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Changes in rainfall amount and frequency do not affect the outcome of the interaction between the shrub *Retama sphaerocarpa* and its neighbouring grasses in two semiarid communities

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Abstract

We evaluated the net outcome of the interaction between the shrub *Retama sphaerocarpa*, our target plant, and different herbaceous neighbours in response to changes in the magnitude and frequency of rainfall events during three years. The experiment was conducted in natural and anthropogenic grasslands dominated by a perennial stress-tolerator and ruderal annual species, respectively. In spite of the neutral or positive effects of neighbours on water availability, neighbouring plants reduced the performance of *Retama* juveniles, suggesting competition for resources other than water. The negative effects of grasses on the photochemical efficiency of *Retama* juveniles decreased with higher water availabilities or heavier irrigation pulses, depending on the grassland studied; however, these effects did not extend to the survival and growth of *Retama* juveniles. Our findings show the prevalence of competitive interactions among the studied plants, regardless of the water availability and its temporal pattern. These results suggest that positive interactions may not prevail under harsher conditions when shade-intolerant species are involved. This study could be used to further refine our predictions of how plant-plant interactions will respond to changes in rainfall, either natural or increased by the ongoing climatic change, in ecosystems where grass-shrubs interactions are prevalent.

Keywords

competition; pulse-driven ecosystems; roadside slope; shrub encroachment; *Stipa tenacissima* L.

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Supplementary material

Appendix A. Kml file with the coordinates of the study sites

1. Introduction

The study of the dynamics of plant-plant interactions along gradients in water availability has been a major topic in dryland ecology during the past decade (e.g. Brooker et al., 2008; Pugnaire et al., 2011). The relationship between plant-plant interactions and water stress gradients is particularly complex in arid and semiarid environments (Holmgren et al., 1997; Goldberg and Novoplansky, 1997), that experience marked, and sometimes erratic, fluctuations in water availability (Schwinning and Sala, 2004; Miranda et al., 2011). The natural temporal dynamics of water availability is likely to be affected by the global changes in climate in semiarid environments, with forecasted reductions in the amount of rainfall and increases in the frequency of high intensity showers in the Mediterranean Basin (IPCC, 2007). Temporal changes in rainfall availability should be considered, therefore, in any study of plant-plant interactions and community dynamics in water-limited ecosystems. This is necessary to be able to better predict the response of plants to natural and future changes in rainfall patterns (Tielbörger and Kadmon, 2000; Miranda et al., 2011).

Grass-shrub interactions are particularly instructive for studying the effects of these rainfall patterns on the outcome of plant-plant interactions because the two growth forms differ markedly in their distribution and abundance, root architecture, and water acquisition strategies (Sala et al., 1989; Scholes and Archer, 1997). While grasses tend to use the water derived from small rainfalls more efficiently, shrubs generally perform better after continuous and large rainfall events, which recharge deeper soil profiles (Sala et al., 1989; Schwinning and Sala, 2004). These grass-shrub interactions are thus likely to be affected by the reduction in rainfall amount and the increases in the frequency of large rainfall events that are predicted under future climate change scenarios. Altered rainfall will also likely affect the degree of niche partitioning and enhance coexistence among the two groups (Ogle and Reynolds, 2004). Additionally, and in spite of their differential use of water sources, facilitation and competition between grasses and shrubs does exist (e.g. Tielbörger and Kadmon, 2000; Soliveres et al., 2012) either mediated by water or by other resources such as light or nutrients (Holmgren et al., 1997; Scholes and Archer, 1997). These grass-shrub interactions might also be sensitive to alterations in water availability; for example, longer summer droughts reduce the recruitment of shrub species (López et al., 2008), and grasses may foster the survival and recruitment of shrub seedlings under moderate, but not extreme, drought conditions (Maestre and Cortina, 2004; Anthelme et al., 2007). The potential responses of these interactions to changes in rainfall amount and frequency may cause abrupt shifts in the sign of grass-shrub seedlings interactions and in the communities dominated by these plant types. However, while the response of grass-shrub interactions across contrasting water regimes is relatively well studied (reviewed in Brooker et al., 2008; Pugnaire et al., 2011), the response of these interactions to larger and multiple level gradients in temporal patterns and abundance of current or future rainfall is poorly understood (Tielbörger and Kadmon, 2000; Miranda et al., 2011). The study of responses across such multi-level gradients is needed in order to improve our predictions and therefore theoretical models of the relationship between plant-plant interactions and abiotic stress (Lortie, 2010).

