

Additive effects of a potentially invasive grass and water stress on the performance of seedlings of gypsum specialists

Matesanz, Silvia^{1*}; Escudero, Adrián^{2,3} & Valladares, Fernando^{1,2,4}

¹Instituto de Recursos Naturales, CCMA, CSIC, Serrano 115, ES-28006 Madrid, Spain;

²Unidad de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, ES-28933 Móstoles, Spain; ³E-mail adrian.escudero@urjc.es;

⁴E-mail valladares@ccma.csic.es; * Corresponding author; Fax +34 915640800; E-mail silvia@ccma.csic.es

Abstract

Question: What is the combined effect of two drivers of local biodiversity changes (presence of a potentially invasive species and seasonal drought) on the performance of seedlings of plants from gypsum habitats under experimental conditions?

Location: A controlled microcosm reconstruction of natural assemblages of gypsum plant communities from central Spain.

Methods: We evaluated the effects of a potentially invasive grass (*Lolium rigidum*) and water stress on the survival, height growth and biomass of five woody species (*Colutea hispanica*, *Gypsophila struthium*, *Thymus lacaitae*, *Lepidium subulatum* and *Helianthemum squamatum*) from semi-arid gypsum ecosystems. Seedlings of the five species were grown with or without the potential invader and under three watering regimes: early stress – simulating an advanced summer, late stress – simulating the characteristic timing of current summer drought and well-watered.

Results: Seedling survival and performance were negatively affected by the presence of the potential invader. Early stress had larger impacts on the gypsum species than late stress. No interactions were found between factors for any of the study variables, and responses to both factors were found to be species-specific.

Conclusions: The lack of interactions between factors indicates that the presence of the potentially invasive grass and water stress had additive effects in our study system. The negative impact of early water stress draws attention to the possible consequences of the advances of summer drought predicted for Mediterranean ecosystems. Finally, the differential responses found for the study species suggest that plant communities will not respond as a unit to global change, leading to significant changes in species composition and dominance.

Keywords: Biological invasion; Climate change; Competition; Global change; Gypsum habitat; Microcosm; Water stress.

Abbreviations: RY = Relative yield; SWC = Soil water content.

Nomenclature: Tutin et al. (2001).

Introduction

Global change is leading to changes in biodiversity through climate change and local drivers such as habitat transformation and fragmentation, pollution, overexploitation and the dispersal of invasive species (Vitousek et al. 1997; Sala et al. 2000; Sala 2001; Anon. 2005). Impacts of individual drivers in ecosystems are widely studied, but few studies evaluate empirically the interactions among different biodiversity drivers acting simultaneously (but see Zavaleta et al. 2003 or Maestre & Reynolds 2006). There is potential for additive or synergistic interactions among components of global changes (Vitousek et al. 1997; Chapin et al. 2000; Chapin et al. 2001), so knowledge on the resulting effects of several co-occurring drivers in different ecosystems is needed (Sala et al. 2000; Dukes et al. 2005; Dermody 2006).

Mediterranean ecosystems are recognized for their high and valuable biodiversity (Cowling et al. 1996), as well as their vulnerability (Lavorel et al. 1998; Chapin et al. 2001; Mooney et al. 2001). The semi-arid gypsum outcrops of the Iberian Peninsula constitute one of the most threatened Mediterranean habitats (Gómez-Campo 1987; Anon. 1992; Palacio et al. 2007). This is due to the relatively low mobility of their substrate-dependent flora and the narrow distribution range of these species (Meyer 1986; Romão & Escudero 2005).

Species invasion and local climate change are two drivers operating in these endangered communities (Anon. 2005). Mediterranean plant populations and communities have suffered fragmentation due to human activities (e.g. agricultural practices, Luzuriaga et al. 2006), and this triggers the invasion of certain plant species (Rejmánek et al. 2005; Peters et al. 2006). In addition, restored sites may be a source of exotic seeds (D'Antonio & Meyerson 2002; Gelbard & Belnap 2003) and invasion by commercial species from restored areas into adjacent natural gypsum communities is already occurring (Matesanz pers. obs.).

Reduction of water availability due to climate change

could hinder germination and seedling establishment, thereby affecting the most critical stages of plant cycle in these water limited communities (Harrington 1991; Eliason & Allen 1997). The most likely future climate scenario for this region is reduced mean annual rainfall with a more pronounced drought during spring and early summer (Christensen et al. 2007). Therefore, information on the effects of water stress at various times on the early life stages of these species is crucial. Additionally, because climate change may increase the incidence of invasive species, understanding the potential impacts of global change on natural systems requires empirical information on the effects of these two important drivers alone and together.

