



A field test of the stress-gradient hypothesis along an aridity gradient

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Keywords

Arid; Competition; Facilitation; Importance; Intensity; Interactions; Species richness

Abbreviations

R_{Ib} = relative interaction intensity (effect of the interaction on biomass); R_{If} = relative interaction frequency (effect of the interaction on species richness). R_{II} (Armas et al. 2004); I_{imp} = importance of the interaction on biomass (Seifan et al. 2010); SGH = stress-gradient hypothesis; SIR = severity–interaction relationship.

Nomenclature

Castroviejo et al. (1986)

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Introduction

The stress-gradient hypothesis (SGH) is a general conceptual model that predicts how competitive and facilitative interactions among plant species change along gradients of herbivory and environmental stress (Bertness & Callaway 1994). The hypothesis accounts for both the importance of the interaction, i.e. the impact of one species on another expressed as a proportion of the impact of the whole environment on the species (Brooker et al. 2005), and the intensity of the interaction, i.e. the magnitude of the effect of one species on another (Armas et al. 2004).

Abstract

Aims: The stress-gradient hypothesis (SGH) predicts how plant interactions change along environmental stress gradients. We tested the SGH in an aridity gradient, where support for the hypothesis and the specific shape of its response curve is controversial.

Location: Almería, Cáceres and Coimbra, three sites in the Iberian Peninsula that encompass the most arid and wet habitats in the distribution range of a nurse shrub species – *Retama sphaerocarpa* L. (Boiss) – in Europe.

Methods: We analysed the effect of *Retama* on its understorey plant community and measured the biomass and species richness beneath *Retama* and in gaps. We estimated the frequency (changes in species richness), importance and intensity of the *Retama* effects, and derived the severity–interaction relationship pattern, analysing how these interaction indices changed along this aridity gradient.

Results and conclusions: The intensity and frequency of facilitation by *Retama* increased monotonically with increasing environmental severity, and the importance tended to have a similar pattern, overall supporting the SGH. Our data did not support predictions from recent revisions of the SGH, which may not apply to whole plant communities like those studied here or when interactions are highly asymmetrical. Facilitation by *Retama* influenced community composition and species richness to the point that a significant fraction of species found at the most arid end of the gradient were only able to survive beneath the nurse shrub, whereas some of these species were able to thrive in gaps at more mesic sites, highlighting the ecological relevance of facilitation by nurse species in mediterranean environments, especially in the driest sites.

The original SGH predicts an increase in the frequency and importance of facilitation with increasing environmental severity (Bertness & Callaway 1994; Brooker & Callaghan 1998) and is widely supported by empirical evidence (Callaway et al. 2002; Kawai & Tokeshi 2007; Brooker et al. 2008; le Roux & McGeoch 2010, and references therein). However, some experimental data (Maestre & Cortina 2004; Michalet et al. 2006; Kawai & Tokeshi 2007) and a recent meta-analysis based on data from arid environments (Maestre et al. 2005; but see Lortie & Callaway 2006) have challenged this hypothesis, leading to suggestions for refining the model (Maestre

et al. 2009). These authors predict that the outcome of plant interactions, or the severity–interaction relationship pattern, would not always increase as environmental severity increases and could have a hump-shaped pattern (see Appendix S1), depending on the strategy of the interacting species (stress tolerators or competitors, sensu Grime 1977) and whether the main factor of stress is a resource or not. Malkinson & Tielbörger (2010) also proposed that physiological responses are not linear with respect to environmental stress gradients, and suggested that in some circumstances the importance of facilitation may be lower at both ends of the stress gradient. Finally, early meta-analyses highlighted that the outcome of the interaction along productivity gradients may vary depending on the plant response variable selected (Goldberg et al. 1999).

The SGH has been tested in different habitats and climates (Callaway 2007); however, predictions may be less consistent at the driest end of an aridity gradient (Maestre et al. 2005; Lortie & Callaway 2006) where negative interactions could prevail (Tielbörger & Kadmon 2000; Maestre & Cortina 2004; but see Holzapfel et al. 2006). In dry hot environments, plant communities are limited by water, and the effects of drought are exacerbated by high temperature. Facilitation would then occur only if benefits from enhanced nutrient availability or shade exceed competition for water (Holmgren et al. 1997) or if the benefactor species can increase the availability of water to the facilitated species (Zou et al. 2005; Maestre et al. 2009). Hacker & Gaines (1997) and Michalet et al. (2006) also suggested that plant interactions would affect the species richness–productivity gradient model (Grime 1973); facilitation might promote diversity at medium- to high-environmental severity levels by expanding the realized niche of stress-intolerant, competitive species into physically demanding environments. However, if the positive effects of benefactors wane when environmental conditions become extremely severe, plant diversity would be reduced at the most stressful end of the gradient (Michalet et al. 2006).

Since climate models predict an increase in aridity and temperature in extended inter-tropical areas including Southern Europe (IPCC 2007), in particular under Mediterranean climate, it is critical to clarify SGH predictions and the shape of the severity–interaction relationship, as they could help forecast future changes in the biodiversity, structure, function and assembly of communities affected by global change (Callaway 2007).

Some uncertainties regarding the SGH could be related to methodological issues. For example, most tests of the SGH have considered two, rarely three, levels of environmental severity and very few have used a gradient stretching through the whole range of environmental tolerances

of a species (Choler et al. 2001; Holzapfel et al. 2006). In the particular case of aridity gradients, if the whole distribution range is not considered, the test may not include the most severe end of the gradient, which is where controversy arises regarding SGH predictions.

