

Consequences of facilitation: one plant's benefit is another plant's cost

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Summary

1. Facilitation is known as the positive effect of one species (benefactor) on associated neighbouring species (beneficiaries). Although the beneficial part of this interaction has received considerable research interest, there is a gap of knowledge on the bidirectional nature of these interactions; in particular, the physiological and fitness consequences for both beneficiaries and benefactors.

2. Alpine cushion plants are generally strong benefactors, increasing species richness and abundance on a global scale, and provide a suitable system to study the physiological effects of bidirectional interactions and its consequences for reproduction. Current knowledge suggests that species improve their fitness when associated to a benefactor cushion species, whereas cushions may receive predominantly negative feedbacks.

3. We measured physiological and reproductive traits of the cushion species *Arenaria tetraquetra* ssp. *amabilis* and three other forbs (*Eryngium glaciale*, *Lotus corniculatus* ssp. *glacialis* and *Plantago nivalis*) in the dry Sierra Nevada Mountains, southern Spain. All four species were studied either when growing alone or when the three forbs were associated with the cushion plant.

4. The three forb species improved their water status when associated with the cushion, and *Lotus* and *Plantago* significantly increased their seed set. In contrast, *Arenaria* showed poorer water status and reduced flower density and seed set with increasing cover of beneficiary species. There was a clear relationship between physiological and reproductive traits in *Arenaria* growing without beneficiaries but not in *Arenaria* with beneficiaries. Control cushions (without beneficiary species) showed increased seed set and seed mass with increasing photosynthetic and water use efficiencies, respectively, the latter being positively related to leaf nitrogen content. In contrast, cushions with a large cover of beneficiary species did not show such relations. The missing relationship between physiological and reproductive traits for these facilitating cushions indicates that reproductive output in heavily colonized cushions may not be directly related to the plant physiological status and the availability of resources.

5. Our results revealed the antagonistic behaviour underlying the interaction between beneficiary species and facilitating cushion plants, similar to parasitic interactions. They also provide indications for changes in cushions' resource allocation pattern in response to colonization by beneficiaries.

Key-words: alpine, antagonistic interactions, bidirectional, competition, functional traits, physiological traits, reproduction, resource allocation pattern

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Introduction

By modifying their local environment, plants may create new niche space, which attracts some species while excluding others (Schöb, Butterfield & Pugnaire 2012). These sorting processes based on the species' impacts and

requirements form the basis of plant–plant interactions (Gause 1934; Callaway 1995) and do generally co-occur in plant communities (Callaway, Nadkarni & Mahall 1991; Callaway & Walker 1997; Pugnaire & Luque 2001; Dickie *et al.* 2005; Schöb *et al.* 2010; Armas & Pugnaire 2011; Schöb, Armas & Pugnaire 2013a). Nevertheless, most single-site studies focus solely on either the attraction, i.e. facilitation, or exclusion, i.e. competition, as if they were independent phenomena (Callaway & Walker 1997). For example, most facilitation studies that examined the positive effect of a benefactor species on its beneficiaries usually neglected the bidirectional nature of the interaction (Bronstein 2009), overlooking the competitive nature of resource interactions between benefactor and beneficiary species (Callaway 2007) or other species growing in association (Aguiar & Sala 1994; Armas, Pugnaire & Sala 2008; Schöb, Armas & Pugnaire 2013a).

Facilitation is most apparent in severe environments where stressful conditions limit plant growth (Callaway 2007; He, Bertness & Altieri 2013). Here, the impact of stress-tolerant species often improves micro-environmental conditions for other species through local amelioration of the prevailing stress factors (Schöb, Butterfield & Pugnaire 2012). For example, cushion plants improve growth conditions in highly mobile alpine gravel habitats by providing a stable micro-environment with improved soil resource conditions and reduced temperature fluctuations (Reid, Lamarque & Lortie 2010). It is the compact growth form of cushions that accrues organic material and acts as a nutrient pool and water storage (Körner 2003). Consequently, nitrogen and phosphorus concentrations, but also soil moisture, have been shown to be significantly higher under cushions compared to the poorly developed neighbouring bare ground (Nuñez, Aizen & Ezcurra 1999; Cavieres, Quiroz & Molina-Montenegro 2008). This local impact of cushions on the environment creates conditions suitable for a range of species otherwise unable to grow in adjacent, nude soil, but in turn may exclude species preferring open areas (Schöb, Butterfield & Pugnaire 2012). The outcome is a mosaic of plant assemblages with only some species overlapping between areas under the influence of the cushion and neighbouring open areas, thereby increasing community-scale plant diversity (Cavieres & Badano 2009; Butterfield *et al.* 2013).