We conducted a microcosm experiment to evaluate the interacting effects of water stress and the presence of a fast growing, potentially invasive grass in an assemblage of woody plant species naturally growing and co-occurring on gypsum habitats in continental Mediterranean regions. We used a microcosm approach recreating natural soil and climatic conditions. This type of approach has been frequently used to simulate community plant responses, and provides a set of potential responses to global change drivers under controlled, but realistic, conditions (Maestre et al. 2006; Maestre & Reynolds 2006). We tested the following three hypotheses: (1) individually, potentially invasive species and water stress each negatively affect the survival and performance of gypsum species' seedlings; (2) these effects are interactive; (3) responses to the two factors are expected to be species-specific.

Methods

Experimental conditions

The experiment was conducted in a greenhouse in Madrid (Spain) from March to August 2004. We conducted a complete randomized factorial experiment with two factors, potentially invasive species (two levels: present and absent) and water treatment (three levels: well watered, early stress and late stress), with ten replicates for each combination. Five target species were selected: *Colutea hispanica* (Fabaceae), *Helianthemum squamatum* (Cistaceae), *Lepidium subulatum* (Brassicaceae), *Gypsophila struthium* ssp. *struthium* (Caryophyllaceae) and *Thymus lacaitae* (Lamiaceae). These species are small chamaephytes, all but *C. hispanica* are gypsophytes (i.e. soil endemics, plants that are restricted to gypsum soils), and all co-occur in the gypsum outcrops of the Tajo valley in the central Iberian Peninsula (40°26'23" N; 3°41'14" W) (Rivas-Martínez & Costa 1970; Costa 1974). Optimum soil moisture

conditions for germination and seedling establishment of these species in central Spain are in the early spring when moisture requirements are met by autumn plus spring rainfall (Escudero et al. 1997).

Lolium rigidum is a rapidly growing annual grass. Its seeds generally germinate during autumn and winter, but occasionally in summer. *L. rigidum* inhabits mainly wastelands, roadsides and annual crops and it is included in several lists as a noxious weed (Villarías 2002). Its invasive success in these conditions is attributed to its high genetic variability, seed production and seed survival over summer and autumn (Tutin et al. 2001; Blanco Moreno 2004). It is widely distributed in the Mediterranean Basin, where it is occasionally reported from natural gypsum areas (e.g. in the Ebro valley by Guerrero-Campo et al. 2006), although its presence in undisturbed gypsum sites is still marginal (Rivas-Martínez & Costa 1970; Izco et al. 1986; Ferrandis et al. 2005). This species is increasing in Mediterranean type ecosystems as a consequence of recent agricultural and restoration practices (Matesanz & Valladares 2007) as it is frequently a major component of seed mixes used in restoration of motorway slopes (Matesanz et al. 2006). Thus, this species is being spread over extensive areas and over a wide range of soils, including gypsum outcrops.

We established three moisture environments based on soil water content (SWC), which was measured daily with a soil mixture sensor (ThetaProbe, Delta-T Devices, Cambridge, UK). In the well watered treatment, SWC values were kept above 15%; in the early and late stress treatments, SWC values were maintained near or below 10% (Fig. 1). We chose this level of water stress because it has been shown to have noticeable effect on seedling performance in Mediterranean conditions (Valladares et al. 2005). Drought in the early stress treatment started 45 days after the beginning of the experiment, whereas late stress started 75 days after the beginning of the experiment, both water stress treatments lasted two months (Fig. 1). We compensated for increasing water demand in the microcosms containing *L. rigidum* by adjusting the watering intensity. Mean SWC over the course of the experiment for the well watered treatment ($18.79 \pm 0.40\%$) was significantly greater than for the water stress treatments, which did not differ significantly from each other ($9.44 \pm 0.28\%$ for early stress and $10.48 \pm 0.47\%$ for late stress; $F = 426.96$; $df = 1, 152$; $P < 0.001$). Mean SWC did not differ between *L. rigidum* treatments ($F = 1.345$; $df = 1, 152$; $P = 0.25$; Fig. 1), and no significant interactions were found between water and *L. rigidum* factors ($F = 0.169$; $df = 1, 152$; $P = 0.69$).