Here, we report an observational study where we tested the net effect of a leguminous shrub, *Retama sphaerocarpa* L. (Boiss), on its understorey community along an environmental gradient extending from the moistest to the driest habitats within the whole distribution range of the species in Europe (Castroviejo et al. 1986). As far as we know, there is just one other test of the SGH on a gradient that includes the maximum range where the nurse/benefactor species naturally occur (Holzapfel et al. 2006). The overall results of this study support the original SGH, but the authors found that the potential benefactor species actually did not act as a nurse.

According to the SGH, the potential severity–interaction relationship patterns, and the specific functional traits of *Retama*, we expected that (a) there would be an increase in the intensity, importance and frequency of net facilitation by *Retama* on its understorey community as environmental severity increases; thus the severity–interaction relationship pattern would be monotonic and positive with increasing aridity and decreasing productivity. Following predictions of Hacker & Gaines (1997) and Michalet et al. (2006) regarding the effect of biotic interactions on the species richness–productivity gradient model (Grime 1973), we expected that (b) where facilitation prevails, it may expand the realized niche of some species that are unable to survive in the open but thrive under the nurse species. This in turn, (c) would lead to distinct understorey communities, differing in composition and richness from plant communities growing outside the canopy.

We quantified facilitation in terms of biomass and species richness of the subordinate plant community by comparison with the community of nearby gaps between shrubs.

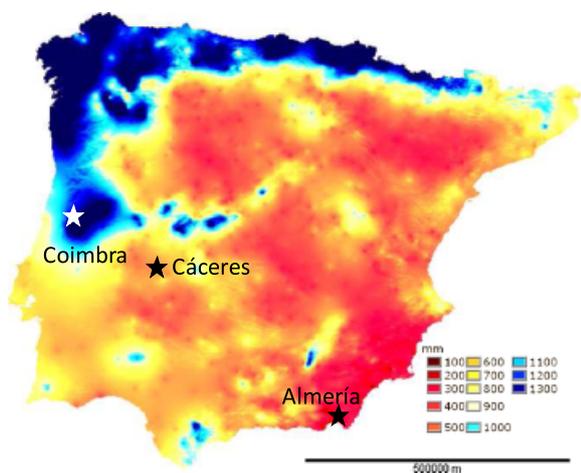
Methods

Field sites, species and data collection

Retama sphaerocarpa (L.) Boiss (*Retama*, hereafter) is a large leguminous shrub, widespread in the Iberian Peninsula and northwest Africa. It has an open canopy with photosynthetic stems, a dimorphic root system with shallow lateral roots and tap roots that can reach 30-m deep (Haase et al. 1996). *Retama* roots are able to lift water from deep, wet layers and release it to shallow, dry soils (Prieto et al. 2010), contributing to its nurse syndrome sensu Niering et al. (1963). *Retama* facilitates establishment and growth of many other species under its canopy

Table 1. Climate gradient across the Iberian Peninsula. Data are based on a 30-year series (1971–2000). Legend: Mean annual temperature (T), minimum annual temperature (Tm), maximum annual temperature (TM), mean annual precipitation (R), aridity index (R/PET where PET is potential evapotranspiration) and cumulative rainfall (R'2008–09) from September 2008 until sampling date (March 2009 for Almería, May 2009 for Cáceres and Coimbra). Data of mean annual precipitation in the Iberian Peninsula, modified from Atlas Climático Digital de la Península Ibérica. Departments of Biología Animal, Vegetal i Ecología and Geografía. Universidad Autónoma de Barcelona, Spain (<http://opengis.uab.es/wms/iberia/index.htm>).

	(–) Water/Productivity gradient (+)		
	Dry/Low	Medium	Wet/High
	Almería	Cáceres	Coimbra
Altitude (m)	630	351	147
T (°C)	17.9	16.1	15.3
Tm (°C)	10.9	10.8	10.4
TM (°C)	24.8	21.4	20.4
R (mm)	240	523	979
R/PET	0.30	0.62	1.26
R 2008–09 (mm)	213.4	295.7	679.3



in dry ecosystems due to the amelioration of microclimatic conditions and increases in mineralization rate, organic matter, nutrients – particularly N and P – and fine soil particles under the canopy (Pugnaire et al. 1996; Moro et al. 1997; Rodríguez-Echeverría & Pérez-Fernández 2003), in an interaction which outcome may change rapidly, depending on abiotic conditions (Pugnaire & Luque 2001).

Sampling was conducted at three locations along a gradient of environmental stress in the Iberian Peninsula, Almería, Cáceres and Coimbra. These three sites include the driest and wettest points of the climate gradient in which *Retama* can grow, thus spanning all its distribution range. The three points have annual rainfall means of 245, 523 and 979 mm (for Almería, Cáceres and Coimbra, respectively), the two mesic sites having two and four times higher rainfall than the driest site (Table 1). Selected sites also represent a gradient of decreasing temperature and aridity, i.e. the ratio of mean annual precipitation to potential evapotranspiration. Climate data were obtained from the nearest weather stations to the field sites, all within 10 km of the sampling sites (Spanish and Portuguese meteorology agencies).

