

Variability in functional traits mediates plant interactions along stress gradients

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Summary

1. Environmental gradients may influence a plant's physiological status and morphology, which in turn may affect plant–plant interactions. However, little is known about the relationship between environmental variation, physiological and morphological variability of plants and variation in the balance between competition and facilitation.

2. Mountain ranges in dry environments have opposing altitudinal environmental gradients of temperature and aridity, which limit plant growth at high and low elevations. This makes them particularly suitable for exploring the relationships between environmental conditions, plant phenotype and plant–plant interactions. We hypothesized that different environmental stressors will differently affect the physiological status of a nurse plant. This, then, manifests itself as variation in nurse plant morphological traits, which in turn mediates plant–plant interactions by altering microhabitat conditions for the nurse and associated species.

3. In an observational study, we measured a series of functional traits of *Arenaria tetraquetra* cushions as indicators of its physiological status (e.g. specific leaf area, relative water content) and morphology (e.g. cushion compactness, size). Measurements were taken along the entire elevation range where *A. tetraquetra* occurs. Furthermore, we analysed how these functional traits related to soil properties beneath cushions and the number of associated species and individuals compared with open areas.

4. Cushions at high elevation showed good physiological status; they were compact and large, had higher soil water and organic matter content compared with open areas and showed the strongest facilitation effect of the whole elevation gradient – that is, the highest increase in species richness and abundance of beneficiaries compared with open areas. Physiological data at low elevation indicated stressful abiotic conditions for *A. tetraquetra*, which formed loose and small cushions. These cushions showed less improved soil conditions and had reduced facilitative effects compared with those at high elevation.

5. Synthesis. Functional traits of the nurse species varied distinctively along the two opposing stress gradients, in parallel to the magnitude of differences in microenvironmental conditions between cushions and the surrounding open area, and also to the facilitation effect of cushions. Our data, therefore, provides a strong demonstration of the generally overlooked importance of a nurse plant's vigour and morphology for its facilitative effects.

Key-words: elevation gradient, facilitation, intraspecific variability, morphology, nurse, phenotypic variability, physiological status, plant fitness, plant–plant interactions, stress-gradient hypothesis

Introduction

Plant–plant interactions are important forces structuring plant communities, and the balance between competition and

facilitation responds to variation in environmental stress and disturbance. The stress-gradient hypothesis predicts an increasing frequency, intensity and importance of positive interactions with increasing levels of abiotic stress and disturbance (Bertness & Callaway 1994; Brooker & Callaghan 1998; but see Maestre *et al.* 2009). However, the shape of the severity–interaction relationship has recently been hotly debated in terms of its patterns at the extreme ends of the stress gradient (Michalet *et al.* 2006; Malkinson & Tielbörger 2010), resource versus non-resource stressors (Maestre *et al.*

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2009) and its application at the species vs. community level (Armas, Rodríguez-Echeverría & Pugnaire 2011).

Gradients of environmental stress and disturbance are also important drivers of morphological variability in plants, for example along elevation gradients (Milla, Giménez-Benavides & Montserrat-Martí 2008). Previous studies suggest that morphological variability could be a mediator of plant–plant interactions (Michalet *et al.* 2011; Cranston *et al.* 2012). For example, Crutsinger, Strauss & Rudgers (2010) illustrated in a Californian coastal dune system that two different phenotypes of *Baccharis pilularis* shrubs significantly differed in their facilitation intensity. They showed that this was caused by differences in morphological traits, which in turn influenced local environmental conditions. Physiological regulation of a plant's vigour (i.e. the physiological status of a plant) could be a driver of morphological differences along environmental gradients, resulting in phenotypic and functional trait variability (Milla, Giménez-Benavides & Montserrat-Martí 2008). In turn, this might alter plant–plant interactions. However, such variation in functional traits has not been incorporated into explorations of the changing role of facilitation across environmental gradients, even though it might influence strongly the net outcome of plant–plant interactions.

Elevation gradients in mountain systems have often been used to test the stress-gradient hypothesis, assuming higher elevations to be more stressful for plants (e.g. Callaway *et al.* 2002; Cavieres *et al.* 2002; Kikvidze *et al.* 2005, 2011; Antonsson, Björk & Molau 2009; le Roux & McGeoch 2010). Physical stress at high elevations is mainly caused by low air temperature, low partial pressure of CO₂ and high UV radiation (Körner 2007), along with extremely thin soils and low nutrient availability (Huber *et al.* 2007). However, plants in mountain systems in dry environments may suffer in addition from water stress at low elevations (Cavieres *et al.* 2006). Low elevation sites in arid mountains experience low precipitation, high summer temperatures and high potential evapotranspiration, all negatively affecting plant water balance (Mooney, Hillier & Billings 1965) and limiting plant growth (Noy-Meir 1973). Despite the fact that water is usually not limited in the high alpine belt of dry mountains (Körner 2003), it often limits plant growth at the lower alpine belt because of soil desiccation during the growing season (Mooney, Hillier & Billings 1965; Reverter *et al.* 2010). Therefore, in dry and warm environments, plant growth is limited by severity gradients that change in an opposing manner with elevation, and which are expected to influence the physiological status and morphology of plants, the structure of plant communities and plant–plant interactions.

