

## Testing the role of functional trait expression in plant-plant facilitation

Stephni van der Merwe\*, Michelle Greve, Bernard Olivier, Peter Christiaan le Roux

Department of Plant and Soil Sciences, University of Pretoria

\* **Correspondence:** Stephni van der Merwe. Email: [Email: stephni.vdm@gmail.com](mailto:stephni.vdm@gmail.com)

### Abstract

1. Positive biotic interactions between plant species may strongly affect species and community level patterns, but the processes through which benefactor species alter the performance of interacting species (via, for example, beneficial mechanisms like resource provisioning) are still inadequately understood. One poorly explored potential explanation is that plant-plant facilitation could occur through the impact of benefactor species on the functional trait expression of beneficiary species. Indeed, plant species that affect local conditions can modify functional trait expression of interacting species, thereby improving their performance and resulting in a facilitative interaction. However, the response of intraspecific trait variation to biotically-driven microhabitat modification, and its role in determining the outcome of plant-plant interactions, has rarely been explored.

2. Here, we test whether growing with benefactor species affects the expression of functional traits of eight species, encompassing different plant growth forms, in two contrasting study systems. This is achieved by using a paired sampling approach to compare values of seven functional traits of conspecific individuals growing within and adjacent to cushion plants (i.e. benefactor species which are known to strongly alter microhabitat conditions and to have positive effects on some of the focal species). In addition, we test whether the effect of biotic interactions on functional trait expression changes along elevational gradients, as the outcome of biotic interactions is expected to vary with elevation.

3. Contrary to predictions, in both systems intraspecific trait variation was not well explained by the biotic interaction with the cushion plant species or the variation in abiotic conditions associated with elevational gradients. Where biotic interactions did affect functional trait expression and bivariate trait relationships, traits responded variably between species, suggesting that context-specificity may be a constraint to predicting how intraspecific trait variation responds to plant-plant interactions, adding to the growing body of literature that challenges the generality and predictability of the drivers of intraspecific trait variation.

4. This research, therefore, suggests that benefactor species' facilitative process is likely not through an impact on intraspecific trait expression, and that instead other processes may be more important

for translating beneficial microhabitat modification or increased resource availability by benefactor species into positive impacts on beneficiary species.

**Keywords:** context-specific, drivers of functional traits, elevational gradient, facilitation, facilitative mechanisms, functional trait expression, intraspecific trait variation, stress gradient hypothesis

## Introduction

The mechanisms through which plant-plant interactions benefit interacting species have broadly been attributed to abiotic amelioration, associational defence, and increased resource availability (Pugnaire et al. 2011; Michalet & Pugnaire 2016). However, the processes through which these mechanisms translate to facilitative effects for beneficiary species remain largely unresolved (Michalet & Pugnaire 2016). A poorly explored process of plant-plant facilitation may occur through the influence of microhabitat modification by benefactor species on the expression of functional traits of beneficiary species (i.e. in response to abiotic amelioration or resource provisioning; Schöb et al. 2012; Garcia-Cervigon et al. 2015). Functional traits are proxies for plant physiology, morphology, and phenology and can thus represent important plant functions such as light capturing (e.g. leaf area), defence (e.g. leaf toughness and thickness), competitive ability (e.g. plant height), and photosynthetic rate (e.g. chlorophyll or nutrient content; Perez-Harguindeguy et al. 2013). Such traits vary intraspecifically (Siefert et al. 2015) and can thus reflect the response of individuals to the biotic and abiotic attributes of their environment (Westoby et al. 2002; Schöb et al. 2013). Where individual plants adjust resource allocation in response to their environment, the change in resource allocation will be reflected as trade-offs in functional trait expression (Schöb et al. 2012) associated with resource-use, abiotic stress, and defence (Westoby et al. 2002), representing a shift towards more conservative or acquisitive functions to maximize individual fitness (Ceriani et al. 2009).

Variation in functional traits, both within and between plant species, may be driven by a range of environmental factors (Westoby et al. 2002; Schöb et al. 2013; Stark et al. 2017), including geology, nutrient availability, abiotic stress, and exposure to sunlight, at both coarse and fine scales (Perez-Harguindeguy et al. 2013; Siefert et al. 2015). Functional traits may thus be predicted to respond to microenvironmental modification caused by interacting species (Butterfield & Callaway 2013; Garcia-Cervigon et al. 2015). As a result, species growing in close proximity to benefactor species and, therefore, experiencing different microenvironmental conditions to the adjacent substrate, are expected to exhibit different trait values than plants growing away from benefactor species (as well as different ranges of trait values; Schöb et al. 2012). More specifically, since local conditions associated with benefactor plants are more favourable than the surroundings (e.g. Cavieres et al.

2007), functional trait values that represent lower abiotic stress and more resource-acquisitive states (i.e. less conservative states), such as high specific leaf area (SLA; Poorter et al. 2009; Wang et al. 2017), are expected for species growing within more favourable microhabitats. Indeed, the limited available evidence suggests that functional traits within species may shift to more resource-acquisitive states in response to the favourable microenvironmental conditions created by benefactor species (Schöb et al. 2012; Garcia-Cervigon et al. 2015; Chacón-Labela et al. 2016), improving species performance and resulting in a positive outcome from the interaction (Schöb et al. 2013). Furthermore, at the community level, species also affect functional trait variation and changes in species composition (Schöb et al. 2012; Siefert et al. 2015; Dolezal et al. 2019). However, the link between plant-plant interactions and intraspecific trait variation (ITV) at the species level has rarely been examined (Butterfield & Callaway 2013; Garcia-Cervigon et al. 2015).

