

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

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Abstract

Question: What is the combined effect of two drivers of global change (presence of a potentially invasive species and climate change) 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 (40°26'23'' N; 3°41'14''W).

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 semiarid 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 was 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.

Nomenclature: Tutin et al. 2001

Keywords: competition, climate change, global change, gypsum habitats, interactions between environmental factors, biological invasions, microcosm, water stress

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Introduction

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

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

Species invasions and climate change are two escalating drivers already
operating in these endangered communities (Millennium Ecosystem Assessment 2005).
24 Mediterranean plant populations and communities have already suffered strong
fragmentation due to human activities (e.g. agricultural practices, Luzuriaga et al. 2006),

and this promotes plant invasions (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, personal observation).

Reductions 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: i) individually, the potentially invasive species and water stress each negatively affect the survival and performance of gypsum species' seedlings; ii) these effects are interactive; iii) responses to the two factors are expected to be species-specific.

Methods

Experimental conditions

3 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
6 (three levels: well-watered, early stress and late stress), with 10 replicates for each
combination. Five target species were selected: *Colutea hispanica* (Leguminosae),
Helianthemum squamatum (Cistaceae), *Lepidium subulatum* (Cruciferae), *Gypsophila*
9 *struthium* subsp. *struthium* (Caryophyllaceae) and *Thymus lacaitae* (Labiatae). These
species are small camephytes, 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
12 the Tajo valley in central Iberian Peninsula (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 autumn plus spring rainfall
15 meet moisture requirements (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
18 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
21 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
24 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 water environments based on soil water content (SWC), which was measured daily with a Soil Mixture Sensor (ThetaProbe, Delta-T Devices, Cambridge, United Kingdom). 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* (Compositae), a gypsum 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 (5 cm. approximately) 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 Romao & 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, USA). Air mean temperature during the experiment (days and nights included) was 20.25 ± 0.41 °C, ranging from 11.38 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, in 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 hr in order to calculate the aboveground dry biomass. Also, the roots in each microcosm were carefully separated from the soil and washed with deionized water to determine 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 analysis of variance (ANOVA) with height or aboveground 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 measurement 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 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). It was calculated as:

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

where $A_{\text{with invasive}}$ is the aboveground biomass of the target species when *L. rigidum* is present, and $\bar{A}_{\text{without invasive}}$ is the mean aboveground biomass of the target species when the *L. rigidum* is absent. As competition with *L. rigidum* increases, the value of 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 homocedasticity were checked to meet the assumptions of the ANOVA.

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Results

Seedling survival

3 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*
6 *lacaïtae* 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
9 (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.*
lacaïtae), the lowest survival rates were in the treatment that had *L. rigidum* and were
12 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 the absence of
L. rigidum, *C. hispanica*'s survival rate was the same in all water treatments, *H.*
15 *squamatum*'s survival rate 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.

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Height and biomass

We found a significant, negative impact of *L. rigidum* on the height of most gypsum
21 species, although the extent of the impact differed among the species (Table 1 and Fig.
3). *G. struthium*, *T. lacaïtae* and *L. subulatum* responded negatively to the presence of
L. rigidum beginning on day 60, while *C. hispanica* and *H. squamatum* did not show a
24 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 strongest 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 (Figure 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.

Aboveground 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. lacaitae* 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 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 was 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 versus 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 ($F = 0.49$; $P = 0.953$).

Discussion

Our results revealed a negative and additive effect of the two global change 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 III et al. 2001; Duarte et al. 2007). This potential has not been demonstrated, however. 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 aboveground biomass).

Invasive species colonizing new habitats can impact species and ecosystems through different mechanisms, including competition, changes in ecosystem processes, and allelopathy (Hierro & 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 aboveground 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.

Since the number of seedlings was the same for both the potentially invasive species and each of the gypsum species in our experimental setting, 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 and 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 likely 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 III 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*).

3 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
6 prepared to cope with water limitations, although changes in the timing of drought,
particularly advances in the onset of drought, can be very detrimental. Even though the
lack of interactions between drivers in our study suggests that the resulting effect of
9 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
12 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
15 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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Table 1. Effects of the presence of *L. rigidum* and water stress on the height of target species (F and *p*-values of factorial ANOVA).