Environmental gradients comprising crossed stress factors provide an excellent system to analyse the interplay between environmental gradients and the functional traits of nurses, and its consequences for plant–plant interactions and overall community dynamics. We addressed these questions in a dry, high mountain system in southeast Spain using a community dominated by the cushion species *Arenaria tetraquetra* ssp.

amabilis. Cushion plants are well-known autogenic ecosystem engineers (*sensu* Jones, Lawton & Shachak 1994), which profoundly modify the physical environment (Badano & Cavieres 2006) and affect community assembly processes (Schöb, Butterfield & Pugnaire 2012). Differences in species composition between cushions and open areas may result from facilitation or competition by the cushion (Reid, Lamarque & Lortie 2010). We assessed the effect of *A. tetraquetra* on species richness and the number of beneficiary individuals along an elevation gradient, which comprised the full altitudinal growth range of the cushion. We measured differences in microenvironmental conditions between cushion-occupied vs. unoccupied areas to reveal potential facilitation mechanisms. We expected that the strength of facilitation would be related to differences in cushion morphology, which itself would change in response to changes in the opposing stress factors along the gradient. We also measured a series of plant functional traits as proxies for the physiological status of *A. tetraquetra*. These enabled us to test for drivers of change in cushion morphology along the gradient. Furthermore, in congruence with the stress-gradient hypothesis (Bertness & Callaway 1994; Brooker & Callaghan 1998), we expected that the strength of facilitation by the nurse would be correlated with the two opposing severity gradients. Specifically, we hypothesized that facilitation would be weakest at intermediate elevations, where both stress factors have intermediate levels, whereas it will be stronger towards the lower end of the elevation gradient due to resource-related stress (drought) and towards the higher end due to non-resource-related stress (low temperatures). We, therefore, implicitly assumed that the effect size of plant–plant interactions mainly reflects the most limiting stressor at a given point in the gradient.

Materials and methods

STUDY AREA

The study was conducted in the north-western part of the Sierra Nevada Mountains, south-eastern Spain. The study area consisted of five sites on a single slope up to the Veleta peak (3396 m; 37°03' N/ 03°21' W), at elevations of 2315, 2520, 2720, 3010 and 3240 m. The distance between the two neighbouring sites was c. 1.5–2 km. The two highest elevation sites are characterized by open grasslands and plants with basal rosettes such as *Festuca clementei*, *Erigeron frigidus* or *Jasione amethystina*, whereas the three lower sites are over moderately developed soils characterized by species such as *Festuca indigesta*, *Leontodon boryi* or *Sideritis glacialis* (Valle 2003). In Pradollano (2507 m a.s.l.) close to our 2520 m site, mean annual rainfall is 690 mm, and mean annual temperature is 3.9 °C (Rivas-Martínez & Rivas-Saenz 1996–2009). The study area is, therefore, characterized by a Mediterranean climate including a relatively hot and dry summer spanning the whole growing season. Correlations between mean annual temperature or precipitation and elevation in the northern side of the Sierra Nevada range revealed a temperature decrease of 0.61 °C every 100 m and an increase in precipitation of 33.3 mm across the same elevation interval (Delgado Calvo-Flores *et al.* 1988), thereby resulting in a crossed climatic gradient along the elevation gradient (Fig. 1a).

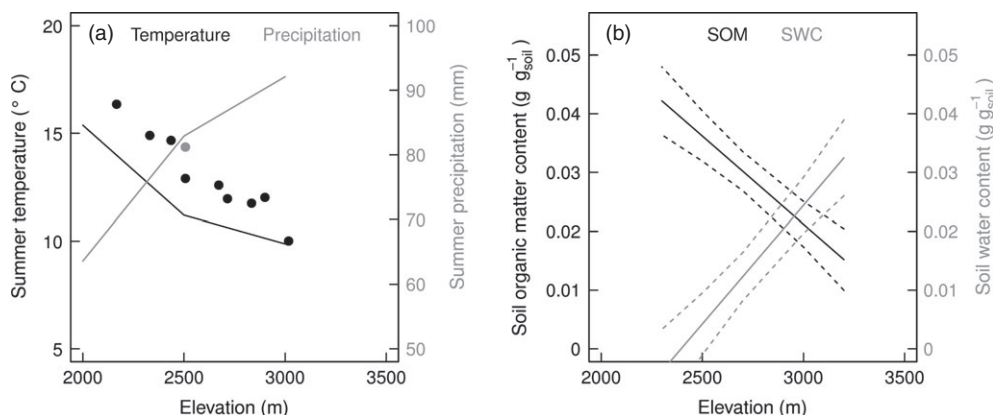


Fig. 1. The two opposing climatic gradients in dry mountains (a) consisting of summer (June–September) mean temperature (black) and summer mean precipitation (grey; Delgado Calvo-Flores *et al.* 1988) running in parallel to two opposing resource gradients (b) consisting of soil organic matter (SOM; black) and soil water content (SWC; grey) in open areas. Lines in (b) for SOM and SWC are model-predicted means (solid lines) and 95% CIs (dashed lines) of generalized linear regression models with log link function ($n = 38$). Dots in (a) represent mean summer temperature (black) and summer precipitation (grey; only one value available) measured at different sites in the study area [Sources (year of measurements): Environment Ministry, Madrid (1975–1989); Cetursa Sierra Nevada S.A., Pradollano (1999, 2000, 2004, 2005); Institute of Astrophysics of Andalusia, Granada (2005–2008, 2010)].

