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Altered rainfall patterns reduce plant fitness and disrupt interactions between below- and aboveground insect herbivores

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Abstract. Evidence is accumulating of the disruptive effects of climate change on species interactions. However, little is known about how changes in climate patterns, such as temporal shifts in rainfall events, will affect multitrophic interactions. Here, we investigated the effects of changes in rainfall patterns on the interactions between root herbivores, a plant, and its associated aboveground insects in a semiarid region by experimentally manipulating in the field rainfall intensity and frequency. We found that a shift in rainfall severely constrained biomass acquisition and flowering of the plant *Moricandia moricandioides*, resulting in fitness reduction. Importantly, enhanced rainfall affected the interactions between below- and some aboveground herbivores, disrupting the positive effects of root herbivores on chewing insects. The shifts in precipitation had also plant-mediated consequences for planthoppers, the dominant sapsuckers in our study system. A combination of mechanisms involving biomass acquisition and plant defenses seemed to be responsible for the different responses of insects and their interactions with the plant. This study provides evidence that altered rainfall patterns due to climate change affect not only trophic groups differentially but also their interactions.

Key words: altered rainfall; Brassicaceae; climate change; herbivory; root herbivore; semiarid environment; simulated precipitation.

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Introduction

Climate change is considered one of the most significant global change drivers (Tylianakis et al. 2008, Valladares et al. 2015). In the last decade, many studies have shown the impact of climate change on species diversity and distribution (Parmesan 2006, Siepielski et al. 2017). Climate change also alters species coexistence and biotic interactions (see Valladares et al. 2015 for a review), with consequences at community and ecosystem levels affecting even evolutionary trajectories (Parmesan 2006, Siepielski et al. 2017). However, understanding and predicting how biotic interactions will respond to climate change remains a great challenge (Tylianakis et al. 2008, Barnett and Facey 2016), since both disrupting (Durant et al. 2007, Memmott et al. 2007, Singer and Parmesan 2010) and strengthening effects of climate change on species interactions have been observed (O'Connor 2009). This variety of results arises partly since responses to climate change differ between trophic levels (Voigt

et al. 2003, Tylianakis et al. 2008, González-Megías and Menéndez 2012).

Climate change models predict altered precipitation patterns and an increased number of extreme precipitation events in the future (IPCC 2014). In the Mediterranean region, rainfall is expected to change seasonality, with peaks of rainfall shifting from autumn–winter to late spring–early summer, and to show more frequent extreme events (IPCC 2014). According to Solomon et al. (2007), climate models for drylands also forecast an increase in temperature and significant alterations in rainfall patterns by the late 21st century. Because arid and semiarid ecosystems represent 41% of the terrestrial surface and account for more than 25% of global soil organic carbon (Safriel and Adeel 2005), it is imperative to gain a clear understanding of the responses of these biomes to climate change (Maestre et al. 2013, Nielsen and Ball 2015). Although arid and semiarid plants are up to a certain point resilient to changes in rainfall frequency and intensity (Miranda et al., 2009), the effects vary among plants depending on life cycle, rooting system, phenological state, and physiological and morphological readiness for water uptake and growth (Ogle and Reynolds 2004, Reynolds et al. 2004, Yahdjian and Sala 2010, Zhang et al. 2019).