All assemblages had the same number of individuals (36) and all species had the same number of individuals (6). In the non-invasive treatment, *L. rigidum* was replaced by *Launea resedifolia* (Asteraceae), a gypsum

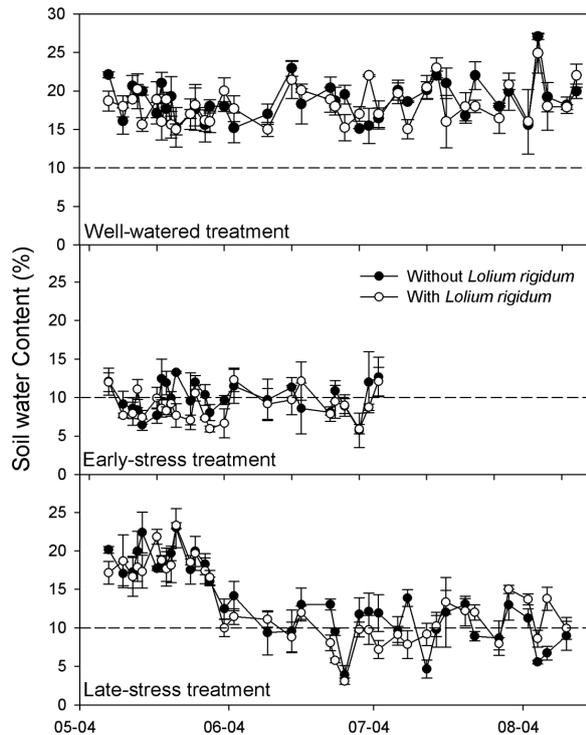


Fig. 1. Water soil content throughout the experiment (%). Well watered microcosms were always maintained above 15% and water stressed microcosms were maintained below 10%.

species, to maintain the number of seedlings. Seeds were sown on 15 March into 3-litre pots filled with soil collected in natural gypsum outcrops in Madrid Province. To avoid germination of seeds existing in the substrate, the upper layer of the substrate of each pot (ca. 5 cm) was sieved (5 mm pore diameter) and sterilized in an autoclave (121 °C) twice. Soil physical properties, such as an extremely hard physical crust, re-appeared within a few days after irrigation (see also Romão & Escudero 2005). Consequently, soil physical conditions rapidly mimicked natural conditions. The seeds were collected in 2001 from field plants growing near Aranjuez (south of Madrid Province, 40°00'49.03" N, 3°36'37.11" W). *L. rigidum* seeds were provided by Intersemillas S.A (Valencia, Spain). Greenhouse air temperature was recorded every 5 min throughout the experiment with a data logger (HOBO model H08-006-04; Onset, Pocasset, MA, US). Mean air temperature during the experiment (days and nights included) was 20.25 °C ± 0.41 °C, ranging from 11.38 °C to 30.41 °C.

Measurements and data analysis

Mortality censuses were conducted on the assemblages on days 45, 60, 75, 105 and 135 of the experiment. Seedling height (length of the stem) was measured on three individuals of each species in each census. Finally, on day 105, coinciding with the end of the early stress treatments, four microcosms of each combination were randomly selected and all the individuals of each species were clipped and dried in the oven at 65 °C for at least 48 h in order to calculate the above-ground dry biomass. Also, the roots in each microcosm were carefully separated from the soil and washed with deionized water before determination of total root biomass.

We used the Kaplan-Meier product-limit method to estimate the survival function of each species in each treatment and the Cox-Mantel test to test for differences in survival functions among species for each combination of factors and among factors for each species. We determined survival rate in the non-stressed plants at two different times: survival at day 105 to compare with survival of plants at the end of the early stress treatment, and survival at day 135 to compare with survival of plants at the end of the late stress-treatment. To assess the effect of the presence of *L. rigidum*, we performed a factorial ANOVA with height or above-ground biomass as dependent variables, *L. rigidum* (two levels: present and absent) and water treatment as categorical predictors, and the number of seedlings per microcosm as a covariate to control for the number of surviving seedlings. This was done for each gypsum species separately. Where different measurement dates were available, the analyses were performed for each date separately. A two-way ANOVA was performed to assess differences in total root biomass among treatments, with *L. rigidum* presence and water treatment as categorical predictors. Finally, we used relative yield (RY) as a competition intensity index to quantify the proportional decrease in plant performance due to competition with *L. rigidum* (Grace 1995; Silvertown & Charlesworth 2001; Vila et al. 2004):