The net outcome of plant-plant interactions typically varies with environmental severity, generally becoming more positive under increasing abiotic stress in alpine environments (as proposed by the stress gradient hypothesis; Bertness & Callaway 1994; He et al. 2013). Indeed facilitation is more often evident in abiotically-stressful environments, where many plants may be dependent on the alleviation of abiotic stresses and disturbances by benefactor species (Cavieres et al. 2007; Liczner & Lortie 2014). As a result, sampling along environmental gradients can be used to examine variation in the outcome of plant-plant interactions under a range of abiotic conditions (see e.g. Armas et al. 2011; He et al. 2013). Elevational gradients are often used in such studies, as elevation is a good proxy for multiple environmental variables (including, e.g., temperature and moisture; Körner 2007). For the same reasons functional traits may also vary with elevation, and previous studies have shown elevational variation in leaf traits (e.g. Schöb et al. 2012; Read et al. 2014). Elevational gradients can thus provide efficient study systems to explore the covariation between biotic interactions and functional trait expression, both of which may be expected to vary strongly with increasing environmental stress (Körner 2007; see also Schöb et al. 2013).

Cushion-forming plant species are common in montane and polar environments globally and have been shown to strongly modify microhabitat conditions, especially in these abiotically stressful environments (Cavieres et al. 2002), to the benefit of species growing within them (Cavieres et al. 2002; Badano et al. 2010; le Roux & McGeoch 2010). The low-growing, compact growth form of cushion plants typically traps heat, providing a warmer and more thermally buffered microclimate (Badano & Cavieres 2006; Nyakatya & McGeoch 2008), whilst retaining moisture (Cavieres et al. 2007; Butterfield et al. 2016), reducing wind speed locally (le Roux & McGeoch 2010), and increasing soil nutrient content (Nunez et al. 1999). As a result, cushion plants are frequently resource-rich habitat patches with distinct microclimates that reduces abiotic stress for beneficiary species and may have

strong positive impacts on the biomass, abundance, and population structure of beneficiary species (e.g. le Roux et al. 2008b). Cushion plants also affect plant community composition and functioning (Reid et al. 2010; Schöb et al. 2012). Under high stress conditions, species tend to adopt more resource-conservative states, indicated by investment in defensive traits, such as higher leaf dry matter content, increased leaf toughness, and thicker leaves (Perez-Harguindeguy et al. 2013). In contrast, due to the ameliorated microhabitat and increased resource availability provided by cushion plants in these environments, beneficiary species may be expected to shift trait expression towards more acquisitive states (regardless of species' life history strategy) when growing within cushion plants, especially at higher elevations. Therefore, cushion plants and their co-occurring species are a good model system for examining functional trait variation (and trade-offs between functional traits) in beneficiary species in abiotically-extreme environments (Arredondo-Nunez et al. 2009; Reid et al. 2010; Schöb et al. 2013; Dolezal et al. 2019).

Here, we test whether interacting with benefactor species affects the expression of functional traits of eight focal species, encompassing different plant growth forms, along two elevational gradients. Additionally, we examine whether the microhabitat modification caused by benefactor species also alters trade-offs in functional trait expression in these species. A paired sampling approach was used to compare functional trait values of conspecific individuals growing within and adjacent to a cushion plant. In addition, we test whether the effect of biotic interactions on functional trait expression changes along elevational gradients, as the outcome of biotic interactions is expected to vary with elevation. Furthermore, by examining multiple traits and trait relationships in several species and in two contrasting study systems, we test the generality of the effect (or lack thereof) of microhabitat modifications by benefactor species on functional traits at the species level. We hypothesised that trait values representing less abiotically stressed and more resource-acquisitive states would be higher in cushion plants and at lower elevations where less stress is generally experienced by plants, resulting in decreased investment in defensive traits.

## **Material and methods**

### *Study site and species*

This study was conducted in two different study systems: species- and nutrient-poor sub-Antarctic tundra on Marion Island ("MI" hereafter; 46°52'34" S, 37°51'32" E), and species-rich montane grassland in the Drakensberg Mountains, South Africa (specifically within Golden Gate Highlands National Park; "GG" hereafter; 28°30'21" S, 28°37'0" E). Marion Island covers approximately 290 km<sup>2</sup> and has a cool, thermally-stable, oceanic climate with mean annual precipitation of c. 2000

mm (Smith 2002). The island's geology is dominated by smoothed pre-glacial "grey" lava and rough post-glacial "black" lava (Gremmen & Smith 2008), with sampling in this study restricted to grey lava. There are no large herbivores on the island, and herbivory by invertebrates is limited. Golden Gate Highlands National Park covers c. 340 km<sup>2</sup> of grassland, with isolated patches of shrubland and Afrotemperate forest, and has pronounced variability in topography, elevation and climate (Daemane et al. 2010). The annual rainfall in GG is c. 780 mm (Daemane et al. 2010). Several species of large herbivores occur within Golden Gate Highlands National Park, but there was no evidence of extensive herbivory at any of our study sites (possibly due to cushion plant species at GG being limited to rocky and exposed sites; Momberg et al. 2018). The study systems both span a range of elevations (MI: 0 to 1230 m a.s.l.; GG: 1837 to 3099 m a.s.l.), with higher elevations at both sites being more abiotically stressful due to lower temperatures and higher wind speeds than lower elevations (le Roux & McGeoch 2010; van der Merwe 2018).

A dominant and widespread cushion plant species was selected in each study system. On MI, *Azorella selago* (Apiaceae) is a compact, dome-shaped, long-lived, and low-growing plant species, with the greatest elevational range of any vascular plant on the island (from sea level to c. 840 m a.s.l.; le Roux & McGeoch 2008a). *Azorella selago* acts as a benefactor species, positively affecting beneficiary species, probably because its cushion form ameliorates extreme environmental conditions (Cavieres et al. 2002; le Roux & McGeoch 2010) and increases resource availability (Buyens 2017; see also Cavieres et al. 2007 for congeneric species). Because of this, *A. selago* is important in vegetation succession on MI and is considered a keystone species (le Roux & McGeoch 2008b; le Roux & McGeoch 2010). *Euphorbia clavarioides* (Euphorbiaceae) is a locally common species in GG, occurring on exposed, rocky ridges, and eroded slopes in the Drakensberg Mountains and other high elevation grasslands in South Africa (Momberg et al. 2018). *Euphorbia clavarioides* is also low-growing and compact, but differs from *A. selago* as it is a succulent species. The richness and diversity of vascular plant species is significantly higher on *E. clavarioides* cushion plants than the adjacent substrate, highlighting the positive effects of this species at the community level (Buyens 2017).

