Research article

Intraspecific competition replaces interspecific facilitation as abiotic stress decreases: The shifting nature of plant–plant interactions

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ARTICLE INFO

ABSTRACT

Plant–plant interactions change depending on environmental conditions, shifting from competition to facilitation when the stress is high. In addition to these changes, the relevance of intraspecific compared to interspecific interactions may also shift as abiotic stress does. We inferred intra- and interspecific plant–plant interactions of the cushion plant *Hormathophylla spinosa* as related to the dominant shrub *Juniperus sabina* in two sites with contrasting abiotic conditions (a slope with high-stress conditions vs. a valley bottom with milder conditions) in a Mediterranean high mountain. Specifically, we studied the spatial patterns and several variables related to plant performance (plant size and form, non-structural carbohydrate – NSC – concentrations and radial growth) of *H. spinosa*.

The spatial pattern varied depending on site conditions. *H. spinosa* plants were positively associated with juniper in the high-stress slope site, probably through higher establishment rates due to the amelioration of soil conditions. In contrast, in the milder valley site *H. spinosa* establishment occurred mostly in open areas. Age structure, inferred from annual rings, reflected a massive establishment event in the whole study area which occurred 30–50 years ago. Canopy variables and radial growth were density dependent: both were negatively affected by the high density of *H. spinosa* individuals in the valley, but favoured by junipers on the slope. Interestingly, NSCs showed the opposite pattern, suggesting lower investment in growth by *H. spinosa* plants in the valley than on the slope.

Our results reinforce the strong links existing between intra- and interspecific relationships and the need to include both when studying the influence of abiotic conditions on plant–plant interactions. This approach enabled us to detect that the direction and intensity of plant–plant interactions may shift at different ecological levels. Particularly interesting was the finding that optimal sites at the population level may not necessarily be the sites showing maximum individual performance.

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Introduction

Together with competition, facilitation is one of the major forces driving community structure (Connell and Slatyer, 1977; Bertness and Callaway, 1994; Callaway and Walker, 1997). Positive interactions between plants can affect local species richness and promote changes in the interaction networks among the facilitated species (Soliveres et al., 2011), and their consequences go beyond community organization. Facilitation may have evolutionary consequences in terms of group selection (McIntire and Fajardo, 2011) or the phylogenetic structure of communities (Valiente-Banuet and Verdú, 2007). In fact, the inclusion of positive interactions into

evolutionary theory has already been proposed by Kikvidze and Callaway (2009), who suggest that they are the underlying mechanism driving major evolutionary transitions such as that explained by the serial endosymbiotic theory (Margulis et al., 2000).

Understanding the balance of positive and negative plant–plant interactions (i.e., facilitation and competition) on species coexistence has been a major focus in plant ecology over the last decade (Brooker et al., 2008; Maestre et al., 2009). Under mild environmental conditions competition is considered to be the leading interaction, because increased resource availability enhances biomass production and reduces the presence of suitable sites for establishment (Connell and Slatyer, 1977). Conversely, under harsh environmental conditions nurse plants can facilitate seedling establishment by ameliorating abiotic conditions (Kitzberger et al., 2000; Núñez et al., 2009), by increasing soil nutrient levels (Anthelme et al., 2012) or by protecting from herbivory (Rousset and Lepart,

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2000). Thus, the net balance between positive and negative interactions may well depend on the environmental context. This is the core idea of the Stress Gradient Hypothesis (SGH) (Bertness and Callaway, 1994), which postulates that facilitative interactions would be dominant in harsh environments, shifting to competitive ones as abiotic conditions ameliorate (Maestre et al., 2009; Soliveres et al., 2010). However, identifying the changes in biotic interactions is usually not so straightforward. Facilitation and competition act simultaneously on a given set of individuals (Holmgren et al., 1997; Callaway and Walker, 1997), and the net observable outcome is a result of the combination of both positive and negative interactions (Brooker and Callaghan, 1998; Holzapfel and Mahall, 1999). Nurse and facilitated species may have also reciprocal effects that can be quantified separately (Holzapfel and Mahall, 1999) and the effect of facilitated species on their nurse plants can often be negative (Holmgren et al., 1997). Moreover, a given level of environmental stress does not have the same effect on all the species of a community, and this depends on the level of stress tolerance of each species (Holmgren et al., 1997; Choler et al., 2001; Soliveres et al., 2011). When stress levels are critical for the less tolerant species, facilitation would allow them to endure the stress and thus helping to expand their distribution range. Nevertheless, the environmental conditions are sometimes so extreme that biotic interactions cannot overcome their effect (Choler et al., 2001; Soliveres et al., 2011). There is empirical evidence supporting the SGH in different plant communities subjected to stressful conditions such as semiarid steppes (Pugnaire and Luque, 2001) and alpine and arctic tundra (Choler et al., 2001; Callaway et al., 2002; Pellissier et al., 2010), but other studies have shown this hypothesis to be inconsistent (Maestre and Cortina, 2004; Maestre et al., 2005; Gross et al., 2010; Soliveres et al., 2011). As a consequence, the debate on the suitability and general applicability of the SGH is still open, and further field studies are still necessary in order for it to be better understood (Callaway, 2007; Sthultz et al., 2007; Maestre et al., 2009).

A major challenge when testing the SGH is the dynamic behaviour of plant performance in response to the changing levels of stress that result from at least two major sources of temporal variability, namely climate variation (Wang et al., 2008) and plant ontogeny (Callaway and Walker, 1997; Miriti, 2006). Climatic conditions at a regional scale change through time, thereby modifying the stress experienced by plants and thus influencing the balance between competition and facilitation (Kitzberger et al., 2000; Sthultz et al., 2007). In relation to ontogeny, adult plants may initially enhance the establishment of other individuals of the same or different species, but as individuals grow this relationship may become negative (Callaway and Walker, 1997; Rousset and Lepart, 2000; Núñez et al., 2009). Since temporal changes in the levels of stress may induce transient changes in the strength and direction of biotic interactions at different scales (from monthly during a whole growth season to annually during several consecutive years), it is therefore necessary to include a temporal perspective in the SGH framework (Callaway and Walker, 1997; Brooker and Callaghan, 1998; Holzapfel and Mahall, 1999). Although this could be achieved by monitoring plants through their entire life-cycle to estimate, for instance, their survival rates, it would require long periods of study prior to reaching any conclusive results, particularly in the case of long-lived species. The retrospective study of radial growth using ring-width series is a valid, albeit incomplete, alternative to this method. It can provide a temporal framework for evaluating the SGH in perennial plants (Soliveres et al., 2010), since it can be used to quantify past growth changes in species with long lifespans (Fritts, 1976).

