

# Temporal dynamics of herbivory and water availability interactively modulate the outcome of a grass–shrub interaction in a semi-arid ecosystem

Santiago Soliveres, Pablo García-Palacios, Andrea P. Castillo-Monroy, Fernando T. Maestre, Adrián Escudero and Fernando Valladares

*S. Soliveres (santiago.soliveres@urjc.es), P. García-Palacios, A. P. Castillo-Monroy, F. T. Maestre, A. Escudero and F. Valladares, Área de Biodiversidad y Conservación, Depto de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnología, Univ. Rey Juan Carlos, ES-28933 Móstoles, Spain. SS, PG-P and FV also at: Inst. de Recursos Naturales, Centro de Ciencias Medioambientales, C.S.I.C., Serrano 115, ES-28006 Madrid, Spain.*

The study of plant–plant interactions along grazing and abiotic stress gradients is a major research topic in plant ecology, but the joint effects of both stressors on the outcome of plant–plant interactions remains poorly understood. We used two different factorial experiments conducted in a semi-arid Mediterranean steppe to assess: 1) the role of the perennial grass *Stipa tenacissima*, a low-palatability species, providing protection from rabbit herbivory to the shrub *Retama sphaerocarpa* (experiment 1), and 2) the effects of environmental amelioration provided by *Stipa* on the recovery of *Retama* after rabbit damage under two contrasted levels of water availability (experiment 2). In the experiment 1, water stress worked as an indirect modulator of herbivore protection by *Stipa*. This species protected *Retama* seedlings from rabbit herbivory during the wetter conditions of spring and winter, but this effect disappeared when rabbit pressure on *Retama* increased during summer drought due to the decrease in alternative food resources. In the experiment 2, *Stipa* exerted a negative effect on the survival of *Retama* seedlings during the three years of the experiment, regardless of inter-annual differences in rainfall or the watering level applied. This negative effect was mainly due to excessive shading. However, *Stipa* increased *Retama* recovery after initial rabbit impact, overriding in part this negative shade effect. Conversely, *Stipa* impact on the Fv/Fm of *Retama* seedlings depended on the intra-annual water dynamics and its experimental manipulation, overall contradicting predictions from the stress–gradient hypothesis. The complex interactions found between herbivory, microclimatic amelioration from *Stipa*, and water availability as drivers of *Retama* performance illustrate the importance of considering the temporal dynamics of both biotic and abiotic stressors to fully understand the outcome of plant–plant interactions.

Interactions among plants can be negative, neutral, or positive, depending their direction and magnitude on the level and type of the stressors governing a given community (reviewed by Callaway 2007). Important efforts have been devoted during the past two decades to understand the dynamics of plant–plant interactions along abiotic stress or herbivory gradients, highlighting how the presence of multiple abiotic stressors or different levels of consumer pressure importantly affect the outcome of plant–plant interactions (Baumeister and Callaway 2006, Graff et al. 2007). However, and despite their common co-occurrence in nature, the joint effects of both herbivory and water stress on the outcome of these interactions remain largely ignored (Ibañez and Schupp 2001, Veblen 2008, Smit et al. 2009). Considering both abiotic stress and herbivory together is crucial to understand the role of plant–plant interactions in dryland ecosystems, where these stressors are major factors influencing plant community dynamics (Fischer and Turner 1976, Whitford 2002).

The stress–gradient hypothesis (SGH; Bertness and Callaway 1994), a framework in which most studies focused on plant–plant interactions rely on, predicts a continuous increase in the frequency of positive interactions with increases in either consumer pressure or abiotic stress. However, several studies suggest that positive plant–plant interactions may collapse under extremely high levels of both consumer pressure and abiotic stressors directly related to resources, such as water or light (Graff et al. 2007, Maestre et al. 2009). These studies caused the generality of SGH predictions to be challenged (Maestre et al. 2005, 2006, Lortie and Callaway 2006). As a result of this debate, predictions from the SGH have been refined to consider the effect of different abiotic stressors and the ecological strategy of the species involved, and to introduce consumer pressure as a major factor affecting plant–plant interactions along abiotic stress gradients (Maestre et al. 2009, Smit et al. 2009). Furthermore, in arid and semi-arid areas water availability is characterized by a strong inter- and intra-annual variability,

with marked temporal dynamics that profoundly affect ecosystem functioning (Whitford 2002). These temporal dynamics add complexity to the response of plant-plant interactions to abiotic stress (Goldberg and Novoplansky 1997, Pugnaire and Luque 2001, de la Cruz et al. 2008), and may also modulate the effect of herbivores on vegetation (Veblen 2008). Thus, they must be specifically considered when studying the relationship between plant-plant interactions and different environmental biotic and abiotic stressors in arid ecosystems.

Herbivory is also a major driver of plant-plant interactions in these regions (Baraza et al. 2006, Graff et al. 2007, Veblen 2008). Unpalatable nurse plants may protect their understorey vegetation from herbivory damage by hiding more palatable plants under their canopies, or by sharing their defenses against herbivores (Baraza et al. 2006, Veblen 2008), a process commonly referred to as “associational resistance” (sensu Hay 1986). This protection against herbivory can strongly affect vegetation dynamics and biodiversity in grazed systems (Veblen 2008). Alternatively, the improvement on water status that nurse plants usually provide to their target plants may also positively affect target plant recovery from herbivory damage (Rand 2004, Acuña-Rodríguez et al. 2006). However, even in dry environments, shade casted by nurse plants can lead to light limitation for the protégée plants (Seifan et al. 2010, Soliveres et al. 2010). The joint effect that both shade and an improved water status provided by nurse plants have on the recovery from herbivory of the protégée plants will depend on the relative importance of water and light as limiting factors for plant performance and how herbivory affects their uptake (Wise and Abrahamson 2005, 2007). Hence, the final outcome on the protégée plants response to herbivory is difficult to generalize and predict, so more studies along these lines are needed to refine predictions on how plant communities respond to different levels of abiotic and biotic stressors (Graff et al. 2007, Smit et al. 2009).