Variable: Height	Day 45		Day 60		Day 75		Day 105		Day 135	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<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 x 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 x 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 x St	0.492	0.611	0.303	0.739	3.322	0.071	0.092	0.912	0.422	0.524
<i>Thymus lacaitae</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 x 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 x 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 aboveground biomass of five gypsum species.

Variable: Aboveground biomass	<i>Colutea hispanica</i>		<i>Gypsophila struthium</i>		<i>Lepidium subulatum</i>		<i>Thymus lacaitae</i>		<i>Helianthemum squamatum</i>	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<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 x St	0.463	0.637	1.760	0.201	0.94	0.409	0.05	0.953	0.154	0.859

3

Table 3. Relative yield (RY, mean \pm standard error) 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 (RY) = $\bar{A}_{L. rigidum} / \bar{A}_{no L. rigidum}$	Well watered conditions	Early stress	Late stress
<i>Colutea hispanica</i>	0.86 \pm 0.13 (a)	0.72 \pm 0.09 (a)	0.74 \pm 0.08 (a)
<i>Gypsophila struthium</i>	0.12 \pm 0.007 (c)	0.16 \pm 0.03 (c)	0.09 \pm 0.002 (c)
<i>Lepidium subulatum</i>	0.17 \pm 0.06 (c)	0.21 \pm 0.06 (c)	0.10 \pm 0.006 (c)
<i>Thymus lacaitae</i>	0.45 \pm 0.09 (b)	0.34 \pm 0.04 (bc)	0.25 \pm 0.03 (bc)
<i>Helianthemum squamatum</i>	0.62 \pm 0.05 (b)	0.53 \pm 0.16 (ab)	0.42 \pm 0.06 (b)