Sampling was carried out in Rambla del Saltador (37°08'N, 2°22'W) in Almería (the dry end of the gradient), in an almost flat valley near Aliseda (38°25'N,

6°41'W) in Cáceres (the intermediate position) and near Coimbra (40°20'N, 08°25'W) at the wettest end of the gradient. Field sites were selected so as to include soils and topographic characteristics among sites that were as similar as possible (see Appendix S2 for more details on soil and field site characteristics).

Seven adult, similar-sized *Retama* shrubs were randomly selected in each field site. The absence of significant differences in mean size of *Retama* individuals among sites was confirmed using one-way ANOVA ($13.91 \pm 1.37 \text{ m}^2$; $F_{2,18} = 0.03$, $P = 0.98$). Maximum canopy diameter and its perpendicular were measured for each shrub and used to calculate projected canopy area as an ellipse. Both annual and perennial species growing underneath each *Retama* individual and in equal-sized plots in an adjacent open area were recorded at the time of sampling. To estimate plant biomass of annuals we randomly placed one $20 \times 20 \text{ cm}$ quadrat in open areas and near the main trunk on the western aspect beneath each *Retama* individual, and clipped all above-ground biomass of annual species at ground level. Plants were dried at 70 °C for 48 h and weighed. Plant productivity of each field site was estimated as the above-ground biomass of the annual community in gaps per unit of area. Sampling was carried out in spring 2009 at the time of peak biomass in each site

(mid-March in Almería, beginning of May in the two other sites).

For each field site, we calculated the intensity and importance of the effect of *Retama* shrubs on their understorey community as a function of biomass, and the frequency of facilitation as a function of species richness under *Retama* and in open areas. We used the relative interaction index, RII (Armas et al. 2004), to assess interaction intensity (RII_b) and frequency (RII_f) using plant biomass and species richness, respectively. RII is a useful metric because plant species in the herbaceous community differ among field sites and can have disparate sizes irrespective of resource availability, making plant biomass not directly comparable between different sites or species. RII is defined as

$$RII = \frac{B_w - B_o}{B_w + B_o} \quad (1)$$

where B_w is mass (RII_b) or number of species (RII_f) in the community under *Retama*, and B_o is the mass (RII_b) or number of species (RII_f) in the community growing in a nearby gap. Samples were paired between each *Retama* shrub and its neighbouring open area.

We used the I_{imp} index (Seifan et al. 2010) as a metric of interaction importance (sensu Brooker et al. 2005). This index assesses the relative contribution of neighbours to the deviation from the optimum performance. It is defined as

$$I_{imp} = \frac{N_{imp}}{|N_{imp}| + |E_{imp}|} \quad (2)$$

where N_{imp} is the neighbour contribution to plant performance and E_{imp} is the environmental contribution to annual plant community performance. N_{imp} and E_{imp} are:

$$N_{imp} = B_w - B_o \quad (3)$$

and

$$E_{imp} = B_o - MB_{\pm w} \quad (4)$$

where $MB_{\pm w}$ is the maximum value of the community biomass regardless of the microsite (i.e. the maximum value can be from gaps or under *Retama*).

Both RII and I_{imp} have defined limits $[-1, +1]$, are symmetrical around zero, and are negative when competition prevails, positive when facilitation prevails and 0 when the net balance of the interaction is neutral or has no importance for plant performance.

Statistical analyses

Differences in all measured variables among sites were tested using one-way ANOVA at a significance level of $P < 0.05$, using location as a fixed factor, after homoge-

neity of variances was checked using Levene's test. Post-hoc differences were tested using Tukey's HSD tests. We tested whether RII values and I_{imp} within each site were different from zero (i.e. neutral interaction or no interaction) using a t -test for single means. Relationships among abiotic characteristics and interaction indices were tested with correlation analyses using the Pearson product-moment correlation coefficient. We ran a factor analysis with all abiotic parameters and productivity in order to avoid multicollinearity among predictor variables, and obtained one single PCA axis. We then used generalized additive mixed models (GAMMs; Wood 2006) to test for the effects of environmental stress (the scores of PCA axis) on RII or I_{imp} , including site as random factor and productivity as covariate, and with Gaussian distribution of errors and identity link. GAMMs were fitted using a spline smoother in R using the gam and mgcv package (R Development Core Team, Vienna, Austria; Wood 2006). Compared with generalized linear models (GLMs), the main difference is that GAMMs are data-driven rather than model-driven and thus do not assume any *a priori* relationship – linear or non-linear; i.e. this nonparametric category of models allows determination of the shape of the response curves from the data instead of fitting an *a priori* parametric model that is limited in its available shape of response (Wood & Augustin 2002; Caprio et al. 2009).

For each field site, a correspondence analysis (CA) was performed to examine similarities between communities under *Retama* and in open areas. Differences between number of species growing exclusively under *Retama* or in gaps and among the three sites were analysed by binary logistic regression. The binomial variable had a value of 1 for species growing exclusively in a microsite, and 0 for species that grew in both microsites. Significance of pairwise comparisons was then corrected with a Bonferroni correction.

All analyses, except GAMMs, were performed with SPSS v 17.0 (SPSS Inc., Chicago, IL, USA) or CANOCO v. 4.5 (Microcomputer Power, Ithaca, NY, USA). Results throughout the text, tables, and figures are presented as mean \pm 1 SE.