All five sites were situated in siliceous gravel habitats with gentle slopes facing north-west and containing the cushion-forming species *Arenaria tetraquetra* ssp. *amabilis* (Bory) H. Lindb. fil. (Caryophyllaceae), a species endemic to the Sierra Nevada Mountains (see Fig. S1 in Supporting Information). *Arenaria tetraquetra* cushions appeared haphazardly dispersed over the study sites and showed no obvious microsite preferences. Large open areas surrounded each selected cushion. The relative cover of *A. tetraquetra* was determined by five 30-m linear transects: the proportion of intercepted transect length of *A. tetraquetra* cushions gave a measurement of cover, which ranged between < 1 and 4%. The relative cover of all other vascular plants in open areas was visually estimated in each sampled open plot, and varied between sites from *c.* 3% to 14% on average, and was generally higher at low elevation than at high elevation. Our five sites encompassed the whole elevation range of *A. tetraquetra*, which was estimated to grow between 2400 and 3300 m a.s.l. (Díaz de la Guardia 2009). Both cushions and open areas were colonized by small annuals and (predominantly) by perennial herbs and grasses with a high frequency of endemism to the Sierra Nevada Mountains (> 40%; C. Schöb, pers. obs.).

DIVERSITY SAMPLING

We sampled 30 cushions and 30 paired neighbouring open areas at 2315, 2520 and 3010 m, whereas at 2720 and 3240 m, the number of pairs was 85 and 100, respectively, giving 550 samples in total. At each site, all samples were taken within an area of *c.* 500 m². Hereafter, we will refer to each sampled cushion and its paired open area as plots. Samples of open areas were equal in size and shape to that of cushions and were randomly placed at *c.* 50 cm distance to the respective cushion. Plot size was determined by measuring the largest cushion diameter and its perpendicular length, and its area was calculated as $\pi \times ((\text{diameter}_1 + \text{diameter}_2)/4)^2$. Therefore, plot size varied between samples due to variability in cushion size but was always the same between a cushion and its paired open plot. Average plot size was 174 ± 5 cm² (mean \pm 1 SE). In July 2010, at the peak of the growing season, the number of species and the number of individuals (or shoots for clonal species) of all vascular plant species were

recorded within the whole area of both cushions and open plots. We quantified plant communities in each plot using both the number of species and the number of individuals: the effects of nurse plants on community assembly have been shown to influence the composition of species but also the number of co-occurring individuals of the same and different species (Schöb, Butterfield & Pugnaire 2012). The number of species ranged between 0 and 9 per plot, and the number of individuals ranged from 0 to 85 per plot. The total number of species recorded over all sites was 41 (see Table S1 in Supporting Information).

MICROENVIRONMENTAL DATA

Between 20 and 21 July 2010, we sampled 15 cm³ of soil from the uppermost 10 cm beneath cushions and in open areas in six pairs of cushions and open plots per site (10 pairs at 2720 and 3240 m) and determined soil water (SWC) and soil organic matter (SOM) content. Soil samples were stored in sealed containers and fresh mass determined immediately after sampling. Gravimetric SWC ($\text{g g}_{\text{soil}}^{-1}$) was measured by mass loss after drying at 105 °C for 48 h. SOM ($\text{g g}_{\text{soil}}^{-1}$) was determined in the same soil samples by mass loss after ignition at 400 °C for 20 h.

At three sites (2315, 2720 and 3240 m), we recorded air temperature with iButtons (Maxim Integrated Products, Sunnyvale, CA, USA). Three temperature sensors per site were randomly placed in open areas 5 cm above-ground and protected from direct solar radiation. Temperature was recorded at 1-h intervals. For subsequent analyses, we calculated mean, maximum and minimum daily temperatures during August 2010.

PLANT FUNCTIONAL TRAITS

We assessed variation in 10 functional traits of *A. tetraquetra* cushions that were expected to respond to environmental conditions along the elevation gradient, and which potentially affect interactions with other plants. We used five traits as proxies of physiological status: (i) leaf dry matter content, LDMC (g kg^{-1}), measured as the ratio between leaf dry mass and fully rehydrated fresh mass; (ii) specific

leaf area, SLA ($\text{m}^2 \text{kg}^{-1}$), measured as the ratio between fresh leaf area and leaf dry mass; (iii) relative water content, relative water content (RWC) (%), determined as the ratio between plant water content in the field and water content after rehydration at full turgor; (iv) effective quantum yield ($\Delta F/F_m'$), the yield of photochemical energy conversion at photosystem II (PSII) reaction centres; (v) apparent electron transport rate, ETR ($\mu\text{mol m}^{-2} \text{s}^{-1}$), the efficiency of electron transportation at PSII. All these traits are considered indicative of the response of plant physiological status to local environmental conditions (Mooney, Hillier & Billings 1965; Noy-Meir 1973; Körner 2003; Reverter *et al.* 2010).

We also measured five functional traits for use as proxies of plant morphology: (i) lateral spread, measured as canopy diameter (cm) and used as a proxy for plant size; (ii) cushion thickness, determined by penetrating the cushion with a metallic rod and measuring the mean distance (cm) between the tip of the outer leaves and the soil underneath the plant at five random locations within the cushion; (iii) branch density (branches cm^{-2}), calculated from the number of terminal branches counted in one randomly placed 2 cm \times 2 cm quadrat per cushion; (iv) terminal branch length, measured as the distance (mm) between the bottom of the terminal leaf rosette and the terminal branching (see also Fig. S1d); and (v) leaf density (leaves cm^{-2}), calculated as the average number of leaves per terminal branch of ten randomly selected branches multiplied by the number of branches cm^{-2} . The area of individual leaves of *A. tetraquetra* was generally very small ($2.6 \pm 0.2 \text{ mm}^2$) and showed low variability, both within and between sites. Due to the low responsiveness of this trait in our study system, it was not considered in analyses. High values of branch and leaf density and low values of terminal branch length indicate high cushion compactness. Compactness, lateral spread and thickness together indicate cushion size and biomass. These functional traits were, on the one hand, considered dependent on the physiological status of the plant (Milla, Giménez-Benavides & Montserrat-Martí 2008), but on the other hand, they were considered to influence microenvironmental conditions within the cushion (Crutsinger, Strauss & Rudgers 2010; Michalet *et al.* 2011). All these traits were measured on 10 cushions per site between 16 and 18 July 2010.