The interaction between benefactor cushion species and associated beneficiary species has been addressed in detail from the beneficiaries' point of view, i.e. the impact of the benefactor on growth and survival of beneficiaries (e.g. Cavieres *et al.* 2005; Cavieres, Quiroz & Molina-Montenegro 2008). However, there is little knowledge on the consequences of this coexistence for the benefactor, i.e. the impact of the cushion-associated species on the micro-environment (e.g. resources, soil biota) and how the spatial association with beneficiaries affects the physiology, growth and fitness of the benefactor (but see Michalet *et al.* 2011; Cranston *et al.* 2012; C. Schöb, R. Michalet, L.A. Cavieres, F.I. Pugnaire, R.W. Brooker,

B.J. Butterfield *et al.* unpublished). In other words, whereas the unidirectional effect of facilitative interactions has received considerable interest, the bidirectional nature of these interactions remains largely unaddressed (Bronstein 2009). Consequently, facilitation is not yet defined in terms of feedbacks between interacting species and does include the whole range of bidirectional interactions, from mutualism (+/+) to commensalism (+/0) and antagonism/parasitism (+/–; Stachowicz 2001; Callaway 2007; Brooker & Callaway 2009).

Current evidence on reciprocal interactions including facilitation suggests mostly negative effects of beneficiaries on benefactors due to resource competition (Callaway 2007). There is evidence of decreased reproductive output of the benefactor in response to the presence of beneficiary species, showing a fitness cost for being cooperative (Flores-Martínez, Ezcurra & Sánchez-Colón 1994; Holzapfel & Mahall 1999; Armas & Pugnaire 2009; Michalet *et al.* 2011; Cranston *et al.* 2012; C. Schöb, R. Michalet, L.A. Cavieres, F.I. Pugnaire, R.W. Brooker, B.J. Butterfield *et al.* unpublished). However, the underlying mechanisms have hardly been addressed so far. To our knowledge, only Holzapfel & Mahall (1999) and Armas & Pugnaire (2009) analysed physiological traits that could clarify the reasons for reduced fitness for the benefactor in response to the association with beneficiary species. Both studies were performed in dry environments and showed a poorer water status of shrubs growing with their associates, an understory of annual species (Holzapfel & Mahall 1999) or a co-occurring shrub species (Armas & Pugnaire 2009), indicating resource competition. In yet another dry environment, and in contrast to such antagonistic interactions, Pugnaire, Haase & Puigdefábregas (1996) reported mutualistic interactions between the beneficiary species *Marrubium vulgare* and its benefactor shrub *Retama sphaerocarpa* expressed as better water and nutrient status, and increased productivity, of both species when growing in association compared to growing alone [see also instances in Holzapfel & Mahall (1999) and Armas & Pugnaire (2005)]. The improved performance of both species was correlated with higher organic matter and water content in soils where both species co-occurred compared to soils lacking either species, suggesting that the combined impact of co-occurring species on their environment can result in mutual benefit and outweigh the likely existence of competition for resources. Therefore, our knowledge of bidirectional interactions in facilitative systems is both ambiguous and scarce. However, a definition of facilitative interactions based on their reciprocity as mutualistic, commensalistic or antagonistic is essential under some circumstances (Brooker & Callaway 2009), such as for research on evolutionary consequences of facilitation (Bronstein 2009).

In a dry alpine mountain area, we studied physiological and reproductive traits of the cushion species, *Arenaria tetraquetra* ssp. *amabilis*, and three co-occurring forb species either growing alone or in association with each other. We

hypothesized improved fitness of the three forbs when associated to cushions related to improved water status, i.e. assuming that the stress-tolerant cushion is able to ameliorate the most limiting stress factor in our study system (Schöb *et al.* 2013b). In addition, we expected simultaneous negative and positive effects of these species when associated to the cushion. On the one hand, we expected poorer water status of the cushion and reduced photosynthetic efficiency due to competition for water and shading respectively. On the other hand, associated species may also improve the cushion's physiological status by increasing the availability of nutrients. Overall, we hypothesized prevailing negative interactions resulting in reduced reproductive output and overall reduced fitness of cushions when associated with other species.

Materials and methods

STUDY SITE AND SPECIES

The study was conducted in two areas of approximately 0.5 ha each in the northern aspect of the Sierra Nevada Mountains, Granada province, south-eastern Spain. The sites (A, B) were situated at 2990 m (37°04' N 03°22' W) and 2680 m elevation (37°05' N 03°23' W), respectively, approximately 2 km apart. In Pradollano (2507 m), approximately 1.5 km north of site B, mean annual rainfall is 690 mm and mean annual temperature is 3.9 °C (Rivas-Martinez & Rivas-Saenz 1996–2009). Both study sites are in an alpine gravel habitat on gentle slopes facing north-east. The rather uniform habitats are dominated by the cushion-forming species *Arenaria tetraquetra* ssp. *amabilis* (Bory) H. Lindb. fil. (Caryophyllaceae). Individual cushions were haphazardly distributed over the study area and surrounded by large open areas. *Arenaria* is a foundation species *sensu* Ellison *et al.* (2005) having significant impacts on its local environment resulting in locally stable and distinct environmental conditions compared to open areas surrounding the cushions (Schöb, Butterfield & Pugnaire 2012). *Arenaria* has deep reaching taproots and its canopy forms compact cushions of short and highly ramified branches and very small leaves (Blanca *et al.* 2009). In an earlier study on *Arenaria* next to our field sites, we observed around 25 terminal branches per cm² and 10 leaves per terminal branch, with a terminal branch length of 8 mm (Schöb *et al.* 2013b). Individual cushions usually produce numerous, insect-pollinated flowers, with a high ratio of pollination by ants (Gómez *et al.* 1996).