The initial formulation of the SGH was done at the interspecific level, thereby highlighting its importance on driving community structure (Bertness and Callaway, 1994; Callaway and Walker,

1997). However, intraspecific interactions should also be considered in the framework of the SGH, since conspecific competition is a major force driving population dynamics (Connell and Slatyer, 1977) that could ultimately affect the net outcome of interspecific interactions at the community level. Intraspecific facilitation occurs between plants at different stages of their life cycle (e.g. Callaway, 1995; Fajardo et al., 2006), but it can also occur among individuals of the same cohort (Goldenheim et al., 2008; Fajardo and McIntire, 2011; McIntire and Fajardo, 2011). Nevertheless, the impact of intraspecific interactions within SGH has remained almost unexplored (but see Tielbörger and Kadmon, 2000; Madrigal-González et al., 2012).

The aim of our study was to evaluate how abiotic conditions modulate intraspecific interactions in the cushion plant *Hormathophylla spinosa* (L.) P. Küpfer, at the same time as considering its interspecific interactions with a local dominant co-occurring species, the shrubby Savin juniper (*Juniperus sabina* L.). We assessed whether these interactions varied locally by comparing not a gradient *sensu stricto*, but two sites with contrasting levels of abiotic stress (valley bottom vs. slope) that are representative of mild and harsh conditions in a Mediterranean mountain area. Studies on the magnitude and shift of biotic interactions are particularly relevant in Mediterranean plant communities (Maestre et al., 2005; Matesanz et al., 2009) due to the critical role of drought stress in plant establishment (Olano et al., 2011) and the positive effects of nurse plants on water availability (Padilla and Pugnaire, 2006). Moreover, since trends of increasing aridity due to rising temperatures and more frequent severe droughts are expected for Mediterranean areas under different global warming scenarios (Gao and Giorgi, 2008), most research has focused on plant–plant interactions in water-constrained communities such as semiarid steppes (Escudero et al., 2000, 2005; Maestre et al., 2001). However, less effort has been directed towards understanding this process in Mediterranean high mountains (e.g. Gómez-Aparicio et al., 2004; Cavieres et al., 2006), despite the fact that in contrast with alpine regions they are high-altitude areas that are constrained by both low temperatures and water deficit (Giménez-Benavides et al., 2007; García-Cervigón et al., 2012).

To test the SGH simultaneously at intra- and interspecific levels, we developed an observational approach combining several techniques of analysis. We carried out spatial analyses that are commonly used to infer patterns related to positive or negative associations between plants (e.g. Schenk et al., 2003; Fajardo et al., 2008), allowing inferences regarding patterns and processes (Tirado and Pugnaire, 2005). To evaluate the status of the target plants, we used complementary measures of plant performance (Lortie and Callaway, 2006) including canopy form, non-structural carbohydrate concentrations in wood and radial growth. We also included a temporal perspective by reconstructing the historical patterns of the two species' association. Specifically, we aimed to evaluate if (i) interspecific interactions shift from positive to neutral or even negative when comparing a stressful vs. a mild site, and (ii) increased intraspecific competition outweighs the benefits of improved abiotic conditions in the site with lower abiotic stress level (see Table 1 for a more detailed explanation).

Material and methods

Study site and target species

The study site was located near Javalambre peak (40°07′ N, 1°01′ W), in the Iberian mountain range, Teruel, eastern Spain (Fig. 1a). The site is above the tree line (1940–1965 m a.s.l.), and vegetation is dominated by large prostrate Savin junipers (*J. sabina*). The substrate is mainly composed of shallow, stony

Table 1
Specific objectives, related hypotheses and expected patterns, and the methods and analyses used to test them.

Objective	Hypotheses and expected patterns	Methods and analyses
Testing the SGH at the interspecific level.	In the more stressful site (slope) *H. spinosa* would appear aggregated and associated with juniper canopies due to nurse effects on establishment. In the mild site, the spatial pattern of *H. spinosa* would be either random or negatively associated with juniper canopies.	• Univariate point patterns analysis based on the pair-correlation function ($g(t)$) to compare spatial patterns in slope vs. valley sites. • χ^2 test to assess and compare *H. spinosa*–juniper potential associations between contrasting sites (slope vs. valley) and microsites (within vs. outside juniper canopy).
Checking if the inclusion of intraspecific interactions improves the vision of the study system provided by the analysis of interspecific ones.	In the mild site (valley) *H. spinosa* density would be higher than in the more stressful site (slope) leading to higher intraspecific competition. This effect may outweigh the amelioration of abiotic conditions in the valley, with individual plant performance being higher on the slope than in the valley.	• ANOVA to compare intraspecific interactions between sites and microsites. • GLM to study the combined effect of intraspecific interactions, site and microsite on plant canopy form and on non-structural carbohydrate concentrations in wood. • LMM to quantify how radial growth is related to site and microsite.

Abbreviations: GLM, General Linear Models; LMM, Linear Mixed Models; SGH, Stress Gradient Hypothesis.

soils developed on limestone bedrock. Climate is continental and Mediterranean with a marked summer water stress period (García-Cervigón et al., 2012). Mean summer and winter temperatures are 17.1 °C and −0.2 °C, respectively, and estimated total precipitation is 520 mm, with a summer drought period lasting from July to August (Ninyerola et al., 2005).