We conducted two simultaneous field experiments to test the effects of rabbit herbivory and water availability on the interaction between the tussock grass *Stipa tenacissima* (the nurse) and seedlings of the leguminous resprouter shrub *Retama sphaerocarpa* (the protégée) in a semi-arid Mediterranean steppe. *Stipa* has an overall low palatability (Ben Salem et al. 1994), and therefore could provide herbivory protection for shrub seedlings by associational resistance. Furthermore, the positive effect that microclimatic amelioration provided by *Stipa* has on the survival of Mediterranean shrub seedlings is well known (Maestre et al. 2001, 2003). Since water stress and the impact of herbivores can prevent *Retama* establishment in these environments (Espigares et al. 2004, Rueda et al. 2008), it is likely that the protection from herbivores and the microclimatic amelioration provided by *Stipa* canopies can play a key role improving *Retama* colonization in semi-arid grasslands. We tested the following hypotheses: 1) *Stipa* provides protection from rabbit herbivory to *Retama*, enhancing its survival when rabbits are present; 2) shade provided by the *Stipa* canopy enhances *Retama* water status increasing its survival, but this positive effect wane during extremely dry years due to the overwhelming effect of competition for water, 3) regardless of plant water status, light reduction produced by *Stipa* will decrease *Retama* seedling recovery from rabbit herbivory; and 4) facilitation of *Retama* seedlings by

*Stipa* will collapse under extreme levels of stress produced by the joint action of herbivore damage and drought stress.

## Methods

### Study area

We conducted the study in the Aranjuez Experimental Station, located in the center of the Iberian Peninsula (40°03'60"N, 3°54'91"W). The climate is semi-arid Mediterranean, characterized by cold winters and a strong summer drought, with average annual precipitation and temperature of 388 mm and 15°C, respectively (1994–2005; Marqués et al. 2008; Supplementary material Appendix 1). The soil is classified as xeric haplogypsid (Marqués et al. 2008). Vegetation is an open steppe dominated by *Stipa* (this species accounts up to 90% of the total perennial cover), with a perennial plant cover of 24%. Sparse adult individuals of *Retama* and small shrubs such as *Lepidium subulatum* and *Helianthemum squamatum* are also present. The study site has a high diversity of annual plants, which reach their production peak in spring and constitute an important part of plant productivity during this period (Peco et al. 2009).

The study area harbours a high density of rabbits *Oryctolagus cuniculus*, as suggested by the high number of visual contacts and the number of warrens and latrines found (Soliveres unpubl.). Domestic livestock or other large herbivores are absent, and thus rabbits are the only herbivores affecting vegetation there. Rabbit activity tracks seasonal variation in vegetation productivity. These animals feed near their burrows to avoid predation during spring and winter, when their preferred food – mainly annuals – is abundant; however, during summer drought - when annuals dry out and food is scarcer -, rabbits increase their exploration to obtain enough food to survive (Rueda et al. 2008). The selection of woody seedlings as a food resource by rabbits increases during summer (Maestre et al. 2001). Rabbit browsing, in the case of *Retama* seedlings, may suppose the virtual removal of all the aboveground biomass.

### Experimental design

In December 2006, we planted 371 two-year old *Retama* seedlings, with a mean height of  $27 \pm 2$  cm (mean  $\pm$  SE,  $n = 20$ ), by using manually-dug holes of  $20 \times 20 \times 20$  cm. The planted seedlings came from central Spain (viveros Barbol, Madrid, Spain), and were maintained in the original nursery until the week prior to planting. In the nursery, seedlings were grown under optimal conditions (full sunlight, fertilised 1:1 peat:coco-peat substrate, watering to field capacity each week). We selected these two-year old seedlings to ensure they had enough size and reserves to resist rabbit damage at least once (see experiment 2). The selection of these two-year old seedlings does not underestimate potential facilitative effects of *Stipa* because *Retama* seedlings were grown under optimal conditions prior to their plantation, and thus they were still sensitive to water stress and to the environmental amelioration provided by *Stipa*. We randomly planted these seedlings on two different microsites: Tussock and Open. The former microsite was located <15 cm from

the upslope face of a *Stipa* tussock (ca 1 m width and 80 cm height). Open microsites were located in bare ground areas, > 50 cm away from any perennial plant. One month after planting, rabbits browsed some of the seedlings, and we set up two parallel experiments then. In the first experiment (hereafter experiment 1) we did not give the seedlings any protection from further grazing. This allowed us to test the protection against herbivores provided by *Stipa* canopy. In the second experiment (hereafter experiment 2) we evaluated the joint effects of contrasted levels of water availability and *Stipa* environmental amelioration on the recovery of *Retama* seedlings after rabbit browsing.

#### **Experiment 1. Nurse plant protection against herbivores**

From the 371 *Retama* seedlings planted, we left a total of 195 seedlings without any protection from herbivores (the remaining plants after experiment 2 was set up). From these seedlings, 103 and 92 were located in Tussock (refuge) and Open (control) microsites, respectively. We scored which of these plants had been browsed by rabbits and which of them were able to resprout and survive after the virtual removal of their aboveground biomass (i.e. the effect of rabbit damage) during January, March and September 2007. Because of the high number of rabbits present in the study area and their repeated browsing, no plants remained alive after September 2007, so we stopped monitoring then.