Results

Our three field sites encompassed a gradient of increasing water availability and decreasing annual temperature, ranging from arid to sub-humid conditions always within a mediterranean climate (Table 1). Plant productivity increased almost four-fold from the driest end to the wettest end of the gradient (Table 2).

Species richness in the driest site, Almería, was higher (76 species) than in the other two sites (43 and 39 species for the intermediate and wettest site, respectively; Table 2).

Table 2. Site productivity (as above ground biomass of the herbaceous community in gaps), importance (I_{imp}) and relative intensity (RII) indices of the interaction of *Retama* on the biomass (RII_b) and species number (RII_r) of the herbaceous community at each site, number of species exclusively found under *Retama*, in gaps, and total number of species sampled in both microhabitats. Data are mean value \pm 1 SE ($n=7$). Values in the same row with different superscript letters are significantly different; RII values with '+' indicate that they are different from zero (\dagger , $P=0.07$), therefore the interaction between *Retama* and its understorey community was significant and positive. The last column shows the resulting F values from ANOVA (** $P < 0.001$; *** $P < 0.01$; $ns P > 0.05$). Resulting χ^2 values from binary logistic regressions for the number of species are shown in Appendix S4.

	(-) \rightarrow Water/Productivity gradient \rightarrow (+)			$F_{2,18}$
	Almería	Cáceres	Coimbra	
Above-ground biomass (g m^{-2})	67.23 \pm 18.43 ^a	144.14 \pm 9.36 ^b	253.32 \pm 41.52 ^c	17.58***
I_{imp}	0.20 \pm 0.03 ^{a+}	0.06 \pm 0.03 ^{b†}	0.06 \pm 0.03 ^b	5.02**
RII _b	0.67 \pm 0.08 ^{a+}	0.18 \pm 0.05 ^{b+}	0.04 \pm 0.09 ^c	18.38***
RII _r	0.15 \pm 0.03 ^{a+}	0.13 \pm 0.03 ^{a+}	0.04 \pm 0.05 ^a	2.14 ^{ns}
# species exclusively under <i>Retama</i>	11.1 \pm 1.2 ^a	2.5 \pm 0.5 ^b	1.6 \pm 0.6 ^b	–
# species exclusively in gaps	4.0 \pm 0.5 ^a	1.5 \pm 0.3 ^a	2.1 \pm 0.7 ^a	–
# Total species	30.1 \pm 1.1 ^a	11.1 \pm 0.5 ^b	8.0 \pm 1.3 ^b	–

Twenty-one per cent of the species from the species pool in Almería were exclusively growing in the understorey of *Retama*, this percentage being twice as high as in the other sites, which had similar values (Appendix S3). The number of species found exclusively in gaps and their contribution to the species pool found within each location (7.0–11.6%) was, however, similar across sites (Table 2, Appendix S3), and the interaction field site \times microsite was significant (Appendix S4). Moreover, 50% of the taxa found in at least two sites in the gradient – Almería and one other site – were exclusively found under *Retama* at the most arid end, whereas they preferred gaps or grew indistinctively in either microsite at the other two more mesic sites (Table 4 and Appendix S5). Only *Pipthaterum miliaceum* did not follow this trend, as it grew exclusively in gaps at the most arid site but was also present in the other field sites (Table 4 and Appendix S5).

The intensity and importance of the effect of *Retama* on biomass (RII_b, I_{imp}) and species richness (RII_r) of understorey communities showed that facilitation was a significant force shaping the structure of the herbaceous communities growing underneath *Retama* in the arid and intermediate sites, facilitation being strongest at the driest site (Table 2). By contrast, *Retama* shrubs did not have any significant effect on the understorey community at the moist end of the gradient (Table 2). RII_b values differed among all sites, while I_{imp} and RII_r were similar in the middle and wettest sites.

The abiotic gradient was represented by the first axis of the factor analysis (PCA), which explained 89.2% of the variance of all environmental variables (variable loadings: $R=0.99$, $R/PET=0.99$, $R/08-09=0.97$, $T=-0.93$, productivity=0.83). GAMMs significantly explained the variation in RII_b and RII_r as a function of the abiotic gradient, and the trend was marginally significant for I_{imp} , after removing the effect of field site (to avoid possible pseudo-replication effects). In no case did pro-

ductivity have a significant effect on such trends ($P > 0.05$ for all indices). All relationships increased positively and monotonically with increasing abiotic severity (Fig. 1, but see the model significance for I_{imp}). There was a strong and negative correlation between the intensity (RII_b) or the importance (I_{imp}) of the interaction and the mean annual precipitation and aridity index (Table 3). There were also positive correlations between the two interaction indices and mean annual temperature. Similar relationships were found between the frequency of facilitation (RII_r) and environmental variables, although correlations were only significant for rainfall, aridity index and cumulative rainfall (Table 3). Overall, these results suggest that the intensity and importance of the facilitative effects of *Retama* on the biomass and species richness of its understorey community increased with decreasing water availability and increasing temperature, ranging from neutral to positive.

Correspondence analysis (CA) gave different results in each site, showing a gradient of similarity between plant communities under and outside *Retama* canopies that followed the water availability and productivity gradients. CA clearly distinguished communities associated with *Retama* shrubs from those in gaps in Almería, the driest site (Fig. 2a). There, plant communities in open spaces were more homogeneous than those found under *Retama* shrubs. In Cáceres, the intermediate site, plant communities under *Retama* canopies grouped together but there were no clear differences in species composition under and outside *Retama* shrubs (Fig. 2b). This lack of discrimination between plant communities was more obvious in the wettest and most productive site of the gradient (Fig. 2c).