$$RY = A_{\text{with invasive}} / \bar{A}_{\text{without invasive}} \tag{1}$$

where $A_{\text{with invasive}}$ is the above-ground biomass of the target species when *L. rigidum* is present, and $\bar{A}_{\text{without invasive}}$ is mean above-ground biomass of the target species when *L. rigidum* is absent. As competition with *L. rigidum* increases, RY decreases. We calculated this index for all microcosms within each water treatment. Two-way ANOVA and Tukey's HSD test were performed to assess the differences in RY across water environments and species. Prior to all the analyses, normality and homoscedasticity were checked to meet the assumptions of the ANOVA.

Results

Seedling survival

Seedling survival differed significantly among species ($P < 0.0001$), with *Gypsophila struthium* and *Colutea hispanica* always having survival $> 50\%$, *Helianthemum squamatum* having survival as low as 25% and *Lepidium subulatum* and *Thymus lacaitae* having intermediate values. However, all species had similar survival rates under no stress (well watered and no *L. rigidum*).

Seedling survival also differed significantly among treatments for all the species (Fig. 2). The lowest survival rate of each species was in treatments where *L. rigidum* was present (Fig. 2, open bars). For two of the five species (*G. struthium* and *T. lacaitae*), the lowest survival rates were in the treatment that had *L. rigidum* and were submitted to late water stress (Fig. 2). For *H. squamatum*, survival was higher under early stress and similar under well watered and late stress conditions. In

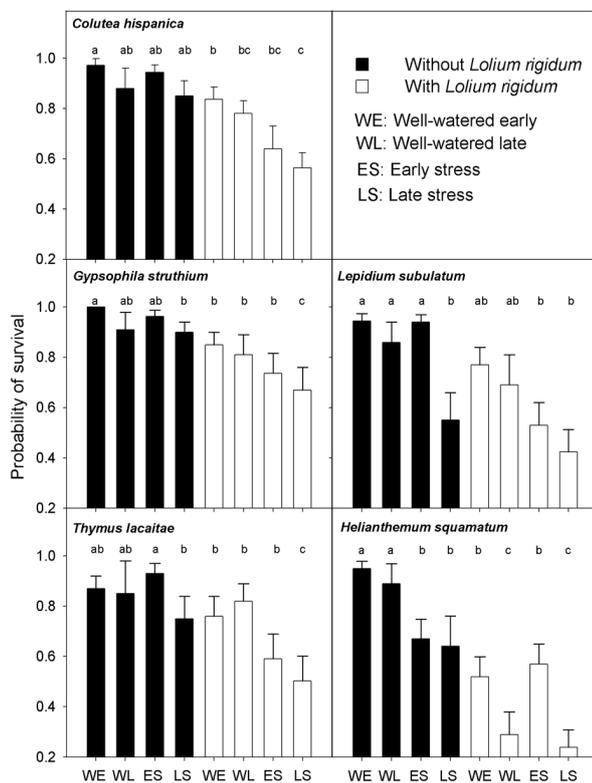


Fig. 2. Final survival proportion for each gypsum species in the *Lolium rigidum* × water treatment combinations. Well-watered-early refers to the survival of plants growing with no water limitation until day 105, and well-watered-late refers to the same plants until day 135. Letters above bars indicate homogeneous groups (Cox-Mantel test; $P > 0.05$).

the absence of *L. rigidum*, survival rate of *C. hispanica* was the same in all water treatments, survival rate of *H. squamatum* was greatest in the well watered treatment and similarly low in both stress treatments. For the remaining species, survival varied among water treatments without *L. rigidum*, but generally was lowest under late water stress.

