	+	+	+	n.s.	+	n.s.	+	+	+
	Elevation	a	b	a	a	ab	a	a	ab	a	ab	a
Total	<i>Azorella</i>	n.s.	n.s.	+	+	+	—	—	n.s.	+	+	+
	Elevation	***	**	n.s.	***	**	n.s.	***	*	n.s.	**	n.s.

Sign.: significance of the variation between the two conditions (*post hoc* Tukey tests on paired differences). Effect of *Azorella* (one sample *t*-tests): +, positive; —, negative; n.s., not significant. Effect of elevation: one-factor ANOVA.

****P* < 0.001, ***P* < 0.01, **P* < 0.05.

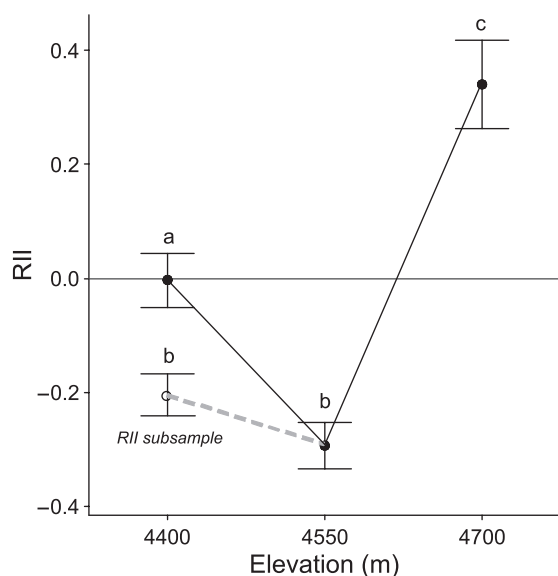


Fig. 3. Outcome of plant–plant interactions (RII) at community level along an altitudinal gradient. Mean values with bars representing 95% CI. Common letters indicate absence of differences among treatments (*post hoc* Tukey tests). Subsample values of RII (unfilled circles) were added for site 4400 to mitigate the effects of a local disturbance.

Species-specific responses of beneficiaries

A significantly higher proportion of species was facilitated by *Azorella* at site 4700 (50%, Fig. 4) than at sites 4550 (12%) and 4400 (25%; Fig. 4). The most facilitated species included *Myrosmodes* sp. (e.g. $RII_{4700} = 1$, $n = 20$), *Huperzia crassa* (e.g. $RII_{4400} = 0.56$, $n = 23$) and *Oreomyrrhis andicola* (e.g. $RII_{4550} = 0.72$, $n = 22$). At the same time, more species were spatially disassociated from

Azorella at site 4550 (42%) than at sites 4400 (25%) and 4700 (6%). Intraspecific competition was particularly strong among *Azorella* plants at sites 4400 ($n = 20$, $RII = -1$; Fig. 4) and 4550 ($n = 15$, $RII = -1$, $n = 49$). *Werneria* spp. in particular were found preferentially outside *Azorella* (e.g. *Werneria nubigena*: $RII_{4550} = -0.83$; *Werneria pumila*: $RII_{4400} = -0.55$, $n = 22$). The other cushion-forming species identified at our study sites displayed either neutral interactions with *Azorella* (site 4400: *Plantago rigida*, *Nototriche hartwegii*, *Baccharis caespitosa*; site 4550: *P. rigida*, bryophytes, *N. hartwegii*; site 4700: *Xenophyllum rigidum*, *B. caespitosa*, *Draba aretioides*) or were inhibited by its presence (site 4400: bryophytes, *Lucilia kunthiana*; site 4550: *L. kunthiana*, *B. caespitosa*; site 4700: bryophytes).

The nine species found at all sites showed a wide array of interaction outcomes with *Azorella*, which could be classified into three groups (RII index; Fig. 5). In the first group, species were more facilitated at site 4700 than at the two other sites (*post hoc* Tukey tests, $P < 0.001$). In the second group, species were more facilitated by *Azorella* at sites 4400 and 4700, and tolerant to its presence at the intermediate site 4550 (one-sample *t*-tests did not differ from 0, $P < 0.001$). In the third group, the species were never facilitated by *Azorella* (one-sample *t*-tests greater than 0, $P < 0.001$).