Studies based on regional climate projections and expected seasonal changes can also provide valuable insights into climate change effects on multitrophic interactions (Jamieson et al. 2012). Precipitation changes due to climate change are predicted to have short-term impacts on insect communities, likely having cascading effects through the food web (Barnett and Facey 2016, Torode et al. 2016). Climate change has also the potential to disrupt belowground–aboveground interactions, such as altering root herbivore effects on plants and consequently on aboveground organisms (van der Putten et al. 2009). It is well known that under drought conditions, root herbivores increase plant susceptibility to aboveground herbivory (Zvereva and Kozlov 2012, McKenzie et al. 2013, Guyer et al. 2018). Root herbivory can decrease water and nutrient uptake and therefore reduce rates of photosynthesis, deplete stored resources in the roots, and cause photoassimilates to be diverted for root regrowth and repair (Blossey and Hunt-Joshi 2003, Johnson et al. 2016). Plant strategies to deal with root herbivores are diverse and can indirectly affect other above- and/or belowground herbivores. Plants can regrow damaged tissue, invest in chemical defenses, allocate nutrients from roots to aboveground tissues, and phenologically escape by dispersing seeds earlier (Newingham et al. 2007, Robert et al. 2014). Studies regarding the effects of precipitation changes on interactions between root herbivores, plants, and aboveground organisms have mostly focused on summer drought simulation scenarios (Johnson et al. 2011, Tariq et al. 2013). However, little is known yet about the effects of shifts in precipitation patterns on such interactions. Moreover, only a handful of studies investigated these complex interactions by manipulating precipitation in the field (Barnett and Facey 2016, Wade et al. 2017).

In the *Moricandia moricandioides* system, a Brassicaceae herb inhabiting semiarid environments, root herbivory has been shown to modulate induced plant defenses and the interaction between the plant and aboveground organisms (González-Megías and Müller 2010). In addition, shifts in rainfall patterns in this system have been shown to provoke changes in the strength and/or the sign of the interactions between below- and aboveground organisms, for example, disrupting the positive effect of detritivores on the abundance of chewing and sucking insects (González-Megías and Menéndez 2012). In the present study, we focus on understanding the impact of changes in rainfall patterns on the interactions between root herbivores, its host plant, and its associated aboveground herbivorous insects. We experimentally manipulated rainfall intensity and frequency during late spring/early summer period, based on future projections for the study area, and measured the effects of changing rainfall patterns on trophic interactions and the underlying mechanisms. We predicted that (1) altered rainfall pattern will negatively affect the reproductive success of the semiarid herb *M. moricandioides* due to reduced soil water holding. Changes in the timing and magnitude of water pulse events, such as extreme rainfall events, can provoke a greater fraction of water to be lost as runoff (Siteur et al. 2014); (2) plant responses to altered rainfall will be mediated by the interaction of root herbivores with the aboveground

insect community; and (3) the underlying mechanisms will be associated with plant strategies to deal with root herbivores, including increased chemical defense and nutrient reallocation.

Materials and Methods

Study system

The experiment was conducted at Barranco del Espartal, a seasonal watercourse located in the semiarid Guadix-Baza Basin (southeastern Spain). The climate at the study area is continental Mediterranean with pronounced temperature fluctuations (ranging from −14°C to up to 45°C) and high seasonality. Annual precipitation rarely exceeds 300 mm.

The plant species *Moricandia moricandioides* (Boiss.) Heywood (Brassicaceae) is highly abundant in this habitat and was used as a study system. *Moricandia moricandioides* plants are distributed in monospecific stands. This species germinates in autumn, grows as a vegetative rosette during winter, and produces reproductive stalks in spring when it receives sufficient amount of water. The stalks remain photosynthetically active during the entire season (González-Megías and Müller 2010). The plants produce glucosinolates, which are the characteristic defense compounds occurring in the order Brassicales (Fahey et al. 2001). After having reproduced, the vast majority of individuals die during summer (approx. 93% of the individuals).

The aboveground insect herbivores associated with *M. moricandioides* include specialist and generalist species belonging to several trophic guilds (see González-Megías and Müller 2010). One of the most abundant root herbivores is *Cebrio gypsicola* Graells (Coleoptera: Cebrionidae), representing ~25% of belowground macroinvertebrate biomass (Doblas-Miranda et al. 2007). We found 0.95 ± 0.2 larva/plant of *C. gypsicola* when sampling random individual during the study years.