H. spinosa is a cushion plant from the western Mediterranean Basin that grows at altitudes of up to 3400 m a.s.l. It is a species inhabiting sites with stony and rocky soils (Küpfer, 1993). *J. sabina* is the dominant shrub above the tree line and up to 2750 m a.s.l in calcareous Mediterranean high mountains, being widely distributed along central and southern Europe, northern Africa and western Asia (López González, 2004). A single individual of this prostrate juniper species can cover areas of up to 0.1 ha, being a keystone species of mountainous ecosystems with a large number of woody and herbaceous species establishing within its canopy
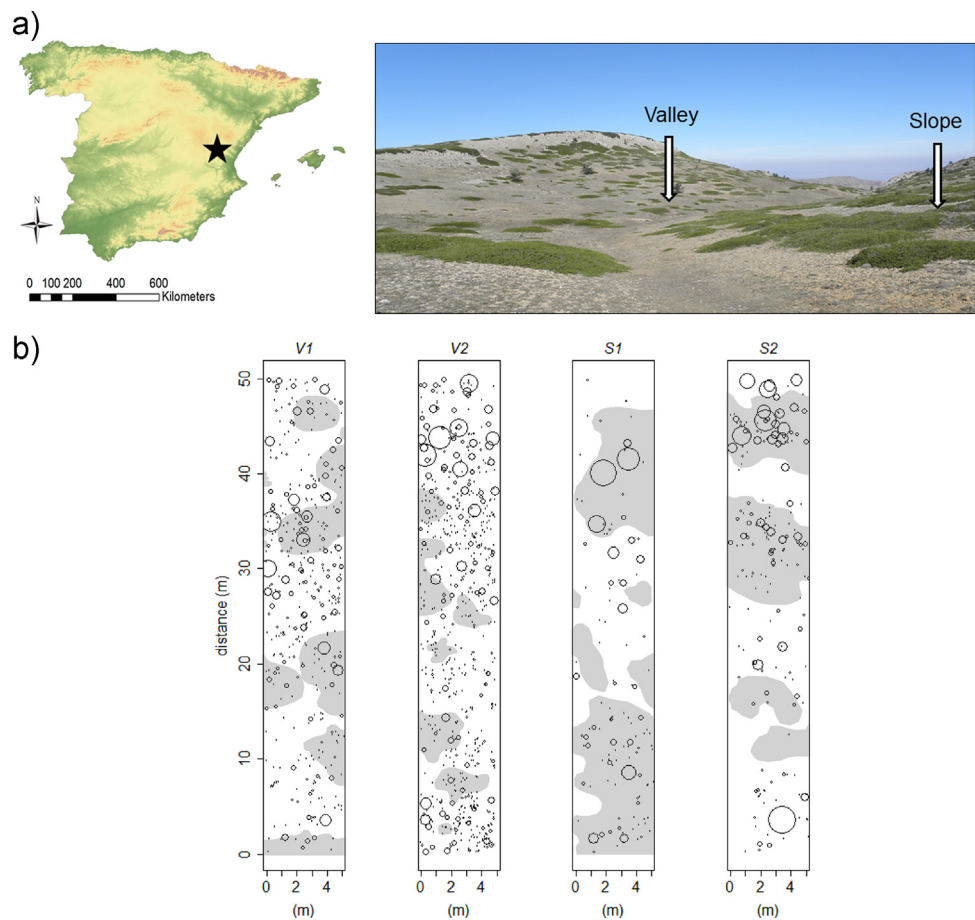

Fig. 1. View and location of the study area in Javalambre, Teruel, eastern Spain. (a) Location of transects. (b) Location of *H. spinosa* plants inside the sampled rectangular transects (V1, valley transect 1; V2, valley transect 2; S1, slope transect 1; S2, slope transect 2). The size of symbols is proportional to the canopy diameter of plants. Grey areas represent surfaces covered by *J. sabina*.

(García-Cervigón, pers. obs.). This may be due to protection from herbivory but also to the better microenvironmental conditions provided by soils developed beneath *J. sabina* plants, which have higher organic matter, N and P contents, higher soil moisture and lower maximal temperatures (Verdú and García-Fayos, 2003). *H. spinosa* and *J. sabina* coexist and are widely distributed in Iberian Mediterranean mountains.

Field and laboratory work

We selected two contrasting sites: a valley bottom (hereafter referred to as valley) with relatively favourable conditions (deep soils, increased moisture and wind protection) and the nearest west-facing slope (hereafter referred to as slope) where abiotic conditions were harsher, due to shallower soils and higher run-off. This sampling scheme does not comprise a true abiotic gradient, but conditions are contrasting enough to enable comparison between a favourable and a less favourable site for the target species. Moreover, both sites are close enough to ensure that other possible confounding factors such as climate or historical human management have been similar. As a proxy of the abiotic stress degree we measured soil depth, considered as the distance between the soil surface and the bedrock, by performing 80 random measures at each site, 40 of them beneath *J. sabina* and the other 40 on the open ground, using a metal stick and a centimetre ruler. Two rectangular transects (5 m × 50 m) were randomly placed at each site (valley transects V1 and V2; slope transects S1 and S2, these two located in the direction of the maximum slope). This transect size is particularly appropriate for the species under study since it tends to encompass several juniper individuals and a large number of *H. spinosa* plants. For each *H. spinosa* individual we recorded its position, microsite (*i.e.*, within or outside the area occupied by a juniper canopy) and size (plant height and two perpendicular canopy diameters). Additionally, we mapped the area covered by juniper individuals in each transect (Fig. 1b). *H. spinosa* size data were used to calculate two variables reflecting the canopy form and size: slenderness (height divided by mean canopy diameter) and area (considering the horizontal canopy projection as an ellipse). These measures were taken for all individuals in each transect. However, to estimate their age, we collected all plants in the two transects located on the slope, but only half of them (from the first 25 m of the transect) in both transects in the valley site, due to the elevated plant density there. Age was estimated by taking a transversal section from the root collar, sanding it carefully with sandpapers of progressively finer grains until rings were clearly visible, and counting growth rings under a binocular lens (Nikon SMZ800).

In order to know whether *H. spinosa* established after or before the nearest juniper, at each site we randomly sampled 20 additional *H. spinosa* plants that were growing within the area of a juniper canopy, as well as the largest juniper branch contiguous to each *H. spinosa* individual. Ages of *H. spinosa* individuals and juniper branches were again estimated by counting annual rings in basal sections.

To obtain an estimate of *H. spinosa* individual reserve levels, we collected 40 additional plants from each site, 20 per microsite (within and outside juniper canopy areas), and measured non-structural carbohydrate (NSC) concentrations in the wood. NSC levels have been used as a proxy for carbon balance in plants under different scenarios as response to disturbances (Clarke et al., 2013), successional dynamics (Olano et al., 2006) or environmental gradients (Körner, 2003a). Since secondary growth is under stronger environmental constraints than photosynthesis (James et al., 1994), high NSC levels at high altitudes would be due to lower growth (sink) activity (Körner, 2003a; Fajardo et al., 2012), and we would

therefore expect a negative relationship between growth and NSC levels. NSCs were measured using the anthrone method (Morris, 1948; see Olano et al., 2006 for a detailed description of the methodology). We collected wood samples from the main stem 1 cm below root collars, which were frozen immediately after being collected to avoid the degradation of NSCs. Before the chemical procedure, samples were debarked, oven-dried at 80 °C for 48 h and finely ground.

Finally, to estimate how radial growth varies through time we measured the annual rings of 80 *H. spinosa* individuals, 40 per site and 20 per microsite. To measure annual rings we took photographs of the sanded samples under a binocular lens at 10–63 magnifications depending on the samples' size and analyzed them with the ImageJ v.1.44 software (available at http://rsb.info.nih.gov/ij; developed by W. Rasband, NIH, Bethesda, MD). Photographs were converted into greyscale images and we traced a radius from pith to bark, visually delimited each annual ring and measured the ring widths along two radii per plant. Since some stems were partially rotten, we used a final sample size of 20 and 15 plants located within juniper canopy areas and 18 and 17 plants outside juniper canopy areas at the valley and slope sites, respectively.

