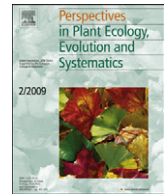




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Research article

Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation

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ABSTRACT

Plant–plant interactions are largely influenced by both environmental stress and ontogeny. Despite the effects of each of these factors on the overall outcome of these interactions has received considerable attention during the last years, the joint effects of both factors as drivers of such outcome are poorly understood. We used the combination of spatial pattern analysis, fruit production surveys, carbohydrate assays, sowing experiments and dendrochronological techniques to explore the interaction between *Stipa tenacissima* (nurse) and *Lepidium subulatum* (protégée) in two different slope aspects. This battery of techniques allows us to study the effects of the nurse plant during the whole life cycle of the protégée, and to assess the role of spatio-temporal variability in abiotic stress as a modulator of ontogenetic shifts in plant–plant interactions. Spatial pattern analyses suggested a net facilitative effect of *S. tenacissima* on *L. subulatum*. This effect was particularly important during the germination, shifting to competition (growth reduction) early after establishment. Competition was gradually reduced as the shrub aged, suggesting niche differentiation. The magnitude of competition was reduced under low rainfall levels in south-facing slopes, whereas this response was observed due to other abiotic factors in north-facing slopes. Our results highlight the crucial effect that positive interactions at early life-stages have to determine the long-term outcome of a given plant–plant interaction, and the existence of multiple shifts between facilitation and competition along different life-stages of the protégée. They also show how these ontogenetic shifts are modulated by abiotic factors, which differ among slope aspects. These findings may help to refine conceptual and theoretical models about shifts between facilitation and ontogeny under current climate change scenarios.

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Introduction

The analysis of the spatio-temporal variation of facilitative and competitive interactions along abiotic stress gradients has become a major research topic in community ecology during the last two decades (Kikvidze, 1996; Maestre and Cortina, 2004; Kikvidze et al., 2006; Brooker et al., 2008). In arid and semi-arid areas, such variations are particularly important, as water, which is the most limiting factor, shows a strong spatio-temporal variability (Whitford, 2002; Holmgren et al., 2006). These

environments are characterized by a high inter-annual variability in rainfall distribution, and as a result, plant recruitment limited to particularly rainy years (Holmgren et al., 2006). Furthermore, water availability also experiences a strong spatial variation between slope aspects: radiation and temperatures, and thus water stress, are higher in south-facing slopes, while in north-facing slopes water stress and evapotranspiration are lower (Friedman et al., 1977; Bellot et al., 2004; Aragón et al., 2008; Pueyo and Alados, 2007). These spatio-temporal changes in water availability have been pointed as a major factor defining the final outcome of plant–plant interactions in drylands (Tielbörger and Kadmon, 2000; Pugnaire and Luque, 2001; Gómez-Aparicio et al., 2004; Miriti, 2007).

The relationship between abiotic stress and the final outcome of plant–plant interactions is further complicated by the ontogenetic changes that plants experience throughout their life cycle, which can strongly modulate facilitation/competition shifts (Miriti, 2006; Schiffrers and Tielbörger, 2006; Armas and Pugnaire,

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2009). Short-term studies, which form the core of facilitation/competition research (see Callaway, 2007 for a review), are insufficient to fully understand the magnitude of ontogenetic shifts in **plant-plant** interactions, but long-term studies are often logistically prohibitive because of economic and temporal constraints. Some studies have overcome these limitations by using annual plants (Schiffers and Tielbörger, 2006), or by sampling specific temporal windows of the plant life cycle (Armas and Pugnaire, 2005, 2009; Miriti, 2006; Valiente-Banuet and Verdú, 2008). These approaches in isolation are insufficient to test **ontogenetically driven** facilitation/competition shifts along the whole plant life, particularly in long-lived perennial plants, and to assess the effects of spatio-temporal changes in abiotic stress on such ontogenetic shifts. These problems can be circumvented using dendrochronological techniques, assigning annual rings to calendar years (Schweingruber, 1988). Since xylem acts as conductive area for water and nutrients within a plant (e.g. Dyer and Bailey, 1987; Bascietto and Scarascia-Mugnozza, 2004), this technique can reconstruct investment in secondary growth along plant life, and therefore, act as a measurement of plant performance in each year during its whole life.