Experimental setup

To explore the individual and combined effects of a future scenario of rainfall changes and root herbivores on the interaction between *M. moricandioides* plants and aboveground insects, we conducted a field experiment in 2013. The experiment consisted of a split-plot design with two factors and two levels per factor. The two factors were rainfall, in which late spring/early summer rainfall was enhanced, and root herbivores, in which the absence/presence of this guild was manipulated. The enhanced rainfall treatment was applied to 10 randomized blocks with five blocks per level. Each block had 20 plants located at 30 cm apart from each other (200 plants in total). A similar experimental design has been previously tested in the study area manipulating rainfall and other belowground organisms (see González-Megías and Menéndez 2012).

During the winter of 2012–2013, seeds of *M. moricandioides* collected from the study area were germinated in pots with soil from the study area and grown in a common garden. Plants without reproductive stalk were kept in these pots until beginning of May when they were moved to the field. The absence of rain during early spring constrains plant flowering during that period (authors' previous observation). Therefore, we started our experiment in May because we were interested in measuring the response of the plants to the delay of rainfall from early to late spring (a period with also higher temperatures). Once in the field, plants were repotted using mixed soil from the study site from which macroinvertebrates were removed by hand. The pots consisted of fiberglass mesh cylinders (10 × 15 cm) of 1 mm mesh size to inhibit the entrance or escape of belowground macroinvertebrates. These pots were then buried with the upper surface level with the ground. During the first week in the field, all plants were watered and net-covered to ensure their establishment.

The two levels of the rainfall treatment (R) were a control without manipulation (R−) and an enhanced rainfall level (R+). The rain level applied was based on a projected precipitation model in which rainfall during late spring/early summer was increased compared with current conditions (a delay in precipitation scenario), but it was concentrated in few events that were more extreme in intensity (lower frequency of rain episodes but higher rainfall quantity per event; Table 1). This scenario was designed according to the future predictions for this type of Mediterranean dryland ecosystems (Solomon et al. 2007, IPCC 2014). To simulate this future climatic scenario, we calculated the mean precipitation in the study area of the last 15 years during two periods, (A) the beginning of spring (March–

Table 1. (A) Total precipitation (precip; mm), rainy days, and precipitation per day (mm) observed in the study area during the previous 15 yr (2003–2012) during two periods (mean ± SE); (B) supplemented (Supp) and natural (in parentheses) precipitation, rainy days, and precipitation per day during the experimental year 2013.

Metric	Period A	Period B	Total	Del period 1	Del period 2	Total
(A)						
Total precip	91.89 ± 12.24	31.12 ± 5.02	123.93			
No. of rainy days	17.90 ± 1.54	5.60 ± 0.82	23.5			
Daily precip	5.00 ± 0.40	5.60 ± 0.98	5.3			
(B)						
Supp precip				64 (+20.40)	32 (+0.30)	117
Rain events				4 (+8)	2 (+1)	15
Supp precip/rain event				16 (+2.27)	16 (+0.30)	16

Notes: Delayed periods corresponded to the rainfall treatment (R+) in which blocks assigned to this treatment received natural precipitation plus the supplemented precipitation (simulating delayed rainfall of periods A and B predicted by IPCC for the study region). Period A, March to mid-May; Period B, mid-May to June. Delayed (Del) period 1, mid-May to June; Delayed period 2, July.

mid-May) and (B) late spring to the beginning of summer (mid-May–June; Table 1). For our experiment, we define two alternative periods: (1) Delayed period 1, in which plants associated with R + treatment were supplemented with water in mid-May to late June to the level of the mean precipitation recorded for the period A, and (2) Delayed period 2, in which plants associated with R + treatment were supplemented with water in July to the level of the mean precipitation recorded for the period B. The amount of water needed to be added to plants under the R + treatment was around 123 mm. Because it was impossible to predict natural precipitation during 2013 (the year we carry out the experiment), we roughly calculated the amount of water to be added based on the predicted precipitation for that particular time of the year of the previous 15 yr. The total final amount of water received by the experimental plants in the R + treatment during the delayed periods was similar than the expected. The frequency of the rain events was reduced by 36.2% of the recorded events during the last 15 yr. The occurring natural precipitation during the experiment was taken into account to adjust the amount of precipitation supplemented as explained above. August was not included in the experiment because no plants survive either in natural or under experimental conditions to this month. To simulate cloud cover during the experimental rainfall events, we covered the blocks with a net that filtered radiation by 50%. This net was used during the day of the experimental rainfall event and the day after, simulating the mean reduction of solar radiation that has been observed under natural rainfall conditions in the study area. The net was located more than 1 m above the top of the plants to ensure no effect on insect visitation. Environmental data were provided by a weather station in the study area (Embalse del Negratín, Confederación Hidrográfica del Guadalquivir, Granada).