Statistical analyses

Hypothesis 1. Testing the SGH at the interspecific level

In order to test whether the juniper presence and site affected soil characteristics we compared soil depth between sites and microsites through an Analysis of Variance (ANOVA).

We explored whether the spatial patterns of *H. spinosa* plants varied between the two sites by using univariate point pattern analyses. We preferred univariate over bivariate analyses to study the relationship between *H. spinosa* and juniper because each juniper individual covered large areas of the transects (see Fig. 1b): the representation of each juniper plant with a single point would mean analysing the interspecific effect only as related to the centre of each juniper individual, not to all its surface. To describe the small-scale spatial pattern in each transect, and to visually compare the patterns in the two studied sites, we used the pair-correlation function $g(t)$, which is a useful second-order statistic to characterize patterns at small spatial scales (Diggle, 2003). Values of $g(t)$ higher and lower than one indicate clustered or regular spatial patterns, respectively. We considered the null hypothesis of a homogeneous Poisson process for complete spatial randomness of *H. spinosa* plants. To test if the represented spatial pattern departs significantly from random we used Monte Carlo test with 999 unrestricted permutations. The analyzed distance range was from 0.25 m to 2.5 m, establishing a maximum limit equal to half the minor axis of each transect (i.e. 2.5 m). We used a numerical approach which uses an underlying grid of cells (0.25 m side in our case) and does not require edge-effect correction (Wiegand and Moloney, 2004). Finally, we calculated a goodness of fit test and its associated probability level to summarize the deviation between the observed pattern and the theoretical results across the distances tested in each analysis (Diggle, 2003). The spatial analyses were done using the software Programita (Wiegand and Moloney, 2004).

To determine whether juniper affects the spatial distribution of *H. spinosa*, we compared plant density per microsite in both sites. We performed χ^2 tests for comparing the expected and observed number of individuals per microsite according to the surface of each transect covered by juniper. We checked the ages of *H. spinosa* individuals and contiguous juniper branches and analyzed the age structure at each microsite in the four transects. We used 5-year age classes to take into account the uncertainty in age estimation associated with possible missing or false rings, since samples were not cross-dated (Fritts, 1976).

Hypothesis 2. Including intraspecific competition in the SGH predictions

Intraspecific competition in *H. spinosa* was estimated by using the modified influence index of Woods (2000) in a radius of 50 cm around each focal plant:

$$\text{Influence index} = \sum_{\text{dist} \leq 50\ \text{cm}} \frac{\text{Area}_n}{\text{dist}_{n,f}}$$

where Area_n is the canopy area of an individual neighbouring plant, and $\text{dist}_{n,f}$ the distance between the neighbouring plant (n) and the focal plant (f), considering only plants in the same microsite. Since the belowground influence area of a plant is proportional to its volume (Casper et al., 2003) and the volume for *H. spinosa* plants ranged from 2.80 to 89.34 dm³, we selected a radius of 50 cm as a compromise between considering the maximum lateral root spread area (assuming the general equation obtained by Casper et al., 2003; Appendix 1) and avoiding larger distances that could respond to ecological factors other than intraspecific competition. High values of the influence index indicate the presence of large neighbouring individuals located at short distances, whereas low values indicate the presence of few small neighbouring conespecifics. The resulting parameter was log-transformed to fit normality of residuals due to their asymmetric distribution biased to low values. We compared the influence index values in the different site × microsite combinations through an ANOVA and a Tukey post-hoc test. These analyses were performed with the spatstat package (Baddeley and Turner, 2005) in R environment (R Development Core Team, 2011).

We used General Linear Models (GLM) to test the effect of intra- and interspecific interactions on several estimates for *H. spinosa* performance: plant form (slenderness and canopy area) and reserve levels (NSC concentrations). Canopy form was related to the nominal factors site and microsite, and to quantitative variables plant age and the log-transformed influence index, a proxy of intraspecific interaction. Influence index was log-transformed since a preliminary analysis suggested a logarithmic relationship between influence index and response variables. We used the gamma distribution to normalize and homogenize residuals. We related NSC concentrations to the same nominal factors and to plant age as a covariate (intraspecific interaction was not considered because the samples for NSC analysis were not collected within transects), using the Gaussian distribution. Finally, we used Linear Mixed Models (LMM, McCullagh and Nelder, 1989) to assess the combined effect of site and microsite and their interaction on *H. spinosa* radial-growth trends during the last 21 years. This time interval was selected to include most *H. spinosa* individuals while minimizing the effect of age trends in young plants. We considered site, microsite and year as fixed effects (year as covariable), and the identity of *H. spinosa* individuals as random effect. Since we only considered one random effect, we checked if its inclusion improved the beyond optimal model for fixed effects (*i.e.*, that with the more complex structure), in which we did not include the triple interaction. We estimated both models (with and without random effect) with the restricted maximum likelihood method (REML) and selected the optimal one by comparing them with the Akaike Information Criterion (AIC; see Bolker et al., 2009 for a summary of model selection methods and their advantages and main concerns). In a second step, we searched for the best structure of the fixed component by fitting all potential models that included the selected random effect structure and estimating them with the Maximum Likelihood (ML) method. We compared all possible models with AIC values and the final model was then refitted using REML to obtain estimates of factor effects. Since the residuals of the final model showed heterogeneity in the variances between groups

by site, we incorporated an identity variance structure (varIdent) to satisfy model assumptions (Zuur et al., 2009). LMM analyses were performed with the nlme package (Pinheiro et al., 2011) in R environment (R Development Core Team, 2011).

Results

Testing the SGH at the interspecific level

We compared soil depth within and outside the juniper canopy to evaluate how abiotic factors depend on microsite conditions and found that soils were deeper within the juniper canopy (mean depth ± SE = 11.8 ± 0.6 cm) than outside it (8.7 ± 0.7 cm; F = 10.389; P = 0.002). The interaction between site and microsite on soil depth was significant (F = 7.669; P = 0.006); at the slope site, soils were deeper within (12.6 ± 0.9 cm) juniper canopies than outside them (6.8 ± 1.0 cm), whereas at the valley site, there were no significant differences in soil depth between microsites (11.0 ± 1.0 cm within vs. 10.6 ± 0.8 cm outside juniper canopies).