Although it is known that the outcome of plant-plant interactions may be affected by the interaction between abiotic stress and the ontogeny of the target species (Goldberg et al., 2001), there is a lack of studies evaluating the simultaneous effects of abiotic stress, both in space and in time, and ontogeny as drivers of on the outcome of plant-plant interactions (but see Schiffers and Tielbörger, 2006; Sthultz et al., 2007). Improving our understanding on the interacting effects of these factors will allow us to further refine current conceptual and mathematical models aiming to predict how plant-plant interactions change along stress gradients (Michalet, 2007), and to increase the precision of our estimates about how plant individuals and communities will respond to ongoing climate change (Brooker, 2006). In this study, we combine spatial pattern analyses, sowing experiments, dendrochronological and reproductive surveys, and carbohydrate assays to explore the relationship between the tussock grass *Stipa tenacissima* L. (Poaceae; the nurse plant) and the shrub *Lepidium subulatum* L. (Brassicaceae; the protégée plant) in two slope aspects (north and south) with contrasting abiotic stress environments. This combination of approaches provides us with a continuous set of data, suitable for testing the presence of ontogenetic facilitation/competition shifts throughout the entire life cycle of the protégée. Furthermore, we aimed to assess the role of spatio-temporal changes in abiotic stress (differences between abiotic factors controlling plant growth and survival among slope aspects, and differences between water availability among years) as a modulator of these shifts. We tested the following hypotheses: (i) the germination and survival of *L. subulatum* seeds and seedlings will be higher under *S. tenacissima* than in adjacent bare ground zones because of the improvement of environmental conditions under the canopy of this nurse plant (Maestre et al., 2001, 2003; Barberá et al., 2006); (ii) the outcome of the interaction will shift from facilitation to competition with shrub age, resulting in less growth and fruit production of *L. subulatum* when growing under the canopy of *S. tenacissima* (Miriti, 2006); (iii) given that both *S. tenacissima* and *L. subulatum* are primarily stress-tolerant species (Pugnaire et al., 1996; Palacio et al., 2007), and that water deficit (a resource-related stress) is the main limiting factor, we expect to find facilitative interactions mainly under moderate abiotic stress levels, (Maestre et al., 2009a). This will happen because the positive effect of microclimatic amelioration is limited after a threshold under high levels of abiotic stress, where competition, mainly for water, overcomes the positive effect of this amelioration and competition arises

again (Maestre and Cortina, 2004); and (iv) the magnitude of facilitation and competition is modulated by spatio-temporal changes in climatic conditions.

Methods

Target species

L. subulatum is a dwarf summer deciduous shrub linked to gypsum outcrops. It is distributed along the Western Mediterranean where it coexists with *S. tenacissima*. Seeds are small, exhibiting atelechory (Escudero et al., 2000) and forming a small permanent seed bank (Caballero et al., 2008). Emergence concentrates in winter, but spans to June, with densities ranging from 19 to 700 seedlings m^{-2} (Escudero et al., 2000; J.M. Olano, unpublished data). Plant recruitment bottlenecks occur during the first summer after germination, when survival rates range from 0.1% to 10%, with high survivorship linked to especially favourable years (Escudero et al., 2000; J.M. Olano, unpublished data). Annual survival rates increase sharply afterwards, reaching 71–95% for adults, depending mainly on autumn and spring conditions. Flowering starts at 2–4 years (M. Eugenio, personal communication). Primary growth occurs in two pulses, from mid February to June, and from September to November, respectively (Palacio and Montserrat-Martí, 2005; Palacio et al., 2007). Secondary growth also occurs in spring. Flowering and fruiting stages last from April to June. A detailed description on the natural history of *S. tenacissima* and the grasslands it forms is given in Maestre et al. (2009b).

Study area

Three sites were selected in central Spain for this study: Aranjuez (40°03'60"N, 3°54'91"W; 545 m.a.s.l.); Tielmes (40°24'89"N, 3°25'26"W; 595 m.a.s.l.); and Noblejas (40°0'36"N, 3°37'11"W; 526 m.a.s.l.). Their climate is Mediterranean semi-arid, with average annual precipitation of 388 mm, characterized by a high inter-annual variability and a characteristic strong summer drought. Mean annual temperature is 14.6 °C, ranging from 25 °C in July to 5.6 °C in January (Data from National Meteorological Service, 1994–2005. Marqués et al., 2008). The three sites were located on gypsum-rich soils, classified as *Typic Gypsiorthid* (Tielmes and Noblejas) and *Xeric Haplogypsid* (Aranjuez; Soil Survey Staff, 1994). Vegetation was in all cases an open steppe dominated by *S. tenacissima*, and contained shrub species like *L. subulatum*, *Retama sphaerocarpa* (L.) Boiss. and *Helianthemum squamatum* (L.) Dum. Cours. Perennial plant cover is below 45% in all cases.

Experimental design

At each site, experimental plots in north- and south-aspect slopes were established, with slopes varying between 13° and 22°. Perennial cover was different depending on the slope aspect in all the studied areas: 31% vs. 33%; 24% vs. 42%; and 32% vs. 35% for south- vs. north-aspect slopes in Tielmes, Aranjuez and Noblejas, respectively. Since cover can be a good surrogate of productivity in ecosystems such as those studied (Flombaum and Sala, 2009), and productivity is a good proxy for abiotic stress at the level of entire plant communities (Lortie and Callaway, 2006), we assume that these differences are related to higher levels of abiotic stress in the south- than in the north-facing slopes. This agrees with many studies conducted in arid and semi-arid areas showing higher abiotic stress in south- vs. north-aspect slopes

(e.g. Friedman et al., 1977; Sternberg and Shoshany, 2001; Bellot et al., 2004; Aragón et al., 2008; Pueyo and Alados, 2007). Differences in perennial cover between slope aspects were particularly evident in the Aranjuez site (Appendix A in supplementary material), so this site was selected to carry out the bulk of the fieldwork in this study.

