A global analysis of bidirectional interactions in alpine plant communities shows facilitators experiencing strong reciprocal fitness costs

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Summary

- Facilitative interactions are defined as positive effects of one species on another, but bidirectional feedbacks may be positive, neutral, or negative. Understanding the bidirectional nature of these interactions is a fundamental prerequisite for the assessment of the potential evolutionary consequences of facilitation.
- In a global study combining observational and experimental approaches, we quantified the impact of the cover and richness of species associated with alpine cushion plants on reproductive traits of the benefactor cushions.
- We found a decline in cushion seed production with increasing cover of cushion-associated species, indicating that being a benefactor came at an overall cost. The effect of cushion-associated species was negative for flower density and seed set of cushions, but not for fruit set and seed quality. Richness of cushion-associated species had positive effects on seed density and modulated the effects of their abundance on flower density and fruit set, indicating that the costs and benefits of harboring associated species depend on the composition of the plant assemblage.
- Our study demonstrates 'parasitic' interactions among plants over a wide range of species and environments in alpine systems, and we consider their implications for the possible selective effects of interactions between benefactor and beneficiary species.

Introduction

Positive and negative interactions among organisms are important drivers of population and community dynamics (Roughgarden & Diamond, 1986) and ultimately of evolutionary processes (Thorpe *et al.*, 2011). Negative (competitive) interactions decrease the reproductive success of co-occurring species as a consequence of resource consumption (Weiner, 1988; Stöcklin, 1997), and bidirectional competitive interactions – the reciprocal impacts of the two competing species – have been widely explored, yielding crucial insight into the role of competition in communities (Keddy, 2001). By contrast, studies of positive, facilitative interactions have been dominated by a unidirectional approach. Such studies have shown that net facilitative interactions with benefactor species (i.e. species that induce a positive effect) improve growth conditions for beneficiary species (i.e. species that perform better under the influence of benefactors), and that this facilitative effect often results in higher productivity and improved fitness for the beneficiaries (Callaway, 2007). However, although the mainly unidirectional focus of facilitation research has improved our understanding of facilitative interactions among plants (Callaway, 2007), the net bidirectional nature of such interactions is poorly understood (Bronstein, 2009).

This has important consequences for considering the ecological and evolutionary impacts of facilitation. In particular, facilitation may have quite different evolutionary consequences for both benefactor and beneficiary species depending on the reciprocal effects of the beneficiary species, and thus the type of overall two-way interaction taking place, that is, commensalism, mutualism, or parasitism (Stachowicz, 2001; Callaway, 2007; Bronstein, 2009; Brooker & Callaway, 2009). For example, depending on the type of feedback effects of beneficiaries, selective forces may act on different traits. In mutualistic interactions (+/+, following the nomenclature of Odum (1968)), evolution should select for superior facilitative traits in both partners at the expense of their competitive traits, whereas in cooperative–antagonistic interactions (+/−) evolution should select for traits that promote tolerance of, or resistance to, colonization by the competitive beneficiary species (Bronstein, 2009). Consequently, an important first step for an increased understanding of the evolutionary consequences of facilitation is quantifying the full nature of the reciprocal effects of facilitators and their associated beneficiary species (Bronstein, 2009).

A few experiments have examined the impacts on benefactors of beneficiaries, and they have demonstrated a wide range of feedback effects from beneficiaries. Working in desert and alpine systems Holzapfel & Mahall (1999), Michalet *et al.* (2011), Cranston *et al.* (2012) and Schöb *et al.* (2013) showed strong negative effects of beneficiary species on their nurse plants' reproduction. Similarly, McAuliffe (1984), Valiente-Banuet *et al.* (1991) and Flores-Martínez *et al.* (1994) reported species-specific negative feedback effects of beneficiary species on their benefactors. These antagonisms might be considered examples of parasitic (+/−) interactions. By contrast, Pugnaire *et al.* (1996) found a positive feedback effect of *Marrubium vulgare* on its benefactor *Retama sphaerocarpa* in a semiarid shrubland (also see instances in Holzapfel & Mahall, 1999), and hence an overall mutualistic (+/+) interaction. Finally, in semiarid communities dominated by *Stipa tenacissima*, Armas & Pugnaire (2005) revealed counterbalancing positive and negative effects of *S. tenacissima* on its nurse shrub *Cistus clusii*, resulting in net neutral feedback effects of the grass on the shrub, which is an example of a commensalistic (+/0) interaction. Thus, the few available cases indicate that the feedback effects of interactions that are positive in one direction can range widely from negative to positive in the opposite direction. Nevertheless, given the close co-occurrence of species, which all depend on the same resources, negative feedback effects are most likely and have most often been observed in empirical research to date (Callaway, 2007). The available body of literature, however – in particular the occurrence of context-specificity in terms of benefactor species and environment – suggests that an integrated approach to assessing the feedback effects of beneficiaries, using a widely distributed system involving many nurse plant species from different families and conducted over large spatial scales, including different environments with different predominating limiting factors for plant growth, would provide a great deal of insight into the general patterns of beneficiary feedback effects in the context of facilitation.