At each site, abundant plant species growing on the respective cushion plants and the neighbouring substrate were selected for sampling (hereafter "focal species"; only locally abundant and widespread species were chosen to ensure adequate sample sizes). On MI only the grass *Agrostis magellanica* commonly occurred with *A. selago* across a range of elevations. In contrast, in GG several species commonly occurred with *E. clavarioides* across a range of elevations, of which seven species with a range of growth forms were selected: three grasses (*Harpochloa falx*, *Microchloa caffra* and *Cymbopogon pospischilii*; Poaceae), two succulents (*Delosperma cooperi* and *Ruschia putterillii*;

**Table 1.** Summary of the effect of microhabitat type and elevation on the expression of seven different functional traits by eight species. Only significant predictor variables are indicated, where functional traits are presented as either lower or higher in association with cushion plants, and increasing ( $\uparrow$ ) or decreasing ( $\downarrow$ ) with elevation. “C” = growing in cushion plants, “S” = soil, i.e. growing away from cushion plants, “E”= elevation. Superscripts indicate biomes: <sup>a</sup> = sub-Antarctic tundra, <sup>b</sup> = montane grassland. Results presented in brackets were significant before, but not after, the Benjamini-Hochberg adjustment for multiple comparisons was applied. For detailed results refer to Tables S1 and S3 in the Supporting Information.

Species	Family	Growth form	n (pairs)	Specific leaf area	Leaf area	Plant height	Chlorophyll content	Leaf thickness	Leaf toughness	Leaf dry matter content
<sup>a</sup> <i>Agrostis magellanica</i>	Poaceae	Graminoid	275	-	-	C<S	-	-	-	-
<sup>b</sup> <i>Cymbopogon pospischilii</i>	Poaceae	Graminoid	29	-	-	-	-	[ $\uparrow$ E]	[ $\uparrow$ E]	-
<sup>b</sup> <i>Microchloa caffra</i>	Poaceae	Graminoid	42	[ $\downarrow$ E]	[ $\downarrow$ E]	-	-	[C>S]	[C>S]	[C>S]
<sup>b</sup> <i>Harpochloa falx</i>	Poaceae	Graminoid	30	-	-	-	-	-	-	-
<sup>b</sup> <i>Delosperma cooperi</i>	Aizoaceae	Succulent dwarf shrub	21	-	[ $\downarrow$ E]	-	-	-	-	-
<sup>b</sup> <i>Ruschia putterillii</i>	Aizoaceae	Succulent dwarf shrub	33	-	-	$\downarrow$ E	-	-	-	-
<sup>b</sup> <i>Oxalis obliquifolia</i>	Oxalidaceae	Geophytic herb	37	[C<S]	[C>S]	-	[ $\uparrow$ E]	C>S	C>S	[C>S]
<sup>b</sup> <i>Senecio rhomboideus</i>	Asteraceae	Herb	53	-	-	-	-	-	-	-

Aizoaceae), and two herbs (*Senecio rhomboideus*; Asteraceae and *Oxalis obliquifolia*; Oxalidaceae; Table 1).

### *Field sampling*

Elevational gradients are frequently used to study variation in individual species and communities, in part because such gradients can be used as proxies for variation in temperature and other abiotic factors (e.g. geomorphological disturbances, wind exposure, and moisture; Read et al. 2014; Körner 2007). Sampling on MI was conducted in April 2016 in three elevational transects running along exposed ridges dominated by *A. selago* cushion plants in fellfield vegetation (the dominant vegetation type on the island; Gremmen & Smith 2008). To select sites that differ in abiotic stress levels, transects were separated by a minimum of 3.5 km, and were sampled at four elevations (c. 75, 150, 250 and 350 m a.s.l.), which includes elevations above and below the c. 100 m a.s.l. threshold where the impact of *A. selago* on *A. magellanica* shifts from competitive to facilitative (le Roux et al. 2010). One transect did not include a c. 75 m a.s.l. site, due to inaccessible topography. Sampling in GG was conducted in January 2016 across an elevational gradient of 1975 – 2316 m a.s.l., with three populations of *E. clavarioides* selected within each of three broad elevational bands (i.e. nine *E. clavarioides* “sites”; c. 1950, 2050 and 2150 m a.s.l.) where all focal species occurred abundantly. All cushion plants sampled were approximately hemispherically shaped, with diameters typically between c. 30 – 80 cm (*A. selago*) and c. 15 - 35 cm (*E. clavarioides*).

Functional trait sampling of all beneficiary species in both systems was conducted following a paired sampling design where a pair of conspecific individuals were sampled in two microhabitat types: (1) one individual rooted firmly within a cushion plant’s canopy (“cushion” hereafter), and (2) one individual rooted in the adjacent substrate (“soil” hereafter). For each individual sampled within a cushion plant, the closest conspecific individual (not more than 1 m away) was sampled in the adjacent substrate. The paired sampling approach was used to minimize potential confounding differences in other environmental conditions (including air temperature and incident solar radiation that could potentially influence traits; see e.g. Poorter et al. 2009) between individuals within the same pair. Only mature individuals were selected for sampling. All pairs at a sampling site were collected on the same day (following Perez-Harguindeguy et al. 2013). Leaves (still attached to twigs, where appropriate) were collected from each individual and kept cool until processing.

### *Functional trait data*

Standardized protocols for sampling and processing plant functional traits were followed (Perez-Harguindeguy et al. 2013). Functional traits were selected which were expected to show

variation between growing in cushion plants and in the adjacent substrate, and to changing environmental conditions across elevational gradients (see e.g. Perez-Harguideguy et al. 2013; Schöb et al. 2013; Siefert et al. 2014).