The root herbivore treatment consisted of a control with no root herbivore (RH−) and the addition of one larva of *C. gypsicola* (RH+). Each of the 20 plants within each block was allocated randomly to one of the two root herbivore levels. Two weeks after the plants were moved to the field, one larva of *C. gypsicola* (second or third instar) was added to the soil of each plant assigned to the RH + treatment. In previous experiments with the same root herbivore, a very high larvae recovery rate was achieved at the time of plant harvest (~90%, González-Megías and Müller 2010), confirming the reliability of this methodology in recording root herbivory effects.

Data collection

Herbivorous insect abundance.—To score the abundance of aboveground herbivores, the number of naturally occurring individuals of each insect species found on each experimental plant was recorded three times per week after the setup of the experiment (from 2 May 2013), resulting in a total of 31 surveys across which

numbers were summed per species. Insect species were assigned to trophic guilds (see González-Megías and Müller 2010 for a similar procedure).

Plant trait measurements.—We recorded the number of open flowers per plant three times per week to calculate flowering phenology (number of days with open flowers). At the end of the experiment (29 July 2013), we counted the number of reproductive stalks and the total number of flowers and fruits produced by each plant. All fruits were taken to the laboratory where the total number of seeds per fruit was counted for each plant. The entire shoots of each plant were collected individually from the field and oven-dried at 40°C for 72 h (until complete desiccation). Shoot tissue was weighed to calculate aboveground biomass. C and N concentrations and their ratio in leaves were determined for plants using a CHN Elemental Analyser. The following variables were used in our analysis: (1) for plant performance: aboveground biomass and the number of flowers; (2) for plant phenology: the number of days the plant display flowers (flowering duration); (3) for plant quality: glucosinolate concentration and C/N ratio; and (4) for plant fitness: plants that produced flowering stalks (as a binary variable) and the total number of seeds produced by the plant.

To quantify glucosinolate (GLS) concentrations of the aboveground plant tissue, the youngest leaf of one stem of each of the experimental plants was collected before leaf senescence (a total of 173 plants; R − RH− = 42 plants, R − RH+ = 43 plants, R + RH− = 43 plants, and R + RH+ = 45 plants). Leaves were stored and immediately freeze-dried, and the dried material was ground and extracted three times in 80% methanol after the addition of *p*-hydroxybenzyl GLS (sinalbin) as an internal standard. GLS extraction was done following previously established methodology (Müller and Sieling 2006, González-Megías and Müller 2010). Desulfoglucosinolates were identified by comparison of UV spectra and retention times to those identified in earlier studies (Müller and Sieling 2006, González-Megías and Müller 2010). Peaks were integrated at 229 nm, response factors of 1 for aliphatic and 0.26 for indolic GLSs considered, and areas related to the internal standard (response factor 0.5) and sample dry weight for calculation of concentrations.

Statistical analyses

Analyses were performed at two levels: (1) with all plants in the experiment: to determine the effect of each experimental factor (rainfall and root herbivores) on plant success and aboveground herbivore abundance, and (2) with flowering plants only (plants that produced reproductive stalks): to determine whether the effect of experimental factors varied once the plant initiated reproduction.

Linear and generalized linear mixed models for treatment effects.—Because this experiment was designed from the beginning as a full factorial experiment with two levels using blocks (a typical split-plot design), we analyzed our data using linear mixed models. The success or failure of plants to produce reproductive stalks was also analyzed as a binomial variable when all experimental plants were included in the analysis.