Height and biomass

We found a significant, negative impact of *L. rigidum* on the height of most gypsum species, although the extent of the impact differed among the species (Table 1 and Fig. 3). *G. struthium*, *T. lacaitae* and *L. subulatum*

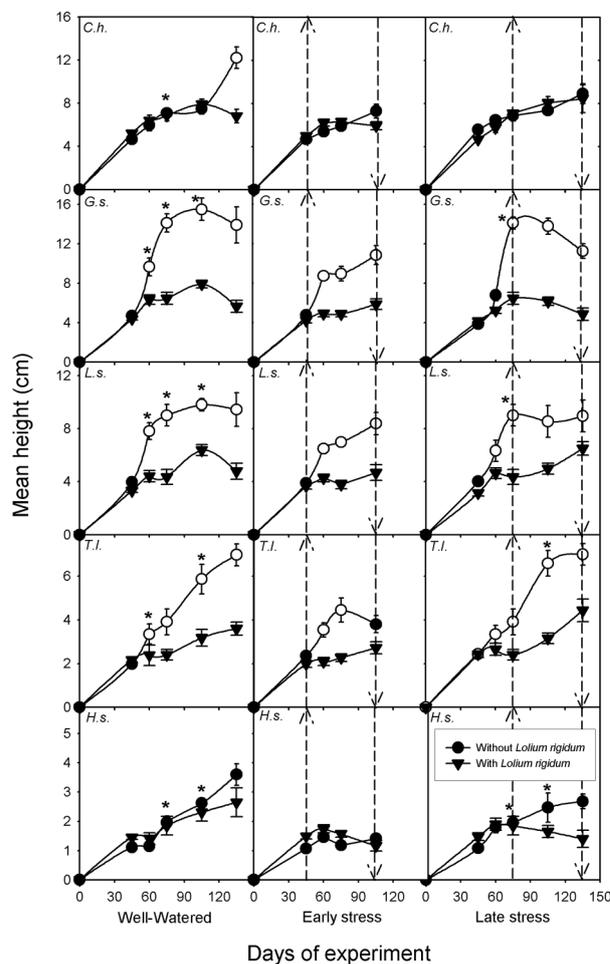


Fig. 3. Mean height of each gypsum species (in rows) over time. Left, central and right panels show well watered, early stress and late stress treatments, respectively. If symbols for *Lolium rigidum* treatments within the same date differ in colour, height differs significantly ($P < 0.05$) between treatments. An asterisk indicates water treatment(s) where seedling height was significantly higher within a date. Arrows facing up show the start of early or late stress, and arrows facing down show the end. Note that y-axis scales differ among species.

Table 1. Effects of the presence of *Lolium rigidum* and water stress on the height of target species (*F*- and *P*-values of factorial ANOVA); bold = significant.

Variable: Height	Day 45		Day 60		Day 75		Day 105		Day 135	
	<i>F</i>	<i>p</i>								
<i>Colutea hispanica</i>										
<i>Lolium rigidum</i> (L)	3.080	0.081	3.169	0.078	0.316	0.575	1.867	0.175	1.856	0.184
Stress (St)	0.399	0.671	0.501	0.608	9.980	0.002	2.277	0.109	2.784	0.106
L × St	0.508	0.602	0.151	0.860	1.088	0.299	1.595	0.209	2.772	0.107
<i>Gypsophila struthium</i>										
<i>Lolium rigidum</i> (L)	1.906	0.169	40.99	< 0.001	113.3	< 0.001	77.58	< 0.001	24.73	< 0.001
Stress (St)	0.027	0.870	5.526	0.005	36.61	< 0.001	8.753	< 0.001	1.757	0.198
L × St	1.965	0.143	1.714	0.185	11.07	< 0.001	1.620	0.204	0.004	0.947
<i>Lepidium subulatum</i>										
<i>Lolium rigidum</i> (L)	3.971	0.048	17.34	< 0.001	93.32	< 0.001	28.31	< 0.001	5.043	0.038
Stress (St)	0.084	0.919	4.026	0.021	8.819	0.003	3.660	0.037	0.536	0.473
L × St	0.492	0.611	0.303	0.739	3.322	0.071	0.092	0.912	0.422	0.524
<i>Thymus lacaïtae</i>										
<i>Lolium rigidum</i> (L)	0.041	0.839	7.469	0.007	22.05	< 0.001	13.85	< 0.001	6.980	0.019
Stress (St)	3.054	0.083	3.807	0.026	0.228	0.796	5.913	< 0.001	3.515	0.081
L × St	1.170	0.313	2.023	0.138	0.445	0.641	2.746	0.066	0.509	0.487
<i>Helianthemum squamatum</i>										
<i>Lolium rigidum</i> (L)	12.52	< 0.001	5.941	0.016	1.151	0.286	1.694	0.197	3.153	0.084
Stress (St)	0.008	0.992	0.768	0.382	11.02	0.001	9.546	< 0.001	0.31	0.584
L × St	0.320	0.726	0.116	0.892	2.079	0.152	0.512	0.601	1.997	0.316

Table 2. Effects of the presence of *L. rigidum* and water stress on above-ground biomass of five gypsum species.