Seven functional traits were sampled: (1) plant height (cm), measured as the minimum distance between the upper boundary of the main photosynthetic tissue of the plant and the ground level; (2) specific leaf area (SLA;  $\text{m}^2 \text{kg}^{-1}$ ), measured as the one-sided area of a leaf divided by its oven-dry mass; (3) chlorophyll content ( $\text{mg m}^{-2}$ ), measured as the amount of chlorophyll a and chlorophyll b per square meter with a handheld chlorophyll meter (CCM 300, Opti-Sciences; Hudson, USA); (4) leaf area (LA;  $\text{mm}^2$ ), measured as the one-sided area of a leaf; (5) leaf dry matter content (LDMC;  $\text{mg g}^{-1}$ ), measured as the oven-dry mass of a leaf divided by its rehydrated fresh mass; (6) leaf thickness (mm) measured with a digital calliper; and (7) leaf toughness (force to punch; N), measured with a digital force gauge (Sauter FH-S; Balingen, Germany). High values of SLA, LA and chlorophyll content represent more resource acquisitive states, i.e. lower abiotic stress levels and more rapid acquisition of resources, while lower values suggest resource conservative states (Wright et al. 2004; Poorter et al. 2009). Plant height is also considered to be linked to lower abiotic stresses and resource acquisition (e.g. responding negatively to stresses like wind exposure and positively to shading; Moles et al. 2009; Perez-Harguideguy et al. 2013). The opposite is true for LDMC, leaf toughness and leaf thickness, which represent species' tolerance of abiotically severe environments, thus high values are considered indicative of resource conservation and defence (Onoda et al. 2011; Perez-Harguideguy et al. 2013).

The seven functional traits were measured on all focal species. Two fully expanded and undamaged leaves were collected from each individual. One leaf was used to measure SLA, LA, LDMC, chlorophyll content, and leaf thickness; leaf toughness was measured on a separate leaf due to the destructive nature of the toughness measurement. Chlorophyll content was measured three times per leaf, with the mean value used in analyses. All leaf samples were processed within 24 h of harvesting, after rehydration (following Garnier et al. 2001). The fresh mass of each leaf was recorded subsequent to rehydration, after which every leaf was scanned using a flatbed scanner at 300 dpi resolution. After scanning, leaves were oven-dried at 60 °C for c. 96 h. Leaf mass was obtained at 1 mg resolution (Radwag PS 200/2000C/1 microbalance; Miami, USA). Leaf area was quantified using ImageJ software v. 1.41 (Wang 2016).

### *Statistical analyses*

General linear mixed effect models were fitted to each species-by-trait combination to determine how elevation (as a proxy for abiotic conditions) and the biotic interaction with the cushion plant (i.e. the two different microhabitat types) affect the expression of each functional trait. A second



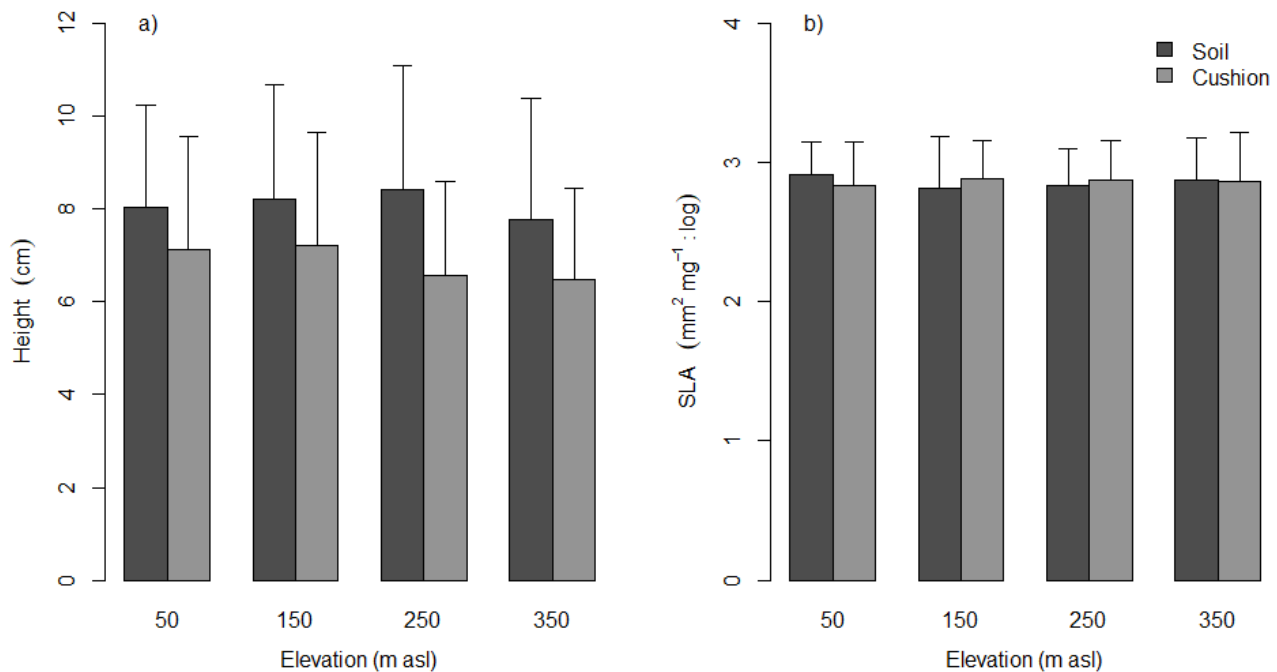
set of models (“combined models”) was run with the GG data where all seven species’ functional trait data was analysed together to test whether there was a general trend for each functional trait across species. In all models, pair identity (soil vs cushion pair), and site identity were included as random effects (with pair nested within site) to account for the spatial clustering of samples. In the combined models (i.e. the analysis that combined all species data), species was additionally added as a random effect. Overall model significance was determined by comparing models to null models containing only the random effects, and only significant models were considered further (i.e. coefficients significantly different from zero were only examined further where the overall model was also significant; see Table S1 and S2 in the Supporting Information). Where necessary, functional trait values (i.e. response variables) were transformed prior to analyses using log or square root transformations to meet model assumptions. For all models marginal  $R^2$  values were calculated to reflect the proportion of variation in the data that is explained by fixed effects (Nakagawa & Schielzeth 2013). Adjusted significance levels were calculated for each set of analyses using the Benjamini-Hochberg method (Benjamini & Hochberg 1995) to reduce the probability of type I errors.