Discussion

A decrease in net primary productivity is commonly considered an indication of stress at the community level

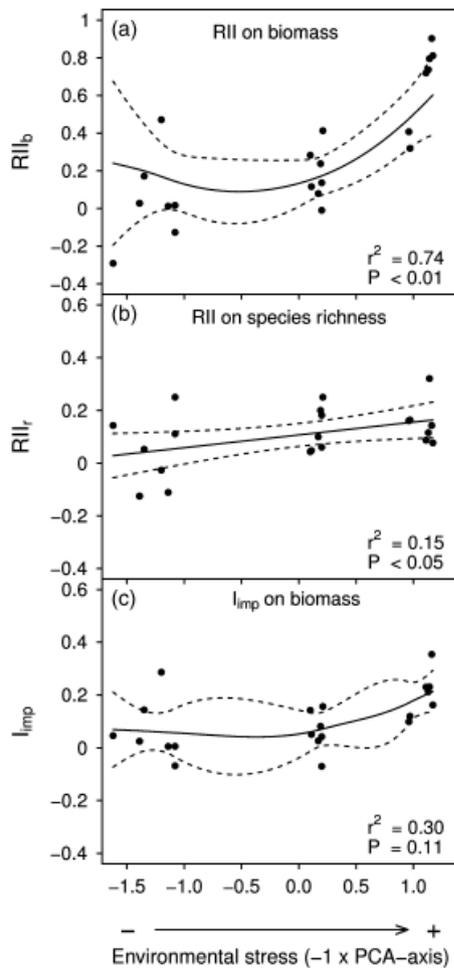


Fig. 1. Intensity (RII) or importance (I_{imp}) of the effects of *Retama* on the understorey community, either on plant biomass (RII_b , I_{imp}) (a and c) or on species richness (RII_r) (b), as a function of environmental stress (predictor), after removing the effect of field site (random factor) and with productivity as a covariate. Solid line shows the resulting best-fit model after performing GAMMs. Dotted lines show 95% confidence intervals, and r^2 and P values inside the panels correspond to the GAMM fit.

(Grime 1977; Bertness & Callaway 1994), particularly in grassland and annual communities. Therefore, the productivity gradient considered here reflects a stress gradient. Our results show that facilitative effects of *Retama* shrubs on biomass and species richness of the understorey community increased monotonically in intensity and, to a less extent, in importance with increasing environmental severity, i.e. increasing aridity and mean temperature, supporting our first hypothesis and the stress-gradient hypothesis (Bertness & Callaway 1994; Brooker & Callaghan 1998).

Sampling in our experiment was only performed in three sites along the gradient. Nonetheless, they encompass the driest and wettest locations of the geographic range of *Retama*, which are the two extreme points in the

Table 3. Linear correlation between the interaction indices and mean annual rainfall (R), aridity index (R/PET), cumulative rainfall from September 2008 to March or May 2009 (R'08-09), and annual temperature (T). The interaction indices correspond to: the importance (I_{imp}) on biomass, and relative intensity (RII_b for biomass or RII_r for species richness) of the interaction of *Retama* on the understorey community.

	I_{imp}	RII_b	RII_r
R (mm)	-0.47*	-0.74***	-0.43*
R/PET	-0.45*	-0.72***	-0.44*
R 2008–09 (mm)	-0.37 ^{ns}	-0.64***	-0.43*
T (°C)	0.58**	0.81***	0.38 ^{ns}

environmental severity gradient. Although the number of sites is limited, they include the two extremes and one middle site on the environmental gradient, and thus allow testing whether the shape of the SGH in our aridity gradient is monotonic and positive – the original prediction – or shows a different pattern, e.g. a hump-shaped curve (Appendix S1). Since GAMM analyses do not assume any *a priori* relationship among variables (i.e. linear or non-linear) but render the best-fit model once the effect of field site is removed, this approach allows testing for all possible severity–interaction relationship outcomes (Malkinson & Tielbörger 2010).

Other empirical evidence focusing on whole communities or pair-wise interactions also corroborate the severity–interaction monotonic increase proposed by the SGH (e.g. Callaway et al. 2002; Holzapfel et al. 2006; Brooker et al. 2008; le Roux & McGeoch 2010), even in arid gradients (e.g. Holzapfel et al. 2006). However, some studies and models focused on such dry environments predict hump-shaped severity–interaction patterns (e.g. Maestre & Cortina 2004; Maestre et al. 2009). This discrepancy between our results and other tests of the SGH may be due to several factors. Environmental severity in our study was unlikely caused by a single factor, as water (resource) and temperature (non-resource) are usually coupled (Callaway 2007; de Boeck et al. 2010, 2011). As Maestre et al. (2009) acknowledged, in hot arid environments it is difficult to distinguish water stress from high temperature, and in this situation facilitation might be expected to generally increase with increasing stress. Moreover, competition for water between *Retama* and annual species is highly asymmetric as they have quite disparate root systems that decrease competition through niche complementarity (Pugnaire & Luque 2001). Differences in rooting depth and the ability to use different water sources constitute mechanisms of species co-existence and could be exceptions to the models proposed by Maestre et al. (2009), which is particularly important in arid environments where the interacting species may not