#### **Experiment 2. Joint effects of herbivory and abiotic stress on the outcome of the interaction**

This experiment had three factors: 1) microsite (Tussock vs Open), 2) herbivory (*Retama* seedlings whose aboveground biomass was completely eaten by rabbits during the first month in the field vs unbrowsed seedlings), and 3) irrigation (watered vs non watered seedlings). Since inter-annual variability is of crucial importance in dry environments, and it may strongly affect the results found and our conclusions (Ibañez and Schupp 2001), we conducted this experiment during a three year-period (and therefore under three contrasted environmental conditions) to add confidence to the results found. We set up 22 replicates per each combination of treatments (176 seedlings in total). All the seedlings from this experiment were protected from further herbivory after the first month in the field using a metallic mesh. The diameter of the openings in the mesh was 5 cm, which casted no detectable shade to the seedlings, and did not confound the effects of any of the factors in the experiment. In this experiment we monitored the variables described below.

We conducted a spatial analysis of browsed seedlings using the spatial analysis by distance indices (SADIE) methodology (see Perry 1998 for details). The spatial pattern of herbivory damage by rabbits was random (SADIE's aggregation index [ $I_a$ ] = 0.95,  $p = 0.56$ ,  $n = 176$ ). Thus, we do not expect unmeasured variables with spatial structure (e.g. soil depth, distance to a rabbit burrow or slope position) to influence seedling response to the assayed treatments. We did not measure seedling attributes that could influence rabbit behaviour (e.g. plant C/N ratio, initial plant height). However, the large number of seedlings randomly assigned to each treatment, and the fact that rabbit damage was equally intense (i.e. complete removal of aboveground biomass) regardless of the microsite considered, should control for the experimental

noise that any unmeasured factors potentially affecting rabbit behavior could have on the results of this experiment.

The irrigation treatment consisted in eight supplementary pulses of water, once every month, between April and July in both 2007 and 2008. The wettest and driest periods of the study area are spring (from March to May) and summer (from June to September), respectively. Thus, the irrigation treatment affected both wet and dry periods. In each monthly watering, we applied an amount of water equivalent to the 50% of the median rainfall registered during the past 30 years for this month, irrespectively of the rainfall registered (i.e. 6, 9.3, 10.9 and 5.7 mm for April, May, June and July, respectively; Supplementary material Appendix 1). With this irrigation scheme we aimed to evaluate the joint effects of reducing the summer drought (summer drought was longer in unwatered plants because of the lack of rainfall in June and July) and increasing precipitation during the wet season (spring rainfall was less abundant in unwatered plants) on the outcome of the interaction studied.

### **Field monitoring of experiment 2**

#### **Plant performance measurements**

We monitored seedling height and survival after each summer, which is the most critical season for seedling survival in Mediterranean semi-arid regions (Maestre et al. 2001, 2003). We measured these variables in September 2007, 2008 and 2009. Height was well correlated with aboveground biomass, as demonstrated by an allometric relationship performed with seedlings of contrasted sizes and ages (Spearman correlation:  $\rho = 0.65$ ,  $p < 0.0001$ ,  $n = 45$ ), and thus was used as our surrogate for seedling biomass in the field.

We measured photochemical efficiency by using the in situ chlorophyll fluorescence parameter Fv/Fm; it is calculated from photosystem II (PS II) fluorescence signals as the ratio between the variable (Fv) and the maximum (Fm) fluorescence signal, which are obtained from a short light pulse after 20 minutes of dark adaptation. Fv is the difference between Fm and the minimal fluorescence signal right before the saturating light pulse, being Fm the light that plant cannot absorb when its absorption capability has been collapsed by a previous pulse. Fv/Fm was determined with a pulse-modulated fluorometer. This variable is an estimator of the overall plant stress (Maxwell and Johnson 2000), and has been widely used as an indicator of plant stress in numerous studies in semi-arid areas (Pugnaire et al. 1996, Maestre et al. 2001, 2003, Aragón et al. 2008). Furthermore, small changes in the concentration of chlorophyll in leaf tissues associated with water limitation, which can be tracked by measuring Fv/Fm, can be crucial during important stages of plant life (Aragón et al. 2008). Although the use of Fv/Fm can be problematic for the detection of water stress in some species (Resco et al. 2008), it is a good proxy for plant stress in our case because the canopy structure, lack of leaves and the high tolerance of *Retama* to solar radiation minimizes photoinactivation and downregulation in this species (Valladares and Pugnaire 1999), a confounding factor that could mask the relationship between Fv/Fm and water stress. We randomly selected eight seedlings per treatment ( $n = 64$ ) for Fv/Fm measurements. We measured Fv/Fm in four key moments for seedling performance in Mediterranean

semi-arid environments: during the wettest period of spring (April–May), in the middle and at the end of the summer drought (July and September, respectively) and during the coldest month (December–January). Fv/Fm was measured in the same plants from May 2007 to September 2009.

### Soil moisture measurements

We measured soil moisture by time-domain reflectometry (TDR; Topp and Davis 1985). In eight randomly selected planting holes per treatment, we vertically installed 10 cm long probes ( $n = 64$ ). We chose this soil depth because the vast majority of root biomass of the planted seedlings concentrates near the soil surface (the pots where *Retama* seedlings were grown had ca 15 cm. depth), and because *Stipa* tussocks also concentrate the majority of their roots in the upper layers of the soil (Puigdefábregas et al. 1999). We conducted a site-specific calibration between time domain reflectometry measurements and gravimetric soil moisture ( $R^2 = 0.84$ ,  $p < 0.0001$ ,  $n = 68$ ) to assess for the validity of these measurements in gypsum soils and to convert them in soil gravimetric moisture data. We measured soil moisture in the same plants in all the samplings, and in the same sampling periods as Fv/Fm surveys. When these measurements coincided with irrigation pulses, soil moisture was measured at least one week after the irrigation. With this approach we avoided giving too much importance to the occasional influence of irrigation in our measurements, and assessed more realistically the soil moisture available for *Retama* seedlings during a given period. In addition to these measurements, and to further evaluate the effects of irrigation on soil water availability, we measured soil moisture one day after the irrigation pulse was applied on June 2007 in the planting holes of 10 undamaged plants (the eight replicates selected for the previously described soil moisture measurements plus two extra replicates) of each of the four combinations between microsite and irrigation ( $n = 40$ ). We measured soil moisture only in unbrowsed plants because we were only interested in assessing for differences in soil moisture between the irrigation treatments applied and the microsites tested.