Discussion

Validation of the SGH in a tropical alpine environment

The SGH has been validated in a wide array of temperate alpine and sub-polar environments along thermal stress gradients (e.g. Callaway et al. 2002). By evidencing increasing intensity and importance of positive interac-

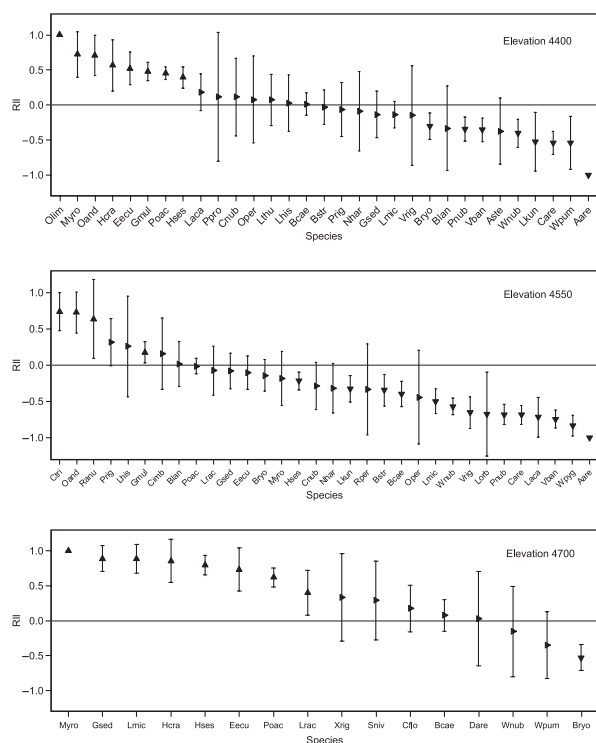


Fig. 4. Intensity of interactions (RII) between *Azorella* and other species at three elevations. Different types of triangles indicate facilitation (upward), neutral effect (horizontal) or inhibition (downward) by *Azorella*. Error bars represent 95% CI. Aste, unidentified Asteraceae; Aare, *Azorella aretioides*; Bcae, *Baccharis caespitosa*; Blan, *Bromus lanatus*; Bryo, Bryophytes; Bstr, *Bartsia stricta*; Care, *Carex cf. tristicha*; Cnub, *Castilleja nubigena*; Ctri, *Cerastium trianae*; Cflo, *Cerastium floccosum*; Cimb, *Cerastium imbricatum*; Dare, *Draba aretioides*; Eecu, *Erigeron ecuadoriensis*; Gmul, *Geranium multipartitum*; Gsed, *Gentiana sedifolia*; Hcra, *Huperzia crassa*; Hses, *Hypochaeris sessiliflora*; Laca, *Lysipomia acaulis*; Lhis, *Lachemilla hispidula*; Lkun, *Lucilia kunthiana*; Lmic, *Lupinus microphyllus*; Lorb, *Lachemilla orbiculata*; Lrac, *Luzula racemosa*; Lthu, *Loricaria thuyoides*; Myro, *Myrosmodes sp.*; Nhar, *Nototriche hartwegii*; Oand, *Oreomyrrhis andicola*; Olim, *Oritrophium limnifolium*; Oper, *Oritrophium peruvianum*; Pnub, *Plantago nubigena*; Poac, Poaceae; Ppro, *Pernettya prostrata*; Prig, *Plantago rigida*; Ranu, *Ranunculus sp.*; Rper, *Ranunculus peruvianus*; Sniv, *Senecio nivalis*; Vban, *Viola bangii*; Vrig, *Valeriana rigida*; Wnub, *Werneria nubigena*; Wpum, *Werneria pumila*; Wpyg, *Werneria pygmaea*; Xrig, *Xenophyllum rigidum*.

tions among plants along an altitudinal gradient, our data are the first to contribute to validating the SGH in a tropical alpine system. However, we also found stronger facilitative effects at 4400 m than at 4550 m, suggesting that the outcome of plant–plant interactions along altitudinal gradients may be driven by factors other than climate drivers (e.g. temperature) alone. At 4400 m, a natural habitat disturbance *sensu* Grime (1979) negatively impacted the vegetation cover directly and through generating higher stresses in soil moisture and soil nutrients (N, P, Ca, Mg), which are generally involved in plant biomass accumulation