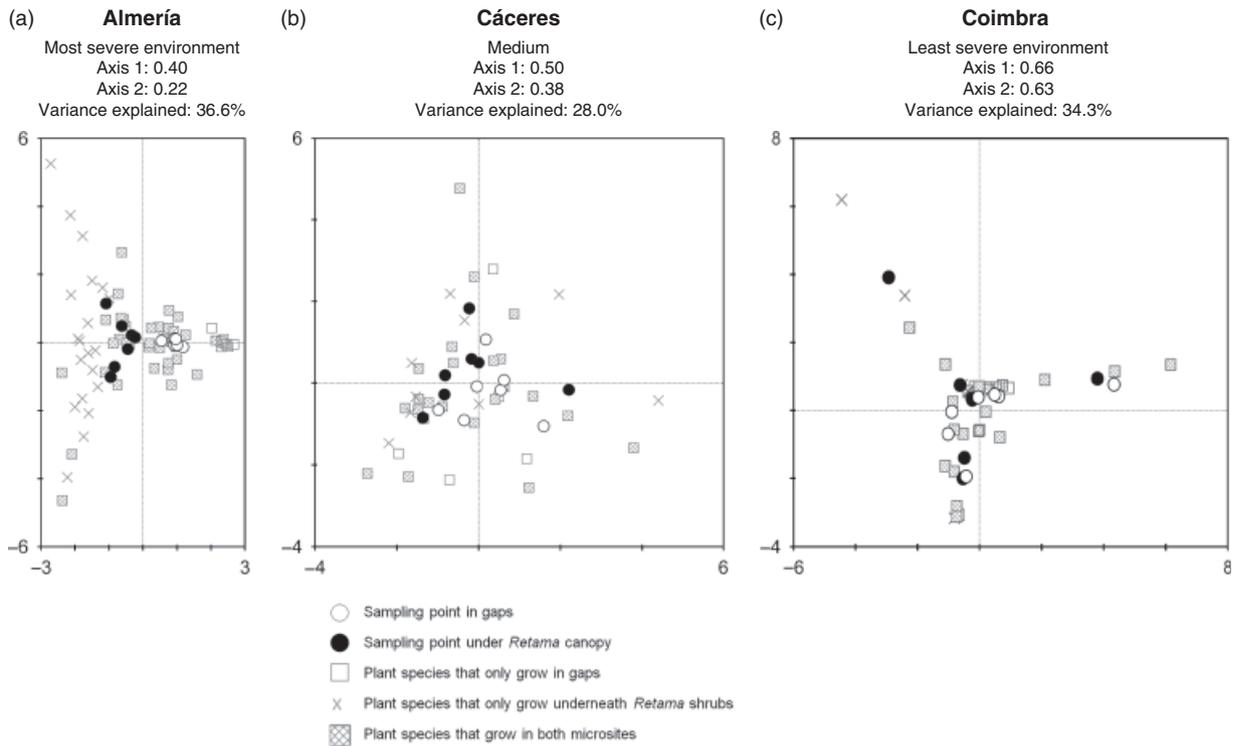


Fig. 2. Plots from correspondence analyses (axes 1 and 2) performed with presence/absence data of species from the herbaceous community found in Almería (a), Cáceres (b) and Coimbra (c). Eigenvalues of axis 1 and 2 and the cumulative variance explained are included above each plot. Circles represent each sampling point, grey symbols represent plant species.

compete for water and the benefactor species ameliorates conditions for neighbours.

Predictions of Maestre et al. (2009) also focus on pairwise interactions, but to be compared with the original formulation of the SGH, they must ultimately be extended to communities (Maestre et al. 2009), as attempted in our experiment. In some cases, competition between pairs of species has been shown to increase with water stress at the patch scale (Pugnaire & Luque 2001; Maestre & Cortina 2004) but the net effect of neighbours at the community level in dry environments tends to be positive (Lortie & Callaway 2006 and references therein; Michalet et al. 2006). Nevertheless, future studies should include measurements of fitness for all beneficiary species along the stress gradient, as it will improve our understanding of the mechanisms behind our observed SGH outcome (Malkinson & Tielbörger 2010). Finally, in contrast with most of the above studies, we did not find evidence of competition in the wettest end of the gradient. Although competition is assumed to be most important in mesic sites, other outcomes might be possible, depending on the specific attributes of the co-occurring species and the characteristics of the studied microsites (Holmgren & Scheffer 2010).

As aridity increased, so did community richness, being highest at the arid end of the gradient. We did not find a

humped-back or unimodal relationship between species richness and productivity, as predicted by Grime (1973) and others. The high species richness at the arid end of the gradient reflects the local species pool rather than being a consequence of plant–plant interactions. The species pool is determined by evolutionary history and dispersal limitations (Zobel & Pärtel 2008), and can be modulated by the physiological tolerance of the species and biotic interactions (Lortie et al. 2004). Species pools are expected to be large in unproductive habitats due to shorter generation time and hence higher diversification rates (Zobel et al. 2011).