### Statistical analyses

As all *Retama* seedlings used in experiment 1 died at the end of the monitoring, we did not perform any statistical comparison with the survival data at this period. However, we used the log-rank statistic of the Kaplan-Meier procedure to compare the shape of the survivorship and number of browsed plants curves between Tussock and Open microsites. We analyzed the survival of *Retama* seedlings from experiment 2 separately for each year by using a hierarchical log-linear analysis, with microsite, irrigation and herbivory as fixed factors. To assess for the effects of the factors assayed during the different environmental conditions characterising each year, only those seedlings that survived the previous summer were taken into account for this analysis (for example, to analyze survival of 2008, we only considered those seedlings alive after the summer of 2007). With this approach, we were able to assess the consistency of treatment effects over the years. It also avoids the potential 'dragging' that an extremely strong effect of a particular treatment during a given year may have on the overall net results (i.e. we could detect if herbivory

had strong effects in 2007, but not in the rest of years). Survival data from one year to another were not correlated (Spearman correlation:  $\rho < 0.4$ ,  $p > 0.2$  in all the cases) and thus independency is expected. Since browsing by rabbits removed most aerial biomass of planted seedlings, and initial seedling height after rabbit impact was not measured, we did not consider such height as a covariate in further statistical analysis.

We analyzed gravimetric soil moisture (obtained from TDR data), Fv/Fm and seedling height data by using repeated measures ANOVA, with microsite, irrigation and herbivory as fixed factors. Data were square-root transformed to reach normality and homocedasticity assumptions when necessary. We found significant interactions between time and the treatments evaluated when analyzing soil moisture and Fv/Fm data (data not shown). These interactions can lead to the misinterpretation of the effects of the fixed factors (Quinn and Keough 2002), and are of biological importance, as intra-annual dynamics in water availability can strongly affect the effects of nurse plants (Goldberg and Novoplansky 1997, de la Cruz et al. 2008). To properly assess the effect of the assayed treatments, and to explore how the intra-annual dynamics in water availability modulates them, we grouped both soil moisture and Fv/Fm data for the three study years in wet/dry periods (periods with soil moisture values above and below 10%, respectively) and analyzed them separately using repeated measures ANOVA. We established this 10% value to separate wet/dry periods because it corresponds to a biological threshold that separates periods when most plant activity concentrates (those with soil moisture  $> 10\%$ ) in semi-arid environments (Noy-Meir 1973, Valladares et al. 2005). These analyses could lack independency because results obtained in dry periods were not completely independent from those coming from wet periods. However, by pooling the data from the three study years together (we conducted two separate RM ANOVA, one with dry and another for wet periods data from the three study-years) only consistent results for the three years may result significant and this lack of independency disappears. Furthermore, with this approach we removed the interactions with the assayed treatments and time, avoiding the confounding effect that the strong temporal variability in water availability could have on the interpretation of the main treatment effects (Quinn and Keough 2002).

We evaluated differences in soil moisture between watered vs. unwatered plants (soil moisture measures after the irrigation pulse of June 2007) using a two factor (microsite and irrigation) ANOVA. These data followed the assumptions of this analysis, and thus were not transformed. We conducted all statistical analyses using SPSS 13.0 for Windows.

## Results

### Experiment 1. Nurse plant protection against herbivores

#### *Survival and herbivore damage of unprotected plants*

Fewer plants were browsed by rabbits when growing under *Stipa* canopies than in Open microsites during spring (Fig. 1A; log-rank test,  $p < 0.0001$ ). Rabbit predation intensity on *Retama* seedlings increased during summer drought.

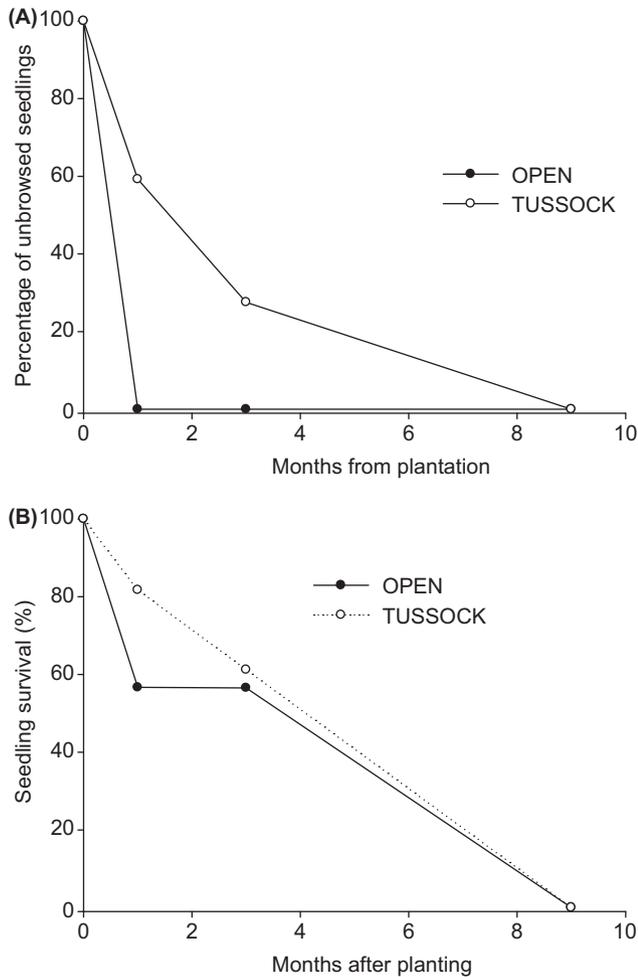


Figure 1. Dynamics of herbivore damage (A) and overall survivorship (B) of unprotected *Retama sphaerocarpa* seedlings growing under the canopy of *Stipa tenacissima* (Tussock) and in bare ground areas (Open) from December 2006 to September 2007.