Variable: Above-ground biomass	<i>Colutea hispanica</i>		<i>Gypsophila struthium</i>		<i>Lepidium subulatum</i>		<i>Thymus lacaïtae</i>		<i>Helianthemum squamatum</i>	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
<i>L. rigidum</i> (L)	7.14	0.016	52.31	< 0.001	59.83	< 0.001	22.72	< 0.001	4.073	0.066
Stress (St)	2.081	0.155	1.713	0.210	0.189	0.838	0.09	0.917	1.358	0.294
L × St	0.463	0.637	1.760	0.201	0.94	0.409	0.05	0.953	0.154	0.859

responded negatively to the presence of *L. rigidum* from day 60, while *C. hispanica* and *H. squamatum* did not show a significant response (Fig. 3). In general, a negative effect of water stress was also found (Table 1). The height ranking for the species was well watered > late stress > early stress, showing that early stress had the greatest effect on height (Table 1, Fig. 3, asterisks). Species-specific responses to water treatment were also found (Table 1). *G. struthium* and *L. subulatum* showed the most consistent significant differences among water treatments over time (Fig. 3). No significant interactions

between *L. rigidum* presence and water treatment were found for any species (Table 1). The total number of seedlings per microcosm did not have a significant effect for any species or measurement date.

Above-ground biomass was significantly higher in the microcosms with no *L. rigidum*, but was not affected by water treatment (Table 2, Fig. 4). As with height, *G. struthium*, *T. lacaïtae* and *L. subulatum* were the most responsive species to the presence of *L. rigidum* (Fig. 4) and the number of seedlings per microcosm did not have a significant effect for any species. In contrast, *RY*

Table 3. Relative yield (*RY*, mean ± SE) values for all the species across water treatments. The letter code indicates homogeneous groups (ANOVA, Tukey’s HSD test) across species, with the same letter within a column denoting no significant difference.

Relative Yield (<i>RY</i>) = $A_{L. rigidum} / \bar{A}_{no L. rigidum}$	Well watered conditions	Early stress	Late stress
<i>Colutea hispanica</i>	0.86 ± 0.13 (a)	0.72 ± 0.09 (a)	0.74 ± 0.08 (a)
<i>Gypsophila struthium</i>	0.12 ± 0.007 (c)	0.16 ± 0.03 (c)	0.09 ± 0.002 (c)
<i>Lepidium subulatum</i>	0.17 ± 0.06 (c)	0.21 ± 0.06 (c)	0.10 ± 0.006 (c)
<i>Thymus lacaïtae</i>	0.45 ± 0.09 (b)	0.34 ± 0.04 (bc)	0.25 ± 0.03 (bc)
<i>Helianthemum squamatum</i>	0.62 ± 0.05 (b)	0.53 ± 0.16 (ab)	0.42 ± 0.06 (b)

values showed that competition varied among water treatments (Table 3; $F = 3.33$; $df = 2, 45$; $P = 0.048$), with late stressed microcosms having significantly greater competition than well watered microcosms (Tukey's HSD, $P = 0.02$). The competitive pressure of *L. rigidum* also differed among species ($F = 41.28$; $df = 4, 45$; $P < 0.001$). No interactions between presence of *L. rigidum* and water treatment were found ($F = 0.46$; $P = 0.875$). *G. struthium* and *L. subulatum* had the lowest *RY* values in all the water environments, followed by *T. lacaitae*, agreeing with the results found for height and biomass (Table 3). Finally, microcosms with *L. rigidum* had significantly more root biomass (15.90 ± 2.37 vs. 4.60 ± 0.49 g; $F = 37.14$, $df = 1, 17$, $P < 0.001$) than those without, and the early water stress treatment had the lowest root biomass of the water treatments ($F = 5.40$, $df = 2, 17$, $P = 0.015$). No interaction between presence of *L. rigidum* and water treatment was found ($F = 0.49$; $P = 0.953$).