In this study, we aimed to test the response of grass-shrub interactions to water pulses differing in amount or frequency in two semiarid communities from central Spain: a natural *Stipa tenacissima* L. steppe (hereafter called “natural grassland”) and an annual-dominated grassland located in a motorway slope (hereafter called “anthropogenic grassland”). Studies focusing on both natural and novel ecosystems can provide insights that allow us to improve predictions of the response of plant communities to differing water regimes (Brooker, 2006). Our focal target species was the leguminous shrub *Retama sphaerocarpa* (L.) Boiss. (hereafter *Retama*) and our focal neighbour species were the stress-tolerator tussock grass *Stipa tenacissima* (syn. *Macrochloa tenacissima* (L.) Kunth.) in the natural grassland, and several ruderal annual species in the anthropogenic grassland. The study of the factors affecting *Retama* establishment is of particular interest because it allows us to improve our knowledge of plant community dynamics and restoration in semiarid Mediterranean environments. In these environments, *Retama* is a keystone species, due to its positive effects on soil fertility (e.g. Caravaca et al. 2003) associated with facilitative effects on other plant species (Pugnaire et al., 1996a). We manipulated the amount and frequency of available water to create a gradient in water stress, and tested the following hypotheses: i) Both *Stipa* and annual species will have positive effects on the performance of *Retama* seedlings due to their microclimatic amelioration (Holmgren et al., 1997; Goldberg and Novoplansky, 1997); ii) *Stipa* and annual species will differ in their effect on *Retama* seedling survival under heavy water shortage. Specifically, *Stipa* could reduce *Retama* seedling survival as the increased competition for water might outweigh the positive environmental buffering promoted by shade in this ecosystem (Holmgren et al., 1997; Maestre and Cortina, 2004). Conversely, as annuals die during summer, their positive effects on *Retama* seedling survival will persist under the driest conditions because of the lack of strong competition and the positive effects expected from the shade produced by their dry tissues (Soliveres et al., 2012); and iii) Less frequent but heavier irrigation events will reduce competition between *Retama* and both *Stipa* and annual species due to the water reaching deeper soil layers and promoting niche segregation (Sala et al., 1989; Ogle and Reynolds, 2004).

2. Material and methods

2.1. Study area

Both the natural and anthropogenic grasslands selected for this study are located in the center of the Iberian Peninsula (about 19 and 44 km South from Madrid [Spain] for the anthropogenic and natural grasslands, respectively; see Appendix A in the Supplementary material for the exact location) and share the same climate and soil type. The climate is semiarid Mediterranean, with an average annual precipitation and temperature of 388 mm and 14 °C, respectively, and with a strong summer drought (Aranjuez weather station; 1994-2005 period; Marqués et al., 2008). Both communities are located on gypsum-rich soils, classified as *Xeric Haplogypsid* (Marqués et al., 2008), although in the anthropogenic grassland the original substrate has been altered by the mixture with gravel and components from external sources during the construction of the motorway (García-Palacios et al., 2010). Vegetation in the natural grassland is an open steppe dominated by *Stipa tenacissima*, with a perennial plant cover of 24%. Vegetation in the anthropogenic grassland is dominated

by annuals, with a mean cover of 75% and with *Bromus rubens* L., *B. diandrus* Roth., and *Medicago sativa* L. as the most abundant species (16, 14 and 14% of the total cover, respectively; García-Palacios et al., 2010). Hereafter we refer to these as herbaceous annuals for simplicity.

2.2. Experimental design

In December 2006, 176 two-year old *Retama* juveniles, with a mean height of 27 ± 2 cm, were hand planted in each site in 20×20×20 cm holes. All plants were watered with 0.5 l of water when planted, to enhance establishment. These juveniles were obtained from a nursery in central Spain (viveros Bárbol, Madrid) and were grown in Forest Pot 300 containers (Nuevos Sistemas de Cultivo S.L., Girona Spain) with a volume of 300 ml and a depth of 18 cm. We randomly assigned these juveniles to two different microsites: “Neighbour” and “Open”. Because of the heterogeneous patch-interpatch structure of the natural grassland, and the homogenous herbaceous cover of the anthropogenic grassland, these microsite types were defined differently in the two ecosystems. Neighbour microsites were located: 1) upslope and adjacent to *Stipa* tussocks of ca. 1 m width and over 80 cm height (< 15 cm from the edge of the north face of the tussock, where facilitative effects of this species on target shrubs have been found, e.g. Maestre et al., 2003) in the natural grassland, and 2) in a vegetation patch of 50 cm diameter in the anthropogenic grassland. The patch consisted of several annual species forming a vegetation cover of 75-100% (in spring) with a height of approx. 40 cm. These were either located in bare ground areas at least 80 cm away from any perennial plant (natural grassland), or placed in sites where all aboveground vegetation within circles of 80-cm diameter surrounding the target seedling was clipped monthly (anthropogenic grassland). We acknowledge that we could not prevent competition of their roots for water just by clipping the aboveground material on neighbouring grasses. However, we could not remove roots because of the important effect that this might have on soil infiltration and therefore on our watering treatment. Moreover, with repeated aerial clipping, non-resprouter species were effectively removed and, eventually, the treatment should also importantly reduce the reserves and resprouting ability of resprouter species. Hence, we believe that our treatment effectively reduced competition between herbaceous plants and our target species and that root competition should be negligible within our Open microsites.