LDMC and SLA were determined in leaves of five cushions per site collected between 9 and 10 August 2010. Following Cornelissen *et al.* (2003), we selected one small branch per cushion containing mature and healthy leaves and fully rehydrated them before determining leaf area and saturated mass of two leaves. Dry mass was measured after 72 h in the oven at 70 °C. LDMC and SLA are generally associated with photosynthetic capacity, relative growth rate and leaf longevity (Cornelissen *et al.* 2003), but are regulated by construction costs related to plant water and carbon economies, respectively. LDMC generally decreases with greater water availability and lower likelihood of physical damage (Cornelissen *et al.* 2003) and often shows no response to temperature variation (Albert *et al.* 2010). By contrast, SLA increases in response to increasing temperatures and decreasing light availability across a broad range of species (Poorter *et al.* 2009) and is positively correlated with soil nitrogen availability (Ordoñez *et al.* 2009) and negatively correlated with leaf longevity and resource-use efficiency (Wright *et al.* 2004). Thus, whilst LDMC and SLA are associated with similar biological processes, they may vary independently in response to different environmental drivers.

Relative water content measurements were performed on branches from eight cushions per site between 20 and 21 July at midday (11.00–13.00, solar time) under cloudless conditions and with cushion surface air temperatures of 21 ± 0.2 °C (mean \pm 1 SE). Time of sampling, temperature and their interaction term did not affect measurements. Fresh branch mass was determined immediately after

sampling in the field, saturated mass was measured after 30 h of rehydration in the dark, and dry mass was measured after 72 h at 70 °C. Accordingly, plant water content in the field was calculated as the difference between field fresh mass and dry mass, and water content at full turgor was calculated as the difference between saturated mass and dry mass. RWC was used as an indicator of plant water status at the time of sampling.

Effective quantum yield ($\Delta F/F_m'$) and ETR were determined in 10 randomly selected cushion plants per site measured with a saturation-pulse fluorometer (Mini-PAM Photosynthesis Yield Analyzer; Walz Mess- und Regeltechnik GmbH, Effeltrich, Germany) under ambient radiation and steady-state conditions. Measurements were carried out in the centre of the cushion on areas with no apparent leaf damage and with similar appearance amongst all cushions. F_m' is the maximum fluorescence of the leaf adapted to ambient irradiation induced by application of a saturating light pulse ($12\,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) closing all PSII reaction centres. $\Delta F = F_m' - F$, where F is the steady-state fluorescence (Schreiber & Bilger 1993). ETR was calculated after Krall & Edwards (1992) combining the effective quantum yield and the corresponding photosynthetically active radiation (PAR):

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

where 0.5 takes into account that about 50% of all absorbed photons reach PSII, and 0.84 is considered the most common leaf absorbance coefficient for C3 plants (Björkman & Demmig 1987). Effective quantum yield and ETR measurements were performed between 20 to 21 July at midday (11.00–13.00, solar time) under cloudless conditions and at air temperatures close to the cushion surface of 21 ± 0.2 °C (mean \pm 1 SE). Time of sampling, temperature and its interaction term did not affect the measurements. Both fluorescence measures are indicators of the efficiency of photosynthesis at the time of sampling either taking differences in incoming PAR into account (i.e. ETR) or not (i.e. effective quantum yield), and can be considered rough measurements of leaf stress.

DATA ANALYSIS

We quantified the absolute and relative difference in SWC and SOM between cushions and open areas, where the relative difference was calculated by dividing absolute differences by the corresponding values in open plots. The difference in the number of species and individuals between cushions and open areas was calculated using the relative interaction index (RII; Armas, Ordiales & Pugnaire 2004). RII was calculated for each paired cushion and open plot as

$$\text{RII} = (N_c - N_o)/(N_c + N_o) \quad \text{eqn 2}$$

where N_c is the number of species or individuals in cushions, and N_o is the corresponding value in the open plot.

For all statistical analyses, we applied a model selection approach using the Akaike Information Criterion (AIC), where lower AIC values indicate a better fit of the corresponding model based on the concept of parsimony, that is, by balancing complexity and power of a model (Johnson & Omland 2004). In addition, we calculated Akaike weights giving the likelihood of each model to be the best model in comparison with all the competing models tested. As some of our models, in particular those including functional traits, include a high number of explanatory variables, we preferred a model selection approach over traditional null hypothesis testing. It allowed us to compare several competing models and to select the best model that included only those variables, which substantially improve the model fit. Furthermore, it allowed us to rank models that included different

combinations of variables, thereby measuring the relative support for each competing model.

The relationships between either SOM or SWC and the presence/absence of cushions and elevation were tested with generalized linear models with a Poisson error structure and a log link function. The relationships between either the number of species or the number of individuals and the presence/absence of cushions were tested with generalized linear models with a Poisson error structure, a log link function and elevation included as a covariate. Comparisons of the number of species and individuals along the elevation gradient were omitted due to unequal plot sizes at different elevations. Linear regression was used to test whether absolute or relative differences in SOM, SWC, number of species (RII_{species}) and number of individuals ($RII_{\text{individuals}}$) between cushions and open areas varied along the elevation gradient.