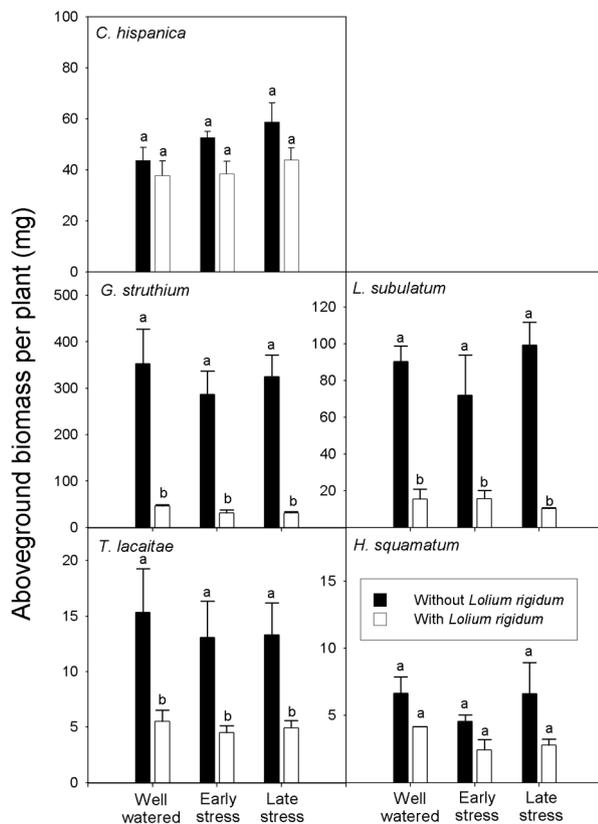


Fig. 4. Above-ground biomass per plant in the *Lolium rigidum* × water treatment combinations. Different letters indicate significant differences among treatments. Note that scales differ among species.

Discussion

Our results revealed a negative and an additive effect of the two biodiversity drivers studied: presence of a potentially invasive species and water stress. These drivers elicited species-specific responses, but contrary to our expectations, no interactions between the drivers occurred. Although the net effect of different global change drivers acting simultaneously is still poorly known (Sala et al. 2000), several authors have pointed towards potential interactions among drivers (Chapin et al. 2001; Duarte et al. 2007). However, this potential has not been demonstrated. Zavaleta et al. (2003) found that the combination of four different types of environmental changes resulted in non-interacting, additive effects on plant diversity in a mediterranean grassland and Dukes et al. (2005) had similar results when assessing the impacts of combined global changes on grassland productivity. Our results concur with these findings, as interactions between the invasive species and water stress were not significant regardless of the study variable (i.e. survival, height or above-ground biomass).

Invasive species colonizing new habitats can impact species and ecosystems through different mechanisms, including competition, changes in ecosystem processes, and allelopathy (Hiero & Callaway 2003; Levine et al. 2003; Orr et al. 2005). In our study the presence of the potentially invasive species *L. rigidum* significantly decreased seedling survival of all gypsum species. Similar results were reported by Brown & Rice (2000), who found that perennial seedling survival decreased with increasing densities of *Vulpia myuros*, an invasive annual grass and by Orr et al. (2005), who demonstrated a reduction in survival of native tree seedlings in the presence of an invasive grass congeneric with the one studied here (*Lolium arundinaceum*). Allelopathic effects on seedling survival and growth in several tree and annual species have been reported for *Lolium rigidum* and other species from the genus *Lolium* (San Emeterio et al. 2004; Orr et al. 2005). In our study, height and above-ground biomass of the gypsum species responded negatively to the presence of the invasive species, resembling the survival patterns. Previous studies suggest that perennial species from arid environments are good competitors once established, but that they have difficulties competing with introduced annual plants at the seedling stage (van Epps & McKell 1983; Escudero et al. 1999). Similar findings were reported by Corbin & D'Antonio (2004), who found that the presence of exotic annual species reduced the productivity of perennials native to California grasslands in the first growing season, while in subsequent seasons this effect became smaller.

The number of seedlings was the same for both the potentially invasive species and each of the gypsum

species in our experimental setting and we suggest that the effects of *L. rigidum* may be larger in field conditions, as the adverse impacts of invasive species have been related to their capacity to quickly become abundant (Vila et al. 2004). This is also supported by the mismatch of the timing of emergence between *L. rigidum* and the gypsum species in field conditions (summer-autumn for the former, winter-spring for the latter), which could represent an advantage for the potentially invasive species in that it may establish and grow before spring. With wetter autumns and warmer winters, such as those expected for some areas of the Mediterranean region (Christensen et al. 2007), the different phenology of this potential invader might become even more advantageous.