We explored the relationship between plants with cushion morphologies and their associated species in alpine plant communities using 14 different cushion species at 35 sites in four continents and a sub-Antarctic island, and experimentally tested the feedback effects of species growing within the cushion canopy on the performance of facilitating cushion species. Alpine cushions are widely studied nurse plant systems (Reid *et al.*, 2010; Cranston *et al.*, 2012; Schöb *et al.*, 2012; Butterfield *et al.*, 2013; Cavieres *et al.*, 2013), and their often substantial facilitative effects are commonly manifest as a higher density and diversity of species within the compact cushion canopy compared with surrounding open areas (Cavieres *et al.*, 2013). The mechanisms by which cushion species facilitate neighbors include stabilizing otherwise dynamic substrates, increasing soil fertility, buffering temperature extremes and providing shelter from the wind (Körner, 2003; Cavieres *et al.*, 2006). Furthermore, the global distribution of cushion plants in alpine systems allows for the study of reciprocal feedback effects of the species associated with cushions over a wide geographic and environmental range and across a very broad phylogenetic group of benefactor species (Butterfield *et al.*, 2013). This provides the potential to reveal general regional patterns that go beyond studies of local sites or particular species (Fraser *et al.*, 2013). In order to avoid the appearance of *a priori* assumptions about the types of relationships that are occurring between cushions and other species in their communities, rather than beneficiary and benefactor species (which assumes a type of relationship is occurring), here we refer throughout to cushion plants and 'cushion-associated' species (i.e. those plant species that grow within the cushions). This latter term does not necessarily imply that the associated species are gaining a net benefit from being within the cushions.

We collected correlative and experimental data on the relationship between cushion plant fitness and assemblages of cushion-associated species. We quantified fitness of cushions by measuring their reproductive output in terms of flower density, fruit set, fruit density, seed set, seed density, seed mass and seed viability, both in cushions with intact cushion-associated species and, at selected sites, in cushions where all above-ground biomass of cushion-associated species was removed repeatedly for two consecutive years. As reproduction by seed is an important fitness component of pioneer species, we considered reproductive traits as the most suitable variables for estimating plant fitness for alpine cushion plants in a short-term study. Cushion-associated plant species assemblages were characterized by biomass, cover, number of individuals, and species richness. This approach allowed us to relate cushion reproductive success in terms of flower, fruit or seed density to the abundance (biomass, cover, number of individuals) of associated species, and to quantify the fitness consequences of cushions for harboring other plants. We expected changes in the reproductive output of cushion plants when growing with associated species as a result of direct interactions for resources or indirect interactions for pollinators or pollen (Weiner, 1988; Feldman *et al.*, 2004; Mitchell *et al.*, 2009). However, it further allowed us to dig deeper into the mechanisms of these interactions by evaluating the relative impact of cushion-associated species at different stages of cushion reproduction (flower density, fruit set, seed set, seed mass and seed viability) where the interaction may switch between competition and facilitation (Stephenson, 1981; Lee, 1988; Feldman *et al.*, 2004). Furthermore, we could assess the part that composition (i.e. the number of species) of cushion-associated species may play in their feedback effect on cushions. Even though these additional analyses of the interaction are not directly relevant in assessing the overall type of interaction between cushions and their associated species, they will indirectly help in narrowing down pathways where adaptation may occur. Based on current empirical evidence indicating predominantly negative feedback effects of beneficiaries on benefactors, we hypothesized that there would be a net cost of facilitation for cushions. We therefore expected the bidirectional interaction between cushions and their associates to be a form of parasitism. In particular, we hypothesized that cushion-associated species would compete for resources and pollinators, thereby negatively affecting each step during cushion reproduction, that is, from flower density over fruit set to seed set and seed viability. By contrast, as seed mass is often canalized during plant development (Harper *et al.*, 1970; Violle *et al.*, 2009), we expected this trait at least to show no significant response to the interaction. We further expected that the species composition of cushion-associated plant assemblages may modulate the overall impact these assemblages have on cushion reproduction.

Materials and Methods

Observational approach

Data were collected from 34 alpine sites in Europe, North and South America, Asia, and a sub-Antarctic island (Supporting Information, Table S1). These sites were dominated by cushion plants and included 13 species of cushions belonging to eight different families. At one site (Sierra Nevada, N-low1), two cushion species were sampled, therefore resulting in 35 datasets in total. The generally large populations of cushions (> 100 individuals of one species) at the study sites were commonly found in low-productivity gravel habitats in alpine belts with gentle slopes and usually within a delimited area of *c.* 0.3 km^2. Vegetation at the study sites was generally discontinuous, with patches formed by cushion plants that were frequently colonized by other vascular species that grew within their canopies. In the open areas surrounding cushions, plant cover was generally < 10% in total.

For each dataset, between 20 and 150 cushions were sampled (mean ± SE = 60.8 ± 7.0), resulting in a total of 2127 individual cushions. For each cushion plant species at a site, we haphazardly selected individuals of similar size within relatively homogeneous habitat conditions with respect to soil, topography, aspect and elevation. We estimated the cushion area of each cushion from the two perpendicular dimensions of the cushion (calculated as π (mean diameter/2)^2). The reproductive output of adult cushions was quantified with five variables, each describing a different stage of the reproductive cycle. However, not all variables were recorded at all sites (Table S2). First, the number of flowers, fruits, and seeds were determined either for whole cushions or in randomly placed quadrats of variable size and number within the cushion canopy. At a few sites, we additionally determined seed mass and seed viability of 20 cushions per site. The mean value of 20 randomly selected seeds per cushion was used for seed mass determinations. Seed viability was tested by the tetrazolium method (Leist *et al.*, 2003). We soaked 20 randomly selected seeds overnight, cut them longitudinally, and placed one half of the seed in a 1% solution of triphenyl-tetrazolium chloride and distilled water. After incubation at room temperature overnight, seeds with embryos that turned fully red were recorded as viable, whereas unstained or partly colored embryos were considered nonviable. Seed viability was then expressed as the ratio of viable seeds to all tested seeds.