For the five functional traits used as proxies for plant morphology, we applied a principal components analysis (PCA) with Varimax rotation (Quinn & Keough 2002) to reduce the number and multicollinearity of variables. We used a correlation matrix (i.e. zero-centred variables with unit variance) because variables were in different units and had different variances. The principal components (PCs) were, then, used as predictor variables in linear models testing the relationship between cushion morphology and differences in SOM or SWC between cushion and open plots. We included SOM or SWC in open plots as covariates in the corresponding models. These covariates accounted for those changes in the differences in soil properties between the two microhabitats with elevation that are due to changes in SOM or SWC with elevation in the open microhabitat, and are therefore not related to the effect of cushions. The models consisting of all possible combinations of PCs and the covariate were then compared with the control model (a model only including the covariate). If any model including a PC and the covariate performed better than the control model, there was support for a relationship between cushion morphology and the differences in either SOM or SWC between cushions and open plots. This relationship was used as indication for a role of cushion morphology in the potential effects of cushions on microenvironmental conditions. With similar models, we tested for the relationship between cushion morphology and RII_{species} or $RII_{\text{individuals}}$ where we included the number of species or individuals in open plots as a covariate. This relationship was used as an indication for the role of cushion morphology in potential facilitation effects of cushions on species richness and abundance.

Because functional traits were not directly matched to specific values of SOM, SWC, number of species or number of individuals within sites (i.e. these variables were sampled in different cushions within the same sites), we only studied their relationship across sites using permutation tests that randomly attributed to each measurement of a morphological trait a measurement of soil properties or the mean of three measurements of the diversity sampling. Random sampling occurred without replacement and was restricted within sites. Each of these data sets was equal to the minimal size of the observed data set (i.e. $n = 38$ and $n = 50$ for the relationship between functional traits and soil properties and between functional traits and RII_{species} and $RII_{\text{individuals}}$, respectively). With this procedure, we kept differences in functional traits, soil properties, number of species and number of individuals amongst sites but randomized them within sites. We preferred this procedure over calculating mean values per site for each variable in that it enabled us to include more complex models testing for relationships between those variables amongst sites. We performed 1000 randomizations of the data sets and calculated AIC values, correlation coefficients between variables and adjusted R^2 values for

each candidate model for each randomization. The best model was, then, selected according to the lowest mean AIC value (AIC_{mean}). In addition, we calculated Akaike weights for all candidate models based on AIC_{mean} , the mean correlation coefficient (r) between each PC and the response variable as a measure of effect size of each PC on the response variable and mean adjusted R^2 (R^2_{adj}) values for each model as a measure of the goodness-of-fit of the corresponding model. Finally, the relationship between morphological and physiological traits of cushions across sites was revealed by Pearson correlations based on mean values per site ($n = 5$).

All calculations and statistical analyses were performed with the R software version 2.14.0 using the packages *base*, *graphics*, *grDevices*, *lme4*, *psych*, *stats* and *vegan* (R Development Core Team 2011).

Results

MICROENVIRONMENT

In open areas, there was an increase in SWC with elevation, whereas SOM and temperature decreased with elevation. SWC in the lowest site was 1.4% of that in the highest site, whereas SOM decreased by 67% from the lowest to the highest site (Fig. 1b). T_{mean} was lower in the highest site (13.5 ± 0.3 °C vs. 19.9 ± 0.4 °C), in parallel with T_{min} (7.2 ± 0.1 °C at 3240 m and 11.7 ± 0.4 °C at 2315 m) and T_{max} (24.1 ± 0.7 °C at 3240 m and 33.1 ± 2.1 at 2315 m).

Both SOM and SWC changed not only with elevation but also with the presence of cushions, showing higher values beneath cushions than in open areas (Fig. 2a,b). The best model explaining SOM included the two main effects, 'elevation' and 'cushion' ($R^2_{\text{adj}} = 0.31$, Table S2), whereas SWC was best explained with a full model including 'elevation', 'cushion' and their interaction term ($R^2_{\text{adj}} = 0.59$, Table S2). The absolute difference in SWC between cushions and open plots increased with elevation ($R^2_{\text{adj}} = 0.21$), but this pattern was not found for SOM (Fig. 2a,b; statistical results in Table S3a). However, the relative differences between cushions and open areas increased with elevation for SOM ($R^2_{\text{adj}} = 0.10$) but not for SWC (statistical results in Table S3b).

DIVERSITY

Cushions harboured a higher number of species and individuals per plot than open areas at all sites (with the exception of number of individuals at 2520 m; Fig. 2c,d; Table S4). RII_{species} and $RII_{\text{individuals}}$ were lowest at intermediate elevation (2720 m), increased towards higher elevations and slightly increased towards lower elevations (Fig. 2e,f). Accordingly, the best models fitting RII_{species} ($R^2_{\text{adj}} = 0.07$) and $RII_{\text{individuals}}$ ($R^2_{\text{adj}} = 0.15$) consisted of the main effect 'elevation²' (Table S5) indicating a nonlinear relationship.