Observational measurements

In April 2008, five $25 \times 4 \text{ m}^2$ transects were randomly established in each of the experimental plots (30 transects in total). Every *L. subulatum* individual found along the transect band was registered. Those individuals located at distances shorter than 20 cm and larger than 50 cm from the edge of a *S. tenacissima* tussock were considered as growing in association with *S. tenacissima* and in isolation, respectively. These situations are hereafter called Stipa and Open microsites, respectively. This distance has been used as separation between microsites in other studies with *S. tenacissima*, detecting significant differences in both biotic and abiotic features between Stipa and Open microsites (e.g. Maestre et al., 2001, 2003, 2009b). *L. subulatum* individuals growing at distances among 20–50 cm from the edge of a *S. tenacissima* tussock were not considered for further analyses.

Sowing experiment

In October 2007, a seed germination experiment was conducted in the Aranjuez site. It was designed as a fully factorial experiment with two treatments: slope aspect (north vs. south) and microsite (open areas devoid of vascular vegetation, north- and south-face of *S. tenacissima* tussocks). Ten replicates were established per treatment combination (each consisting in a $25 \times 25 \text{ cm}^2$ plot), and 75 commercial seeds of *L. subulatum* were seeded in each replicate (25 holes, 3 seeds per hole). We chose this approach over the alternative neighbor removal approach, because neighbor removal does not erase the facilitative legacy effects of a nurse plant upon soil infiltration and fertility. Seeds were buried at 0.5 cm to avoid ant depredation, irrigated with 40 ml of water and protected from rabbits (*Oryctolagus cuniculus* L.) by using a metallic mesh that did not shade the seeding site. A germination test conducted under controlled conditions revealed that the total germination rate of the pool of seeds employed was 89% after one month in a growth chamber (16 light hours at 20 °C and 8 dark hours at 10 °C). Seed emergence and seedling survival were monitored monthly until July 2008, when all germinated seedlings died during the summer drought. Because of this extreme mortality event and the lack of germination in Open sites, sapling survival data analyses cannot be provided.

Dendrochronological surveys

In June 2007, adult individuals of *L. subulatum* were randomly selected in the Aranjuez site for dendrochronological measurements. These individuals were chosen among those naturally growing under four different conditions, resulting from the combination of two microsites (Stipa vs. Open) and slope aspects (north vs. south); 16 individuals were selected for each combination (64 in total). After harvesting, a section of the stem including the root collar was selected to measure the annual growth ring widths as an indicator of plant growth over the course of its life. Annual rings were dated and measured following standard dendrochronological techniques as detailed in Appendix B in supplementary material. A section of the main root of the same plants was also collected to measure the content of non-structural

carbohydrates using the anthrone method (see Olano et al., 2006 for a full account of the methodology). Two different fractions of non-structural carbohydrates were measured in this study: non-soluble and soluble carbohydrates. In *L. subulatum*, non-soluble carbohydrates are used to overcome respiration rates in the leafless plant during summer drought (Palacio et al., 2007). Therefore, low contents in non-soluble carbohydrates may compromise the ability of this species to survive this critical moment. We interpret differences in this variable between microsites or slope aspects as differences in the ability of *L. subulatum* to respond to summer drought stress. On the other hand, soluble carbohydrates provide a surplus of sugars that can be stored for use under favourable conditions (Chapin et al., 1990), and that are susceptible to be immediately allocated to functions such as growth. Thus, higher contents of soluble carbohydrates in a given combination of microsite/slope aspect may indicate that the plant experiences more benign conditions there. Both soluble and non-soluble carbohydrates inform us about the status of the reserves of the plant, and thus are an integrative measurement of plant performance during the whole year. These variables were measured in June, just before summer drought, and in the main root because this organ and date match with the maximum starch content organ and period of the year for *L. subulatum*, respectively (Palacio et al., 2007).

Fruit/infructescence ratio surveys

In June 2008, ten reproductive *L. subulatum* individuals in each combination of two slope aspects (north vs. south) and microsites (Stipa vs. Open) were randomly selected in the Aranjuez site. Ten infructescences per plant were randomly chosen, and the number of fruits in each infructescence was registered. The canopy area of each sampled individual was calculated using the ellipse formula with the diameters parallel and perpendicular to slope. This measurement was introduced in the analysis as covariate to control for plant size.

Statistical analyses

The frequency of naturally occurring *L. subulatum* individuals in the surveyed plots was analyzed by using a Chi-square goodness of fit test. Our null hypothesis was that *L. subulatum* individuals have a random spatial pattern (depending directly on the cover of each microsite). Data were tested for independence in 6 separate one-way tables (resulting from each combination of site and slope aspect) including only the microsite factor (Stipa vs. Open). A joint analysis that would permit testing the interaction between the factors included in the model was not possible because the relative Stipa/Open microsites cover (and therefore the expected frequencies) in the different site \times slope aspect combinations were not equiprobable, a general assumption of the null hypothesis used when analyzing contingency tables with multiple factors. To calculate the expected frequencies, the number of total plants found in each transect were multiplied per the percentage of cover of each microsite. The sum of predicted/observed frequencies of the five transects per each site \times slope aspect combination was used to run the Chi-square test. To adjust for the increase in Type I error because of multiple testing, the Bonferroni correction was used (corrected α : $0.05/6=0.0083$).