6

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

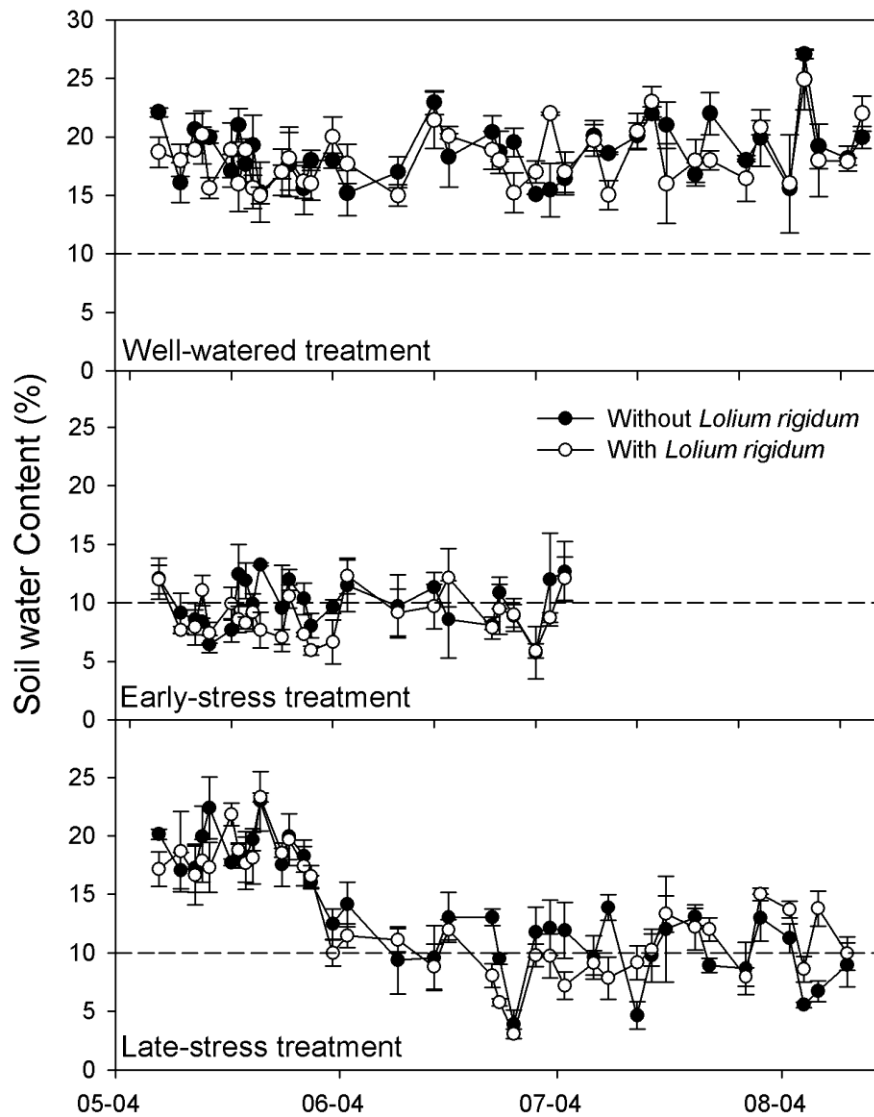


Figure 2. Final survival proportion for each gypsum species in the *L.rigidum* × water treatment combinations. Well watered-early refers to the survival of plants growing with no water limitation until day 105, and well watered-late refers to the same plants but until day 135. Letters above bars indicate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