We related cushion reproductive output to variables characterizing the cushion-associated plant species assemblage. Four variables were determined for the cushion-associated species assemblage in each cushion: number of species, relative cover, number of individuals, and standing biomass. All these variables were measured at approximately the time of flower production of the cushion and across the entire area of each cushion. Relative cover was determined as the visually estimated cushion area covered by the cushion-associated species relative to the entire cushion area. Standing biomass was determined by clipping all aerial biomass of cushion-associated plants, drying it at 80°C for at least 48 h, and weighing. However, not all variables characterizing the cushion-associated plant species assemblage were recorded at all the sites (Table S2).

Removal experiments

At 10 sites and one cushion plant species per site, we experimentally manipulated the effect of cushion-associated species on the cushion by removing all above-ground biomass of the cushion-associated species (Table S3). Overall, eight different cushion plant species from six different families were used for the removal experiment. Cushions were haphazardly selected on a pairwise basis and from the same population as those used for the observational approach (except for the Pyrenees site, which was not included in the observational study because of missing data for cushion size). Cushions forming a pair were similar in size and located in close vicinity to each other in order to reduce the possibility of significant environmental differences among the two cushions. From each pair of cushions we then randomly selected one cushion for the removal treatment, with the other one serving as a control. In the year following the initial removal of all above-ground biomass, all regrowth of plants growing within cushions was removed anew early in the season, and, when necessary, clipping was repeated during the growing season. The reproductive output of the cushions with all associated species removed was compared with that of the cushions with the associated plant species assemblage left intact. There were between 18 and 21 pairs of replicates per site. Even though above-ground clipping did not remove the whole plant, it has frequently been shown to be an efficient method for strongly reducing interactions among plants (Callaway *et al.*, 2002). We therefore preferred this procedure over removal of the whole plant in order to keep disturbance of the cushion minimal, which might otherwise affect cushion reproduction.

Statistical analyses

In the observational approach, we tested the relationship between flower density (i.e. the number of flowers cm^{-2}), fruit set (i.e. the ratio of flowers that turned into fruits), fruit density (i.e. the number of fruits cm^{-2}), seed set (i.e. the number of seeds per fruit) or seed density (i.e. the number of seeds cm^{-2}) of cushions and relative cover, biomass, or number of individuals of the cushion-associated plant species assemblages. We used generalized linear mixed models with Poisson error structure, followed by type-III Wald χ^2 tests, and included the number of flowers, fruits or seeds as response variables. As explanatory variables, we used square-root-transformed values of relative cover, biomass cm^{-2}, or number of individuals cm^{-2} of cushion-associated species. The transformation was applied to make the data more balanced and extreme values less influential compared with untransformed data. The cushion size independent variables were used in order to make them comparable among each other, but also among samples of different sizes. This further allowed us to use the covariate 'cushion area' to specifically control for size-dependent variation in reproductive effort (Samson & Werk, 1986). Furthermore, 'cushion species × site' was included as a random factor that accounted for differences in the mean reproductive output among cushion species and geographical sites. To translate the number of flowers, fruits or seeds into densities (i.e. the number of flowers, fruits or seeds cm^{-2}) and the number of fruits and seeds into rates (i.e. the number of fruits per flower and the number of seeds per fruit) we introduced offset terms into the models. For densities we used the area where flowers, fruits and seeds were sampled as the offset (= flower density/fruit density/seed density); for the number of fruits we further used the number of flowers as the offset (= fruit set); and for the number of seeds we also used the number of fruits as the offset (= seed set). All offset terms were $\log_e$-transformed before inclusion into the models in order to match with the $\log_e$ link-function of the model. For seed mass and seed viability, we used the same model structure but a Gaussian distribution of error terms and no offset variables.

The analyses of the relationship between cushion flower density, fruit set, fruit density, seed set and seed density and the relative cover of the cushion-associated species assemblage were repeated for each cushion plant species and site separately. For these separate analyses we performed generalized linear models with the same model structure, except that the random factor 'cushion species × site' was omitted, followed by type III likelihood χ^2 tests. This first set of additional analyses was used to check if the general finding, in which all datasets were included, was a common result for individual datasets.

In addition, we repeated the original analyses of the effects of relative cover of cushion-associated species assemblages on the reproductive output of cushions, but included richness of cushion-associated species and the interaction term with relative cover as fixed effects. We added one to each value of species richness and then used $\log_e$-transformed values for statistical analyses. We are aware of multicollinearity issues for these analyses as a result of the strong correlation among the two main effects ($r = 0.56$). Therefore, care must be taken with the interpretation of the estimates of individual fixed effects for this set of analyses. This set of analyses was performed in order to check if the cost or benefit of harboring associated plants may depend on the number of species forming this plant assemblage.

In the experimental approach, we tested the effects of neighbor removal on flower density, fruit set, fruit density, seed set, seed density, seed mass or seed viability. We used the same models as described earlier for the observational data. These models included the counts of either flowers, fruits or seeds as response variable; the sampling area in which flowers, fruits or seeds have been counted as offset variables for flower, fruit or seed density and the number of flowers or fruits as offset variables for fruit set or seed set, respectively; the removal treatment as explanatory variable; and 'cushion species × site' as random factor. These statistical analyses based on all pooled datasets were again repeated for each individual dataset using general linear models without random effect.

To test for correspondence of the results of the removal experiment and the observational approach, we performed Spearman rank correlations between model coefficients. For each cushion reproductive trait variable, we related the coefficients of the removal treatment to the corresponding coefficients of relative cover using all the sites where observational and experimental data were available.