Non-metric multidimensional scaling (NMDS; Bray-Curtis distance measure, two dimensions, transformed using Wisconsin double standardisation) ordination and permutational multivariate analysis of variance (PERMANOVA) were used to assess the combined response of all traits to microhabitat type and elevation (and their statistical interaction).

Standard major axis (SMA) regression was used to examine if the interaction with the cushion plant altered trait trade-offs in the focal species, by testing for differences in bivariate trait relationship patterns between individuals growing on the soil and on cushion plants for each species and for each pair of traits individually. Standard major axis regression analyses test for four types of potential differences in the nature of bivariate relationships (Falster 2006). First, it tests for differences in the slopes of bivariate trait relationships between individuals growing on the soil vs on a cushion plant (i.e. differences in trait trade-off patterns between groups; type A shift). A difference in the slope of the bivariate relationship between two traits of individuals growing in cushion plants vs on soil suggests differences in trade-off patterns in the two habitats. If slopes do not differ, SMA regression tests for shifts of traits along the same trait trade-off gradient (i.e. differences in the range of trait values without differences in the slopes of bivariate relationships; either a type B, C or D shift; Falster 2006). Type B shifts occur when one bivariate relationship has the same slope but a different elevation than the other relationship. In contrast, type C shifts occur when the bivariate relationship between two traits has the same elevation and slope, but the values of both traits for the two habitats are significantly separated along the common slope. Finally, type D shifts occur when the bivariate relationship for both habitats have the same slope, but the bivariate relationship for one habitat has

both a different elevation and is significantly separated along the common slope, i.e. both a type B and type C shift (Warton et al., 2006). Differences in bivariate trait patterns (i.e. differences in slopes or trait value ranges) were only considered meaningful if the associated trait-pair showed a significant correlation for at least individuals growing on the soil, or individuals growing on cushion plants.

Statistical analyses were conducted using R statistical software (R Development Core Team 2016), implementing functions from the car (Fox & Weisberg 2011), vegan (Oksanen et al. 2016), MuMIn (Barton 2017), BiodiversityR (Kindt & Coe 2005), MASS (Venables & Ripley 2002), lme4 (Bates et al. 2015), and SMATR (Warton et al. 2012) packages.



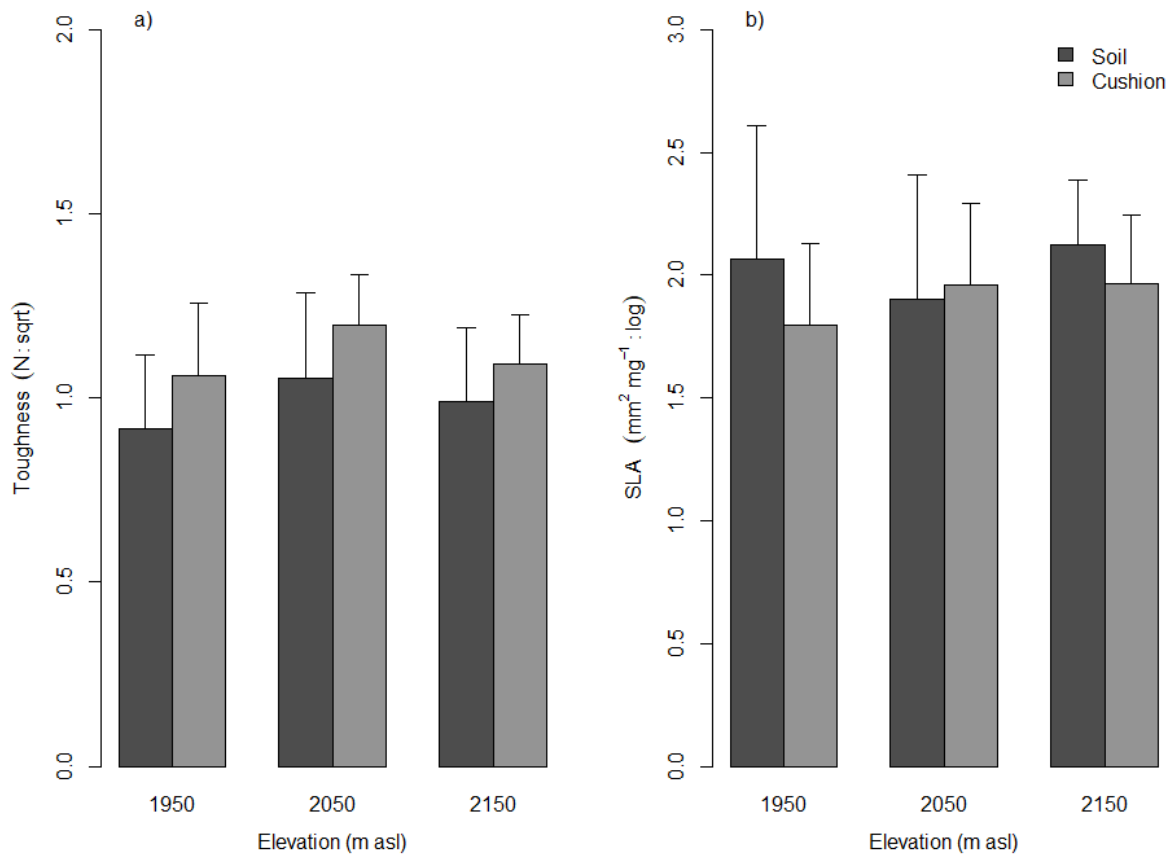
**Figure 1.** Mean functional trait values ( $\pm$  SD) of *Agrostis magellanica* growing in two microhabitat types in sub-Antarctic Tundra: growing within *Azorella selago* cushion plants (“Cushion”) or away from cushion plants in the adjacent substrate (“Soil”;  $n = 275$  per microhabitat type), measured at four elevations for a) plant height, which differed between microhabitat type but not across elevation, and b) specific leaf area, which did not differ across microhabitat type or elevation (see Table S1 in the Supporting Information for statistical details). The midpoint of each elevational band is indicated. Specific leaf area was log transformed.