Because of the high density of rabbits observed at both study sites (S. Soliveres *pers. obs.*), and to avoid seedling predation, juveniles were protected from browsing by surrounding them with a frame of thin wire mesh. This mesh did not shade the juveniles, and thus did not confound the effects of any of the factors studied in the experiment.

The juveniles were randomly allocated to establish a fully factorial experiment with two factors: Microsite (Neighbour vs. Open) and Irrigation. The irrigation treatment comprised four levels, three of which corresponded to different amounts of water applied during eight watering pulses (once every month between April and July in both 2007 and 2008). In each watering event, either 0% (control), 25% (amount 1), or 50% (amount 2) of the median from the last 30 years for the particular month was applied. For amount 1 we therefore added 5, 10, 11, and 5 mm of water per month, whereas for amount 2 we added 11, 20, 22, and 10

mm of water per watering event, respectively. The fourth treatment consisted of applying the same amount of water employed in the +50% treatment, but with two instead of four pulses per year (hereafter +50%-2X; 31 and 32 mm of water applied per watering event). This latter treatment was applied in May and July in both 2007 and 2008. In each of the watering events applied in the +50%-2X treatment, the amounts of water added equaled the sum of April and May, and June and July irrigations applied in the +50%-4X treatment, respectively (Fig. 1). Both irrigation treatments were applied regardless of the natural rainfall received each month (Fig. 1) and over an area of *ca.* 0.25 m². Hence, we applied the fourth amount of water described above in each watering event, proportionally to the amount of rain that would fall in 0.25m² areas. With this design we aimed to evaluate the effects of two characteristic rainfall patterns on the outcome of plant-plant interactions: 1) changes in the length of summer droughts and in rainfall abundance during the growing season (summer drought was longer and spring rainfall was less abundant in less-watered plants [+0% > +25% > +50%]), and 2) changes in the frequency of heavy showers (the magnitude of individual watering events was higher in the 2X than in the 4X treatment, despite both treatments received the same amount of water).

2.3. Monitoring of soil moisture and plant performance

Soil moisture was measured by time-domain reflectometry (TDR; Topp and Davis, 1985) using a Campbell TDR100 system (Campbell Scientific Ltd, Loughborough, UK). In 10 randomly selected planting holes per treatment combination, 10 cm long TDR probes were vertically installed in the topsoil ($n = 80$). We selected this soil depth (0-10 cm) because we expected that most of the interactions between *Retama* seedlings and grass neighbours would occur in the near surface horizons, which contained most of the grass and shrub roots (Scholes and Archer, 1997). Further, water at the top (0-10 cm) soil layer has been identified as one of the main drivers of shrub and grass performance in other semiarid tussock steppes (Cipriotti et al., *in press*) and was related to our own measurements of plant water status (see $\delta^{18}\text{O}$ Results below). A strong relationship between TDR values and soil gravimetric moisture has been found in soils of the study area ($R^2 = 0.84$; $P < 0.001$; Soliveres et al., 2011); thus, this measurement can be considered a good proxy for soil moisture availability. Soil moisture was measured every two months between April 2007 and September 2009, except during the summer months (June - September) when sampling was conducted monthly. These measurements were taken before the irrigation treatment to avoid noise when comparing months with/without irrigation and to avoid overstating the response of soil moisture to the irrigation treatment if this response only lasted a few days.

The outcome of plant-plant interactions can vary widely depending on the performance measure used (Goldberg and Novoplansky, 1997; Maestre et al., 2005). Thus, as recommended when studying these interactions along environmental gradients (Brooker et al., 2008), we used several performance measures to test the effect of neighbours on *Retama* juveniles. Seedling height, root collar diameter and survival were measured at the end of each summer, in September 2007, 2008 and 2009. A strong relationship between standing biomass and root collar diameter has been shown previously for *Retama* juveniles ($R^2 = 0.823$, $P < 0.001$; Soliveres et al., 2011), so root collar diameter was used as our surrogate of

seedling biomass in the field. We considered as dead, any plants with no green and non-flexible tissues and with no resprouting detected during the next sampling period.