This, together with the removal of most aboveground biomass produced after each browsing impact, caused the depletion of the resprouting ability of *Retama* seedlings, and all the plants died during summer regardless of the microsite where they were planted (Fig. 1B). Thus, we did not find differences in survival among microsites during the study period (log-rank test,  $p = 0.466$ ).

## Experiment 2. Joint effects of herbivory and abiotic stress

### Plant performance

*Stipa tenacissima* reduced significantly the survival of *Retama* seedlings during the three years of study (2007:  $G^2 = 12.7$ ,  $DF = 1$ ,  $p = 0.005$ ; 2008:  $G^2 = 9.3$ ,  $DF = 1$ ,  $p = 0.002$  and 2009:  $G^2 = 3.6$ ,  $DF = 1$ ,  $p < 0.06$ , Fig. 2). Survival was 17%, 27% and 15% lower in Tussock than in Open microsites for 2007, 2008 and 2009, respectively. Irrigation increased the survival of *Retama* seedlings during the first summer by 27% ( $G^2 = 10.29$ ,  $DF = 1$ ,  $p = 0.001$ ), but did not affect the negative effect of *Stipa*. Browsing damage did not affect mortality rates *per se* in this experiment, but reduced the negative effect of *Stipa* on *Retama* (microsite  $\times$

herbivory:  $G^2 = 7.7$ ,  $DF = 1$ ,  $p = 0.005$ , Fig. 2). We found a significant interaction between prior herbivory damage and water availability in 2009 ( $G^2 = 8.85$ ,  $DF = 1$ ,  $p = 0.003$ ), being the survival of unbrowsed seedlings higher than that of browsed seedlings when these were unwatered. When watered, seedlings that were browsed showed a higher survival rate than those that were not browsed (Fig. 2).

Herbivory decreased seedling height during the three-years of study (RM ANOVA:  $F_{1,171} = 4.6$ ,  $p = 0.036$ ), an effect that was especially evident in 2008 (Fig. 3). We did not detect any microsite or watering effect, neither any interaction between the treatments evaluated or with time, when analyzing seedling height. *Stipa tenacissima* reduced the Fv/Fm ratio of *Retama*

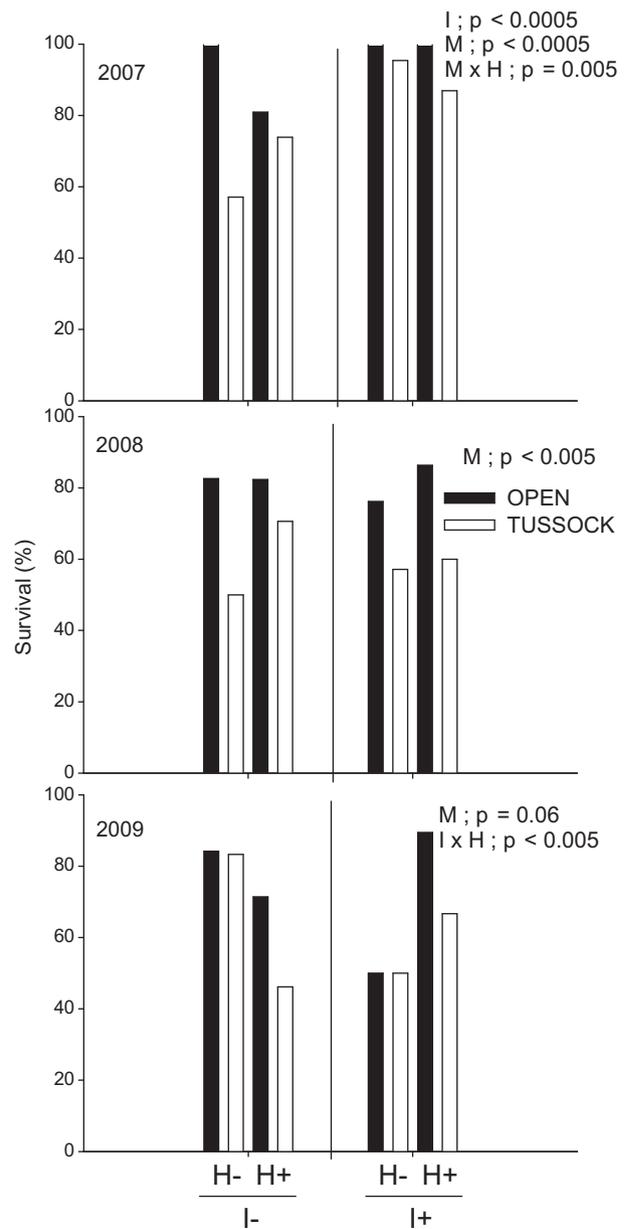


Figure 2. Survival of *Retama sphaerocarpa* seedlings during the three years of study in the eight combinations of treatments evaluated. Open = bare ground areas, Tussock = *Stipa tenacissima* canopies, I- = no irrigation, I+ = irrigation of 50% of the median of April–July period rainfall in four pulses, H- = no herbivore damage, and H+ = seedlings partially eaten by rabbits. Initial  $n = 22$ .