H. spinosa individuals at the slope site displayed an aggregated pattern with significant clustering at specific distances of 0.25 and 1 m from the focal plant (transect S1) or from 0.25 up to 2.5 m (transect S2) (Fig. 2). At the valley site, *H. spinosa* individuals either did not show significant aggregation (transect V1) or presented only short-distance clustering up to 0.5 m (transect V2). Although spatial patterns on transects S1 and V2 may seem similar, the detected clustering on transects S1 and S2 were positively related to the presence of juniper canopies (S1: χ^2 = 23.934, P < 0.001; S2: χ^2 = 32.595, P < 0.001), whereas clustering on transect V2 was negatively associated with juniper canopies (χ^2 = 21.052, P < 0.001). Plants on transect V1 were independent of juniper canopies (χ^2 = 1.553; P = 0.213).

The age structure of *H. spinosa* populations was similar between sites and microsites (Fig. 3), with the oldest individual being 76 years old. Most individuals had established between the 1960s and 1980s, with low establishment rates since then. *H. spinosa* density was three times larger in the valley (2.00 individuals m⁻²), than in the slope (0.64 individuals m⁻²). In the valley, juniper branches were older than neighbouring *H. spinosa* individuals only in 17% of all analyzed cases (Fig. 4a), whereas on the slope all juniper branches were older than neighbouring *H. spinosa* individuals (Fig. 4b).

Including intraspecific competition in the SGH predictions

Influence index differed between sites, being significantly higher in the valley (mean ± SE = 40.12 ± 10.67 cm) than on the slope (15.52 ± 2.81 cm; n = 1286; F = 28.609; P < 0.001). The interaction term between site and microsite also had a significant effect on the influence index (F = 55.780; P < 0.001). In the valley the influence index was larger outside (46.22 ± 12.75 cm) than within juniper canopies (8.84 ± 1.29 cm), whereas the inverse pattern occurred on the slope, with higher influence indices within junipers (19.93 ± 3.91 cm) than outside them (4.85 ± 1.10 cm).

H. spinosa plants slenderness was affected by intra- and interspecific interactions (residual deviance = 34.80%), whereas canopy area responded to intraspecific interactions (residual deviance = 28.69%). Irrespective of the site (i.e. valley vs. slope), plants were more slender within juniper canopies (mean ± SE = 0.98 ± 0.03) than outside them (0.65 ± 0.02; Table 2). The interaction between site and microsite was also significant. *H. spinosa* individuals outside junipers were more slender in the valley (0.70 ± 0.02) than on the slope (0.51 ± 0.03), whereas within juniper canopies the pattern was reversed, with plants being more
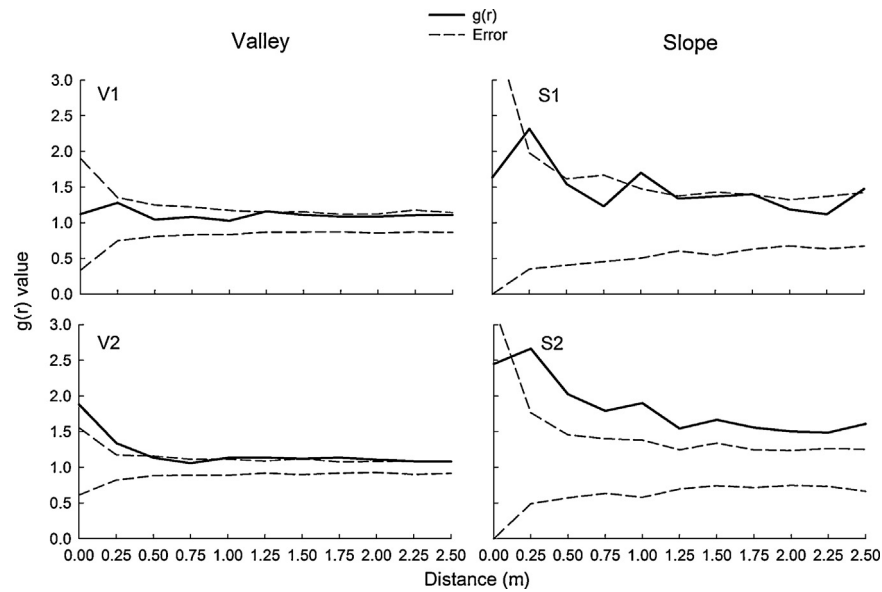
Fig. 2. Values of the univariate pair-correlation function ($g(r)$, continuous lines) calculated as a function of distance (r) and 5th and 95th percentiles based on 999 permutations of the original data (dashed lines) of the four transects in the two sites (valley, transects V1 and V2; slope, transects S1 and S2). Values of the $g(r)$ functions above 1 and the upper percentile envelope indicate significantly aggregated patterns, whereas $g(r)$ values above 1 but inside the envelopes indicate aggregated patterns which do not differ from spatial randomness.
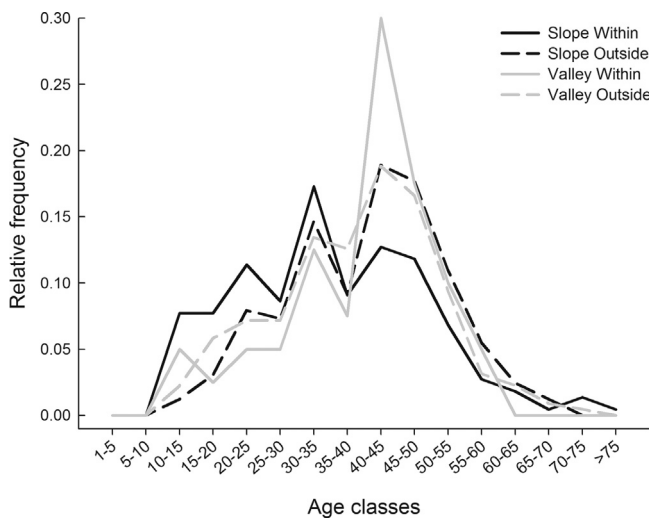

Fig. 3. Age structure (5-year classes) of *H. spinosa* at slope and valley sites and considering each microsite (within or outside juniper canopy).

slender on the slope (1.00 ± 0.03) than in the valley (0.91 ± 0.06). Age decreased plant slenderness, whereas conspecific density increased it. Canopy area differed marginally between the slope ($373.9 \pm 26.73 \, cm^2$) and the valley ($183.6 \pm 11.62 \, cm^2$), with no significant microsite effect. Intraspecific competition and age exerted negative and positive effects on canopy area, respectively.