Both microhabitats, i.e. cushions and open areas, were colonized by annuals but mostly by perennial grasses and forbs. The dominant and most frequent species growing within cushions and in neighbouring open areas were *Eryngium glaciale* Boiss. (Asteraceae) at site A, and *Lotus corniculatus* ssp. *glacialis* (Boiss.) Valdés (Fabaceae) and *Plantago nivalis* Boiss. (Plantaginaceae) at site B. Similar to *Arenaria*, *Eryngium*, *Lotus* and *Plantago* are perennial species with deep taproots in adaptation to the poorly developed soils in this dry environment. *Eryngium* is a perennial plant forming a basal leaf rosette consisting of 3.5–12 cm long, three-lobed and spiny leaves, whereas the flowering stems can be up to 20 cm in height and bear numerous insect-pollinated flowers (Blanca *et al.* 2009). *Lotus* is a prostrate herb with each leaf consisting of five oval, up to 5 mm long leaflets (Blanca *et al.* 2009). It is a leguminous species, which is able to fix atmospheric nitrogen by means of symbiotic *Rhizobium* bacteria (Jarvis, Pankhurst & Patel 1982), and its flowers produce nectar and attract pollinating insects. *Plantago* forms rosettes of lanceolate-shaped, up to 5 cm long,

woolly leaves and short inflorescences containing numerous tiny, wind-pollinated flowers (Blanca *et al.* 2009).

EXPERIMENTAL DESIGN

As control, we selected 20 individuals of every target species per site (*Arenaria* and *Eryngium* at site A, and *Arenaria*, *Lotus* and *Plantago* at site B) growing alone, i.e. apart from each other. At site A the sampling was completed with 20 individuals of *Arenaria* growing in close association with *Eryngium*. *Eryngium* covered $54.3 \pm 4.7\%$ (mean \pm SE) of the *Arenaria* canopy when associated with *Arenaria*. At site B, we additionally sampled 20 individuals of *Arenaria* associated with *Lotus*, 20 individuals of *Arenaria* associated with *Plantago* and another 20 individuals of *Arenaria* associated with both *Lotus* and *Plantago*. *Lotus* and *Plantago* covered $18.6 \pm 1.3\%$ of the *Arenaria* canopy when associated with the cushion and plant cover did not vary significantly between species or when only one or both species grew within the *Arenaria* canopy respectively. Consequently, total relative cover of *Lotus* and *Plantago* in the 20 cushions in which they grew together made up to $36.1 \pm 2.7\%$. Cover of other vascular plant species growing in association with *Arenaria* cushions was $5.4 \pm 0.3\%$ and did not differ between sites or among cushions with associated target species and control cushions without associated target species. For each cushion, we recorded latitude and longitude (± 5 m) using a handheld GPS (Garmin eTrex Vista, Garmin Ltd., Olathe, USA). Cushion size was considerably smaller at site A (166 ± 13 cm²) than at site B (312 ± 19 cm²), but there were no differences between control cushions and cushions with associated target species within sites. On the other hand, cushion thickness (i.e. the distance from the tip of the outermost leaf to the soil underneath the cushion) was significantly higher at site A (65 ± 3 mm) compared to site B (49 ± 2 mm), but again it did not differ significantly among cushions within sites. Diameter and height of the aboveground vegetative parts of *Eryngium* (136 ± 21 mm wide and 99 ± 5 mm high), *Lotus* (11.3 ± 1.0 wide and 8.5 ± 0.6 mm high) and *Plantago* (16.6 ± 1.2 mm wide and 8.9 ± 0.6 mm high) did not vary significantly with microhabitat type (except *Lotus* being wider in the open and *Plantago* being taller in association with *Arenaria*).

PHYSIOLOGICAL TRAITS

The apparent electron transport rate (ETR; $\mu\text{mol m}^{-2} \text{s}^{-1}$) was quantified with a Mini-PAM Photosynthesis Yield Analyser (Walz Mess- und Regeltechnik GmbH, Effeltrich, Germany). Measurements were carried out the 9 and 10 August 2011 between 11 and 13 h solar time under cloudless conditions and ambient solar irradiance. Fluorescence emission was measured on leaves with no apparent damage at three random locations within the canopy area on *Arenaria* and on one randomly selected leaf on *Eryngium*, *Lotus* and *Plantago*. By application of a saturating light pulse ($12000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) that closed all photosystem II reaction centres, we determined the maximum fluorescence of the leaf adapted to ambient irradiance (F_m') and the steady-state fluorescence (F). ETR was then calculated after Krall & Edwards (1992):

$$\text{ETR} = [(F_m' - F)/F_m'] \times \text{PAR} \times 0.5 \times 0.84 \quad \text{eqn 1}$$

where PAR is the photosynthetically active radiation measured simultaneously with the fluorescence measurements, 0.5 is the estimated proportion of absorbed photons that reach photosystem II, and 0.84 is considered the most common leaf absorbance coefficient for C3 species (Björkman & Demmig 1987). ETR is a surrogate of the efficiency of electron transport at photosystem II, where the transport of four electrons is needed for each molecule

of CO₂ to be assimilated. Therefore, ETR is an overall measure of the photosynthetic efficiency and stress experienced by the plant, with lower ETR values indicating reduced electron transport rate and increased stress (Maxwell & Johnson 2000).