## Results

The statistical interaction between elevation and microhabitat type (i.e. the two predictors of intraspecific trait variation) was not significant in any model, and was therefore not included in final models. Functional traits showed limited response to microhabitat type (i.e. to the biotic interaction with the cushion plant species) and elevation (Table 1). On MI, only plant height was significantly

different between microhabitat types, with *A. magellanica* individuals being shorter in the cushion plants than in soil (Fig 1; Table S1). Elevation was not significantly related to variation in any traits at MI (Table S1).

When considering species separately at GG, only leaf toughness and thickness were higher for individuals growing in cushion plants for *O. obliquifolia* (Fig. 2; Table S2). Plant height, SLA, LA, LDMC, and chlorophyll content were not significantly different between microhabitat types for any species in GG. Furthermore, only in *R. putterillii* was plant height significantly related (negatively) to elevation. No other species or traits had a significant relationship with elevation.

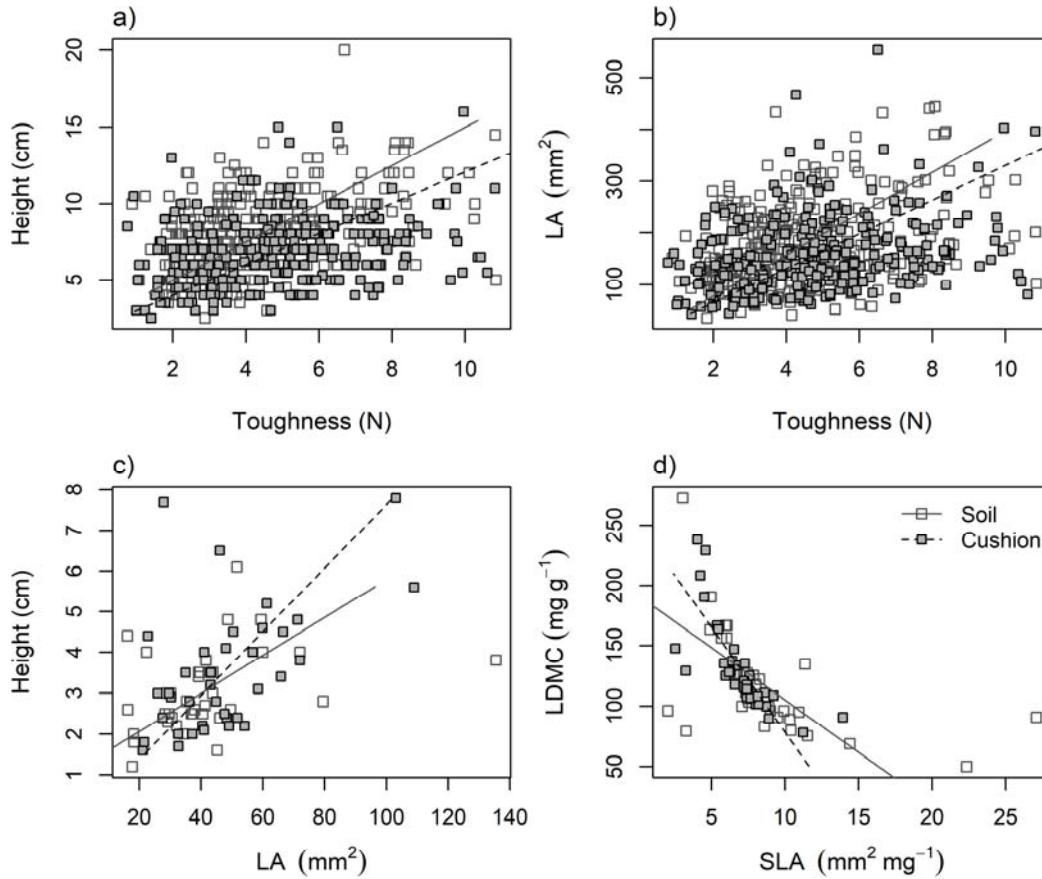


**Figure 2.** Mean functional trait values ( $\pm$  SD) of *Oxalis obliquifolia* growing in two microhabitat types; within *Euphorbia clavarioides* cushion plants (“Cushion”) and away from cushion plants (“Soil”) in each elevational band ( $n = 37$  per microhabitat type) in a montane grassland, measured at three elevations for a) leaf toughness, which varied with microhabitat (but not elevation), and b) specific leaf area, which did not differ between microhabitat or across elevations (see Table S2 in the Supporting Information for statistical details). The midpoint of each elevational band is indicated. Toughness was square root transformed and specific leaf area was log transformed.

When evaluating all seven GG species together to test the generality of patterns across species, LA, leaf toughness, and leaf thickness were significantly higher within cushion plants (Table S3). Only chlorophyll content was significantly affected by elevation (increasing with elevation) in the combined analysis. The statistical interaction between microhabitat type and elevation was not significant.