NSC concentrations in wood were affected by site and microsite (Table 2, $R^2_{adj} = 0.365$; $P < 0.001$) but not by plant age. NSC concentrations (mean $\pm$ SE) were higher in plants growing in the valley ($10.06 \pm 0.53\%$) than on the slope ($6.62 \pm 0.39\%$); they were also higher outside ($8.79 \pm 0.42\%$) than within juniper canopies ($7.89 \pm 0.63\%$). The interaction between site and microsite significantly affected NSC concentrations. At the slope site, plants had higher NSC concentrations outside ($8.11 \pm 0.43\%$) than within juniper canopies ($5.21 \pm 0.45\%$), whereas in the valley there was no significant difference between them ($10.72 \pm 0.79\%$ within and $9.44 \pm 0.70\%$ outside the canopy).

The optimal structure in GLMM fitted to radial growth included the site × position and site × year interactions as fixed factors, individual as random factor and an identity variance structure based on site and individual (Table 3; Appendix 2). All fixed factors and interactions significantly influenced radial growth (Table 4). Ring width was much lower in the valley (mean $\pm$ SE = 0.091 ± 0.008 mm) than on the slope (0.168 ± 0.017 mm) and this difference was stronger

Table 2
Statistics of the General Linear Models used to evaluate differences between sites (valley vs. slope) and microsites (within vs. outside neighbouring juniper canopies) for slenderness, canopy area and non-structural carbohydrate (NSC) concentrations in wood of *H. spinosa* plants. An influence index was calculated in a radius of 50 cm around each focal plant.

		Slenderness ($n = 561$)		Area ($n = 561$)		Total NSC ($n = 82$)	
		F	P	F	P	F	P
Factors	Site	0.016	0.899	3.637	0.057	**27.762**	**<0.001**
	Microsite	**83.704**	**<0.001**	3.122	0.078	**6.111**	**0.016**
	Site*Microsite	**13.366**	**<0.001**	0.142	0.706	**6.245**	**0.015**
Covariates	Age	**47.334**	**<0.001**	**25.050**	**<0.001**	1.946	0.167
	Influence index	**32.160**	**<0.001**	**28.998**	**<0.001**	–	–

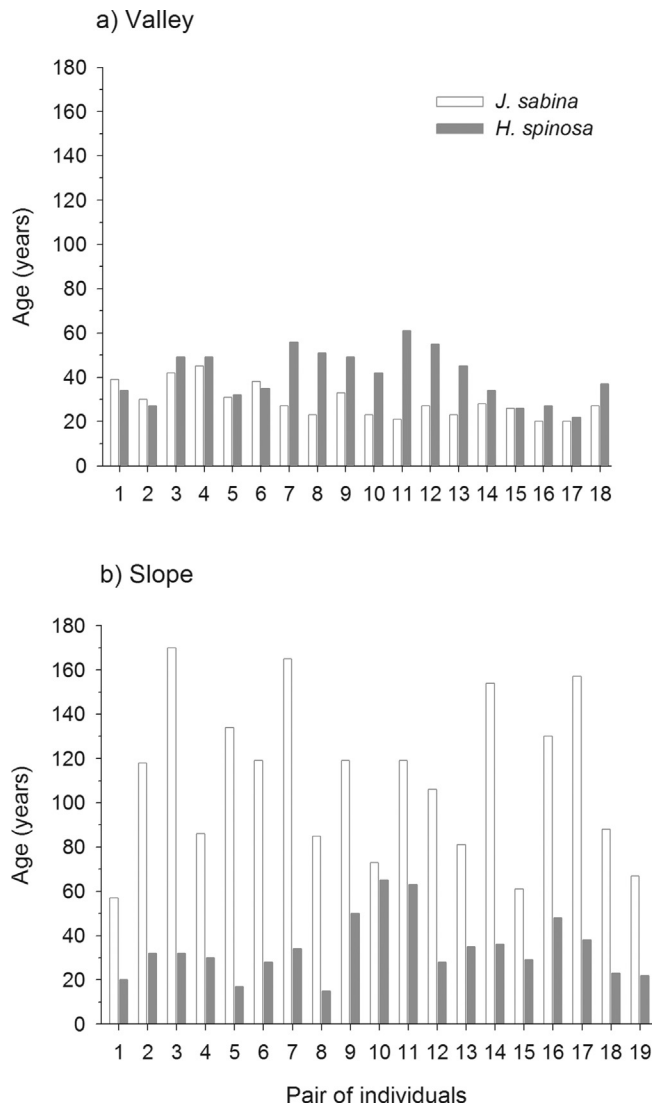Bold values correspond to significant effects ($P < 0.05$).

Fig. 4. Paired ages of *H. spinosa* individuals and the nearest juniper (*J. sabina*) branch at valley and slope sites. (a) At the valley site *H. spinosa* individuals were mostly (15 out of 18) older than junipers, indicating that they established in bare areas that were later colonized by junipers. (b) At the slope site, by contrast, *H. spinosa* plants were always younger than junipers, and thus had established within areas previously occupied by junipers.

for plants located within juniper canopy areas (0.085 ± 0.010 mm vs. 0.196 ± 0.027 mm for valley and slope sites, respectively) than outside them (0.097 ± 0.013 mm vs. 0.144 ± 0.020 mm). Ring width decreased from 1990 to 2010 in all site × microsite combinations, but it was more pronounced at the slope than at the valley site (Fig. 5a) and also outside juniper canopies than within them (Fig. 5b).

Discussion

Our results support the hypothesis that both intra- and interspecific interactions among neighbouring plants are affected by environmental conditions: under lower stress conditions the net effect of intraspecific interactions shifted from neutral to negative, while interspecific interactions changed from exerting a net positive to a neutral or negative effect (Holzapfel and Mahall, 1999; Sthultz et al., 2007; Soliveres et al., 2010; Xu et al., 2010). The

range of analyses performed allowed us to detect the combined effects of intra- and interspecific interactions, abiotic conditions and historical processes on *H. spinosa* spatial patterns and performance. Overall, our findings point to the importance of considering both intra- and interspecific interactions when testing the SGH, despite its initial formulation at an interspecific level (Bertness and Callaway, 1994; Callaway and Walker, 1997). In this case it allowed us to develop a more complete picture of the system than that provided by the analysis of interspecific interactions alone. Although our study presents some limitations, since we only consider interactions between two species and in two contrasting environmental situations rather than across a whole gradient, it provides evidence of the need to consider the intraspecific component when studying plant–plant interactions in relation to stress at the community level.