Relative water content (RWC) was determined as water content in field conditions compared to the water content at full turgor. Samples for each species in isolation or in association with other species were collected between 6:00 and 8:00 h solar time from 9 to 12 August 2011. Fresh mass was determined immediately after sampling in the field, saturated mass was determined after 24 h of rehydration in the dark and dry mass was measured after drying at 70 °C for 48 h. From the same samples, we took one individual leaf per sample (two leaves for *Arenaria*) and determined saturated mass, dry mass and leaf area. With these data, we calculated leaf dry matter content (LDMC) as the ratio of leaf dry mass to fully rehydrated fresh mass (g kg⁻¹) and specific leaf area (SLA) as the ratio of fresh leaf area to leaf dry mass (m² kg⁻¹). RWC reflects the plant water status at the time of sampling, whereas LDMC relates to the long-term plant strategy in response to general water availability. RWC increases with increasing water availability, whereas LDMC decreases (Cornelissen *et al.* 2003). On the other hand, SLA has been shown to increase with increasing temperature and light availability, i.e. conditions improving carbon assimilation (Körner 2003; Poorter *et al.* 2009), and is positively correlated to soil nitrogen availability (Ordoñez *et al.* 2009) and negatively correlated with leaf longevity and resource use efficiency (Wright *et al.* 2004).

At site B and only for *Arenaria*, we additionally determined leaf carbon (C) and nitrogen (N) content (%) and their stable isotope composition (¹²C and ¹³C, ¹⁴N and ¹⁵N) in 40 samples (10 control, 10 with *Lotus*, 10 with *Plantago*, and 10 with both *Lotus* and *Plantago*) randomly selected from all available samples. Leaves were sampled on 11 August 2011, immediately covered with aluminium foil and stored in envelopes until they were oven-dried at 70 °C for 48 h. Analyses were performed with an elemental analyser (PDZ Europa ANCA-GSL, Sercon Ltd, Cheshire, UK) connected to a continuous flow mass spectrometer (PDZ Europa 20–20, Sercon Ltd, Cheshire, UK) at UC Davis, USA. Delta values were calculated as follows:

$$\delta = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \times 1000\text{‰} \quad \text{eqn 2}$$

where R is the ratio of ¹³C/¹²C and ¹⁵N/¹⁴N respectively. Analytical precision was <0.8‰. Isotope ratios of C and N were used to assess water use efficiency and possible N source of the cushion plant respectively. Less negative values of δ¹³C have been shown to indicate increased water use efficiency (Ehleringer, Hall & Farquhar 1993), whereas values of δ¹⁵N close to zero may indicate atmospheric N being fixed by bacteria as an additional N source (Robinson 2001).

REPRODUCTIVE TRAITS

Reproductive output was quantified for all species as number of flowers (i.e. flower heads for *Eryngium* and *Plantago*), number of fruits (i.e. fruit heads for *Plantago*; no data for *Eryngium*), number of seeds (no data for *Eryngium*) and seed mass (no data for *Eryngium*). For *Arenaria* the number of flowers, fruits and seeds were recorded in three randomly placed 16 cm² quadrats. For analyses, we used the mean of the three quadrats per cushion. In *Eryngium*, *Lotus* and *Plantago* we recorded the number of flowers, fruits and seeds for all individuals growing within the cushion and for the same number of individuals in the neighbouring open area. Individuals in gaps were selected according to their proximity to the cushion. Average seed mass of all species was determined by weighing 10 seeds per sample (or the number of seeds available if there were less than 10 seeds). The number of aborted and fertilized fruits was

assessed between the 8 and 9 August 2011 respectively. Flower number was then calculated as the sum of the number of aborted and fertilized fruits. Seeds were collected on 01 September 2011.

STATISTICAL ANALYSES

To assess the difference in physiological and reproductive trait values of *Eryngium*, *Lotus* and *Plantago* either growing isolated or in association with *Arenaria* cushions, we applied generalized linear mixed models with cushion (i.e. control vs. with cushion) and species as fixed factors, and site as random effect. We used a Poisson error structure for the number of flowers, fruits and seeds and a Gaussian error structure for all other variables. Furthermore, for number of fruits and number of seeds we included the previous developmental stage, i.e. number of flowers and number of fruits, respectively, as an offset variable. Therefore, the resulting measurements of reproductive output were number of flowers per quadrat (i.e. flower density), number of flowers that turned into fruits (i.e. fruit set) and number of seeds per fruit (i.e. seed set). Significance of each factor was tested with type-II analysis of variance and F - or χ^2 -tests for normally or non-normally distributed data respectively. Significant differences in traits related to the association of individual species to *Arenaria* were tested by multiple simultaneous comparisons among *a priori* selected linear contrasts.