Hacker & Gaines (1997) and Michalet et al. (2006) suggested that facilitation promotes diversity at medium- to high-environmental severity levels by expanding the realized niche of stress-intolerant species. In our study, some taxa shared by at least two different field sites changed their habitat preferences (living in gaps versus beneath *Retama*) along the gradient. Some species that only grew in gaps (e.g. *Plantago* sp.) in the mesic sites – Coimbra and Cáceres – could only establish under *Retama* at the most stressful site (Almería, Table 4). Similarly, other species (5–7 out of the 10 shared taxa) that had no preference for either of the two microsites in Coimbra or Cáceres only established under *Retama* in Almería. Our results suggest that facilitation by the nurse shrub could have expanded the realized niche of some species,

Table 4. Common taxa found in at least two sites along the environmental gradient and their microsite preference (■: exclusively under *Retama*; □: exclusively in gaps; ☒: both sites). If the same genus had two possible microsite preferences within a site, the notation in brackets is for the less frequent microsite.

Taxon	Water/Productivity gradient		
	Dry/Low	Medium	Wet/High
	Almería	Cáceres	Coimbra
<i>Asphodelus</i> sp.	☒ (■)	☒	
<i>Avena sterilis</i>	■	☒	
<i>Carlina corymbosa</i>	■	☒	
<i>Centaurea</i> sp.	■	☒	
<i>Plantago</i> sp.	■	□	□
<i>Sonchus oleraceus</i>	■	☒	
<i>Medicago</i> sp.	☒ (■)	☒	□
<i>Hordeum</i> sp.	☒	☒	
<i>Eryngium campestre</i>	□	□	
<i>Cistus</i> sp.		□ (☒)	□ (☒)
<i>Geranium</i> sp.		■	■
<i>Sanguisorba</i> sp.		□	□
<i>Pipthaterum miliaceum</i>	□	■	☒

improving their chances of survival at the driest and hottest end of the gradient. Further analyses including more sampling sites, and taking into account evolutionary and dispersal history in each site are needed. Nevertheless, the presence of the nurse in the driest site was an important factor shaping the structure and composition of the community, and to a lesser extent in the other two sites. Twenty-one per cent of all species found in the driest site could only thrive beneath *Retama*, causing the composition of communities associated with *Retama* shrubs to be different from those found in gaps. At intermediate levels of stress – Cáceres, *Retama* still offered safe sites for the establishment of some species that cannot survive in open areas, but the contribution of facilitation by *Retama* was gradually lost as environmental stress decreased, and at the wettest end of the gradient – Coimbra, plant communities beneath *Retama* and in open areas were similar in species composition.

Conclusions

Our data show that facilitation by *Retama* on its associated community increased with increasing aridity and decreasing productivity, lending support to the original formulation of the SGH. The overall stress–interaction pattern was positive and monotonic. A significant fraction of species found at the most arid end of the gradient were only able to survive beneath the nurse shrub, whereas some of these species were able to thrive in more mesic sites without the help of *Retama*. Our results and the stress–interaction pattern found here could be important to im-

prove our ability to predict the impact of a changing environment on the composition and structure of plant communities in mediterranean climates.

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References

- Armas, C., Ordiales, R. & Pugnaire, F.I. 2004. Measuring plant interactions: an index comparison and a proposal. *Ecology* 85: 2682–2686.
- Bertness, M.D. & Callaway, R.M. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9: 191–193.
- Brooker, R.W. & Callaghan, T.V. 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos* 81: 196–207.
- Brooker, R.W., Kikvidze, Z., Pugnaire, F.I., Callaway, R.M., Choler, P., Lortie, C.J. & Michalet, R. 2005. The importance of importance. *Oikos* 109: 63–70.
- Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.J., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F.I., Quiroz, C.L., Saccone, P., Schifffers, K., Seifan, M., Touzard, B. & Michalet, R. 2008. Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* 96: 18–34.
- Callaway, R.M. 2007. *Positive interactions and interdependence in plant communities*. Springer, Dordrecht, NL.
- Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R., Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E.T., Armas, C., Kikvidze, D. & Cook, B.J. 2002. Positive interactions among alpine plants increase with stress. *Nature* 417: 844–848.
- Caprio, E., Ellena, I. & Rolando, A. 2009. Assessing habitat/landscape predictors of bird diversity in managed deciduous forests: a seasonal and guild-based approach. *Biodiversity and Conservation* 18: 1287–1303.
- Castroviejo, S., Laínz, M., López-González, G., Montserrat, P., Muñoz-Garmendia, F., Paiva, J. & Villar, L. 1986. *Flora ibérica. Plantas vasculares de la Península Ibérica e Islas*