Spatial patterns reflected the influence of interspecific interactions. Juniper plants modify the small-scale soil conditions, creating fertility islands by increasing soil depth, improving infiltration, increasing nutrient and organic matter contents and diminishing compaction (Verdú and García-Fayos, 2003; Escudero et al., 2004). At the slope site, improved soil conditions may have enhanced the establishment of *H. spinosa* individuals within juniper canopies, a process already observed for this and other juniper species (Verdú et al., 2004; Montesinos et al., 2007; DeSoto et al., 2010), and thus leading to a clumped pattern of *H. spinosa* plants within juniper canopies. Contrastingly, on the deep soils found in the valley, where it is likely that the juniper canopy did not lead to deeper soils, *H. spinosa* establishment occurred preferentially in open areas. In the valley site, the age comparison between *H. spinosa* plants and the nearest juniper branch indicated that most of the *H. spinosa* individuals appearing within juniper plants had in fact also originally established in open areas that were subsequently colonized by spreading junipers. Consequently, these results supported the SGH predictions (Bertness and Callaway, 1994; Schenk et al., 2003), since under harsh environmental conditions *H. spinosa* was facilitated by juniper through the amelioration of soil conditions, but under mild conditions the interaction between both species was mainly neutral or even negative.

Combining different measurements of plant performance provided a more accurate view about the net response of individuals to intra- and interspecific interactions under contrasting environmental conditions. The more slender habit of *H. spinosa* plants within juniper canopies than outside them was probably due to altered light transmittance through the juniper canopy (Xu et al., 2010). At the same time, the more slender habit of plants growing in the valley than on the slope could be related to negative density-dependent effects resulting from higher intraspecific competition (Goldenheim et al., 2008). This could be the same reason that secondary growth was depressed in the valley, whereas the higher secondary growth within juniper on the slope was possibly due to interspecific facilitation. As expected, NSC levels showed an inverse pattern to secondary growth, and this concurs with previous work showing sink activity (i.e., investment on secondary growth) to be the major factor driving NSC levels in alpine environments (Körner, 2003a; Fajardo et al., 2012). Secondary growth shows higher sensitivity to environmental conditions than photosynthesis (Körner, 2003b), and when secondary growth is limited plants can continue incorporating carbon up to a certain threshold and thus leading to an accumulation of NSC. Secondary growth limitation in alpine environments is mainly driven by low temperatures (Körner, 2003b), but summer water deficit poses an additional constraint in Mediterranean high mountains (Giménez-Benavides et al., 2007; García-Cervigón et al., 2012). In fact, soil conditions have been considered more important than radiation as a limiting factor for plant development in Mediterranean

Table 3

Linear mixed model construction (Zuur et al., 2009) and selection following the Akaike Information Criterion (AIC). Fixed effects are represented by site (valley or slope) and microsite (within or outside juniper canopies) as factors and year as covariable. Random effects comprise the identity of *H. spinosa* individuals (indicated as 1|Individual). BOM: beyond optimal model including the more complex structure of fixed effects without considering the triple interaction = Site × Microsite + Site × Year + Microsite × Year. RANDOM: represents the selected structure of random component = 1|Individual, varIdent (Individual|Site). ∆AIC: increment on AIC values respect to that of the model with lowest AIC; ML: maximum likelihood; REML: restricted maximum likelihood; NPar: number of estimated parameters.

| Model	AIC	∆AIC	NPar
Random component selection, adjusted with REML			
Growth ~ BOM + (1|Individual), varIdent(Individual|Site)	9175.735	0.000	10
Growth ~ BOM + (1|Individual), varIdent(Site)	9443.834	268.099	9
Growth ~ BOM, varIdent(Individual|Site)	9665.735	490.000	9
Growth ~ BOM, varIdent(Site)	9905.453	729.718	8
Fixed component selection, adjusted with ML			
Growth ~ Site × Microsite + Site × Year + RANDOM	9171.651	0.000	9
Growth ~ Site × Microsite + Site × Year + Microsite × Year + RANDOM	9175.735	4.084	10
Growth ~ Microsite + Site × Year + RANDOM	9181.058	9.407	8
Growth ~ Site × Microsite + Year + RANDOM	9181.502	9.851	8
Growth ~ Site × Year + RANDOM	9182.820	11.169	7
Growth ~ Site × Microsite + Microsite × Year + RANDOM	9185.052	13.401	9
Growth ~ Site + Microsite + Year + RANDOM	9190.912	19.261	7
Growth ~ Site + Year + RANDOM	9192.665	21.014	6
Growth ~ Site + Microsite × Year + RANDOM	9194.459	22.808	8
Growth ~ Site × Microsite + RANDOM	9197.262	25.611	7
Growth ~ Site + Microsite + RANDOM	9206.678	35.027	6
Growth ~ Site + RANDOM	9208.418	36.767	5
Growth ~ Microsite + Year + RANDOM	9228.113	56.462	6
Growth ~ Year + RANDOM	9229.215	57.564	5
Growth ~ Microsite × Year + RANDOM	9231.654	60.003	7
Growth ~ Microsite + RANDOM	9243.906	72.255	5
Final model, adjusted with REML			
Growth ~ Site × Microsite + Site × Year + (1|Individual), varIdent (Individual|Site)			9
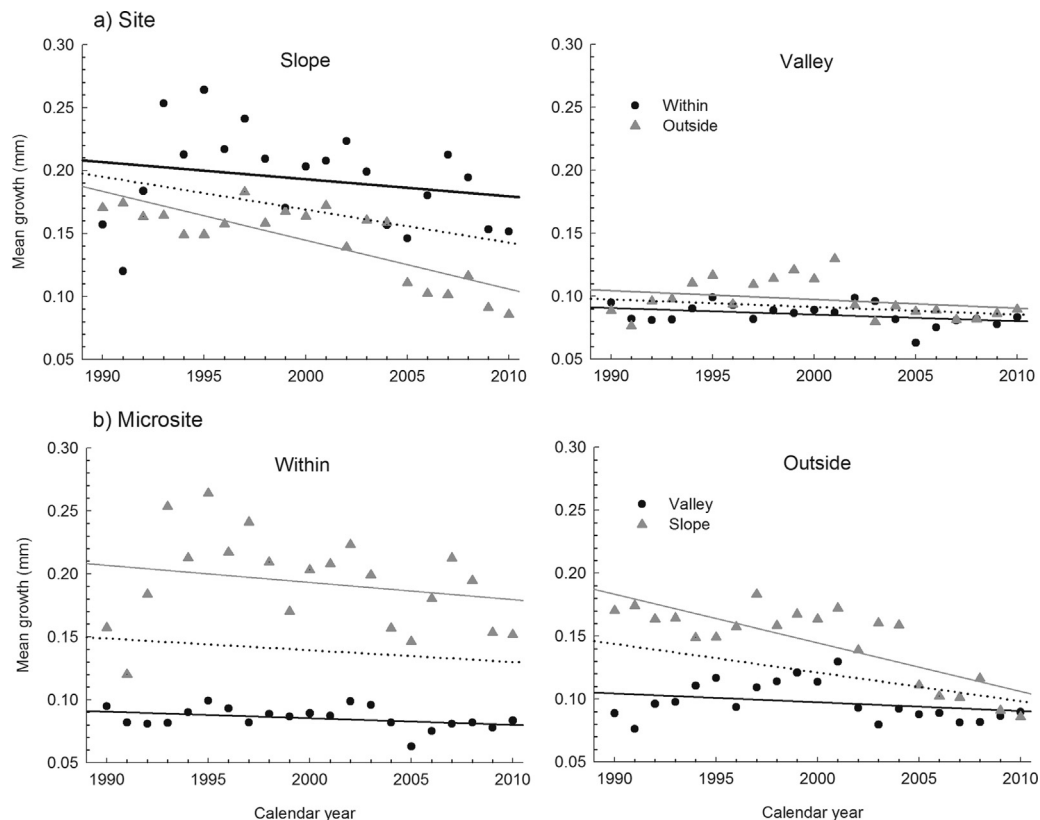
Fig. 5. Trends in annual radial growth (shrub-ring width) of *H. spinosa* individuals as a function of site (slope vs. valley) (a) and microsite (within vs. outside juniper canopies) (b). Means were obtained from 15 individuals within juniper canopies and 17 outside them at the slope, and 20 individuals within and 18 outside juniper canopies at the valley. Continuous lines indicate linear growth trends for each site/microsite combination while dotted lines correspond to mean trends for each graph.