The effects of slope aspect (north vs. south) and microsite (open areas, north face of *S. tenacissima* tussocks and south face of *S. tenacissima* tussocks) on the cumulative number of germinated seeds were tested by using generalized linear models (GLMs). GLMs were run with a Poisson error distribution combined with a

log link function; Type I log-likelihood ratios were used to analyze main effects. As recommended to counteract data over-dispersion and to adjust the statistics properly, the scale parameter was estimated by dividing the square root of the Pearson's Chi-square statistic by the degrees of freedom (McCullagh and Nelder, 1989). Differences between microsites were tested using a post-hoc test based on least-square means.

Growth data obtained from dendrochronological measurements were analyzed using two complementary approaches. First, the ring width data of all individuals measured in a given combination of microsite and slope aspect were grouped according to *L. subulatum* age, independently of recruitment year, and averaged. The average ring width of each of these four groups was used to estimate the effect of *S. tenacissima* on the growth of *L. subulatum* throughout the ontogeny of the later by using the relative interaction index (RII; Armas et al., 2004). This index was calculated as $(G_{st} - G_o)/(G_{st} + G_o)$, where G_{st} and G_o are the average ring widths of *L. subulatum* individuals growing in Stipa and Open microsites, respectively. RII values were obtained from 1- to 12-year old individuals to maintain enough sample size along the whole age range ($n=12-16$ individuals in all the groups for all the ages analyzed; sample size dramatically decreased for older individuals). This 12-year period recovers different ontogenetic stages of the protégée plant: seedling (first year), juvenile (from 2 to 4 years) and adult stage (from 5 years onward). Therefore, it is sufficient to test possible ontogenetic shifts in the sign of the interaction studied. To remove potential autocorrelation derived from repeated measures of ring width, a Prais-Winsten autoregression was performed to test the relationship between the values of the RII index and *L. subulatum* age. This analysis takes into account the non-independence of the samples by estimating a regression equation whose errors follow a first-order autoregressive process (SPSS, 2004). Second, ring width data were averaged for each calendar year, without taking into account the age of each *L. subulatum* individual, to evaluate the effect of climate inter-annual variability on annual growth rates. The RII index was calculated with these data as described above, evaluating the relationship between this index and the rainfall registered in March by using linear regression. March rainfall was selected as the best possible rainfall predictor of abiotic stress because it was highly correlated with the standardized ring width series of *L. subulatum* (Pearson's correlation index: 0.78 and 0.62, $P < 0.05$ for ring widths growth of *L. subulatum* in Open microsites in south- and north-aspect slopes, respectively, see Appendix C in supplementary material). To ensure that no other important rainfall variable was ruled out without the proper test, we evaluated the bivariate correlations between the width growth of *L. subulatum* rings and the rest of possible rainfall indicators of abiotic stress: rainfall of the months, total annual rainfall, monthly rainfall of September–December of the previous year, cumulative rainfall of spring (March–May), and the cumulative rainfall of the two annual pulses of *L. subulatum* growth (February–June and September–November). Of all these correlations, only March rainfall, which is prior to the main pulse of *L. subulatum* primary growth (Palacio et al., 2008), was statistically correlated with the width growth of *L. subulatum* rings (Appendix C in supplementary material). Therefore, we selected rainfall in this month as our surrogate of abiotic stress. Separate regression and autoregression analyses were conducted for north and south slopes. This approach allowed us to test differences in the effects of rainfall on the final outcome of the interaction depending on the variability in the abiotic conditions among the two slope aspects considered.

The relative importance of both ontogeny and abiotic stress as drivers of the outcome of the interaction studied was also explored. For doing this, three sequential analyses were run.

First, the effects of microsite (Stipa vs. Open) and slope aspect (north vs. south) on the growth of *L. subulatum* were evaluated using repeated measures ANOVA. For doing this, ring width data were grouped according to *L. subulatum* age, independently of the calendar year when they were formed. As abiotic conditions are different in north and south slopes due to different irradiation, temperature and water stress levels (Friedman et al., 1977; Bellot et al., 2004; Aragón et al., 2008), separate analyses were run for each slope aspect. This procedure allowed us to test potential differences in the nature of abiotic factors controlling the final outcome of the interaction at each slope aspect. The second step was to introduce the recruitment year as covariate in this analysis to assess the specific weight of climatic variability. *L. subulatum* individuals were of contrasting ages (recruitment dates of individuals analyzed vary from 1981 to 2000), so the ring width corresponding to each individual age may be influenced by the particular abiotic conditions in the period they grew, and therefore a high intra-group variability was expected to be found. As abiotic factors can produce non-random differences between individuals, and these differences could mask the effect of an experimental treatment, its inclusion as a covariate has been recommended to gain power when testing the effects of the factors of interest, particularly when a high intra-group variability is found (see Engqvist, 2005 and references therein). If the effect of this covariate is significant, and changes that of *S. tenacissima*, this would suggest that abiotic stress is modulating the net effects of *S. tenacissima* on the growth of *L. subulatum* during ontogeny. Lastly, the same analysis was performed but changing the covariate to the median of March rainfall during the lifetime of each individual included in the analysis. March rainfall is a key driver of *L. subulatum* growth (Palacio and Montserrat-Martí, 2005; Palacio et al., 2007; Appendix C in supplementary material), and its median would be a good estimator of the water stress level suffered by each individual during its entire life. With this analysis we aimed to differentiate the effects of rainfall from those of unmeasured abiotic factors characterizing each year as modulators of ontogenetic shifts in plant–plant interactions.