PHYSIOLOGICAL TRAITS AND CUSHION MORPHOLOGY

There was a negative relationship between elevation and LDMC ($r = -0.40$) and SLA ($r = -0.22$) and a positive correlation between elevation and RWC ($r = 0.23$), effective

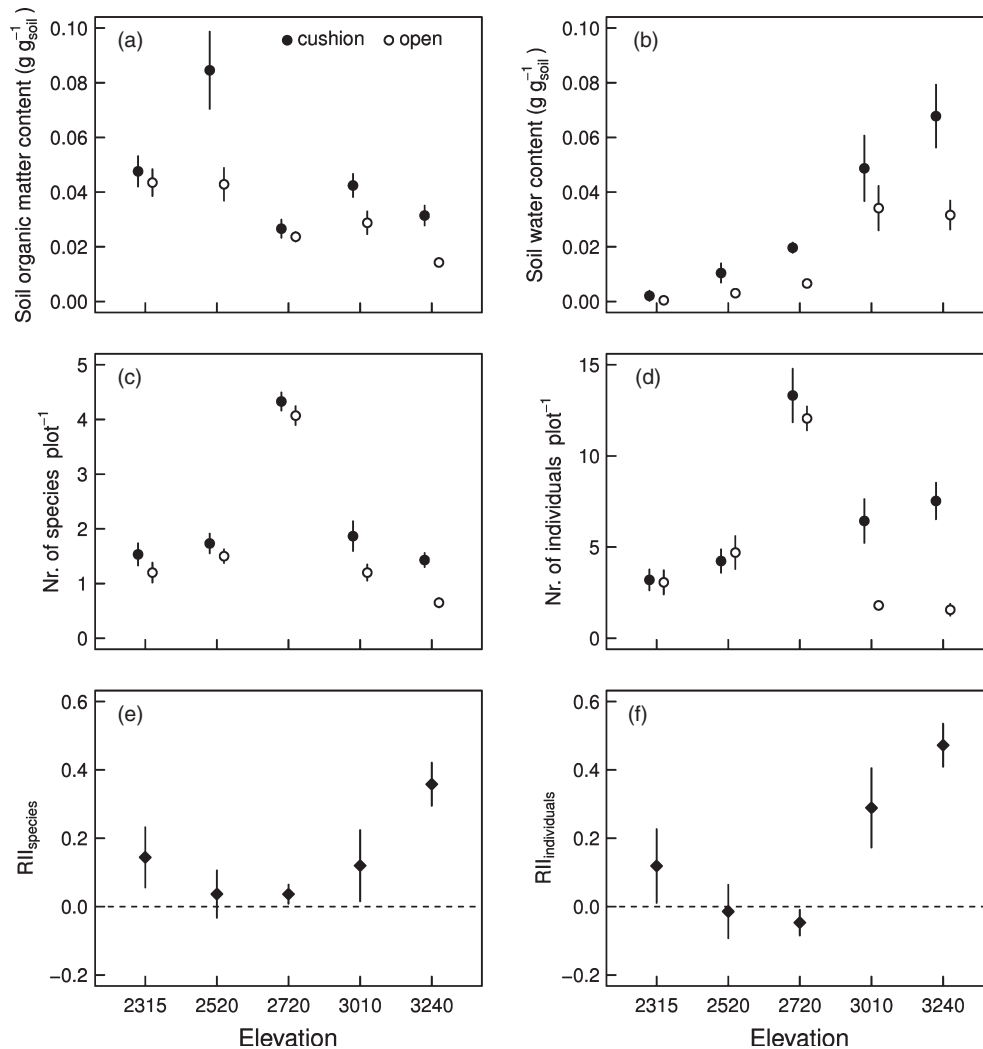


Fig. 2. Soil organic matter content (a), soil water content (b), number of species (c) and number of individuals (d) in cushions (filled circles) and open plots (open circles), as well as RII_{species} (e) and $RII_{\text{individuals}}$ (f) along the elevation gradient. Negative values of RII indicate a lower number of species or individuals in cushions compared with paired open plots, whereas positive values indicate a higher number of species or individuals in cushions compared with paired open plots. Displayed values are means \pm 1 SE; $n = 76$ for soil organic matter and soil water content, $n = 550$ for number of species and individuals and $n = 275$ for RII_{species} and $RII_{\text{individuals}}$. For statistical analyses, see Table S2 (for soil organic matter and soil water content), Table S4 (for number of species and individuals) and Table S5 (for RII_{species} and $RII_{\text{individuals}}$).

quantum yield ($r = 0.20$) and ETR ($r = 0.72$; Fig. S2). LDMC and SLA showed negative correlations with all morphological traits except terminal branch length, whereas RWC, effective quantum yield and ETR showed positive correlations for all traits except terminal branch length (Fig. 3 and Table S6). The strongest relationships were found between morphological traits and ETR ($r > 0.75$ for all morphological traits; Table S6).

CUSHION MORPHOLOGY AND ENVIRONMENT/ DIVERSITY

All functional traits used as proxies for cushion morphology varied with elevation. Cushions were larger ($r = 0.64$), thicker ($r = 0.41$), had higher branch and leaf density ($r = 0.87$ and $r = 0.88$, respectively) and shorter terminal branch length

($r = -0.57$) at high than at low elevations (Fig. 3 and Fig. S2). The first three axes of the PCA with the five morphological traits explained 58%, 24% and 10% of the variance, respectively. The first axis (PC1) represents cushion compactness and size, with strong positive correlations for branch density, leaf density and lateral spread (Fig. 3 and Table S7). The second axis (PC2) represents cushion thickness and branch length with strong positive correlations for thickness and terminal branch length. Positive values on the third axis (PC3) represent small and rather compact cushions with negative correlations for lateral spread and positive correlations for leaf density.