- Baleares. Real Jardín Botánico - Consejo Superior de Investigaciones Científicas, Madrid, ES.
- Choler, P., Michalet, R. & Callaway, R.M. 2001. Facilitation and competition on gradients in alpine plant communities. *Ecology* 82: 3295–3308.
- de Boeck, H.J., Dreesen, F.E., Janssens, I.A. & Nijs, I. 2010. Climatic characteristics of heatwaves and their simulation in plant experiments. *Global Change Biology* 16: 1992–2000.
- de Boeck, H.J., Dreesen, F.E., Janssens, I.A. & Nijs, I. 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. *New Phytologist* 189: 806–817.
- Goldberg, D.E., Rajaniemi, T., Gurevitch, J. & Stewart-Oaten, A. 1999. Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. *Ecology* 80: 1118–1131.
- Grime, J.P. 1973. Competitive exclusion in herbaceous vegetation. *Nature* 242: 344–347.
- Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169–1194.
- Haase, P., Pugnaire, F.I., Fernandez, E.M., Puigdefábregas, J., Clark, S.C. & Incoll, L.D. 1996. An investigation of rooting depth of the semiarid shrub *Retama sphaerocarpa* (L) Boiss by labelling of ground water with a chemical tracer. *Journal of Hydrology* 177: 23–31.
- Hacker, S. & Gaines, S.D. 1997. Some implications of direct positive interactions for community species diversity. *Ecology* 78: 1990–2003.
- Holmgren, M. & Scheffer, M. 2010. Strong facilitation in mild environments: the stress gradient hypothesis revisited. *Journal of Ecology* 98: 1269–1275.
- Holmgren, M., Scheffer, M. & Huston, M.A. 1997. The interplay of facilitation and competition in plant communities. *Ecology* 78: 1966–1975.
- Holzapfel, C., Tielbörger, K., Parag, H.A., Kigel, J. & Sternberg, M. 2006. Annual plant–shrub interactions along an aridity gradient. *Basic and Applied Ecology* 7: 268–279.
- IPCC. 2007. Climate change 2007: the physical science basis. Contribution of Working Group I. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. & Miller, H.L. (eds.) *Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. pp. 849–940. Cambridge University Press, Cambridge, UK.
- Kawai, T. & Tokeshi, M. 2007. Testing the facilitation–competition paradigm under the stress-gradient hypothesis: decoupling multiple stress factors. *Proceedings of the Royal Society B: Biological Sciences* 274: 2503–2508.
- le Roux, P.C. & McGeoch, M.A. 2010. Interaction intensity and importance along two stress gradients: adding shape to the stress-gradient hypothesis. *Oecologia* 162: 733–745.
- Lortie, C.J. & Callaway, R.M. 2006. Re-analysis of meta-analysis: support for the stress-gradient hypothesis. *Journal of Ecology* 94: 7–16.
- Lortie, C.J., Brooker, R.W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F.I. & Callaway, R.M. 2004. Rethinking plant community theory. *Oikos* 107: 433–438.
- Maestre, F.T. & Cortina, J. 2004. Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. *Proceedings of the Royal Society of London. Series B* 271: S331–S333.
- Maestre, F.T., Valladares, F. & Reynolds, J.F. 2005. Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology* 93: 748–757.
- Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97: 199–205.
- Malkinson, D. & Tielbörger, K. 2010. What does the stress-gradient hypothesis predict? Resolving the discrepancies. *Oikos* 119: 1546–1552.
- Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I., Valiente-Banuet, A. & Callaway, R.M. 2006. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters* 9: 767–773.
- Moro, M.J., Pugnaire, F.I., Haase, P. & Puigdefábregas, J. 1997. Mechanisms of interaction between *Retama sphaerocarpa* and its understory layer in a semi-arid environment. *Ecography* 20: 175–184.
- Niering, W.A., Whittaker, R.H. & Lowe, C.H. 1963. The saguaro: a population in relation to environment. *Science* 142: 15–23.
- Prieto, I., Kikvidze, Z. & Pugnaire, F.I. 2010. Hydraulic lift: soil processes and transpiration in the Mediterranean leguminous shrub *Retama sphaerocarpa* (L.) Boiss. *Plant and Soil* 329: 447–456.
- Pugnaire, F.I. & Luque, M.T. 2001. Changes in plant interactions along a gradient of environmental stress. *Oikos* 93: 42–49.
- Pugnaire, F.I., Haase, P. & Puigdefábregas, J. 1996. Facilitation between higher plant species in a semiarid environment. *Ecology* 77: 1420–1426.
- Rodríguez-Echeverría, S. & Pérez-Fernández, M.A. 2003. Soil fertility and herb facilitation mediated by *Retama sphaerocarpa*. *Journal of Vegetation Science* 14: 807–814.
- Seifan, M., Seifan, T., Ariza, C. & Tielbörger, K. 2010. Facilitating an importance index. *Journal of Ecology* 98: 356–361.
- Tielbörger, K. & Kadmon, R. 2000. Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology* 81: 1544–1553.
- Wood, S. 2006. *Generalized additive models: An introduction with R*. Chapman & Hall and CRC Press, Boca Raton, FL, US.
- Wood, S. & Augustin, N. 2002. GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. *Ecological Modelling* 157: 157–177.
- Zobel, M. & Pärtel, M. 2008. What determines the relationship between plant diversity and habitat productivity? *Global Ecology and Biogeography* 17: 679–684.
- Zobel, M., Otto, R., Laanisto, L., Naranjo-Cigala, A., Pärtel, M. & Fernández-Palacios, J.M. 2011. The formation of species

pools: historical habitat abundance affects current local diversity. *Global Ecology and Biogeography* 20: 251–259.

Zou, C.B., Barnes, P.W., Archer, S. & McMurtry, C.R. 2005. Soil moisture redistribution as a mechanism of facilitation in savanna tree–shrub clusters. *Oecologia* 145: 32–40.

Supporting Information

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

Appendix S1. Hypothesized shapes of the severity–interaction gradient.

Appendix S2. Detailed description and pictures of field sites.

Appendix S3. Biomass and species richness of annual plant communities beneath *Retama* and in nearby

open areas, and contribution of species that were exclusively found under *Retama* or in open areas to the total pool of species within each site.

Appendix S4. Results from the binomial logistic regression for the frequency of species only found under *Retama* or in gaps (Microsite) along the environmental gradient (among Sites).

Appendix S5. List of species found in each site.

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