Differences in physiological and reproductive trait values of *Arenaria* cushions when associated or not to other species were tested with generalized linear models with total species cover within *Arenaria* as a fixed factor and cushion area, cushion height and cushion coordinates (latitude and longitude) as covariates. We used again a Gaussian distribution of error terms for all traits except for number of flowers, fruits and seeds, where a Poisson error structure was applied. The same offset variables were applied here for *Arenaria* to finally quantify the relationship between the abundance of species growing in cushions and flower density, fruit set and seed set of *Arenaria*. With the covariates we controlled for the small differences in cushion size and for potential small-scale environmental differences between and within study sites.

To reveal relationships between physiological and reproductive traits in *Arenaria* and potential differences of these relationships between cushions with and without associated target species, we applied covariance analyses, using cushion area, height and coordinates as covariates.

Statistical analyses were performed with R version 2.15.2 (R Core Team 2012) using the libraries car (Fox & Weisberg 2011), lme4 (Bates, Maechler & Bolker 2012) and multcomp (Hothorn, Bretz & Westfall 2008).

Results

The beneficiary species associated with *Arenaria* had overall higher RWC and lower LDMC than individuals living isolated (significant main effect of 'cushion', Fig. 1a,b). However, individual contrasts for each species revealed significant differences in plants growing either with or without cushion only for LDMC of *Plantago*. ETR tended to be higher for *Plantago* in the open ($P = 0.052$), whereas ETR of *Eryngium* and *Lotus* did not vary with microhabitat type (Fig. 1c). SLA showed no variation related to microhabitat type for any of the three species (Fig. 1d). *Lotus* showed increased flower density (Fig. 1e) and higher fruit set (Fig. 1f) in association with cushions whereas both *Lotus* and *Plantago* increased seed set within cushions over that of isolated plants (Fig. 1g). Seed mass did not vary with microhabitat type for both *Lotus* and *Plantago* (Fig. 1h).

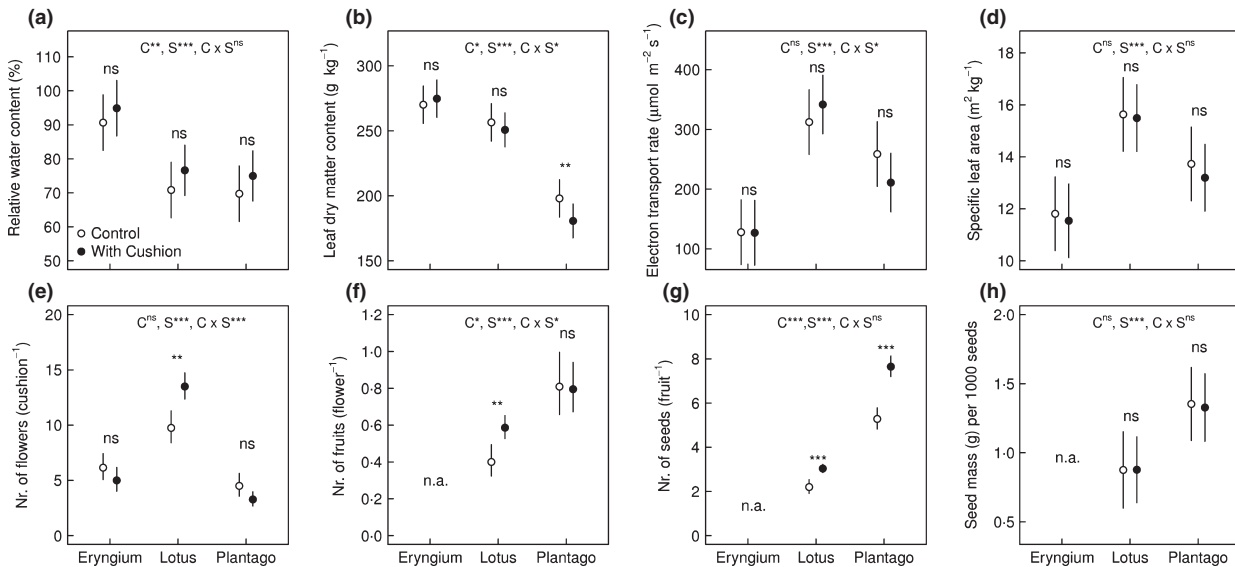


Fig. 1. Traits of *Eryngium glaciale*, *Lotus corniculatus* and *Plantago nivalis* either growing alone or in association with the cushion species *Arenaria tetraquetra*. Displayed are the model predicted relationships with 95% confidence intervals. Statistical models are generalized linear mixed models with the cushion, C, (control vs. with cushion), the species identity, S, (*Eryngium*, *Lotus* and *Plantago*) and their interaction term, C × S, as fixed factors and site as a random factor. Orthogonal contrasts were applied to test for significant effects of cushion association on plant traits of each species separately. For *Plantago* and *Eryngium*, flowers were counted as the number of flower heads, fruits of *Plantago* were counted as the number of fruit heads. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = not significant ($P > 0.05$). n.a. = data not available. $n = 160$, except for number of flowers, fruits and seeds $n = 120$.