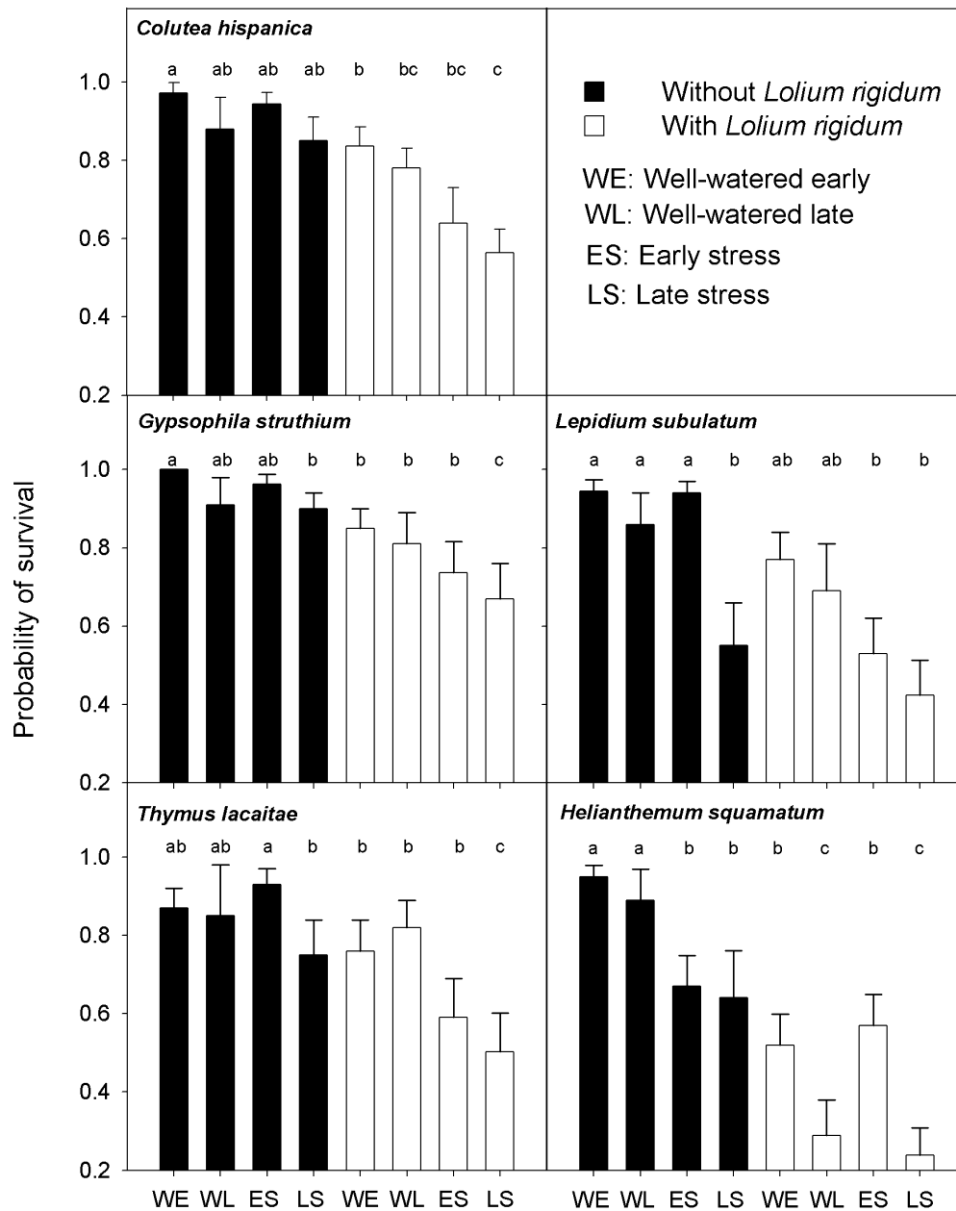


Figure 3. Mean height of each gypsum species (in rows) over time. Left, central and right panels show well-watered, early-stress and late stress treatments, respectively. If symbols for *L. rigidum* treatments within the same date differ in color, height differs significantly ($P < 0.05$) between treatments. 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.

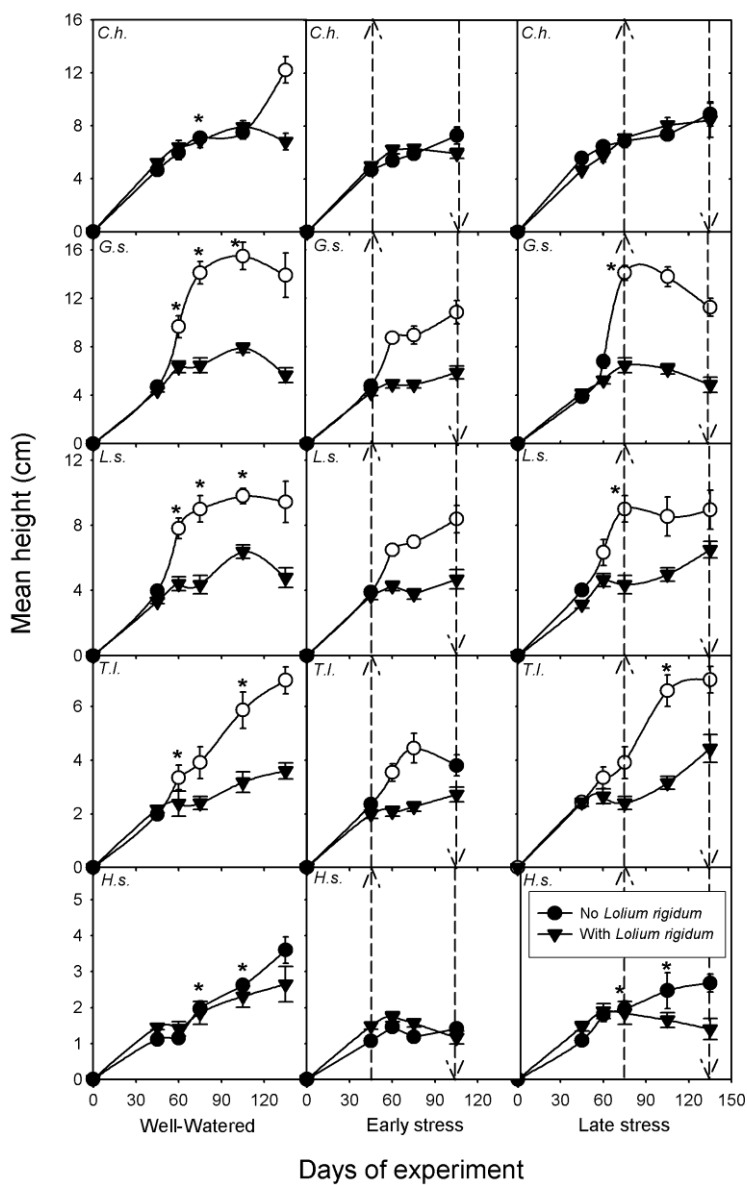


Figure 4. Aboveground biomass per plant in the *Lolium rigidum* × water treatment combinations. Different letters indicate significant differences among treatments. Note that scales differ among species.