We also estimated the potential photochemical efficiency of *Retama* juveniles by measuring the maximum quantum yield of PSII (Fv/Fm) of dark-adapted green stems (30 min) with a pulse-modulated fluorometer (FMS2, Hansatech Instruments, Norfolk, UK). Measurements were taken from 12.00 to 14.00 GMT, during maximum solar radiation. We were interested in changes in the stress level of our target plants, rather than on the natural variability in photochemical efficiency through the year. Thus, we kept incident radiation constant at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ through all these measurements, interpreting changes in the Fv/Fm ratio as changes in plant performance. Fv/Fm has been widely used as an indicator of plant stress in semiarid regions (e.g. Pugnaire et al. 1996b; Maestre et al., 2003; Aragón et al., 2008); small drops in photochemical efficiency have been associated with water limitations during important stages of the lifecycle of woody plants in these environments (Aragón et al., 2008), and have been found to match results with other performance measures (e.g. survival) when evaluating the outcome of plant-plant interactions (e.g. Maestre et al., 2003). Six juveniles per treatment combination and grassland type were randomly selected for these measurements ($n = 48$ per grassland). Different randomly selected plants were measured in each sampling period, which took place on the same dates than TDR surveys (see above).

Finally, green stem tissue from *Retama* juveniles was also sampled for stable carbon and oxygen isotope composition ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$). Plant tissue was taken in June, at the end of the main growing season, during the second year of the experiment (2008). We only sampled green tissue grown during that year. The samples were oven-dried (60°C , 72 h) and ground to fine powder using a ball mill. Stable isotopes are integrated measurements of the physiological status of the plant during the entire growing period of sampled tissues, and therefore improve the interpretation of ecophysiological measurements (Dawson et al., 2002). $\delta^{13}\text{C}$ provides a time-integrated proxy of plant water use efficiency (WUE; the ratio between photosynthesis and stomatal conductance) and has been successfully used to assess the effects of plant-plant interactions on WUE in semiarid environments (see Dawson et al., 2002 for a review). On the other hand, $\delta^{18}\text{O}$ is inversely related to stomatal conductance and transpiration rate. This variable guides the interpretation of $\delta^{13}\text{C}$ data by providing information about stomatal conductance, independent of any effects of photosynthetic rate on $\delta^{13}\text{C}$. Thus, the combined use of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ data allows us to infer long-term effects of environmental factors on the stomatal conductance, photosynthetic capacity, and their relationship with WUE (Scheidegger et al., 2000). $\delta^{13}\text{C}$ analyses were conducted at the University of California Davis Stable Isotope Facility, using a continuous flow isotope ratio mass spectrometer (CF-IRMS; Europa Scientific, Crewe, UK), with the dual-isotope mode interfaced with a CN sample converter. The standard was Pee Dee Belemnite. The oxygen isotope signature is expressed in $\delta^{18}\text{O}$, relative to the internationally accepted standard (Vienna Standard Mean Oceanic Water, VSMOW), and was analyzed in a Heckatech HT Oxygen Analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The long-term standard deviation in measurements against laboratory standard samples is lower than 0.5 ‰ for both isotopes. The water used for irrigation was taken from areas < 5 km to the study areas trying to minimize the confounding effect that the

different isotopic compositions of rainfall and irrigation water may have on plant isotopic composition.

2.4. Statistical analyses

Since the anthropogenic and natural grasslands are likely to differ in many different ways (e.g. different neighbour species and plant community structure and composition, past management history and different manipulative approaches to assess for plant-plant interaction outcomes), separate analyses were performed for each area. Soil moisture (TDR), seedling survival and diameter were measured repeatedly through time in the same samples. Thus, we used Generalized Estimation Equations (GEEs; Liang and Zeger, 1986) to analyze them. GEEs are a quasi-likelihood method based upon Generalized Linear Models (GLMs) that allow for the analyses of repeated measurements designs with flexibility in the distribution and nature of the variables, in the modeling of the temporal covariance and in the inclusion of covariates that change over time (Halekoh et al., 2006). We used link functions for Gaussian distributions of errors to model TDR measurements (square-root transformed), and diameter (square-root transformed), and binomial distribution of errors to model survival. In all cases, we used the autoregressive temporal correlation (CorAR1), that assume a t-1 lag in the temporal correlation among sampling dates (i.e. the measurements of time t are highly related to the measurements of time t-1). We introduced initial seedling height as a covariate in the analyses regarding plant diameter and survival, to control for initial differences in seedling status that could affect our results.