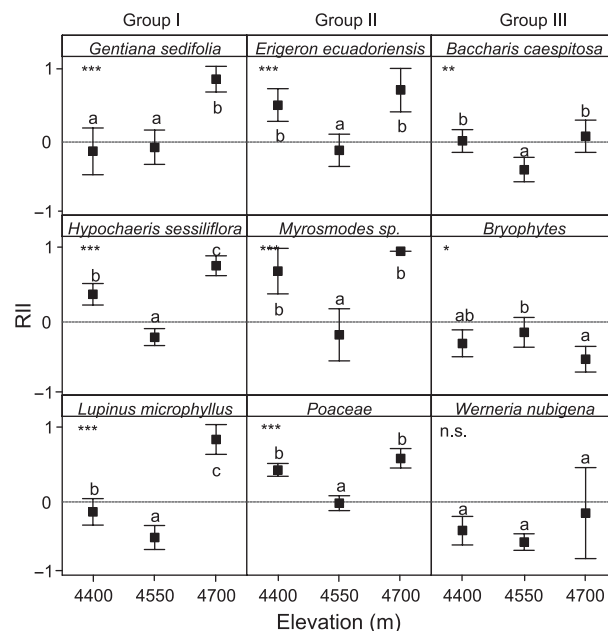


Fig. 5. Individual responses to the presence of *Azorella* of nine species found frequently at all sites. Error bars represent 95% CI. ANOVA on elevation: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s., not significant. Common letters indicate no difference among treatments (*post hoc* Tukey tests; $P < 0.05$).

(Feller et al. 2008). While this result supports the SGH – more stress and/or more disturbance, more facilitation (Bertness & Callaway 1994; Brooker et al. 2008) – it also evidences the co-existence of multiple stress drivers, as observed in other studies assessing facilitation in severe environments (e.g. Cavieres et al. 2006; Anthelme & Michalet 2009).

The relatively high influence of local habitat disturbance on the outcome of plant–plant interactions is likely due to our relatively shallow altitudinal gradient with weak – albeit significant – effects on air temperature. However, it was not possible to extend our gradient as it already covered the entire altitudinal distribution of *Azorella aretioides* in our study area. This problem might be specific to the tropical Andes, where altitudinal distribution of alpine plants is generally narrower than at high latitude environments due to weaker effects of glacial fluctuations (Molau 2004). This stresses both the advantages and the limits of using altitudinal gradients as proxies for abiotic stress gradients (Körner 2007; Malhi et al. 2010).

At the top of our gradient, the presence of species with RII values close to +1 (e.g. *Myrosmodes sp.*, *Gentiana sedifolia*) means that the presence of nurses is a prerequisite for their establishment at this elevation. This supports the opinion that facilitation promotes niche expansion, especially in severe environments (Choler et al. 2001; Bruno et al. 2003), and that environmental changes can

be mitigated by the effects of plant–plant interactions (Brooker 2006).

Species-specific effects drive the outcome of interactions

By providing direct comparisons between beneficiary plants, we think that our approach is relevant to generalize concepts on plant–plant interactions in relation to abiotic stress (see Brooker et al. 2008; Fajardo et al. 2008; Maestre et al. 2009; Quiroz et al. 2009). We acknowledge, however, that confounding factors such as the potential effect of mycorrhizae on plant–plant interactions or the fact that cushions may act as seed traps (although the surface of *Azorella* may not be well adapted for this, Cavieres et al. 2005) may limit the interpretation of our results, and that further manipulative experiments would be needed.

Among the three groups of beneficiary plants, some species were more facilitated at higher elevation. One possible explanation for this pattern is that these species responded both to nutrient and microclimatic stresses: they may take advantage of nutrient/soil moisture enrichment by the nurse plants at sites 4400 and 4700 and avoided sites where *Azorella* negatively affected the microclimate (4400, 4550, see Fig. 2). They may be considered as typical ‘SGH species’ not observed at higher elevation in the area.

The second group included species that seemed to take advantage of the soil nutrient/soil moisture enrichment provided by *Azorella*, but were not affected by changes in microclimate conditions. Therefore, the outcome of the interaction for these species may be purely dependent on a resource factor (nutrients) that may not only run parallel to the altitudinal gradient, but also be affected by local habitat disturbance such as that found at 4400 m.

The third group comprised species that were never facilitated. Bryophytes and *Baccharis caespitosa* are flat cushions and *Werneria nubigena* is a rosette-like species, i. e. they present growth forms similar to that of *Azorella*. This similarity (or low complementarity) among species may explain the absence of observed facilitation (Fajardo et al. 2008; Maestre et al. 2009). Moreover, species in this group were able to establish at higher elevation in the area without nurse species, suggesting that they did not reach their physiological limits (*species-level strain*; Gross et al. 2010).