Our results confirmed the negative effects of water stress on some of the gypsum species studied, which concur with the studies by Escudero et al. (1999, 2000; 2005) and Romão & Escudero (2005) under natural conditions. In these studies, drought was the main cause of mortality of different gypsophyte seedlings in gypsum outcrops of central Spain. Additionally, we found that early stress had greater impacts on the height of the gypsum species than late stress. At least for some of these species, survival in the field is size-dependent, probably as a consequence of the necessity of rooting below the surface crust before summer drought (Escudero et al. 1999, 2000). Consequently, seedlings have greater difficulty overcoming water stress at the beginning of the growing season than later, when their root system is better developed below the crust. This highlights the importance of the change not only in the magnitude and sign of a particular climatic feature (e.g. temperature, rainfall) but also in the timing of such change.

In contrast to Vila et al. (2004), who found that competition with weeds was not significantly higher than competition without them in a review of 32 studies of impacts of weeds on crops, we found that competition for gypsophytes was stronger when *L. rigidum* was present. Furthermore, competition increased significantly with water stress. A possible explanation for this is that the root system of *L. rigidum*, with up to tenfold more biomass than the gypsum species, to a large extent depleted the scant water of the water stressed treatments, disproportionately reducing the water available for the gypsum species. It must be noted that soil water content was kept relatively constant across water stress treatments, so our results suggest that a larger fraction of the available water was taken up by *L. rigidum*. In our study, the presence of *L. rigidum* is likely to have also resulted in limited space availability for gypsum species growth, due to the higher relative growth rate of the annual species compared to the gypsum perennials (Chapin 1980; Garnier 1992). Thus, even though our purpose was to assess the overall effect of this

potentially invasive species on the performance of the gypsum species, rather than to determine the underlying mechanism by which the introduced species is affecting their performance, we suggest that competition was the most important interference mechanism.

We found species-specific responses to water stress, the potentially invasive species or both, thus supporting our third hypothesis. Multispecies, realistic experiments are essential to understand the processes by which global change is operating, as environmental changes are faced by whole communities, not by single species, and contrasting species' responses at the community level may help to predict future changes in species composition or dominance (Lavorel & Garnier 2002; Walther et al. 2002). In our study, the species-specific responses can be grouped in two categories. The first group (*L. subulatum*, *T. lacaitae* and *G. struthium*) showed strong, negative responses, in terms of survival, growth and competitive intensity, to the presence of the potentially invasive species and to water stress. Competition with annuals has previously been reported for seedlings of *L. subulatum* in field conditions (Escudero et al. 2000) and water was recognized as the main limiting resource for seedlings in these systems, accounting for high mortality rates (Harrington 1991; Escudero et al. 1997; Romão & Escudero 2005). The second group includes those species that showed little or no significant responses to either *L. rigidum* presence or water stress in terms of growth (*H. squamatum* and *C. hispanica*) and only a weak response in terms of survival (*H. squamatum*). The latter findings concur with a recent study of *H. squamatum* showing its great ability to maintain high levels of ecophysiological and reproductive performance across contrasting environmental and climatic conditions (Aragón et al. 2007). In the case of *C. hispanica*, its significantly larger seeds compared to the rest of the studied species (20 to 100 times larger) may have conferred an advantage in the early seedling stage addressed in this experiment. These results suggest that, under a global change scenario, the species within a plant community would segregate according to their sensitivity to the new situation, ranging from species with low survival rates and high responsiveness (*L. subulatum*) to species with high survival rates and low responsiveness (*C. hispanica*).

In conclusion, our study reveals that the impacts of the widely planted, potentially invasive annual grass, *Lolium rigidum*, in these plant communities will be more severe than those of water stress. Native gypsum species are generally well prepared to cope with water limitations, although changes in the timing of drought, particularly advances in the onset of drought, can be detrimental. Even though the lack of interactions between drivers in our study suggests that the resulting effect of multiple global changes can be the sum of single-factor effects,

multifactor experiments are strongly recommended because the outcome depends on both the drivers and the response variables that are relevant in each case. In addition, the species-specific responses observed contribute to better predict the future composition and dynamics of these plant communities and to identify especially sensitive species. Although studies on seedlings have obvious limitations, seedling establishment is the most critical stage for these species, and these results could be used as a starting point to further our knowledge on the effects of global change in semi-arid, Mediterranean plant communities.

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