The effects of microsite (Stipa vs. Open) and slope aspect (north vs. south) on the mean fruits/infructescence ratio and on soluble and non-soluble carbohydrates content were evaluated with a two-way ANCOVA, where the size of *L. subulatum* individuals was used as a covariate. GLM analyses were carried out using the GENMOD procedure of SAS 9.0 (SAS Institute, 1985). The remaining statistical analyses were conducted using SPSS 13.0 for Windows (Chicago, IL, USA). The non-soluble carbohydrates content did not meet the ANOVA assumptions (normality and homocedasticity), and was transformed by using the arcsin transformation. The rest of the data met these assumptions, and were not transformed.

Results

L. subulatum was more frequent than expected under *S. tenacissima* canopies than in open microsites in five of the six site \times slope aspect combinations studied (Table 1). The overall cumulative germination rate was very low due to the drought conditions of the study year (Fig. 1). However, it was higher under the canopy of *S. tenacissima* than in open microsites. A significant microsite \times slope aspect interaction was also found, suggesting that the magnitude of the differences between microsites varied with the slope aspect considered. More germination events occurred in the north- than in the south-face of *S. tenacissima* canopies in the south slope ($\chi^2=9.2$; $P=0.002$), but no differences between the north- and south-face of the canopy were found in the north slope ($\chi^2=1.2$; $P=0.277$).

Table 1
Expected and observed frequency of *Lepidium subulatum* individuals found under the canopy of *Stipa tenacissima* (Stipa) and in bare ground areas (Open) in north- and south-facing slope aspects at the three studied sites. Data represent the sum of the number of individuals of the five transects measured in each site × slope aspect combination. Chi-square test results evaluate the effects of microsite (χ^2 and *P*-value) in the various site and slope aspect combinations. Significant results (Bonferroni-corrected α) are in bold.

Site	Slope aspect	Expected frequencies		Observed frequencies		χ^2	<i>P</i> -value
		Open	Stipa	Open	Stipa		
Tielmes	South	75.5	29.5	40	26	17.1	< 0.0001
	North	63.62	23.38	52	9	10.97	0.009
Aranjuez	South	176.95	28.05	137	29	9.05	0.002
	North	63.56	33.44	24	34	24.63	< 0.0001
Noblejas	South	99.44	33.56	89	17	9.27	0.002
	North	146.42	63.58	119	49	8.48	0.004

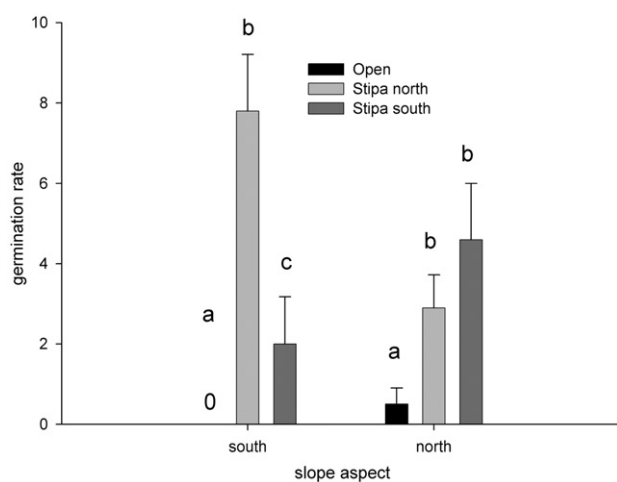


Fig. 1. Cumulative germinations registered in bare ground areas (Open), north face of *Stipa tenacissima* tussocks (Stipa north) and south face of *S. tenacissima* tussocks (Stipa south). Data represent means \pm SE; $n=10$. Different letters indicate significant differences between microsites in each slope aspect (post-hoc test based on the differences of least-square means).

A significant positive relationship between the RII values and *L. subulatum* age was found, suggesting that the negative effect of *S. tenacissima* on the growth of *L. subulatum* decreased as individuals aged (Fig. 2a). This relationship was found in both slope aspects ($R^2=0.83$ and 0.51 for south and north slopes, respectively). A negative linear relationship between the RII values and March rainfall was found in the south slope, suggesting that negative interactions dominated in years of high March rainfall (Fig. 2b). No significant relationships were found in the north slope.