The potential photochemical efficiency (Fv/Fm) was measured repeatedly through time, but using different juveniles; hence, no temporal autocorrelation was expected. The data were heavily skewed and with heterogeneous variance; thus, we analyzed this data using a mixed model with the semi-parametric PERMANOVA approach (Anderson 2001), with time as a random factor and microsite and irrigation as fixed factors. For these analyses, data were initially normalized (subtracting the mean and dividing by their standard deviation), and we used Euclidean distance and 999 permutations. To increase confidence to these results, we also analyzed Fv/Fm data without any previous transformation and Bray-Curtis distance, and also with Generalized Linear Mixed Models (GLMMs) assuming either a normal or a Poisson distribution. All the analyses rendered very similar results, so we applied the PERMANOVA with normalized variables and Euclidean distance.

The $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were analysed separately by using GLMs, with microsite and irrigation as fixed factors and the identity-link function. In all cases, separate analyses were performed for the natural and the anthropogenic grasslands. GEEs, GLMs and GLMMs were conducted using the “geepack” (Halekoh et al., 2006) and “MASS” packages for R version 2.10.1 (R Development Core Team 2009). PERMANOVA analyses were carried out with PERMANOVA+ for PRIMER (PRIMER-E Ltd., Plymouth Marine Laboratory, UK).

3. Results

3.1. Soil moisture

The irrigation treatments produced contrasting results depending on the type of grassland. In the anthropogenic grassland, increasing water supply increased soil moisture by ~18%, particularly for those treatments with a higher frequency of watering (25% and 50%-4X, but not 50%-2X). In the natural grassland, however, the irrigation treatment did not affect soil moisture. The effect of microsite on soil moisture also differed between grasslands. While *Stipa* increased (~19%) soil moisture in the natural grassland, annuals did not affect soil moisture in the anthropogenic grassland (Fig. 2). There were no significant interactions among the factors for soil moisture, with all interaction terms excluded from the most parsimonious model (saturated model vs model without interactions: $\chi^2 = 1.92$; df = 4, $P = 0.75$ for the anthropogenic grassland, and $\chi^2 = 14.1$; df = 4, $P = 0.37$ for the natural grassland).

3.2. Plant performance

Neighbors reduced the survival of *Retama* juveniles in the two grasslands studied (Fig. 3). Neither irrigation nor initial height significantly affected the survival of *Retama* juveniles. Indeed, the difference between the saturated model and the model without these predictors was not significant ($\chi^2 = 14.1$, df = 13; $P = 0.37$ for the anthropogenic grassland, and $\chi^2 = 3.24$, df = 13; $P = 0.99$ for the natural grassland). Similarly, results for root collar diameter showed a negative effect of neighbours in both grasslands, irrespective of irrigation level (Fig. 4). However, for root collar diameter, the Microsite $\times$ Time interaction was highly significant, with an increasing negative effect of neighbours through time. Higher irrigation levels (25%, 50%-4X, but not 50%-2X; *post-hoc* tests not shown), increased root collar diameter in the anthropogenic, but not in the natural, grassland.

$\delta^{13}\text{C}$ was not significantly different between the two study sites, demonstrating the mostly neutral effect of neighbours on the WUE of *Retama* juveniles (Table 1). However, $\delta^{18}\text{O}$ was significantly higher in Open than in Neighbour microsites in the natural grassland ($F_{1,81} = 5.02$; $P < 0.05$), suggesting higher transpiration rates in juveniles growing beneath *Stipa* canopies than in open areas. In the natural grassland, irrigation shifted microsite effects on $\delta^{18}\text{O}$ (Microsite $\times$ Irrigation: $F_{3,81} = 2.65$; $P = 0.05$). While $\delta^{18}\text{O}$ was higher in open microsites when juveniles were irrigated, regardless of the amount and frequency of water pulses, $\delta^{18}\text{O}$ was lower in the open than under *Stipa* in the unwatered plants. Neither Microsite nor Irrigation significantly affected $\delta^{18}\text{O}$ in the anthropogenic grassland.

All the three factors interacted to determine the Fv/Fm of *Retama* juveniles in both grasslands. Neighbours reduced the photochemical efficiency of *Retama* juveniles (Microsite: $F_{1,680} = 10.6$ and $F_{1,643} = 6.6$ for the anthropogenic and natural grasslands, respectively; $P < 0.05$ in both cases). However, these negative effects were only evident during summer; being neutral or slightly positive during the rest of the year (Microsite $\times$ Time: $F_{16,680} = 4.6$ and $F_{16,643} = 4.0$ for the anthropogenic and natural grasslands, respectively; $P = 0.001$ in both cases). Increasing water supply increased the photochemical efficiency of *Retama* juveniles in the anthropogenic grassland, and reduced the negative

effects of annual grasses (Microsite $\times$ Irrigation: $F_{3,680} = 4.8$; $P = 0.01$; Fig. 5). Annuals reduced the photochemical efficiency of the target juveniles in all but the 50%-4X treatment, where the effects of annual grasses were mostly neutral (Fig. 5). In the natural grassland, the effect of irrigation changed through time and with the microsite (Microsite $\times$ Irrigation $\times$ Time: $F_{48,643} = 1.7$; $P < 0.01$). All the irrigation treatments produced similar results during the first year of study, and did not reduce the negative effect of *Stipa*. During the second year, however, the 50%-2X treatment (highest water supply with lowest frequency) significantly reduced the negative effect of *Stipa* on the Fv/Fm of *Retama*, and even turned it to positive during the summer drought. Results for the third year followed a similar trend, but with weaker effects of the factors studied (Fig. 5).