Nutrients, more than microclimate, drive facilitation

The creation of biogenic habitats by nurse plants through the amelioration of non-resource abiotic factors has been relatively well documented in alpine environments, for example, through the mitigation of minimum temperature

(Nyakata & McGeoch 2008; Badano & Marquet 2009), buffering of extreme temperatures (Cavieres et al. 2006; Nyakata & McGeoch 2008; Badano & Marquet 2009; Yang et al. 2010) or shelter from wind (Cavieres et al. 2002; le Roux & McGeoch 2008, 2010). Therefore, stress gradients in alpine ecosystems have been proposed to be preferentially unrelated to resources (Maestre et al. 2009), although amelioration in terms of soil water availability has also been cited as a facilitative process (Cavieres et al. 2008; Yang et al. 2010). Accordingly, alpine environments are thought more likely to corroborate the SGH than resource-driven ecosystems in, for example, arid environments, because plants will not compete for a limiting resource at the extremity of this type of stress gradient (Maestre et al. 2009). Unexpectedly, in our study, we found a negative effect of cushions on the minimum values of air and soil temperature (see Yang et al. 2010 for a similar result) and relative humidity, thereby challenging the proposed mechanistic framework of the SGH. This singularity may issue from the giant, semi-hemispherical shape of *A. aretioides*, which likely promotes soil enrichment but negatively affects microclimate conditions because of stronger exposure to the freezing and desiccating effects of wind. Determining whether this result could be a specificity of tropical alpine systems represents a stimulating line of research.

Studies that attempt to correlate the enrichment of soil by nurse plants with the strength/commonness of facilitation in alpine environments are scarce. The limited empirical evidence either concerns a small number of key soil elements (N, P, K) with few repetitions (Nuñez et al. 1999; Cavieres et al. 2006; Yang et al. 2010) or refers to the amelioration of N levels through N fixers (Chapin et al. 1994). Interestingly, positive effects of cushions on soil nutrient content were suggested to explain in part validation of the SGH in the Himalayas (Yang et al. 2010), essentially through K, and to a lesser extent N, enrichment. Our study supports these results through monitoring a large set of nutrients. Despite the shallowness of our altitudinal gradient, our data therefore suggest that the SGH may be driven through the enrichment of a large range of soil nutrients by cushions in comparison with neighbouring open areas, especially when these nutrients are relatively less abundant (see values outside cushions at 4400 and 4700 m in Table 1). As water-holding capacity of soils in (super)páramos is positively correlated with their proportion of organic matter (Poulenard et al. 2003; Buytaert et al. 2006), it is likely that the increase in soil moisture would be a result of soil nutrient enrichment. This contrasts with the hypothesis of Maestre et al. (2009), who suggest that the outcome of interactions in alpine environments is driven by non-resource-related stress. According to these authors, the outcome of interaction at the end of a

resource gradient with a stress-tolerant nurse (*Azorella*) and stress-tolerant beneficiaries (the majority of our beneficiaries) is likely to be neutral or competitive, which was not the case in our study (facilitation). Therefore, the hypothesis that humid tropical alpine environments may provide such peculiar conditions that they do not match the classical framework of the SGH is reinforced.

Acknowledgements

We are grateful to R.M. Callaway and L. Cavieres for proposing the design used in this study. We would like to thank them, C. Armas, R. Ejrnaes, Z. Kikvidze and one anonymous reviewer for constructive comments that improved the manuscript. M. Launay, E. Cadier, A. Leenaerts, J.P. Almeida and M. Mafla are acknowledged for valuable help in the field; P. Sklenář and D. Carate for contributions to botanical determinations; B. Francou for discussions on the abiotic (micro)environment. We dedicate this work to the kind memory of Charlotte Mazoyer.