Repeated-measures ANOVA showed a negative effect of *S. tenacissima* on the growth of *L. subulatum* individuals (Appendix B in supplementary material), which was particularly evident in the north slope (north: $F_{1,32}=5.535$, $P=0.025$; south: $F_{1,27}=3.875$, $P=0.059$). When recruitment year was introduced as a covariate in this analysis, the effects of microsite became non-significant in both the south (recruitment year: $F_{1,26}=6.713$, $P=0.015$; microsite: $F_{1,26}=0.461$, $P=0.503$) and north (recruitment year: $F_{1,31}=4.243$, $P=0.048$; microsite: $F_{1,31}=3.224$, $P=0.082$) slopes. When the median of March precipitation was used as a covariate, it did not change substantially the effects of *S. tenacissima* on the growth of *L. subulatum* in the north slope (median March precipitation: $F_{1,31}=0.893$, $P=0.352$; microsite $F_{1,31}=4.660$, $P=0.039$), but it did so in the south slope (median March precipitation: $F_{1,26}=3.631$, $P=0.068$; microsite: $F_{1,26}=0.577$, $P=0.454$).

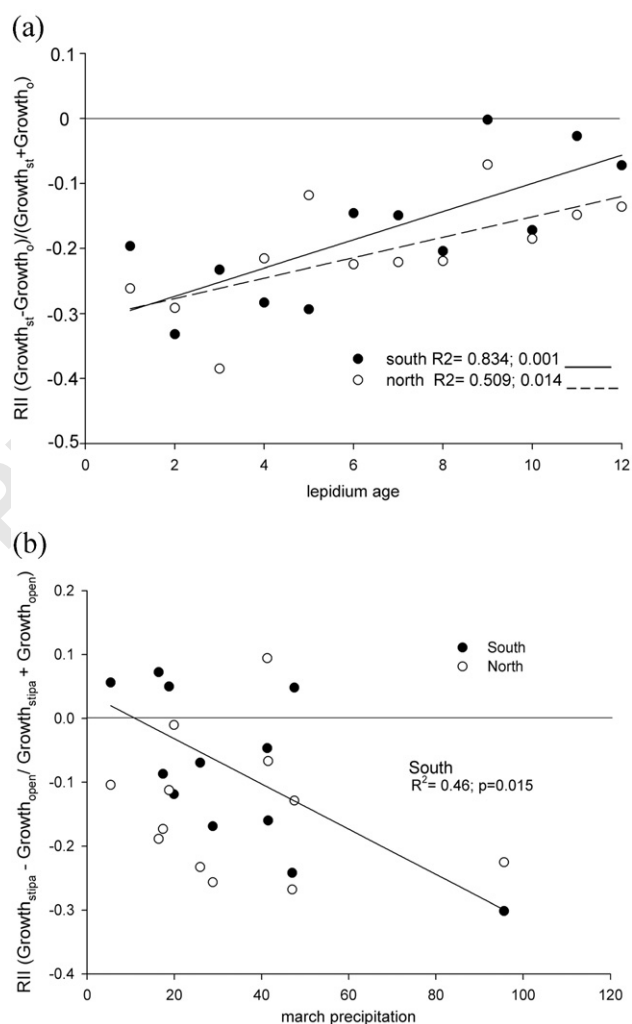


Fig. 2. (a) Relationships between the effect size of *Stipa tenacissima* on the growth of *Lepidium subulatum*, as measured by the RII index, and the age of *L. subulatum*. (b) Relationships between values of this index and the median of rainfall registered in March during the period 1995–2007. Results of significant autoregressions (a) and linear regressions (b) are shown. Each RII value is obtained by averaging growth data from 12 to 16 *L. subulatum* individuals.

Non-structural carbohydrate content was higher in Open than in Stipa microsites ($F_{1,58}=4.44$; $P=0.039$ and $F_{1,58}=4.71$; $P=0.034$, for soluble and non-soluble carbohydrates, respectively; Fig. 3). A higher content of soluble carbohydrates was found in north- than in

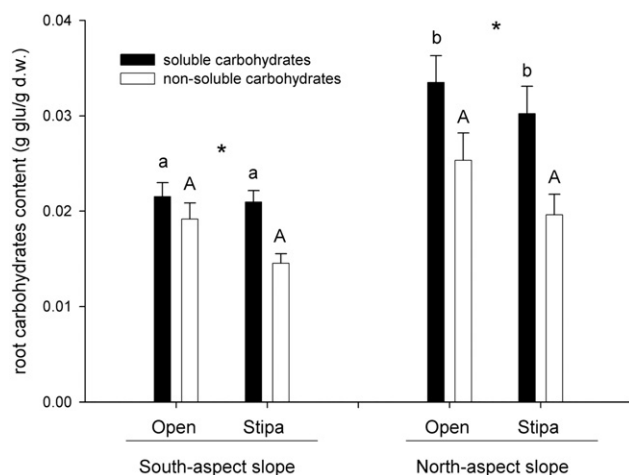


Fig. 3. Soluble and non-soluble root carbohydrates content (black and grey bars, respectively) of *Lepidium subulatum* individuals harvested into two different slope aspects (north vs. south) and growing underneath *Stipa tenacissima* canopy (Stipa) or in areas without perennial vegetation (Open). Data represent means \pm SE; $n=16$. Asterisks mark significant differences in root carbohydrates content between microsites within each slope aspect. Different letters mark significant differences among slope aspects for soluble (normal letters) and non-soluble carbohydrates (capital letters).