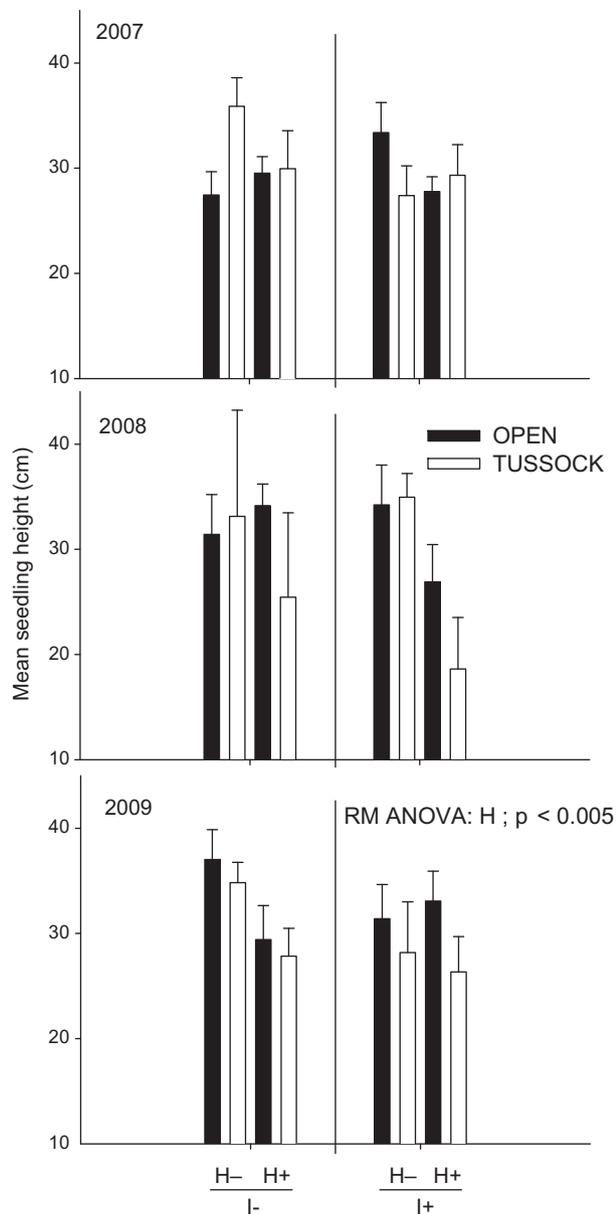


Figure 3. Height of *Retama sphaerocarpa* seedlings during the three years of study in the eight combinations of treatments evaluated (mean  $\pm$  SE; n depended on survival). Rest of legend as in Fig. 2.

seedlings during dry seasons (RM ANOVA:  $F_{1,56} = 9.6$ ,  $p = 0.003$ ), but this effect decreased when plants were watered (microsite  $\times$  irrigation:  $F_{1,56} = 3.9$ ,  $p = 0.05$ ). This microsite  $\times$  irrigation interaction was also found in wet periods, when irrigation reduced the positive effects of *Stipa* on *Retama* Fv/Fm (Fig. 4; Supplementary material Appendix 2A).

#### Soil moisture

2007 was the wettest year of the studied period, with soil moisture levels well above 20% during spring (Supplementary material Appendix 2B). Conversely, 2009 was the driest year, with soil moisture levels below 10% in three of the four periods sampled (Supplementary material Appendix 2B). *Stipa tenacissima* slightly ( $< 2\%$ ) reduced soil water availability during dry periods (RM ANOVA:  $F_{1,56} = 5.02$ ,  $p = 0.029$ ). During wet periods, a significant microsite  $\times$

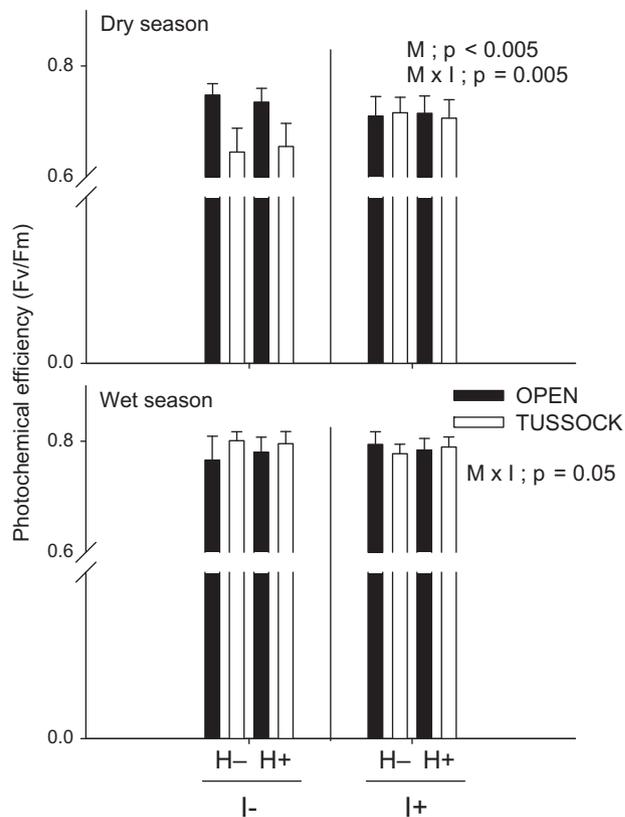


Figure 4. Photochemical efficiency (Fv/Fm) of *Retama sphaerocarpa* seedlings during wet/dry periods (periods above/below 10% gravimetric soil moisture, respectively). Data are means  $\pm$  SE of the three years-study period data pooled by wet/dry seasons;  $n = 8$ . Rest of legend as in Fig. 2.

herbivory interaction was found ( $F_{1,56} = 5.35$ ,  $p = 0.024$ ), with more water available under *Stipa* canopies and Open microsites for browsed and unbrowsed plants, respectively (Fig. 5). Although irrigation increased soil moisture values by an average of 35% after watering (two-way ANOVA,  $F_{1,36} = 8.05$ ,  $p < 0.001$ ), it did not affect soil moisture at the long-term, as this treatment had no significant effects on this variable when analyzing the data gathered during the whole year (repeated-measures ANOVA;  $p = 0.929$ ).