Table 4
Main statistics of the fixed factors for the Generalized Linear Mixed Models fitted to evaluate differences in annual radial growth (last 21 years) of *H. spinosa* plants as a function of site (valley vs. slope), microsite (within vs. outside neighbouring juniper canopies) and time (year, covariable). The identity of *H. spinosa* individuals was included as random factor. All fixed effects are significant ($P < 0.05$).

Model: Growth ~ Site × Microsite + Site × Year + (1|Individual), varIdent (Individual|Site)				
Fixed effects	Estimate	SE	t	P
Intercept	551.479	92.349	5.972	<0.001
Site (valley)	−422.175	102.609	−4.114	<0.001
Microsite (outside juniper)	−4.891	1.577	−3.101	0.003
Year	−0.266	0.046	−5.763	<0.001
Site (valley) × Microsite (outside juniper)	6.103	2.095	2.913	0.005
Site (valley) × Year	0.206	0.051	4.009	<0.001
Random effect	SD	SE		
Individual	4.162	0.109		
Variance function	Multiplication factor	Residual SE		
Site = valley	0.528	0.100		
Site = slope	1.000	0.189		

mountains (Gómez-Aparicio et al., 2004). Our results are consistent with this hypothesis, through the impact on *H. spinosa* secondary growth and NSC levels of the amelioration of the harsh environmental conditions by the juniper on the slope (Maestre et al., 2002; Michalet et al., 2006). Moreover, negative effects of intraspecific interactions in the valley can be interpreted as the result of the enhancement of summer drought stress, due to increased root competition between conspecifics (Robberetch et al., 1983; Deng et al., 2006), resulting in lower growth rates and higher NSC accumulation for individual plants.

Conclusions

Our study highlights the temporal and spatial complexity of facilitative-competitive processes in plant communities from Mediterranean high mountains. At the population level, the more favourable abiotic conditions in the valley, compared to the slope, enabled greater *H. spinosa* establishment there. At the individual level, however, the stronger biotic constraints exerted by neighbouring conspecifics in the valley led to reduced individual growth rates and high NSC accumulation despite the more favourable abiotic conditions. The study of annual rings made possible the interpretation of the origins of plant–plant interactions within a temporal context.

Intra- and interspecific effects should be considered in order to evaluate the outcome of plant–plant interactions as a function of abiotic conditions. In our study case, under mild environmental conditions intraspecific competition was the leading force, whereas under harsher conditions interspecific facilitation became the dominant interaction. The direction, intensity and nature of biotic interactions changed as a consequence of the amelioration in abiotic conditions. The simultaneous analysis of intra- and interspecific interactions allowed us to improve our understanding of a two-species study system, and this should be considered when studying plant–plant interactions at the community level.
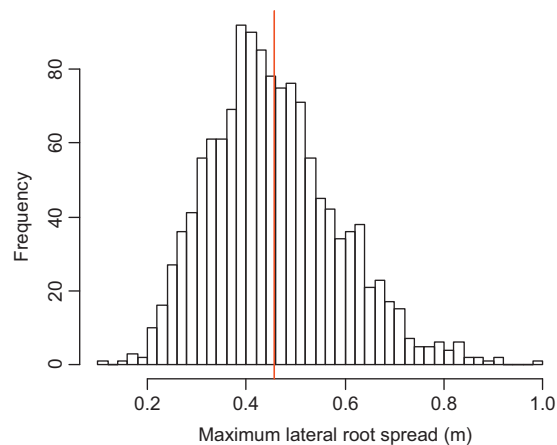
Acknowledgements

Fig. A1. Histogram of the maximum lateral root spread of *H. spinosa* individuals, indicated as the distance from the stem. Red line represents the mean value. Maximum lateral root spread was calculated following the general equation obtained by Casper et al. (2003).
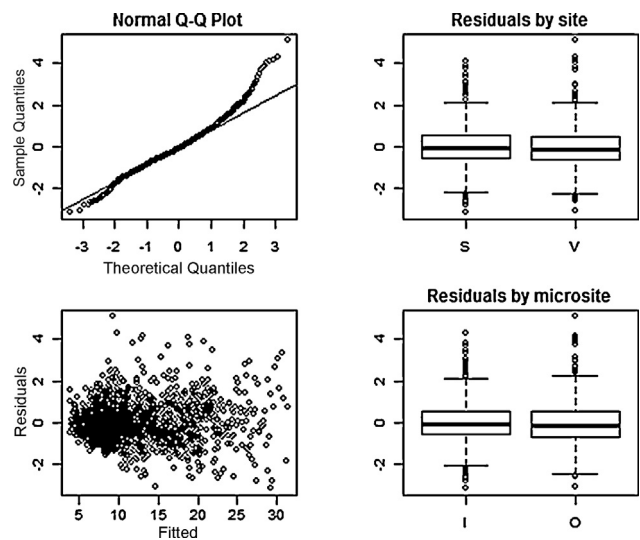

Fig. A2. Residuals analysis of the LMM. Normal *Q*–*Q* plot, predicted vs. residuals plot and two boxplots of residuals by site (S, slope; V, valley) and by microsite (I, within juniper; O, outside juniper), the two assessed fixed factors, are shown.

Appendix 1.

See Fig. A1.

Appendix 2.

See Fig. A2.

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