All statistical analyses were done with R, version 2.15.3 (R Core Team, 2013), using the packages 'car' (Fox & Weisberg, 2011) for type III ANOVAs of general and generalized linear mixed models, 'effects' (Fox, 2003) and 'lattice' (Sarkar, 2008) to predict and plot model fitted values, and 'lme4' (Bates *et al.*, 2011) for general and generalized linear mixed models.

Results

Observational approach

As the cover of associated species increased, there was an overall reduction in the number of fruits and seeds per unit cushion area (Fig. 1d, Table 1), associated with fewer flowers, fewer seeds per fruit, but more fruits per flower (Fig. 1a–c, Table 1). These results for flower density, fruit set, fruit density, seed set and seed

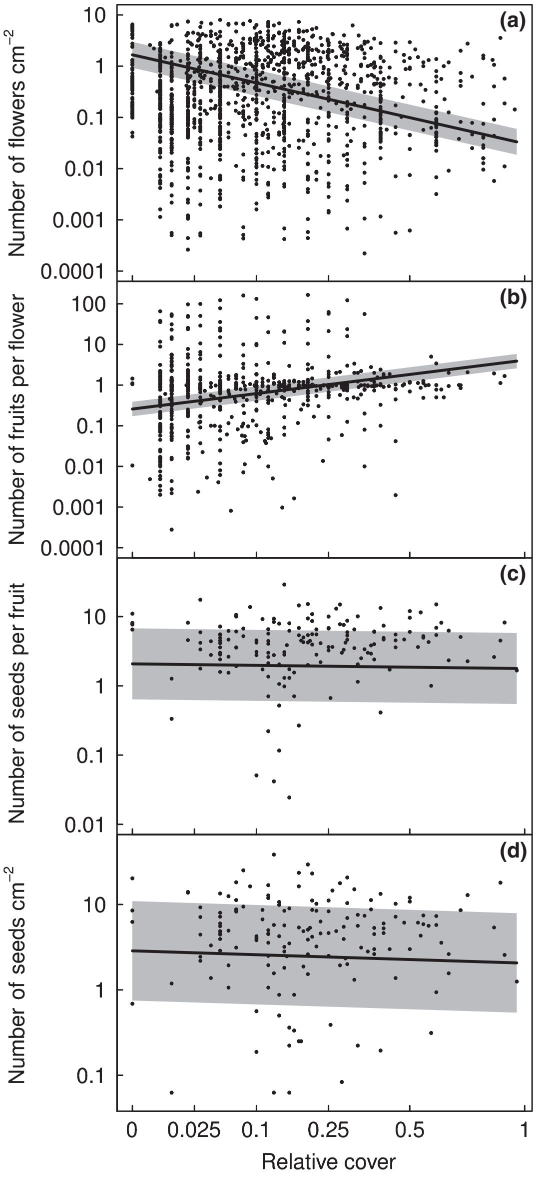

Fig. 1 Reproductive output of cushion plants in relation to the relative cover of cushion-associated plants. Shown are model predicted means and 95% CIs and measured values as dots. Note the square-root scale on the *x*-axis for the relative cover of cushion-associated plants and the log$_e$ scale on the *y*-axis for the number of flowers cm^{-2} (a), the number of fruits per flower (b), the number of seeds per fruit (c), and the number of seeds cm^{-2} (d). See Table 1 for statistical results and Notes S1 and S2 for the results relating reproductive output of cushions to biomass and number of individuals of the cushion-associated plant assemblage, respectively.

Table 1 Type III Wald χ^2-tests of the generalized linear mixed models testing the relationship between relative cover of cushion-associated species, and flower density (i.e. the number of flowers cm^{-2}; $n = 1608$, sites = 25, cushion species = 10), fruit set (i.e. the number of fruits per flower; $n = 1016$, sites = 14, cushion species = 5), fruit density (i.e. the number of fruits cm^{-2}; $n = 1036$, sites = 15, cushion species = 6), seed set (i.e. the number of seeds per fruit; $n = 180$, sites = 5, cushion species = 2), seed density (i.e. the number of seeds cm^{-2}; $n = 180$, sites = 5, cushion species = 2), seed mass ($n = 204$, sites = 7, cushion species = 3), and seed viability ($n = 81$, sites = 5, cushion species = 3) of cushions

Variable	df	χ^2	*P*	Coefficient
Flower density				
Relative cover	1	131 944	< 0.001	−3.99
Cushion area	1	282 654	< 0.001	0.96
Fruit set				
Relative cover	1	31 583	< 0.001	2.77
Cushion area	1	85 336	< 0.001	−1.32
Fruit density				
Relative cover	1	31 254	< 0.001	−2.60
Cushion area	1	1393	< 0.001	−0.16
Seed set				
Relative cover	1	16.89	< 0.001	−0.16
Cushion area	1	68.63	< 0.001	−3.87
Seed density				
Relative cover	1	84.66	< 0.001	−0.33
Cushion area	1	0.01	0.911	0.05
Seed mass				
Relative cover	1	1.90	0.168	−0.00004
Cushion area	1	0.15	0.698	−0.00005
Seed viability				
Relative cover	1	0.50	0.480	−0.16
Cushion area	1	1.51	0.219	−0.40

Cushion area was included as covariate and 'cushion species × site' was included as random factor in all models. See Notes S1 and S2 for the results relating reproductive output of cushions to biomass and number of individuals of the cushion-associated species assemblage, respectively.

density obtained over all pooled datasets held for 73, 43, 73, 20 and 60% of the datasets, respectively, when each dataset was analyzed separately (Tables S4–S8). In contrast to these quantitative measures of cushion reproduction, seed mass and seed viability of cushion plants were not significantly related to the relative cover of associated plants (Table 1). Given the strong and positive association of the relative cover of cushion-associated species with their biomass ($n = 269$, sites = 8, $F_{1,260} = 786$, $P < 0.001$) and number of individuals ($n = 1528$, sites = 25, $F_{1,1502} = 633$, $P < 0.001$) per unit area, the effects of biomass cm^{-2} (Notes S1) and number of individuals cm^{-2} (Notes S2) of cushion-associated plants on the variables of cushion reproduction resemble those of relative cover in direction and significance. The exceptions were a negative relationship between biomass of cushion-associated species and cushion fruit set and no significant relationship between biomass of cushion-associated species and seed set (Notes S1). Furthermore, there was a negative relationship between the number of cushion-associated plant individuals and the seed mass produced by cushions (Notes S2).