References

- Anthelme, F. & Michalet, R. 2009. Grass-to-tree facilitation in an arid grazed environment (Air mountains, Sahara). *Basic and Applied Ecology* 10: 437–446.
- Armas, C., Ordiales, R. & Pugnaire, F.I. 2004. Measuring plant interactions: a new comparative index. *Ecology* 85: 2682–2686.
- Arroyo, M.T.K., Cavieres, L.A., Peñaloza, A. & Arroyo-Kalin, M.A. 2003. Positive associations between the cushion plant *Azorella monantha* (Apiaceae) and alpine plant species in the Chilean Patagonian Andes. *Plant Ecology* 169: 121–129.
- Badano, E.I. & Marquet, P.A. 2009. Biogenic habitat creation affects biomass–diversity relationships in plant communities. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 191–201.
- Bertness, M.D. & Callaway, R.M. 1994. Positive associations in communities. *Trends in Ecology and Evolution* 9: 191–193.
- Brooker, R.W. 2006. Plant–plant interactions and environmental change. *New Phytologist* 171: 271–284.
- Brooker, R.W. & Kikvidze, Z. 2008. Importance: an overlooked concept in plant interaction research. *Journal of Ecology* 96: 703–708.
- Brooker, R., Kikvidze, Z., Pugnaire, F.I., Callaway, R.M., Choler, P., Lortie, C.J. & Michalet, R. 2005. The importance of importance. *Oikos* 109: 63–70.
- Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffrers, K., Seifan, M., Touzard, B. & Michalet, R. 2008. Facilitation in plant communities: the past, the present and the future. *Journal of Ecology* 96: 18–34.
- Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18: 119–125.
- Buytaert, W., Deckers, J. & Wyseure, G. 2006. Description and classification of highly weathered Andosols in the south Ecuadorian páramo. *Geomorphology* 73: 207–221.
- Buytaert, W., Cuesta-Camacho, F. & Tobón, C. 2011. Potential impacts of climate change on the environmental services of humid tropical alpine regions. *Global Ecology and Biogeography* 20: 19–33.
- Callaway, R.M. 2007. *Positive interactions and interdependence in plant communities*. Springer, Dordrecht, NL.
- Callaway, R., Brooker, R., Choler, P., Kikvidze, Z., Lortie, C., Michalet, R., Paolini, L., Pugnaire, F., Newingham, B., Aschehoug, E., Armas, C., Kikodze, D. & Cook, B.J. 2002. Positive interactions among alpine plants increase with stress. *Nature* 417: 844–848.
- Cavieres, L.A. & Badano, E.I. 2009. Do facilitative interactions increase species richness at the entire community level? *Journal of Ecology* 97: 1181–1191.
- Cavieres, L.A., Arroyo, M.T.K., Peñaloza, A. & Molina-Montenegro, M.A. 2002. Nurse effect of *Bolax gummifera* (Apiaceae) cushion plants in the alpine vegetation of the Chilean Patagonian Andes. *Journal of Vegetation Science* 13: 547–554.
- Cavieres, L.A., Quiroz, C.L., Molina-Montenegro, M.A., Muñoz, A. & Pauchard, A. 2005. Nurse effect of the native cushion plant *Azorella monantha* on the invasive non-native *Taraxacum officinale* in the high-Andes of central Chile. *Perspectives in Plant Ecology, Evolution and Systematics* 7: 217–226.
- Cavieres, L.A., Badano, E.I., Sierra-Almeida, A. & Molina-Montenegro, M.A. 2006. Positive interactions between alpine plant species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of central Chile. *New Phytologist* 169: 59–70.
- Cavieres, L.A., Badano, E.I., Sierra-Almeida, A. & Molina-Montenegro, M.A. 2007. Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native plants in the high-Andes of central Chile. *Arctic, Antarctic and Alpine Research* 39: 229–236.
- Cavieres, L.A., Quiroz, C.L. & Molina-Montenegro, M.A. 2008. Facilitation of the non-native *Taraxacum officinale* by native nurse cushion species in the high Andes of central Chile: are there differences between nurses? *Functional Ecology* 22: 148–156.
- Chapin, F.S.I., Walker, L.R., Fastie, C.L. & Sharman, L.C. 1994. Mechanisms of primary succession following deglaciation at Glacier Bay. *Ecological Monographs* 64: 149–175.
- Choler, P., Michalet, R. & Callaway, R.M. 2001. Facilitation and competition on gradients in alpine plant communities. *Ecology* 82: 3295–3308.
- Dullinger, S., Kleinbauer, I., Pauli, H., Gottfried, M., Brooker, R., Nagy, L., Theurillat, J.P., Holten, J.I., Abdaladze, O., Benito, J.L., Borel, J.-L., Coldea, G., Ghosn, D., Kanka, R., Merzouki, A.,