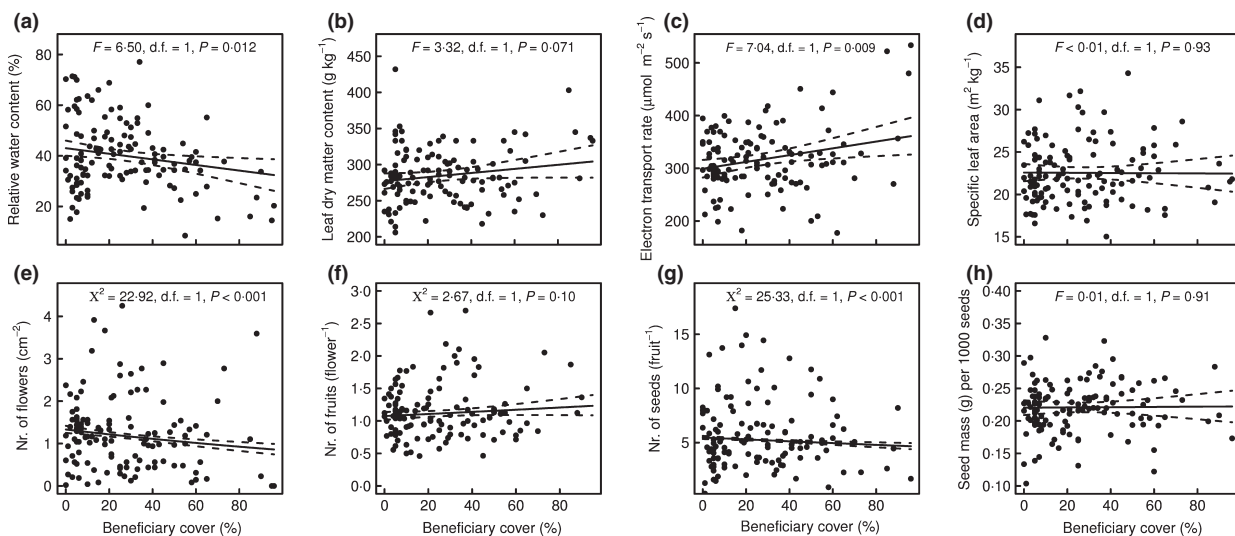


Fig. 2. Traits of the cushion species *Arenaria tetraquetra* in relation to the relative cover of associated beneficiary species. Displayed are the model predicted relationships with 95% confidence intervals and the measured values (as dots). Statistical models are generalized linear models with total associated species cover as independent variable and cushion thickness, cushion area, and cushion location (latitude and longitude) as covariates. $n = 120$.

Arenaria cushions showed decreasing RWC and a tendency towards increasing LDMC with increasing cover of beneficiary species (Fig. 2a,b). ETR increased with increasing beneficiary cover (Fig. 2c), whereas SLA did not significantly change with beneficiary cover (Fig. 2d). The C/N ratio, total N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ did not show any significant relationship to the cover of beneficiary species (see Fig.

S1 in Supporting information). In terms of reproduction, *Arenaria* showed lower flower density (Fig. 2e) and smaller seed set (Fig. 2g), but no significant variation in fruit set (Fig. 2f) with increasing beneficiary cover. Seed mass did not significantly vary with beneficiary cover (Fig. 2h).

Covariance analyses of physiological and reproductive traits of *Arenaria* control individuals and *Arenaria* with

associated beneficiary species revealed that both groups of *Arenaria* tended towards increased seed mass with increasing RWC (d.f. = 1, $F = 3.35$, $P = 0.070$) without any significant difference in this relationship between them (Fig. 3a). In contrast, *Arenaria* control individuals showed a significantly different relationship between seed set and ETR, seed mass and $\delta^{13}\text{C}$, and between N and $\delta^{13}\text{C}$ compared to *Arenaria* individuals with beneficiary species (Fig. 3b-d). Control *Arenaria* individuals had a positive relationship between seed set and ETR ($r = 0.16$) and between seed mass and $\delta^{13}\text{C}$ ($r = 0.74$), whereas for *Arenaria* with beneficiary species these relationships were negative (seed set vs. ETR: $r = -0.22$; seed mass vs. $\delta^{13}\text{C}$: $r = -0.19$). Furthermore, control cushions showed a positive relationship between total N and $\delta^{13}\text{C}$ ($r = 0.62$; Fig. 3d), whereas *Arenaria* with associated beneficiary target species did not show this relation ($r = 0.02$).

Discussion

We found support for our hypothesis of improved fitness of species associated to the cushion-forming *Arenaria tetraquetra*. Depending on the identity of the beneficiary species, an improved performance was found at all measured reproductive stages (flower density, fruit set and seed set).