Differences in the number of species and individuals between cushions and open areas were best explained by PC1 with minor contributions of PC2 and PC3 (Tables S8 and S9). Both RII_{species} and $RII_{\text{individuals}}$ showed positive

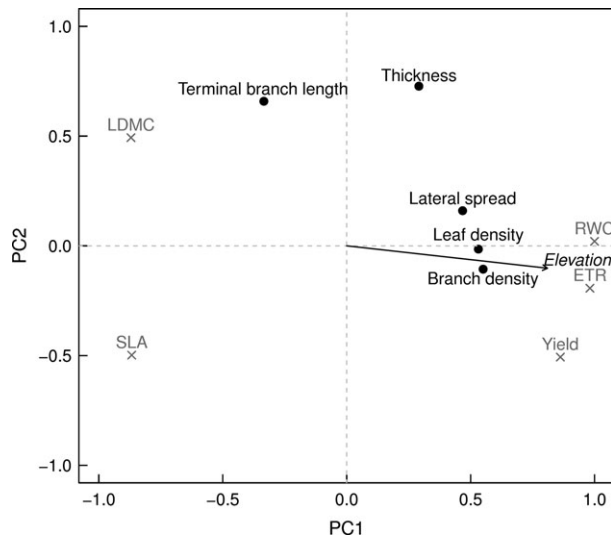


Fig. 3. Variation in functional traits of *Arenaria tetraquetra* ssp. *amabilis*. PCA based on morphological traits of the cushion (black dots), with physiological traits (grey cross) and elevation (black arrow) fitted onto the ordination. The first and second principal component explained 58% and 24% of the variance in the 5 morphological traits, respectively. $n = 50$ for morphological traits as well as effective quantum yield (Yield) and apparent electron transport rate (ETR), $n = 40$ for relative water content (RWC) and $n = 25$ for leaf dry matter content (LDMC) and specific leaf area (SLA).

correlations with PC1 ($r = 0.25$ and $r = 0.36$, respectively). PC1 was also the best predictor for the absolute differences in SOM between cushions and open areas, with minor contributions of PC2 and PC3 (Table S10), whereas the model including PC1 and PC3 best predicted absolute differences in SWC between cushions and open areas (Table S11). PC1 was negatively correlated with differences in SOM between cushions and open areas ($r = -0.12$), whereas all principal components were positively correlated with differences in SWC between cushions and open areas, with a particularly high effect size for PC1 ($r = 0.46$).

Discussion

Our data indicate that functional traits of *A. tetraquetra* play a critical role in its facilitative effects on other species. The cushion phenotype modulated the balance of plant–plant interactions along, and together with, the crossed severity gradients found in the Sierra Nevada Mountains. Specifically, our results suggest that the physiological status of *A. tetraquetra* may affect its morphology, which in turn influences the microenvironmental conditions provided by the cushion and, consequently, modulates its facilitative effect on species richness and the number of associated individuals.

CUSHION TRAITS AND FACILITATION

The cushion life-form is most frequent in nutrient poor and cool environments, particularly on bare soils or in open vegetation in windswept habitats (Körner 2003). In these

environments, the compact and dense canopy of cushions traps and accumulates leaf litter (Körner 1993), mitigates extreme temperatures inside the cushion and increases soil moisture beneath it (Cavieres *et al.* 2006). This creates a favourable microenvironment for decomposers (Schinner 1982a), which probably results in the cushion acting as a fertility island (Körner 2003). However, it is highly likely that all these components of microhabitat amelioration by cushions are highly dependent on their morphology. More compact and larger cushions probably provide a better wind shield than loose and small cushions, thereby retaining leaf litter, ameliorating low temperatures and increasing moisture storage. In line with this, the effects of cushions on SOM and SWC were highest at high elevation where cushions were compact and large, whereas at low elevation, where cushions were loose and small, these effects were smaller. Consequently, cushions may need a certain range of trait values to efficiently improve microhabitat conditions and act as nurses.

The positive link between physiological and morphological traits of *A. tetraquetra* points towards a dependence of the cushion morphology on its vigour. A better physiological status (e.g. higher ETR, effective quantum yield and lower SLA) and an improved water balance (e.g. higher RWC and lower LDMC) went along with compact and large cushions. *Arenaria tetraquetra* developed its optimal physiological status at high elevations. At low elevations – with low water availability and high temperatures – *A. tetraquetra* performed poorly, as indicated by low plant water status and low photosynthetic efficiency. Indeed, Reverter *et al.* (2010) showed that the permanent wilting point for most plants growing in the Sierra Nevada Mountains at 2300 m occurred when gravimetric soil water content dropped below 3%; SWC at our low site, both in open areas and beneath cushions, was below this threshold. Therefore, at low elevations, *A. tetraquetra* was most probably too stressed to develop large cushions with a compact and dense canopy.

The benefit of the microhabitat amelioration (*sensu* Callaway 2007), or the creation of biogenic microhabitats (*sensu* Badano & Marquet 2009) by cushions, is not necessarily restricted to other species but also probably benefits the cushion species itself. Consequently, we assume that strong facilitation occurred under conditions where niche construction (*sensu* Kylafis & Loreau 2011) by *A. tetraquetra* was adequate, as occurred at high elevation. At low elevations, however, *A. tetraquetra* may not have been able to adequately develop its own niche because the most limiting niche factor (i.e. water availability) could not be considerably improved. Overall, these results highlight the importance of niche construction by cushions for their facilitation effect.