- Klettner, C., Moiseev, P., Molau, U., Reiter, K., Rossi, G., Stanisci, A., Tomaselli, M., Unterlugauer, P., Vittoz, P. & Grabherr, G. 2007. Weak and variable relationships between environmental severity and small-scale co-occurrence in alpine plant communities. *Journal of Ecology* 95: 1284–1295.
- Eränen, J. & Kozlov, M. 2008. Increasing intraspecific facilitation in exposed environments: consistent results from mountain birch populations in two subarctic stress gradients. *Oikos* 117: 1569–1577.
- Fajardo, A., Quiroz, C.L. & Cavieres, L.A. 2008. Spatial patterns in cushion-dominated plant communities of the high Andes of central Chile: how frequent are positive associations? *Journal of Vegetation Science* 19: 87–96.
- Feller, U., Anders, I. & Mae, T. 2008. Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. *Journal of Experimental Botany* 59: 1615–1624.
- Grime, J.P. 1979. *Plant strategies and vegetation processes*. Wiley, Chichester, UK.
- Gross, N., Liancourt, P., Choler, P., Suding, K.N. & Lavorel, S. 2010. Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 9–19.
- Hugo, E.A., McGeoch, M.A., Marshall, D.J. & Chown, S.L. 2004. Fine scale variation in microarthropod communities inhabiting the keystone species *Azorella selago* on Marion Island. *Polar Biology* 27: 466–473.
- Jacobsen, D. (2008) Tropical high-altitude streams. In: Dudgeon, D. (ed.) *Tropical stream ecology*. pp. 219–256. Elsevier Science, London, UK.
- Jacobsen, D., Dangles, O., Andino, P., Espinosa, R., Hamerik, L. & Cadier, E. 2010. Longitudinal zonation of macroinvertebrate in an Ecuadorian glacier-fed stream: do tropical glacial systems fit the temperate model? *Freshwater Biology* 55: 1234–1248.
- Kikvidze, Z., Pugnaire, F.I., Brooker, R.W., Choler, P., Lortie, C.J., Michalet, R. & Callaway, R.M. 2005. Linking patterns and processes in alpine plant communities: a global study. *Ecology* 86: 1395–1400.
- Körner, C. 2003. *Alpine plant life – functional plant ecology of high mountain ecosystems*. 2nd ed. Springer, Berlin, DE.
- Körner, C. 2007. The use of ‘altitude’ in ecological research. *Trends in Ecology and Evolution* 22: 569–574.
- Lortie, C.J. 2010. Synthetic analysis of the stress-gradient hypothesis. In: Pugnaire, F.I. (ed.) *Positive plant interactions and community dynamics*. pp. 125–147. CRC Press, London, UK.
- Luteyn, J.L. 1999. *Páramos. A checklist of plant diversity, geographical distribution and botanical literature*. The New York Botanical Garden Press, New York, NY, US.
- Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97: 199–205.
- Maisincho, L. & Carceres, B. 2007. *Glaciares del Ecuador: Antisana y Carihuayrazo*. IRD, INAMHI, EMAAP-Q, Quito, EC.
- Malhi, Y., Silman, M., Salinas, N., Bush, M., Meir, P. & Saatchi, S. 2010. Introduction: Elevation gradients in the tropics: laboratories for ecosystem ecology and global change research. *Global Change Biology* 16: 3171–3175.
- Michalet, R. 2007. Highlighting the multiple drivers of change in interactions along stress gradients. *New Phytologist* 173: 3–6.
- Molau, U. 2004. Mountain biodiversity patterns at low and high latitudes. *Ambio (Special Report)* 13: 24–28.
- Núñez, C., Aizen, M. & Ezcurra, C. 1999. Species associations and nurse plant effect in patches of high-Andean vegetation. *Journal of Vegetation Science* 10: 357–364.
- Nyakata, M.J. & McGeoch, M.A. 2008. Temperature variation across Marion Island associated with a keystone plant species (*Azorella selago* Hook. (Apiaceae)). *Polar Biology* 31: 139–151.
- Onipchenko, V.G., Blinnikov, M.S., Gerasimova, M.A., Volkova, E.V. & Cornelissen, J.H.C. 2009. Experimental comparison of competition and facilitation in alpine communities varying in productivity. *Journal of Vegetation Science* 20: 718–727.
- Poulenard, J., Podwojewski, P. & Herbillon, A.J. 2003. Characteristics of non-allophanic Andisols with hydric properties from the Ecuadorian páramos. *Geoderma* 117: 267–281.
- Quiroz, C.L., Badano, E.I. & Cavieres, L.A. 2009. Floristic changes in alpine plant communities induced by the cushion plant *Azorella madreporica* (Apiaceae) in the Andes of central Chile. *Revista Chilena de Historia Natural* 82: 171–184.
- Ramsay, P.M. & Oxley, E.R.B. 1997. The growth form composition of plant communities in the Ecuadorian páramos. *Plant Ecology* 131: 173–192.
- le Roux, P.C. & McGeoch, M.A. 2008. Spatial variation in plant interactions across a severity gradient in the sub-arctic. *Oecologia* 155: 831–844.
- le Roux, P.C. & McGeoch, M.A. 2010. Interaction intensity and importance along two stress gradients: adding shape to the stress-gradient hypothesis. *Oecologia* 162: 733–745.
- Rundel, P.W., Smith, A.P. & Meinzer, F.C. 1994. *Tropic alpine environments – plant form and function*. Cambridge University Press, Cambridge, UK.
- Seifan, M., Seifan, T., Ariza, C. & Tielboerger, K. 2010. Facilitating an importance index. *Journal of Ecology* 98: 356–361.
- Sklenář, P. 2009. Presence of cushion plants increases community diversity in the high equatorial Andes. *Flora* 204: 270–277.
- Sklenář, P. & Balslev, H. 2005. Superpáramo plant species diversity and phytogeography in Ecuador. *Flora* 200: 416–433.
- Sklenář, P., Luteyn, J.L., Ulloa, C.U. & Jorgensen, P.M. 2005. *Flora Genérica de los Páramos: Guía Ilustrada de las Plantas Vasculares*. New York Botanical Garden, New York, NY, US.
- Stocks, G., Seales, L., Paniagua, F., Maehr, E. & Bruna, E.M. 2008. The geographical and institutional distribution of ecological research in the tropics. *Biotropica* 40: 397–404.
- Venn, S.E., Morgan, J.W. & Green, P.T. 2009. Do facilitative interactions with neighboring plants assist the growth of