This improved fitness is in line with the improved water status, i.e. higher RWC and lower LDMC, of plants associated with *Arenaria* cushions. In contrast, *Arenaria* had reduced fitness when associated with beneficiary species, showing reduced flower density and seed set with increasing abundance of beneficiary species. The reduced fitness of *Arenaria* is in line with their reduced water status (i.e. lower RWC) suggesting that beneficiary species were competing for water with the cushion. Consequently, the bidirectional interaction between *Arenaria* and its associated beneficiary species seems antagonistic, with beneficiary species behaving like parasitic plants, where hosts show reduced reproductive output after parasitic infestation due to resource competition (Silva & Martinez del Rio 1996; Puustinen & Salonen 1999). Finally, *Arenaria* cushions without associated *Eryngium*, *Lotus* or *Plantago* plants (i.e. control cushions) showed positive relationships between physiological traits and reproduction indicating that improved physiological status went along with increased reproduction for these cushions. In contrast, cushions with a high abundance of beneficiaries did not show such positive relationships between plant physiological status and reproductive output. This suggests that cushions without beneficiary species invest resources to reproduction in relationship with their physiological status, whereas the

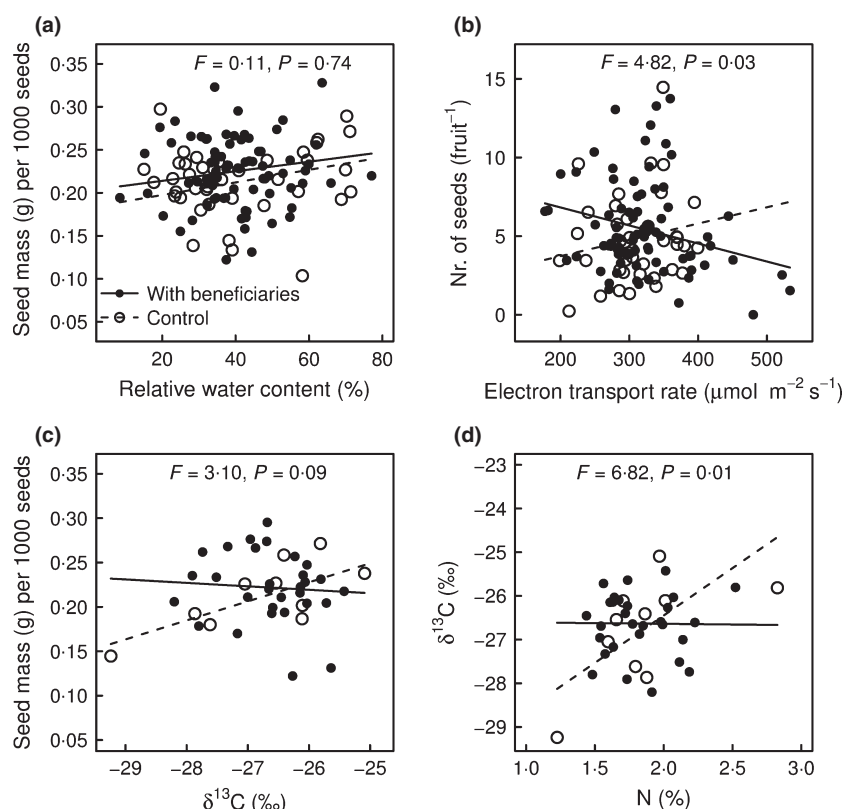


Fig. 3. Relationships between physiological and reproductive traits (a–c) and between physiological traits (d) of the cushion species *Arenaria tetraquetra* with (filled circles, $n = 30$ – 60) or without (Control, open circles, $n = 10$ – 20) the associated beneficiary species *Lotus corniculatus* and/or *Plantago nivalis*. Displayed are the model predicted relationships and the measured values (as circles) for *Arenaria* with (solid line) and without (dashed line) beneficiaries respectively. Slope test results of the ANCOVA testing the difference of the relationship among traits between *Arenaria* control and *Arenaria* with beneficiary species are shown.

resource investment towards reproduction of cushions with a large number of beneficiaries seems to be largely independent of their physiological conditions. This differential use of resources for reproduction does, therefore, indicate a changed resource allocation pattern of cushions when colonized by other species.

ARENARIA FACILITATES ASSOCIATED SPECIES

Cushion plants have a high water storage capacity that help them, and associated species, to overcome drought periods (Körner 2003), in particular in late summer (Cavieres *et al.* 2006). Therefore, the observed water-related facilitation of beneficiary species by *Arenaria* confirms our hypothesis of predominating beneficiary effects through the most limiting resource in our study system (Schöb *et al.* 2013b). Water-related facilitation effects in dry habitats have been frequently observed both in alpine and lowland systems (Cavieres *et al.* 2006; Pugnaire, Armas & Maestre 2011) and mainly include shade, improved soil water storage capacity or hydraulic lift (Pugnaire, Armas & Maestre 2011). The dense canopy of cushion plants does likely reduce evaporation of soil water, but the large amount of organic matter accrued by cushions does certainly improve water storage compared to neighbouring bare soil, which is particularly relevant after long periods of drought (Cavieres *et al.* 2006; Schöb, Butterfield & Pugnaire 2012; Schöb *et al.* 2013b). Therefore, the improved water balance in cushions likely acts as the main driver for improved water relations of plants growing within the cushion canopy. Nevertheless, hydraulic lift through the deep reaching taproots (Prieto, Armas & Pugnaire 2012) of *Arenaria* cannot be excluded as another source of water supply to beneficiary species.