## Discussion

The results of our study highlight the importance of herbivory as a major factor affecting the relationship between plant–plant interactions and abiotic stress. The increase in rabbit pressure during summer drought, indirectly caused by the lack of alternative food resources during this season, overrode the herbivory protection provided by *Stipa* during wetter periods, when rabbit pressure upon *Retama* seedlings was lower. Conversely, the initial loss of biomass produced by rabbit browsing shifted the interaction between *Stipa* and *Retama* from negative to neutral. Our results suggest that the negative effect of *Stipa* on the performance (Fv/Fm) of *Retama* was driven by water availability, but that competition for other resources rather than water modulated the effect of *Stipa* on *Retama* survival. The complex interactions between

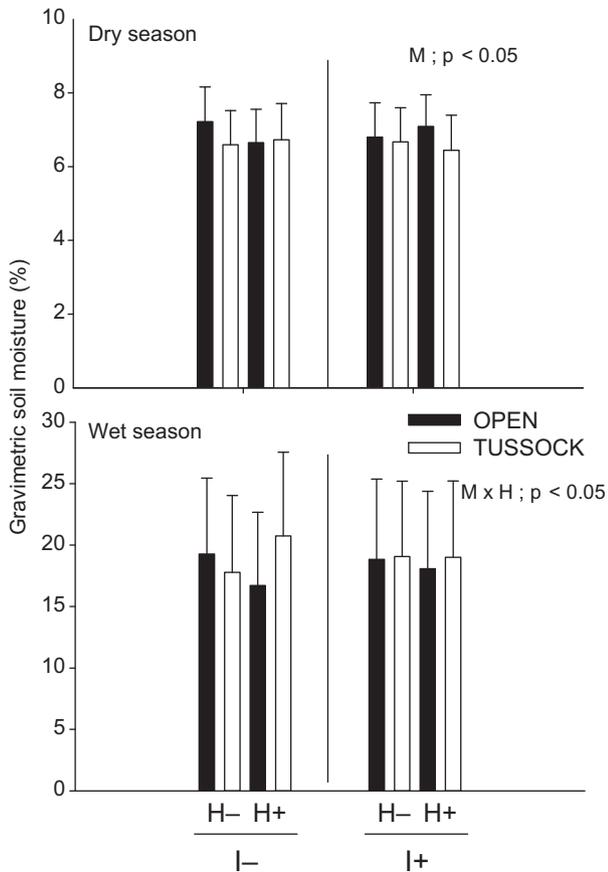


Figure 5. Gravimetric soil moisture (inferred from time-domain reflectometry measurements) during wet/dry periods. Rest of legend as in Fig. 4.

herbivory, abiotic stress and their temporal dynamics as drivers of the outcome of plant–plant interactions highlight the importance of considering these stressors together to fully understand the outcome of plant–plant interactions along environmental gradients (Goldberg and Novoplansky 1997, de la Cruz et al. 2008, Anthelme and Michalet 2009).

### Protection against herbivores by *Stipa tenacissima*

Although *Retama* seedlings were protected from herbivore damage when *Stipa* was present, this effect disappeared during summer drought, when the higher rabbit impact upon *Retama* seedlings overshadowed the refuge effect of *Stipa* (Fig. 1). Annual plants, which provide an important fraction of plant productivity in arid and semi-arid systems (Fischer and Turner 1978), have completed their life cycle before the onset of summer drought in our study area (Peco et al. 2009). Thus, the corresponding increase in rabbit predation upon perennials due to changes in diet produced by the lack of annuals during summer (Rueda et al. 2008) can explain the suppression of this facilitative effect during this season. Similar reductions of facilitative effects under high herbivory pressure have been previously reported (Graff et al. 2007, Smit et al. 2007), and should be common when food resources are less abundant and the same number of herbivores may exert higher pressure on the remaining plants (but see Veblen 2008, Anthelme and Michalet 2009).

### Does shade intolerance of the protégée explain the negative effect of the nurse plant?

In contrast with previous studies using the same nurse plant (Maestre et al. 2001, 2003), we found a net negative effect of *Stipa* on survival of *Retama* seedlings. Plant–plant interactions depend up to a great degree on the identity of the species involved (Callaway 2007), and thus these contrasting results are not fully surprising. Plant competition in drylands is generally attributed to water or nutrients (Whitford 2002). Interestingly, most of the negative effects of *Stipa* on *Retama* were not influenced by increases in water availability, neither were explained by the effect of *Stipa* on this variable. The reduced light availability under the canopy of *Stipa* tussocks (which suppose >80% of incident PAR reduction; Maestre et al. 2003) has been identified as the major driver of the positive effect of *Stipa* on shrub seedlings (Maestre et al. 2003). However, this same shade could be also a key factor affecting the negative outcome of the studied interaction (Soliveres et al. 2010, Seifan et al. 2010). The lack of leaves of *Retama*, and the concentration of the total photosynthetic area on its cladodes, is associated with the high light requirements of this species (Valladares and Pugnaire 1999, Valladares et al. 2003, Espigares et al. 2004), and suggests that a reduction in available light might underlie the outcome observed.

Most plant species adapted to drought are not able to cope with deep shade (Niinemets and Valladares 2006), and therefore, it is likely that species that are more adapted to full sunlight and drought, which are abundant in dry environments, do not benefit from the presence of a nurse plant unless the positive effects of the nurse on the water status of the protégée overcome negative effects promoted by light reduction (Holmgren et al. 1997). The more drought-tolerant the protégé plant is, the less positive the shade effect is expected to be, according to the general ideas discussed in recent revisions of the SGH (Maestre et al. 2009, Malkinson and Tielbörger 2010). The same may happen with different life stages of a given species, as plants are often more shade tolerant early during their ontogeny than in later stages of development, and therefore are more likely to benefit from nurse's shade (Callaway and Walker 1997, Miriti 2006). Our results highlight the species-specific nature of such effects (Callaway 2007), since the same shade that is beneficial for some species (Maestre et al. 2001, 2003) could conceivably be negative for *Retama* or other shade-intolerant plants (Marañón and Bartolomé 1993, Seifan et al. 2010). Thus, more studies involving species with different ecological strategies and drought and shade relative tolerances are needed to improve our understanding on the responses of plant–plant interactions to abiotic stress and herbivory at the entire community level.