- seedlings at high altitudes in alpine Australia. *Arctic, Antarctic and Alpine Research* 41: 381–387.
- Wagnon, P., Lafaysse, M., Lejeune, Y., Maisincho, L., Rojas, M. & Chazarin, J.P. 2009. Understanding and modeling the physical processes that govern the melting of snow cover in a tropical mountain environment in Ecuador. *Journal of Geophysical Research* 114: 1–14.
- Yang, Y., Niu, Y., Cavieres, L.A. & Sun, H. 2010. Positive associations between the cushion plant *Arenaria polytrichoides* (Caryophyllaceae) and other alpine plant species increase with altitude in the Sino-Himalayas. *Journal of Vegetation Science* 21: 1048–1057.

Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Individual of *Azorella aretioides* in our study area, 4700 m a.s.l., massively colonized by various beneficiary species, among which *Senecio nivalis* (white-grey Asteraceae) and *Huperzia crassa* (reddish erected pteridophyte).

Appendix S2. Microenvironmental measurements inside and outside cushions of *Azorella aretioides*, at 4700 m a.s.l.

Appendix S3. Variation in air temperature and rainfall at 15α Glacier. (a) Mean monthly temperature at 4850 m during years 2005, 2006 and 2007, 1.5 m above soil surface (Station ORE IRD-INAMHI, Maisincho & Carceres 2007), (b) mean temperature each 15 min on a 24-h scale at site 4700 m outside cushions (10 cm above soil surface), and (c) mean monthly rainfall amount at 4850 m, 1.5 m above soil surface, from 2000 to 2007; bars represents 95% confidence intervals (Station ORE IRD-INAMHI, Maisincho & Carceres 2007). Despite different height for measurements, stronger day-to-day variation than seasonal variation is easily identifiable.

Appendix S4. Importance of interactions between *Azorella* and other species at three elevations as calculated with the importance index I_{imp} (Seifan et al. 2010). Different types of triangles indicate facilitation (upward), neutral effect (horizontal) or inhibition (downward) by *Azorella*. Error bars represent 95% CI. See Fig. 4 for abbreviations of plant names.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.