Using ordination to consider all species and functional traits simultaneously, there was a very weak, albeit statistically significant, relationship between functional traits and microhabitat type (PERMANOVA:  $p = 0.043$ ,  $R^2 = 0.5\%$ ; Fig. S1) and elevation ( $p = 0.001$ ,  $R^2 = 2.5\%$ ) on MI (Fig. S1). The statistical interaction between microhabitat type and elevation was not significant ( $p = 0.94$ ,  $R^2 = 0.2\%$ ). In GG, there was no significant relationship between microhabitat type and functional traits ( $p = 0.15$ ,  $R^2 = 0.09\%$ ); however, trait values differed significantly between species ( $p = 0.001$ ,  $R^2 = 76\%$ ) and across elevation ( $p = 0.001$ ,  $R^2 = 1.0\%$ ), although the latter explained a negligible amount of variation (Fig. S2).

Standard major axis regression showed significant differences in bivariate trait patterns between the two microhabitat types for only four of the possible 147 trait pairs for the GG species (all of which are for *O. obliquifolia*), and for six of the possible 21 trait pairs for *A. magellanica* on MI (Table S4). All the statistically significant SMA results were for differences in trait trade-offs (i.e. bivariate trait slopes differ between microhabitats; type A patterns; Table S4; Falster 2006). No bivariate trait relationships for any of the species showed significant type B, C, or D shifts. For example, on MI, plants growing in soil had a steeper increase in both plant height (Fig. 3a) and LA (Fig. 3b) with increasing toughness, compared to plants growing in cushion plants. At GG, *O. obliquifolia* had a greater increase in LA with increasing plant height when growing in cushion plants (Fig. 3c), and faster reduction in SLA with increasing LDMC (Fig. 3d) in cushion plants, compared to *O. obliquifolia* growing in the adjacent soil. In all but one of the significant comparisons, the differences in slope were relatively small, with individuals growing on the soil and within cushion plants exhibiting slopes of the same sign (see Table S4 for full results).



**Figure 3.** Standard major axis regression showing differences in bivariate trait relationship patterns between microhabitat types; growing in cushion plants (“Cushion”; dashed line) and away from cushion plants (“Soil”; solid line) for a) leaf toughness and plant height, and b) leaf toughness and leaf area, for *Agrostis magellanica* on MI, and c) leaf area and plant height, and d) specific leaf area and leaf dry matter content, for *Oxalis obliquifolia* in GG. Only significant relationships are presented here (i.e. the slope of the bivariate trait relationships differed significantly between microhabitat type in (a) – (d)). See Table S4 in the Supporting Information for full results.

## Discussion

The results from these two study systems give new insights into the potential for the modification of functional traits by a benefactor species to act as the process driving plant-plant facilitation. First, contrary to predictions, there was a limited response of functional traits to the interaction with cushion plants in both systems, despite cushion plants having strong positive impacts on the biomass, abundance, and population structure of beneficiary species on MI (le Roux et al. 2008b) and on species richness and diversity in GG (Buyens 2017). Second, in the 6 % of species-trait combinations where cushion plants had a significant impact on intraspecific trait variation, the impacts

on functional traits were context-specific. These results are in contrast to other studies that have found microhabitat modification by benefactor plants impact on functional traits at the community level (Schöb et al. 2012; Garcia-Cervigon et al. 2015; Chacón-Labela et al. 2016; Dolezal et al. 2019; see also Cavieres et al. 2005 for a study examining physiological processes directly).

Contrary to expectations, 94 % of species-trait combinations did not respond to the microenvironmental modification by cushion plants (despite all of our focal species showing ITV equal to, or exceeding, typical within-site trait variation; Table S5). This was unexpected, since cushion plants, for example, buffer extreme temperatures (Nyakatya & McGeoch 2008), retain soil moisture (Cavieres et al. 2007), and increase soil nutrient content (Nunez et al. 1999), which should decrease important stresses (especially in montane systems; Körner 2003) for beneficiary species. Indeed, cushion plants worldwide often act as resource-rich habitat patches that ameliorate harsh conditions (Reid et al. 2010), and thus the functional traits expressed by individuals growing within cushion plants were expected to reflect less stressed and more resource-acquisitive states (Westoby et al. 2002), indicated by a shift in functional trait values. Therefore, we did not find support for the hypothesis that the conditions created by the cushion plants change species level functional trait expression to values representative of more resource-acquisitive states. This is in contrast to, for example, Schöb et al. (2012) who found increased ITV and shifts in community level variation in SLA and LDMC in the presence of cushion plants. However, they also concluded that the specific mechanism of facilitation by cushion plants at the community level is likely context dependent (Schöb et al. 2012).

In the species-trait combinations that were significantly related to microhabitat, individuals growing in cushion plants tended to express functional traits values representative of higher abiotic stress and resource conservation than did conspecific individuals growing in the adjacent substrate. For instance, on MI only plant height differed between microhabitat types, with shorter grasses growing on *A. selago* cushion plants. In GG, functional trait expression by *O. obliquifolia* also responded to microhabitat type, with leaves that were tougher and thicker when growing within *E. clavarioides* cushion plants. Leaf toughness and thickness are indicators of resistance to mechanical damage (Perez-Harguindeguy et al. 2013), and, therefore, higher values of these traits may reflect that individuals growing in cushion plants are more exposed to wind (due to an elevated position and smooth surrounding cushion plant surface). This may result in shorter growth and stronger leaves under greater wind exposure. Indeed, wind is a major stress factor for vascular plants in montane environments (Körner 2003) and in the sub-Antarctic (see e.g. Gremmen & Smith 2008), affecting the distribution and performance of species (Smith et al. 2001). The frequent gale-force winds on MI may thus select for tougher or thicker leaves of species growing on the cushion plants, even if cushion plants reduce wind speed locally (le Roux & McGeoch 2010). There might thus be little shelter for

mature (i.e. larger) individuals growing on cushion plants (see also e.g. Mathakutha et al. 2019). Well-protected leaves (i.e. leaves with high toughness and thickness) are typical of a more resource-conservative state (Wright et al. 2004), allowing the plant to function better under sub-optimal conditions (i.e. exhibiting stress tolerance; Pierce et al. 2005; Ceriani et al. 2009). *Oxalis obliquifolia*, the species most strongly affected by its interaction with cushion plants in GG (along with *A. magellanica* on MI), has the shortest stature of the species studied here and could therefore experience a disproportional increase in wind exposure when growing in the elevated position on cushion plants. Therefore, cushion plants may not always offer protection from wind to all interacting individuals, but instead may increase stress for those individuals that are more sensitive to wind exposure and are growing in exposed positions on cushion plants. As a result, even though benefactor species may ameliorate some (or even many) environmental conditions, the stressors that are unaltered (or strengthened) by these biotic interactions may potentially still constrain functional trait expression in beneficiaries from shifting to more acquisitive states.