FACILITATION ALONG OPPOSING STRESS GRADIENTS

Changes in facilitation intensity – that is, the difference in species richness and the number of individuals inside and outside cushions – along the elevation gradient were likely to be a reflection of the opposing stress factors in our study system that affected species richness and abundance both in

and out of cushions. On the one hand, our results suggest that the number of species and individuals associated with *A. tetraquetra* changed with elevation through the effects of the opposing stress factors on cushion morphology – the latter affecting the influence of the nurse on microenvironmental conditions. On the other hand, the opposing stress gradients directly affected species richness and abundance in open areas, that is, a reduced richness and abundance with increasing environmental severity towards both ends of the elevation gradient.

Facilitation intensity was high at the high end of the elevation gradient but not at the low end. This difference in facilitation intensity at the two stressful ends of the gradient could have been the result of differences in the capacity of the nurse to mitigate the different environmental factors affecting the interaction balance (Michalet 2007; Maestre *et al.* 2009). Environmental severity at high elevation was characterized by non-resource-related stress (i.e. low temperature), whereas at low elevation, it was caused by resource limitation (i.e. low water availability). However, low temperature at high elevation may indirectly affect nutrient availability *via* reduced plant productivity and SOM (Körner 2003), especially because SOM is the primary source of soil nutrients in our study system (Sánchez-Marañón *et al.* 2002). A case study performed at our field site showed that SOM is positively correlated with total N (Sánchez-Marañón *et al.* 2002), and similar relationships between SOM and total N have also been shown in other alpine (Rehder 1970; Körner 2003) and water-limited (dry to arid) ecosystems (Whitford, Reynolds & Cunningham 1987). Furthermore, low temperatures and short growing seasons in high elevation sites are associated with low microbial activity, which may result in low decomposition rates of SOM, which would further reduce soil nutrient availability at high elevations (Schinner 1982b; Seastedt, Walker & Bryant 2001). Consequently, at both ends of the elevation gradient, the limiting factors for plant growth may have been resources: nutrients at high elevation and water at low elevation. Nonetheless, at high elevations, soils beneath *A. tetraquetra* cushions showed higher levels of resources (i.e. SWC and SOM) than those in open areas, indicating that cushions likely buffered this resource-related stress at high elevation. This is in line with two previous reports on cushion plants in alpine environments that revealed nutrient enrichment at high elevation sites (Yang *et al.* 2010; Anthelme *et al.* 2012) and provision of moisture in arid environments (Cavieres *et al.* 2006) as drivers of increased facilitation. Consequently, these results indicate that *A. tetraquetra* was able to alleviate both limiting stressors provided that it could develop the appropriate combination of functional trait values needed to improve microenvironmental conditions and act as nurse.

Conclusions

Previous reports showed that different morphotypes of plants can differ in their facilitative effects (Pugnaire *et al.* 1996; Proffitt *et al.* 2005; Crutsinger, Strauss & Rudgers 2010; Michalet *et al.* 2011; Cranston *et al.* 2012). These studies

reported that nurse plants showed morphological variability, which could be the result of age (Pugnaire *et al.* 1996), evolutionary adaptation to local conditions (Michalet *et al.* 2011) or reflect differences in plant's physiological status (Thompson, McNeilly & Gray 1991). However, these studies did not demonstrate the mechanistic link presented here between environmental gradients and plant morphology, and how this may affect the small-scale environmental conditions created by the nurse plant, which finally have a relevant effect on its competitive or facilitative effects on other species. Morphological variability due to either ontogeny (Pugnaire *et al.* 1996), genotypic variability (Michalet *et al.* 2011) or morphological plasticity in response to differences in plant vigour (this study) can strongly interact with different types of stressors or environmental severity and influence the effect of facilitating species. This clearly indicates the need for careful interpretation of changes in the strength of facilitation along stress gradients. Forthcoming manipulative experiments may now be needed to verify the mechanistic links outlined here between environmental conditions, plant physiological status, functional traits and how the resulting plant's phenotype influences habitat modification, the outcome of plant interactions and their effects on plant community dynamics.

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Supporting Information

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

Table S1. Species list and difference in the number of individuals between cushions and open areas for each species at each site.

Table S2. Model selection for the relationship between elevation and cushions to soil organic matter and soil water content.

Table S3. Model selection for the relationship between elevation and the absolute and relative difference of soil organic matter (SOM) and soil water content (SWC) between cushions and open areas.

Table S4. Model selection for the difference in the number of species and individuals between cushions and open areas.

Table S5. Model selection for the relationship between elevation and the difference in the number of species (RII_{species}) and the number of individuals ($RII_{\text{individuals}}$) between cushions and open areas.

Table S6. Correlation between morphological and physiological traits of *Arenaria tetraquetra* cushions.

Table S7. Correlation coefficients and variable loadings for the principal components and five morphological traits.

Table S8. Model selection for the relationship between the difference in species richness between cushions and open areas (RII_{species}) and morphological traits of cushions.

Table S9. Model selection for the relationship between the difference in the number of individuals between cushions and open areas ($RII_{\text{individuals}}$) and morphological traits of cushions.

Table S10. Model selection for the relationship between the difference in soil organic matter (SOM) between cushions and open areas and morphological traits of cushions.

Table S11. Model selection for the relationship between the difference in soil water content (SWC) between cushions and open areas and morphological traits of cushions.

Figure S1. Images of habitat and morphology of *Arenaria tetraquetra* ssp. *amabilis*.

Figure S2. Functional traits of *Arenaria tetraquetra* along the elevation gradient.