When we included species richness of cushion-associated species in the model, seed density was negatively related to an increased cover of cushion-association species and positively to an increased number of species, but without a significant interaction term (Table 2). Over the range of cover and richness values, we observed that the effect of cover (0 → 100% cover = sqrt(1) × −2.02 = −2.02 seeds cm^{-2}) was stronger than the effect of species richness (0 → 17 species = log(17 + 1) × 0.44 = + 1.27 seeds cm^{-2}), confirming the predominant negative effect of cushion-associated species cover on cushion seed density. For the models explaining cushion flower density, fruit set, and fruit density, we obtained significant interaction terms between relative cover and richness of the cushion-associated plant assemblage (Table 2). This indicates that the effect of the abundance of

Table 2 Type III Wald χ^2-tests of the generalized linear mixed models testing the relationship between relative cover and species richness of the cushion-associated species assemblage, and flower density ($n = 1488$, sites = 23, cushion species = 10), fruit set (i.e. the number of fruits per flower; $n = 896$, sites = 12, cushion species = 5), fruit density (i.e. the number of fruits cm^{-2}; $n = 916$, sites = 13, cushion species = 6), seed set (i.e. the number of seeds per fruit; $n = 60$, sites = 3, cushion species = 2), seed density (i.e. the number of seeds cm^{-2}; $n = 60$, sites = 3, cushion species = 2), seed mass ($n = 85$, sites = 5, cushion species = 3), and seed viability ($n = 81$, sites = 5, cushion species = 3) of cushions

Variable	df	χ^2	P	Coefficient
Flower density				
No. of species	1	77 056	< 0.001	−1.27
Relative cover	1	68 269	< 0.001	−6.58
No. of species × relative cover	1	53 243	< 0.001	3.38
Cushion area	1	244 090	< 0.001	0.90
Fruit set				
No. of species	1	8610	< 0.001	0.64
Relative cover	1	48 525	< 0.001	7.06
No. of species × relative cover	1	24 030	< 0.001	−4.07
Cushion area	1	80 195	< 0.001	−1.25
Fruit density				
No. of species	1	6821	< 0.001	−0.46
Relative cover	1	9383	< 0.001	−1.94
No. of species × relative cover	1	43	< 0.001	0.14
Cushion area	1	1748	< 0.001	−0.18
Seed set				
No. of species	1	0.20	0.652	−0.10
Relative cover	1	0.93	0.335	−0.02
No. of species × relative cover	1	0.15	0.700	0.19
Cushion area	1	66.66	< 0.001	−7.03
Seed density				
No. of species	1	34.84	< 0.001	0.44
Relative cover	1	28.01	< 0.001	−2.02
No. of species × relative cover	1	0.70	0.401	0.36
Cushion area	1	80.97	< 0.001	−7.87
Seed mass				
No. of species	1	0.510	0.475	0.00006
Relative cover	1	0.297	0.586	−0.00021
No. of species × relative cover	1	0.004	0.950	0.00001
Cushion area	1	1.229	0.268	−0.00015
Seed viability				
No. of species	1	0.41	0.522	0.12
Relative cover	1	0.05	0.819	−0.21
No. of species × relative cover	1	0.02	0.889	−0.07
Cushion area	1	2.01	0.157	−0.48

Cushion area was included as covariate and 'cushion species × site' was included as random factor in all models.

associated species on flower density, fruit set or fruit density of cushions depended on the number of species forming the cushion-associated plant assemblage. For flower density, our model predicted a less negative relationship between relative cover and flower density, with an increase in the number of species forming the cushion-associated plant assemblage, and a change in the relationship from negative to positive when the assemblage reached eight species or more (Figs 2a, S1a). Nevertheless, flower density of cushions was estimated to be lower for all cushions having associated species, that is, for all combinations of richness and cover of cushion-associated species that were different from zero species and zero cover. By contrast, for fruit set, the modulating effect of species richness was precisely the opposite to the one observed for flower density, that is, a change in the relationship between cushion-associated plant cover and fruit set from positive to negative with increasing species richness, and a turning point when the plant assemblage consisted of seven species (Figs 2b, S1b). Fruit set was therefore estimated to be higher for cushions with associated species than for cushions without associated species for most of the observed cases. In addition, fruit density was estimated to be highest under conditions with no cushion-associated species and was negatively related to both cover and richness of associated species (Fig. 2c). However, our model indicated that the negative relationship between fruit density and relative cover of cushion-associated species became weaker with increasing species richness (Fig. S1c).