4. Discussion

Our study is one of a few to address changes in shrub-grass interactions in response to the high variability in the frequency and amount of water availability in semiarid environments (but see Tielbörger and Kadmon, 2000; Miranda et al., 2011). Moreover, the water levels we chose are based on levels predicted to result from changes in rainfall patterns in response to the ongoing global change. Further, we applied these treatments to two different communities with different herbaceous neighbours. There are few studies reporting shifts in facilitation/competition along environmental gradients where more than two levels of resources are compared (see Brooker et al., 2008; Lortie, 2010 for reviews). Our study, therefore, can lead to a deeper understanding of the exact form of the relationship between plant-plant interactions and abiotic stress (Lortie, 2010). Competition for water or the improvement of water status by neighbours have been invoked as important mechanisms driving competition or facilitation in drylands, respectively (e.g. Holmgren et al., 1997; Maestre et al., 2003). However, we found contradictory effects of neighbours on soil moisture (neutral or positive) and seedling performance (negative), which suggest that competition for factors other than water were more important in the final outcome of the interaction studied. These results are reinforced by the findings that, despite the increase in transpiration rate (measured with $\delta^{18}\text{O}$) in juveniles growing beneath neighbour plants, neither survival, growth, water use efficiency ($\delta^{13}\text{C}$) nor potential chlorophyll fluorescence (Fv/Fm) of *Retama* juveniles were improved by their presence.

4.1. Negative effects of neighbours on shrub performance: water or light competition?

Since *R. sphaerocarpa* is present in environments with much lower rainfall availability (e.g. Pugnaire et al., 1996a), it could be argued that the prevalence of negative interactions found for this species relates to the relative mild conditions of the study. Under the rainfall availability of our study sites, a relatively low stress would be expected for a drought-adapted shrub such as our target species, and therefore positive interactions would be less likely to occur (Holmgren et al., 1997; Brooker et al., 2008). However, we believe that the stress level experienced by our target species in the study sites was sufficiently high (ca. 60% of mortality in both study sites; Fig. 3) to allow positive interactions to occur. Rather than these mild environmental conditions, the well-known shade intolerance of *Retama sphaerocarpa* (Valladares et al., 2003) arises as a suitable potential explanation for the prevalence of negative interactions among the species we studied. This shade intolerance

suggests that light competition is a key factor explaining the contrasting results between water availability and plant performance found in our study. Although we did not measure shade in our experiment and therefore we cannot be certain, it is likely that the canopy of neighbours could shade *Retama* juveniles enough to limit carbon gain (reductions of over 80% in photosynthetically active radiation found for *Stipa*; Maestre et al., 2003), causing the reduction in growth and survival that we measured. Furthermore, previous greenhouse experiments using the same target species support this finding (Espigares et al., 2004).

Surprisingly, this effect remained negative regardless of the watering level applied or the neighbour tested, and contrasts with most current theory, and our own initial hypotheses, regarding plant-plant interactions in drylands. One potential explanation is that our watering treatment did not provide a significant water gradient since it did not markedly affect *Retama* survival or growth. However, changes in rainfall patterns and abundance similar to those applied here are predicted under future climatic scenarios in our study area, and these have been shown to substantially affect plant communities in similar environments (Miranda et al., 2011). The lack of response of our target plant, rather than suggesting an ineffective water gradient, is itself an important result that suggests a high resilience of this species to changing rainfall patterns and scarcer water inputs. How general this result is for other Mediterranean semiarid species clearly deserves further attention. The beneficial effect of shading provided by neighbours is thought to increase with water stress (Holmgren et al., 1997). Our results, however, show that competition remains or even increases, rather than decreases, with abiotic stress when the beneficiary is a shade-intolerant species. These results are supported by three different lines of evidence: i) those studies showing a more detrimental effect of shade under drought than under wetter conditions in Mediterranean environments (Valladares and Pearcy, 2002), ii) those studies showing detrimental effects of shading by neighbours for different species living under arid or semiarid conditions worldwide (USA: Forseth et al., 2001; Australia: Prider and Facelli, 2004; Jefferson and Pennacchio, 2005; Israel: Seifan et al., 2010; Spain: Soliveres et al., 2010; 2012; Chile: Becerra et al., 2011), and iii) by the prevalence of functional traits associated with shade-intolerance in open microsites rather than under the canopy of nurse plants even under the extreme aridity found in desert ecosystems of western USA (Butterfield and Briggs, 2011). The generality of these results is also supported by the consistency in the effects found in this study in spite of the different manipulative approaches (removal/no removal of neighbours vs. open/neighbour microsite selection) used, which are known to affect the outcome of plant-plant interactions (Maestre et al., 2005; Brooker et al., 2008). Neighbour plants may exert a positive effect on the availability of one resource (i.e. water); however, the overall effect of a neighbour on a particular target species will be negative if shading overcomes the physiological limits that this particular target species can withstand, regardless of its effect on water availability (Holmgren et al., 1997; Malkinson and Tielbörger, 2010). In arid and semiarid environments, an important proportion of the species present have developed adaptations to water scarcity and excessive radiation that may illicit a negative response to neighbour plants. Consequently, while positive interactions among plants could be expected under drier conditions for shade-tolerant and drought-sensitive species (Holmgren et al., 1997; Butterfield and Briggs, 2011), negative interactions could be