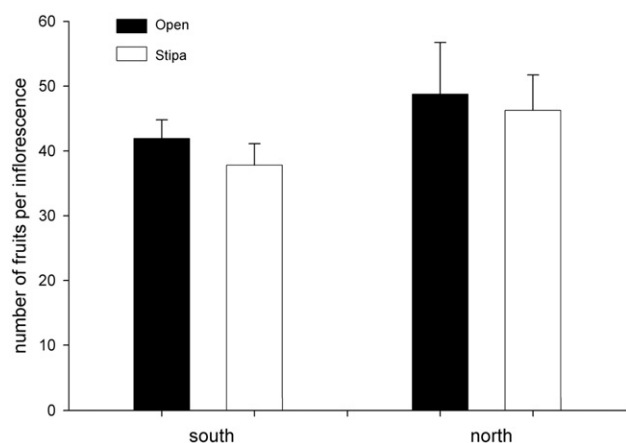


Fig. 4. Number of fruits per infructescence (mean \pm SE; $n=10$) of *Lepidium subulatum* individuals growing into two different slope aspects (north vs. south) and microsites (underneath *Stipa tenacissima* canopy, Stipa, or in areas without perennial vegetation, Open).

south-aspect slopes ($F_{1,58}=28.822$; $P<0.001$), but non-significant effects of slope aspect were found when analyzing non-soluble carbohydrates. The average number of fruits/infructescence ratio was affected by the size of *L. subulatum* individuals ($F_{1,35}=10.703$, $P=0.002$), as larger plants had more fruits. However, no significant effects of slope aspect and microsite were found (slope aspect: $F_{1,35}=0.911$, $P=0.346$; microsite: $F_{1,35}=0.039$, $P=0.845$. Fig. 4).

Discussion

In contrast to studies showing a single facilitation/competition shift throughout the ontogeny of perennial plants (Miriti, 2006; Valiente-Banuet and Verdú, 2008; Armas and Pugnaire, 2009), our results provide evidence of multiple ontogenetic shifts between facilitation/competition during the life cycle of the

protégée. We also found that spatio-temporal changes in abiotic stress modulated these ontogenetic shifts. To our knowledge, these responses have not been described before. While the importance of studying different abiotic stressors and their interaction has been highlighted (Holmgren et al., 1997; Riginos et al., 2005; Baumeister and Callaway, 2006), our study illustrates how the spatial variability of these stressors, even at small spatial scales, and their interaction with ontogeny determine the final outcome of plant-plant interactions.

As expected, we found higher germination rates under the canopy of *S. tenacissima* than in bare ground areas in both slope aspects (Barberá et al., 2006; Schiffrers and Tielbörger, 2006). This effect seems crucial to define the final outcome of the interaction studied, as indicated by the strong net positive effect of *S. tenacissima* on the abundance of *L. subulatum* individuals in most of the situations and sites studied. It is interesting to note, however, that if only two microsites (e.g. open vs. north face of *S. tenacissima* tussocks) would had been considered, as it has been done by most facilitation studies (see Callaway, 2007), the higher positive effect of *S. tenacissima* on the germination of *L. subulatum* found in the south-aspect slope would point to a higher facilitative effect with increases in abiotic stress (Bertness and Callaway, 1994). However, the contrasting results found between slope aspects when evaluating the germination of *L. subulatum* in the north- and south-faces of *S. tenacissima* tussocks suggest a complex interplay between abiotic stress and facilitation, which is strongly influenced by spatial variability in these abiotic stressors (e.g. light availability; Parker and Muller, 1982; Marañón and Bartolome, 1993; S. Soliveres, unpublished data). One possible explanation for these results is that in south-aspect slopes, where water stress is higher than in north-aspect slopes (Bellot et al., 2004; Aragón et al., 2008), seeds predominantly germinate under the north face of tussocks, where the improvement in microclimate by *S. tenacissima* is maximal due to water inputs coming from run-off and to the shadow provided by the canopy of this species (Maestre et al., 2001, 2003). This microsite preference is not so evident in the north-aspect slope, as seed germination in both faces of *S. tenacissima* tussocks was similar. These results seem to suggest a trade-off between microclimatic amelioration in the north face of *S. tenacissima* tussocks (Maestre et al., 2003) and the increase in interference competition with other neighboring plants (Goldberg et al., 2001; Miriti, 2006), as they are less abundant under the south-face of the tussocks (S. Soliveres, personal observation).