Removal experiments

Experimental removal of cushion-associated species resulted in 48% more seeds per unit area compared with control cushions (Fig. 3d), associated with 38 and 24% higher flower and fruit density, respectively (Fig. 3a, Table S9), 57% higher seed set (Fig. 3c), but no significant effect on fruit set (Fig. 3b). Significant positive effects of the removal treatment on flower density, fruit density, seed set and seed density were observed for 89, 60, 67 and 67% of the datasets, respectively, whereas no significant relationship between the removal treatment and fruit set was observed at 100% of the sites (Table S10). Furthermore, experimental removal of cushion-associated species did not translate into significant changes in seed mass ($n = 139$, df = 1, $\chi^2 = 2.57$, $P = 0.11$) or seed viability ($n = 128$, df = 1, $\chi^2 = 2.14$, $P = 0.14$) of cushion plants (Fig. S2).

For seed density, but also for flower density and seed set, there was a tendency towards negative correlations between the model coefficients of the removal treatment and the corresponding model coefficient for relative cover in the observational approach, indicating that the sites with larger negative effects of relative cover on reproductive traits of cushions in the observational approach also tended to have a stronger positive response to the removal treatment ($\rho_{\text{flower density}} = -0.48$, $\rho_{\text{seed set}} = -0.5$, $\rho_{\text{seed density}} = -0.5$). By contrast, for fruit set and fruit density, no such negative correlation was found ($\rho_{\text{fruit set}} = 0.2$, $\rho_{\text{fruit density}} = 0.2$). However, owing to the low number of sites with both experimental and observational data available, none of these correlations were statistically significant.

Discussion

Using information collected across 35 arctic-alpine sites on four continents and a sub-Antarctic island, we found highly consistent and general negative feedback effects of cushion-associated species on cushion facilitators across 14 cushion plant species.

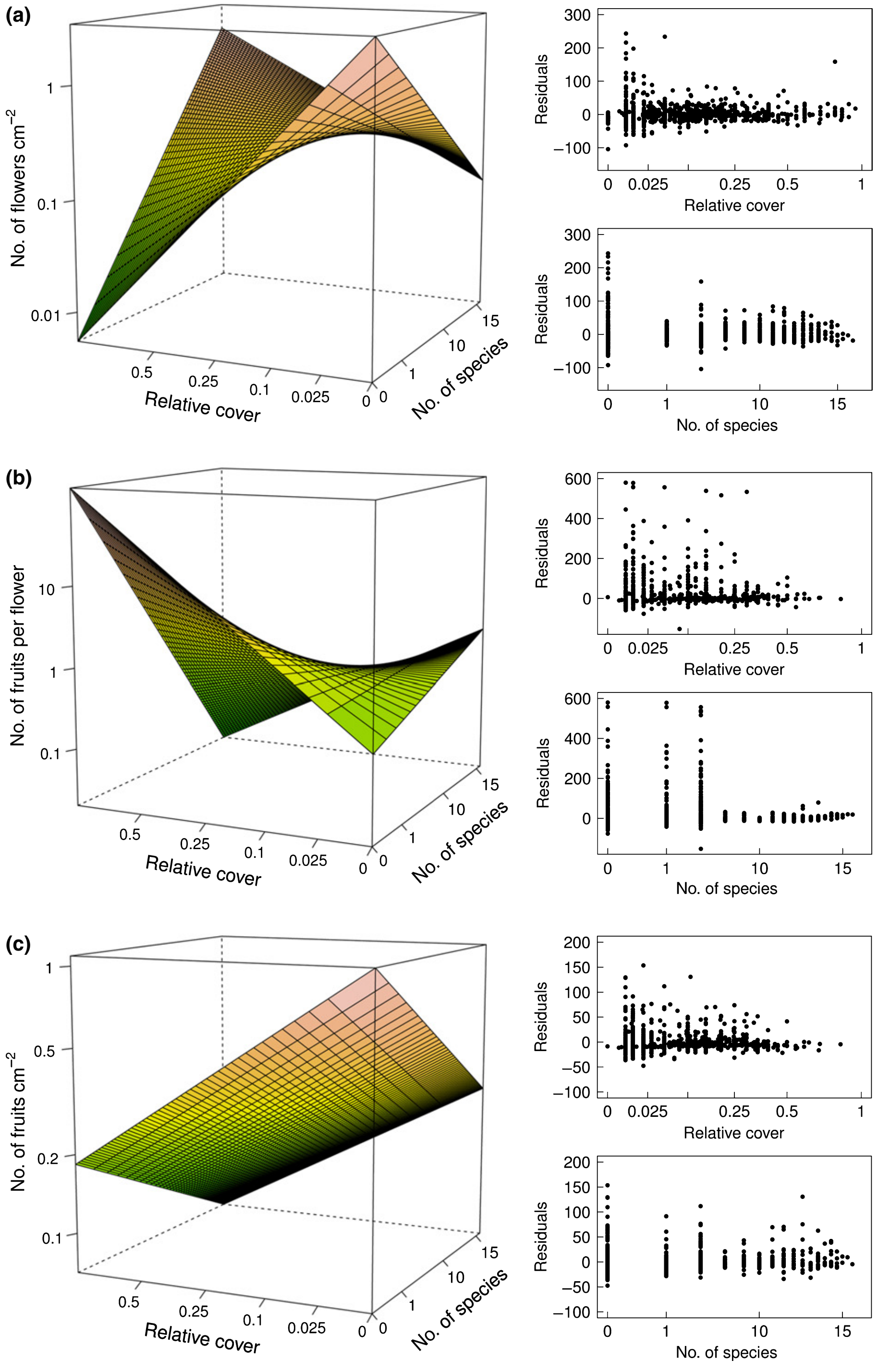

Fig. 2 Flower density (a), fruit set (b), and fruit density (c) of cushions in relation to species richness and relative cover of the cushion-associated plant assemblages. Shown are model predicted means as a response surface and, in separate panels, the corresponding residuals for each predictor variable. Note the square-root scale for the relative cover and the $\log_e$ scale for the number of species of the cushion-associated species assemblage and the number of flowers of cushions cm^{-2}, the number of fruits per flower, or the number of fruits cm^{-2}. See Table 2 for statistical results.