In addition to altered water conditions, improving nutrient cycling or buffering microclimatic conditions could further contribute to the higher fitness of species associated to cushions. In particular, *Lotus* showed higher reproductive output when associated to *Arenaria*, which could be related to an ameliorated microclimate (Schöb *et al.* 2013b). Nodulation of nitrogen fixing bacteria in legumes may depend on the microenvironment (Del Gallo & Fabbri 1991), and nitrogen fixation is particularly sensitive to water shortage (Sprent 1972). Consequently, growing within the cushion may help *Lotus* for nodulation and N fixation, which would likely contribute to its higher fitness in association with *Arenaria*. But also the visitation rate and diversity of pollinators can be higher under improved microclimatic conditions and higher flower density in cushions (Reid & Lortie 2012), which in turn could be responsible for the increased fruit set observed for this insect-pollinated species.

BENEFICIARY SPECIES COMPETE WITH *ARENARIA*

The higher water availability within *Arenaria* cushions likely attracted other species, which then benefited from the

association but in turn deteriorated their host's water status. This antagonistic interaction suggests that the species associated to *Arenaria* act like parasites on their hosts (e.g. Ehleringer, Cook & Tiezen 1986). Since a causal relationship between water availability and reproduction is likely (e.g. Pradhan *et al.* 2012), competition between beneficiary species and *Arenaria* for water probably decreased the cushion's reproductive output (Weiner 1988).

Contrary to competition for water, the nutrient status of *Arenaria* did not change with the presence or abundance of beneficiaries, suggesting that the negative effect of beneficiaries on cushions was mainly linked to water rather than to nutrients. However, if *Arenaria* cushions are split into two groups, one colonized by large amounts of beneficiaries (ranging 15–90% cover) and another with none or very few beneficiaries ($5.4 \pm 0.3\%$ cover) we observe direct and indirect, positive relationships of reproductive traits (i.e. seed set and seed mass) with resource use efficiency (ETR and $\delta^{13}\text{C}$) or nutrient concentration (N) in the control group, whereas no such relationships were found in cushions colonized by a large amount of beneficiaries.

Positive relationships between resource availability and reproductive output have previously been shown for other alpine species (Moulton & Gough 2011). In our case, cushions without beneficiary species showed enhanced reproduction when photosynthetic and water use efficiencies were improved, with a positive correlation between water use efficiency and total N. Overall, these results point to a positive relationship between resource availability, plant physiological status, and reproductive output for cushions growing without associated plants. In other words, cushions without beneficiary species allocate resources to reproduction in direct relation to their availability. In contrast, *Arenaria* cushions with a large cover of beneficiary species showed no such relationship, except for water-related traits (i.e. RWC). The significant effect of water could reflect its relevance for plant reproduction in our study system. However, apart from this, for *Arenaria* cushions with beneficiaries, our results point towards a reproductive effort largely independent on the availability of other resources. In other words, in contrast to *Arenaria* cushions without beneficiaries, cushions with beneficiary species seem to allocate a limited amount of resources to reproduction independently of their availability.

Taken together, these different relationships between reproductive effort of *Arenaria* with or without beneficiaries and resource-related traits could indicate a changed resource allocation pattern in response to the colonization by other species – similar to changes in allocation patterns of hosts in response to parasites or herbivores (Matthies 1995; Pennings & Callaway 2002). Indeed, for such long-lived organisms resource investment into either growth or defence in response to competitive feedback effects may ultimately be better for plant fitness than primary allocation to reproduction and the correspondingly increased risk for mortality (Morris & Doak 1998).

Conclusion

Whereas species associated to cushions showed an improved physiological status along with enhanced reproductive output compared to isolated individuals, this association resulted in an overall negative feedback effect for the cushion. These cushions showed worse physiological status and reduced reproductive output. Consequently, the interaction between *Arenaria* and its beneficiary species seems predominantly antagonistic. However, the differential relationship between physiological status and reproductive output between cushions with and without beneficiary species points towards changes in resource allocation as a consequence of colonization by beneficiary species. It is likely that cushions without beneficiary species invest resources into reproduction in direct relation with resource availability, whereas cushions with beneficiaries do not adapt their reproductive effort to resource availability, probably due to a change in resource allocation towards growth and/or defence traits. However, further research on resource investment of benefactors towards growth, defence and reproduction, and its relationship to facilitative effects, is needed to unambiguously reveal this proposed shift in resource allocation as an adaptive response to the negative feedback effect of beneficiary species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Nutrient content of leaves of the cushion plant *Arenaria*.