We found no evidence that the effect of cushion plants on functional traits changes with elevation, despite both plant-plant interactions and functional traits being expected to vary strongly with increasing environmental stress associated with an increase in elevation (Körner 2007; see also Schöb et al. 2012). Therefore, even though the effect of *A. selago* on the biomass and abundance of *A. magellanica* shifts with elevation on MI (le Roux & McGeoch 2008b), the impact of *A. selago* on ITV does not change with elevation. Functional trait values of only one out of 56 combinations were significantly related to elevation. There was thus no evidence for an overall shift in the plant species resource-use state or a change in response to abiotic stress across the elevational gradient, suggesting that the resource-use strategy remained unchanged with elevation. A similar pattern has been observed for *Primula glaucescens*, a montane species that showed no correlation between leaf traits (SLA and LDMC) and elevation (Ceriani et al. 2009). Consequently, it may be that other abiotic factors, which are not correlated with elevation at these sites, affect species-specific trait expression more strongly (e.g. edaphic conditions; Siefert et al. 2014). This suggests more broadly that environmental conditions correlated with elevation in both study systems are not an important driver of species-specific trait expression. Therefore, measurement of more proximal (i.e. mechanistic) environmental variables may be required to identify the gradients correlated with ITV in these ecosystems (see e.g. Blonder et al. 2018).

Not all species and not all functional traits were affected similarly by microhabitat modifications by benefactor species, even when the two benefactor species possessed the same growth form (as suggested by Callaway 1998; Butterfield & Callaway 2013). In this study, two cushion plant species differed in the impacts on functional traits in those species-trait combinations that were

significantly related to microhabitat. Since *E. clavarioides* is a succulent cushion plant, it may affect plants differently than other commonly studied cushion plants which are typically not succulent (Reid et al. 2010). The tightly packed succulent stems of *E. clavarioides* may limit space for the accumulation of organic matter and for root growth of beneficiary species. Consequently, individuals that grow within *E. clavarioides* might grow tougher, smaller or thicker leaves due to resource limitations (Ceriani et al. 2009; Dolezal et al. 2019), in contrast to the positive effect on resource availability typically suggested for cushion plants (Cavieres et al. 2007). Therefore, the effect of benefactor species on functional traits may be species-specific (Siefert et al. 2014; Schöb et al. 2017), depending on the identity of both the benefactor and the beneficiary species (Callaway 1998). Species-specificity may thus be a key characteristic determining how benefactor species affect ITV (Baruah et al. 2017; Coyle 2017; Roybal & Butterfield 2019).

In agreement with a growing body of literature that challenges the generality and predictability of the drivers of ITV (Coyle et al. 2017; Anderreg et al. 2018; Blonder et al. 2018), trait expression was not well explained by the interaction with the benefactor species or the abiotic conditions associated with elevational gradients. The large amount of unexplained variation could partially be explained by the fact that unmeasured site-specific factors may affect ITV (e.g. microenvironment or neighbourhood effect; Blonder et al. 2018). Indeed, functional traits have been found to respond predictably to environmental conditions not considered here (Stark et al. 2017; Bjorkman et al. 2018). For example, leaf area has been observed to increase along a precipitation gradient (Cochrane et al. 2016). Other biotic interactions, such as competition, may also play a role in driving the observed variation in leaf functional traits. Environmental context is thus an important consideration in understanding the role of biotic interactions in trait-environment relationships (Siefert et al. 2014; Blonder et al. 2018). Indeed, if the effect of benefactor species on functional traits is strongly context-dependent, forecasting shifts in species functioning in response to environmental change will be more challenging (Butterfield & Callaway 2013). More generally, the context-specificity observed here highlights the importance of understanding the drivers of functional trait variation across various systems and spatial scales, if general predictors of responsiveness to environmental change are to be identified (Bjorkman et al. 2018).

In addition to a weak effect on ITV, the biotic interaction with the cushion plants also did not have a strong impact on bivariate trait relationships (with only 10 out of 168 trait pairs significantly affected), highlighting that correlations between functional traits (including well-documented trait trade-offs, e.g., between SLA and LDMC) are not sensitive to changes in local environmental conditions in our study sites. Therefore, examining how bivariate trait relationships are affected by biotic interactions, in combination with the direct effects of biotic interactions on trait expression, revealed



limited effects of the benefactor species on beneficiary functional traits across a range of abiotic conditions. This result again reinforces our finding that facilitation does not shift beneficiary plants to more resource-acquisitive states. Nonetheless, this approach of simultaneously examining trait-environment and bivariate trait-environment relationships may help to disentangle more fully how benefactor species influence beneficiary species in systems where functional traits are more strongly impacted by biotic interactions.

More broadly, these results suggest that the process underlying the positive effects of benefactor species appears not to be through a change in expression of the functional traits of beneficiary plants. The mechanisms proposed for the facilitative effect of nurse plants are typically related to microhabitat modification (Reid et al. 2010), and can be broadly categorized as resource provisioning (le Roux & McGeoch 2004; Mortimer et al. 2008; Pugnaire et al. 2011), abiotic amelioration (Arredondo-Nunez et al. 2009), and associational defence (Michalet & Pugnaire 2016). This research, therefore, suggests that benefactor species' facilitative process is likely not through an impact on ITV, and that instead other processes may be more important for translating beneficial microhabitat modification or increased resource availability by benefactor species into positive impacts on beneficiary species.

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