dominant for shade-intolerant and drought-resistant ones, regardless of the environmental conditions.

4.2. Changes in rainfall frequency and potential for niche segregation

Niche segregation is among the most important factors allowing grass-shrub coexistence in semiarid environments (Sala et al., 1989; Scholes and Archer, 1997). Niche segregation should, therefore, enhance coexistence between *Retama* juveniles and their neighbours since these juveniles are able to reach deep soil layers soon after its establishment (Padilla and Pugnaire, 2007). Niche segregation may also work even if competition among woody juveniles and grasses is mainly driven by light and not by water. Once woody juveniles grow enough to escape from light competition, a shift in the sign of their interaction with neighbours would be expected because the negative effects of shade disappear, but the positive effects on plant water status remain (Soliveres et al., 2010). This would explain the positive interactions found for adult *Retama* individuals and its neighbours (Pugnaire et al., 1996a), or the reduction in competition between *Stipa* and other pioneer shade-intolerant shrubs with the aging of the latter (Armas and Pugnaire, 2005; Soliveres et al., 2010).

Our third hypothesis was a strong reduction of the competition between *Retama* juveniles and their neighbours with heavier watering pulses (the 2X treatment). Contrary to our expectations, however, the differences in the frequency of water pulses (2X vs 4X treatments) did not increase seedling survival nor growth, but only reduced the stress level, of *Retama* juveniles growing beneath grasses in the studied sites. Although the effects found on the stress (Fv/Fm) level experienced by *Retama* juveniles did not affect survival or growth at the short-term, we speculate that it could increase performance of *Retama* juveniles in the longer term (Aragón et al., 2008), releasing them from grass competition and promoting niche segregation.

Considering Fv/Fm measurements, the effects of the two different watering frequencies varied with grassland type. While more frequent water inputs (4X treatment) reduced competition in the anthropogenic grassland, less frequent but heavier water inputs (2X treatment) had the same effects in the natural one. Since we did not measure soil moisture at different levels, we can only speculate about the effect of our watering treatments in recharging the deep and superficial soil profile. However, we believe that the most parsimonious explanation for this differential response between grassland types is due to differences in the life-strategy (annuals vs. perennials) of the neighbours (Gómez-Aparicio, 2009). In the anthropogenic grassland, more frequent, rather than heavier, water pulses (4X vs. 2X treatment) increased the amount of water available for juveniles. This increase in water availability might compensate competition for water and light between annuals and *Retama* juveniles (Soliveres et al., 2012), as suggested by the significant reduction of the negative effects on the Fv/Fm found. In contrast, *Stipa* is not only perennial and therefore competitive during the entire year, but also highly efficient, accessing water from both short and low rainfall inputs, even during summer (e.g. Pugnaire et al., 1996b). Thus, the 4X treatment more likely benefited *Stipa* rather than reducing the competition between this species and *Retama* juveniles in the natural grassland. However, the less frequent but heavier water pulses applied in the 2X treatment could reach deeper soil profiles, where

Stipa is unable to access soil water, and therefore may increase shrub performance (Fv/Fm results; see also Sala et al., 1989; Ogle and Reynolds, 2004).