Stipa tenacissima had a negative effect on the growth and carbohydrate content of *L. subulatum*, suggesting that not only sink activity (growth), but also resource levels (carbohydrate content) are lower in this microsite. Although we cannot statistically differentiate the effect found on *L. subulatum* growth from a neutral one (we do not have error bars as we grouped our data by the four possible treatments combinations and averaged them to calculate RII), there is a clear reduction trend on the strength of the negative effect on growth as *L. subulatum* individuals aged. This effect, together with the positive effect found on germination, points to multiple facilitation/competition shifts along the life cycle of *L. subulatum*. Differences between our results and those from previous studies (Miriti, 2006; Valiente-Banuet and Verdú, 2008; Armas and Pugnaire, 2009) can be explained because our study focuses on a grass-woody plant interaction, while previous studies have focused on woody-woody plant interactions. In a grass-woody interaction, the growth of the woody individuals as they age helps to avoid water and light competition from the grasses (Fowler, 1986; Van Auken, 2000), whereas the benefits of shade and increased soil resources under the canopy of the later still exist (Maestre et al., 2003). This may render competition less important, as effective niche

separation is likely to occur with increasing age (Fowler, 1986; Van Auken, 2000; Armas and Pugnaire, 2005). This is less likely to occur when the nurse and protégée share the same ecological traits (e.g. annuals: Goldberg et al., 2001; Schiffers and Tielbörger, 2006; or shrubs: Miriti, 2006), as they are likely exhibiting greater niche overlap (Maestre et al., 2009a) and the increase of standing biomass can lead to an increase in resource competition (Schiffers and Tielbörger 2006). This difference suggests that differentiation in the traits of the plants involved are likely to play an important role in determining ontogenetic facilitation/competition shifts (Armas and Pugnaire, 2009), and thus should be explicitly taken into account when trying to generalize the results of particular studies.

Apart from the life form of the interacting species, differences in the tolerance to abiotic stress and the competitive ability of the interacting species are crucial when studying plant–plant interactions (Liancourt et al., 2005; Maestre et al., 2009a; Gross et al., in press). In our study, two stress-tolerant species coexist along a temporal stress gradient driven by water availability of each year. As both species are stress tolerant, and water is a resource-related factor, positive interactions are expected to be dominant at intermediate levels of abiotic stress because of the existence of thresholds in both sides of the gradient (Maestre et al., 2009a). This prediction was not met, as we found less competition under more stressful (i.e. lower rainfall) conditions in the south slope, and no relationships between abiotic stress and the outcome of the interaction were found in the north slope. The relationship with rainfall observed in the south slope can be explained by the compensation of the negative effect of shade on growth with the reduction in the water stress experienced by *L. subulatum*, particularly in dry years (Fig. 2b; Holmgren et al., 1997; Hastwell and Facelli, 2003). In our case, it seems that rainfall modulated the trend toward escaping light competition with age, being shade less negative under dry years. This is suggested by the reduction observed in the negative effects of *S. tenacissima* on the growth of *L. subulatum* when rainfall was introduced as a covariate. In the north slope, the ontogenetic trend was the same, but abiotic factors other than rainfall modulated this trend, as indicated by the change in microsite effect along ontogeny when recruitment year, but not rainfall, was introduced in the analysis. Our study shows how the same nurse effect, and its interaction with ontogeny, depends on the spatio-temporal changes in the overall amount of abiotic stress experienced by the interacting individuals, and on the resources driving such stress (Holmgren et al., 1997; Hastwell and Facelli, 2003). Moreover, we show how a longitudinal track of competitive or facilitative interactions and its relationship with climatic conditions can be easily obtained using secondary growth data present in annual rings.

Conclusion

In contrast with previous studies, which found an increase in competition as protégée plants grew (Miriti, 2006; Schiffers and Tielbörger, 2006; Valiente-Banuet and Verdú, 2008), we found a reduction of competition as plant aged. This result may be influenced by differences in ecological traits of species involved in our study comparing to the previous studies, which can result in an effective niche separation between grasses and shrubs, and therefore a reduction of competition (e.g. Armas and Pugnaire 2005; our case) or in a niche overlap, which together with the increase in biomass can lead to higher competition (e.g. Miriti, 2006 for shrubs; Schiffers and Tielbörger, 2006 for annuals).

Because perennial grasses conform an important component of many vegetation formations worldwide (Zimmermann et al., in press), our results provide useful information to refine current

theoretical models about facilitation/competition shifts along ontogeny of perennial species. Our results also provide important insights on how spatio-temporal changes in abiotic stress can modulate multiple facilitation/competition shifts. As rainfall increased competition escape in south-aspect slopes, other factors were more important in north-aspect slopes. Despite the mostly negative effect of the nurse plant on growth and reserve accumulation, the positive effect found on early stages of the **life cycle** of the protégée (germination) may be driving the net positive sign of the interaction, as demonstrated by the spatial aggregation found between studied species. Given the implications of understanding how **plant–plant** interactions change along stress gradients for accurately predicting global change impacts on communities and ecosystems (Brooker, 2006), future studies should pay special attention to the interplay between abiotic stress and ontogeny as joint drivers of facilitation/competition shifts, and more specifically, on the effect of nurse plants in key stages of the **life cycle** under different environmental conditions (Goldberg et al., 2001). This is particularly true when working with long-lived species in stressful environments.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ppees.2010.02.003.

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