Overall, there was broad general evidence of a substantial net fitness cost for the facilitation of other species by species with cushion morphologies, resulting in parasitism as the predominant form of species interaction in our study system. The cost of cushions for hosting other species was expressed as a significant decrease in the number of seeds, associated with a reduced number of flowers and fruits produced per unit area of the cushion. The actual cost (i.e. the reduction in seed production in response to harboring associated species) occurred during flower production and seed set, but not at the fruit set stage. In addition, our results suggest that these feedback effects of plants hosted by cushions further depend on the species richness and composition forming the cushion-associated species assemblage, with monocultures having stronger negative impacts than diverse species assemblages.

The cost of facilitation

High relative cover of cushion-associated species reduced seed density in cushions in both the experimental and observational

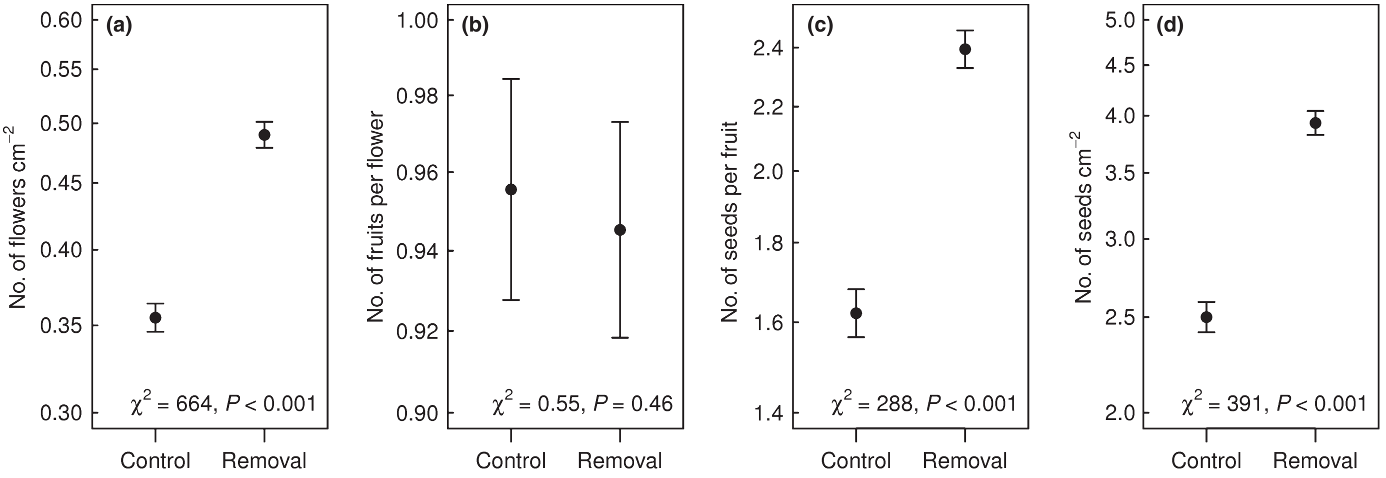

Fig. 3 Response of flower density (a), fruit set (b), seed set (c), and seed density (d) of cushions to experimental removal of plants growing inside cushions. Shown are model predicted values of the effect of the removal treatment ± 95% CIs and Wald χ^2-tests for treatment as a main effect of generalized linear mixed models. Note the log$_e$ scale of the y-axis. See Table S9 for full statistical results.

approaches in the field. However, final reproductive output (i.e. the amount of viable seed produced) does depend on various steps during reproduction, such as the number of flowers, the number of flowers turning into fruits, the number of seeds produced per fruit, and the viability of the seeds produced. Interestingly, our results from both approaches indicate that the cost does mainly occur at the stages of flower formation and seed set, whereas no negative effects of cushion-associated species were observed on fruit set and seed quality of cushions. The overall negative effect of the high abundance of associated plants on flower density corresponds with previous work on the cushion plants *Silene acaulis* (Cranston *et al.*, 2012), *Geum rosii* (Michalet *et al.*, 2011) and *Arenaria tetraquetra* ssp. *amabilis* (Schöb *et al.*, 2013). Reduced flower density is probably the result of competition for resources (Weiner, 1988; Stöcklin, 1997; Schöb *et al.*, 2013), where large amounts of living plant mass simply have stronger negative effects on available resources than small amounts (e.g. intercepting more light, leading to greater shading). This is consistent, too, with evidence that seed set is also reduced by competition for resources (Weiner, 1988; but see Stöcklin, 1997). By contrast, fruit set depends on the rate of flower abortion in response to the availability of resources and on pollination success (Stephenson, 1981; Ayre & Whelan, 1989; Guitián, 1993). Cushion-associated species do probably interfere with the cushions for resources (Schöb *et al.*, 2013) and pollinators (Muñoz & Cavieres, 2008; Wirth *et al.*, 2011; Molenda *et al.*, 2012). Our rather inconsistent and weak results in terms of fruit set do probably indicate, therefore, that different positive and negative effects of cushion-associated species may be involved here. Finally, cushion seed quality measured as seed mass and seed viability showed no response to differences in the assemblages of cushion-associated species, in either the observational or the experimental approaches, which is in line with evidence that both seed mass and viability are generally unaffected by competition (Violle *et al.*, 2009).