4.3. Concluding remarks

Our results show a negative effect of neighbours on the performance of *Retama* juveniles, regardless of changes in the amount and frequency of water pulses. The negative effect on seedling survival, growth or photochemical efficiency contrasted with the neutral or positive effects of neighbours on soil moisture and plant water status (transpiration: $\delta^{18}\text{O}$; WUE: $\delta^{13}\text{C}$). Although we did not measure nor manipulate incident radiation, we believe that these contrasting results provide strong support for the notion that competition was driven by light rather than by water. The results presented here help to refine current predictions of the sign and strength of plant-plant interactions in response to shade/water shortage (Holmgren et al., 1997) and to the ongoing climate change in Mediterranean ecosystems (Brooker, 2006). Our study shows that facilitative interactions involving shade-intolerant species are not expected to increase their frequency or importance under the forecasted scenarios of longer summer droughts, lower rainfalls, or higher frequency of heavy showers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Climatic data (mean monthly temperature, black circles; and monthly rainfall, black bars) obtained from a meteorological station located in the natural grassland. The increment in monthly rainfall promoted by the irrigation treatments applied during 2007 and 2008 is represented by different colors (see legend): +25% = irrigation of 25% of the median of April-July rainfall in four pulses, +50-4X = irrigation of 50% of the median of April-July rainfall in four pulses, and +50-2X = irrigation of 50% of the median of April-July rainfall in two pulses).

Figure 2.

Effect of the factors assayed on soil water availability for the anthropogenic (left) and the natural (right) grasslands during the study period. Data are mean $\pm$ 1SE ($n = 10$). Open and Neighbour microsites are shown with red and blue colors, respectively. The different irrigation treatments are shown in different panels (with a box in the middle showing the treatment with the abbreviations used in Table 1). Statistical results (GEEs), only for those predictors included in the most parsimonious models, are summarized in the panels.

Abbreviations are: M = microsite (neighbour /open), I = irrigation (+0%,+25%, +50%-4X, +50%-2X), T = time, ns = non significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Figure 3. Survival percentage of *Retama sphaerocarpa* juveniles in the anthropogenic (A) and the natural (B) grasslands during the study period. Rest of legend as in Fig. 2.

Figure 4.

Effect of the factors assayed on the root collar diameter of *Retama sphaerocarpa* juveniles in the anthropogenic (A) and the natural (B) grasslands during the study period. Data are mean $\pm$ 1SE (n depending on seedling survival). Rest of legend as in Fig. 2.

Figure 5.

Effect of the factors assayed on photochemical efficiency (F_v/F_m) of *Retama sphaerocarpa* juveniles in the anthropogenic (left) and the natural (right) grasslands during the study period. Data are mean $\pm$ 1 SE ($n = 6$). Statistical results of the PERMANOVA analyses (only the main effects or those significant, for simplicity) are shown. Rest of legend as in Fig. 2.

Table 1

Stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) of *Retama sphaerocarpa* juveniles in the different treatments and study sites. Values are means $\pm$ SE ($n = 12$). Open refer to areas without perennial vegetation, and Neighbour refer to sites under annuals (anthropogenic grassland) or *Stipa tenacissima* (natural grassland). Rest of abbreviations as follows: +0% = unwatered juveniles, +25% = irrigation of 25% of the median of April-July period rainfall in four pulses, +50%4X = irrigation of 50% of the median of April-July period rainfall in four pulses, and +50%2X = irrigation of 50% of the median of April-July period rainfall in two pulses). Asterisk indicates a significant effect of the treatments assayed on the concentration of a given isotope, statistical differences among treatment levels are shown with different letters.

Microsite	Treatment	Anthropogenic grassland		Natural grassland	
		$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)*
Open	+0%	-26.8 ± 0.16	36.8 ± 0.79	-28.4 ± 0.26	$36 \pm 0.78^{\text{ab}}$
	+25%	-27.1 ± 0.22	36.5 ± 1.00	-27.9 ± 0.19	$36.6 \pm 0.70^{\text{ab}}$
	+50%4X	-27.3 ± 0.21	35.7 ± 1.00	-27.6 ± 0.26	$37.6 \pm 0.95^{\text{a}}$
	+50%2X	-26.9 ± 0.2	37.2 ± 0.91	-28.2 ± 0.29	$37.9 \pm 0.72^{\text{a}}$
Neighbour	+0%	-26.8 ± 0.22	37.6 ± 0.64	-27.8 ± 0.22	$37.1 \pm 0.58^{\text{a}}$
	+25%	-26.7 ± 0.23	36.3 ± 0.90	-27.7 ± 0.33	$34.9 \pm 0.71^{\text{ab}}$
	+50%4X	-27.0 ± 0.23	34.9 ± 0.88	-27.8 ± 0.41	$36.6 \pm 0.83^{\text{ab}}$
	+50%2X	-26.6 ± 0.31	36.18 ± 0.69	-28.4 ± 0.29	$34.6 \pm 0.56^{\text{b}}$