### The role of facilitation and resource availability on the recovery of browsed plants

Irrigation increased survival during part of the studied period (Fig. 2) and also the degree of stress experienced by *Retama* seedlings was lower during wet periods. Both results indicate that water was limiting the performance of this species,

regardless of the microsite tested. However, light was also an alternative limiting factor for plant performance in Tussock but not in Open microsites (discussed above). The limited resource model (LRM), which correctly explains most of the relationships between different resource levels and tolerance to herbivory, predicts a differential recovery from tolerance depending on the nature of the limiting resources and the way herbivory damage affect their uptake (Wise and Abrahamson 2005, 2007). Particularly, this model foresees a higher tolerance to herbivory under wetter conditions when water is the only limiting factor (such as in Open microsites), but a lower tolerance under these same wet conditions when herbivory exacerbates the limitation of an alternative resource, light in our case (such as in Tussock microsites). This latter outcome is explained because plants growing under drought are already at their optimum light conditions, and are mainly limited by water availability, while plants growing under *Stipa* canopies are closer to their optimum moisture conditions, but also limited by light. Thus, the latter plants will be much more sensitive to the reduced uptake of light (their alternative limiting resource), and therefore to the loss of biomass produced by herbivory, than the former (Baraza et al. 2004, Wise and Abrahamson 2005, 2007).

Following these predictions, we may expect lower tolerance to herbivory in Tussock than in Open microsites, especially in watered plants, since plants growing beneath the canopy of *Stipa* should be more limited by light, while plants growing in Open microsites should be limited only by water. However, our results did not fully match predictions from the LRM, maybe because our target plant was a resprouter, and therefore its tolerance to herbivory might be affected not only by the amount of resources available, but also by the reserves of each seedling might have (Vesk et al. 2004). In contrast, a higher survival in browsed plants was found when they were watered, regardless of the microsite where they were planted. This finding could indicate an overcompensation of browsing damage by plants when environmental conditions were more benign (Crawley et al. 1998). Why the LRM predictions did not correctly explain the effects of shade provided by *Stipa* on *Retama* recovery after herbivory damage? Shade provided by *Stipa* might increase the water status of *Retama* seedlings by a reduction in transpiration (Holmgren et al. 1997). Thus, it is likely that this improvement in the water status of *Retama* seedlings increased their tolerance to herbivory in a similar way that watering did it in Open microsites. This positive effect in the recovery from herbivory shifted the negative effect that this same shade exerted on *Retama* survival on unbrowsed plants (we found a significant microsite  $\times$  herbivory interaction when analyzing seedling survival). The compensation of resource competition due to herbivory protection has been observed in previous studies in semi-arid environments (Graff et al. 2007). Our results suggest that this compensation also occurs when the nurse plant improves recovery from herbivory, and highlight the complex interaction between water stress and herbivory as drivers of the outcome of an interaction strongly influenced by shade.

### Temporal dynamics in water availability modulate the effect of *Stipa* on *Retama*

In contrast with our initial hypothesis, *Stipa* negatively affected *Retama* survival during the three years of study,

regardless of the inter-annual variation in rainfall availability among years. This may be explained because the studied interaction was mainly driven by light competition, and therefore water availability only played a secondary role in its outcome. However, intra-annual dynamics in water availability and our irrigation treatment modulated the effect of *Stipa* on the stress level experienced by *Retama* seedlings. Goldberg and Novoplansky (1997) proposed a conceptual model to introduce the effect of intra-annual resource dynamics on plant–plant interactions. In their model, nurse plants affected negatively protégée growth during pulses (our wet seasons) due to competition by resources, while increased survival during periods with low nutrient availability (our dry seasons). The final outcome would depend on how much the negative effect on growth during wet seasons affects survival during dry periods, and on the relative importance of plant uptake or abiotic factors affecting resource availability during these dry periods (Goldberg and Novoplansky 1997, Hastwell and Facelli 2003). However, the effects of *Stipa* on the Fv/Fm (our surrogate of plant stress) of *Retama* seedlings during wet/dry seasons found differed from the expected responses arisen from the predictions of Goldberg and Novoplansky (1997). Specifically, we detected a trend towards facilitation and competition during wet and dry seasons, respectively (although it must be considered that we only measured the degree of stress experienced by *Retama* and not its growth or survival seasonally, which could be a better test for this model). Differences produced in the outcome of the interaction studied within these seasonal dynamics varied with irrigation, which overall suggest a reduction of competition intensity at intermediate levels of abiotic stress (watered plants during summer or unwatered plant during spring), but a prevalence of competition in the rest of assayed situations, as suggested by the refined SGH when the two species involved are stress tolerators (Maestre et al. 2009).

### Concluding remarks

Collectively, our experiments indicate the existence of a complex hierarchy of mechanisms affecting the interaction studied (Baumeister and Callaway 2006). In our case, water shortage per se was irrelevant under extremely high herbivory impacts (all seedlings died in experiment 1 but none of them due to drought), but indirectly modulated herbivory impacts by affecting alternative rabbit food resources. Conversely, the initial loss of biomass produced by herbivory was a major factor affecting the outcome of the interaction studied. *Stipa* exerted a negative effect on *Retama* seedlings mainly by light competition, but this shade improved seedlings recovery after herbivory, overriding the negative effects found on unbrowsed plants. Our findings illustrate how the complex interactions between herbivory and water stress jointly influence the outcome of plant–plant interactions. They provide insights to fully understand the interplay between facilitation and competition, and they can be used to further refine conceptual models aiming to predict the outcome of plant–plant interactions along composite stress gradients.

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Supplementary material (available online as Appendix O18993 at <[www.oikosoffice.lu.se/appendix](http://www.oikosoffice.lu.se/appendix)>). Appendix 1–2.