For the purpose of this short-term study we used reproductive output as an indicator of plant fitness. However, we are aware that for perennial plants, such as cushion plants, survival is another key component for their lifetime reproductive success (Morris & Doak, 1998). Cushion plants are long-living organisms and may reach several hundred years of age (Molau, 1997; Morris & Doak, 1998). Consequently, small differences in annual reproduction can easily be compensated by survival. Nevertheless, given the impossibility of studying the lifetime reproduction of such long-living organisms, short-term reproductive success is a good indicator of the status of the cushion plant at the time of the experiment – that is, the time when both reproductive output of cushions and the abundance and richness of the cushion-associated species were recorded.

The effect of species composition

By including species richness of cushion-associated plant assemblages in the models explaining the reproductive output of cushions, we revealed that the effect of cushion-associated plant species assemblages on the cushion probably depends on their richness and composition. Seed density of cushions was also positively related to cushion-associated plant species richness, which at least partially compensated for the negative effect of cushion-associated plant abundance. Similarly, high cushion-associated plant species richness partly compensated for the negative effect of their cover on flower density at high values of cover. In other words, the cost of being a facilitator in terms of reduced flower density increased with increasing abundance of plants associated with cushions, but this cost was mitigated if the high cover of cushion-associated plants was formed by a high number of different species. By contrast, high species richness in most cases reduced fruit set of cushions. This modulating effect of species richness on the effect of cushion-associated plant cover on the reproductive output of cushions could be the result of meaningful ecological processes, but potentially also of methodological artifacts, as multicollinearity between species richness and relative cover may have reduced the accuracy of the estimates of their individual effects. Nevertheless, there is a reasonable possibility of ecologically important positive effects of species richness on flower density of cushions through selective facilitation, or reduced negative effects through increased niche complementarity (Bruno *et al.*, 2003). A higher diversity of plants could further result in accumulation of complementary positive effects, where different cushion-associated species improve different resources or improve a specific resource involving different mechanisms. On the other hand, higher species richness can similarly be associated with negative selection or complementarity effects, such as those observed for flower density under low amounts or relative cover and for fruit set under high amounts of relative cover. Similar to the higher probability of harboring a facilitative species with increasing species richness, so the probability of harboring a very competitive species is increased; complementary resource use among cushion-associated species may also deplete resources more efficiently and reduce their availability for the cushion plant (Fargione & Tilman, 2005). Consequently, even though our study is not designed to disentangle species abundance vs species richness effects, it indicates that the effect size of the negative impact of harboring associated species may strongly depend on the composition and diversity of the associated plant assemblage. However, additional experiments independently manipulating abundance, composition and richness of beneficiary species are needed to differentiate their impact on benefactor fitness unambiguously.

Is there an adaptive response to facilitation?

Bronstein (2009) argued that studies of positive interactions among plants have rarely addressed the evolutionary consequences of facilitation, and that such evolution may occur if beneficiary species have feedback effects on their benefactors. We observed significant and prevailing negative feedback effects of cushion-associated species on cushions (i.e. a parasitism). In this case, Bronstein (2009) envisaged two scenarios of evolutionary consequences: tolerance of the beneficiaries – that is, selection for traits that reduce the negative impact of beneficiaries which would shift the interaction from parasitism towards commensalism; and avoidance of beneficiaries – that is, a selection of traits that reduce infestation by other plants. Given the parasitic nature of the interaction between cushions and their associated species in our study, future studies investigating the coevolution in this system may therefore focus on traits reducing competition between the cushion and its associates, such as an improvement of niche differentiation between the two sets of actors. On the other hand, studies of traits that reduce the establishment of other species, such as a smooth and dense canopy reducing seed trapping, may be envisaged. However, it may be difficult to separate out the element of such traits that is driven by competition, because different driving forces may select for similar traits. In addition, as a result of the likely occurrence of mutually positive interactions when diversity of associated species is high, a third scenario could include mechanisms of cushions to determine selection or complementarity effects of associated species (e.g. through the selection of species with mutualistic interactions). However, it is unclear which traits of the cushion might be involved in such a response. Consequently, there is the potential for evolutionary processes with plant–plant interactions (Ehlers & Thompson, 2004; Valiente-Banuet *et al.*, 2006; Bronstein, 2009; Michalet *et al.*, 2011; Thorpe *et al.*, 2011). We now need to explore in greater detail whether there has been an evolutionary response of either cushion hosts or their associates to these clear costs of facilitation. We also need studies that include other mechanisms of facilitation, such as a reduction in salinity or herbivory, in order to reveal if parasitism is the common type of reciprocal interaction in plant–plant facilitation.

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Supporting Information

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

Fig. S1 Slope changes of reproductive traits on species richness or relative cover.

Fig. S2 Response of seed mass and seed viability to the removal treatment.

Table S1 The study sites and cushion plant species

Table S2 The datasets for the observational approach

Table S3 The datasets for the removal experiments

Table S4 Statistical results of the relationship between plant cover and cushion flower density for each site

Table S5 Statistical results of the relationship between plant cover and cushion fruit set for each site

Table S6 Statistical results of the relationship between plant cover and cushion fruit density for each site

Table S7 Statistical results of the relationship between plant cover and cushion seed set for each site

Table S8 Statistical results of the relationship between plant cover and cushion seed density for each site

Table S9 Statistical results of the experimental removal of cushion-associated species on cushion reproductive output

Table S10 Statistical results of the experimental removal of cushion-associated species on cushion reproductive output for each site

Notes S1 The relationship between biomass of cushion-associated plant assemblages and the reproductive output of cushions.

Notes S2 The relationship between the number of cushion-associated plant individuals and the reproductive output of cushion.

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