Due to the specific requirements of each response variable in terms of data distribution, homoscedasticity, overdispersion, and zero inflation, we used univariate linear mixed models (LMMs) and generalized linear mixed models (GLMMs; Appendix S1) to test the effects of each factor (rainfall and root herbivores) and their interaction on plant variables and insect abundance. These models provide a flexible and accurate approach for analyzing these kinds of data with random effects (Bolker et al. 2009). Treatment effects on herbivore abundance were analyzed at trophic guild level (chewers and sapsuckers). For sapsuckers, we also performed the analysis separately for planthoppers because they represented the vast majority of this guild (Appendix S2). We did not perform analysis on other separate taxonomic groups due to their low abundance (Appendix S2). We fitted the most appropriate distribution for each variable according to the error conformation (Gaussian, binomial, Poisson or Negative Binomial). When variables analyzed with normal distribution were not homoscedastic, we modeled heteroscedasticity using generalized least squares. Block was always included in the model as a random factor nested within rainfall except when overdispersion was found. In those cases, GLMMs with observation-level random effects

were run, which allowed for variation at plant level (Harrison 2014). Zero inflation was also modeled when necessary. Model selection was based on Bayesian information criterion (BIC). All analyses were performed in R 3.1.2 (R Core Team 2014) using nlme (Pinheiro et al. 2014) and lme4 (Bates et al. 2015) packages, and with glmmADMB (Fournier et al. 2012) in the case of zero-inflated models.

Multivariate analysis on chemical defense profile.—We used nonmetric multidimensional scaling (NMDS) to test for dissimilarities in GLS profiles between the treatments. NMDS is an indirect gradient analysis approach that produces an ordination based on a distance or dissimilarity matrix. Treatments were assigned as vectors onto a two-dimension plot, and we utilized Horn dissimilarity, 10,000 permutations, and 100 random starts for assessing significance. The analysis was performed with the R package vegan (Oksanen et al. 2017).

Results

Effects of the experimental treatments on plant traits

Effects on plant performance and fitness.—For all plants, enhanced rainfall reduced aboveground vegetative biomass by 66% (Table 2, Fig. 1A) and removed by more than half the proportion of plants that produced reproductive stalks (Fig. 1B). Enhanced rainfall also reduced the number of flowers (Fig. 1C) and the number of seeds produced per plant (Fig. 1D). There were no significant effects of root herbivores on any of the plant traits analyzed (Appendix S4).

For flowering plants only, enhanced rainfall marginally reduced the number of flowers (Table 3, Fig. 2A) but had no significant effect on any other plant traits. The presence of root herbivores significantly increased flowering duration (Fig. 2B). No experimental effect was observed on the number of seeds when considering only flowering plants.

Effects on plant quality: C/N content and GLSs.—No treatment effects were observed on C content, N content, or C/N ratio in leaves for either all plants or flowering plants only (Appendices S3 and S4).

Ten GLSs were found in *M. moricandioides* leaves, six aliphatic and four indolic. The total GLS concentration was on average 7.73 ± 0.77 μmol/g of dry weight, with a prevalence of aliphatic (89.65%) over indolic GLSs (10.35%). No treatment effects on GLSs were observed when considering all plants (Appendices S3 and S4). The NMDS also showed that treatments explained little of the observed variation in the GLS profiles (Appendix S5).

For flowering plants only, enhanced rainfall induced the production of the main indolic compound, indol-3-yl-methyl GLS (Fig. 2C). No significant effect was observed for total indolic or total aliphatic GLS concentrations (Appendix S3). NMDS results showed that the GLS profile composition of the enhanced rainfall with no root herbivory treatment was significantly dissimilar from

Table 2. LMM and GLMM results for the effect of rainfall (R), root herbivores (RH), and their interaction (R × RH) on plant traits and herbivore abundance for all plants.

Metric	R			RH			R × RH		
	F/χ^2	*P*	Df	F/χ^2	*P*	Df	F/χ^2	*P*	df
Plant traits									
Aboveground biomass	**19.88**	**0.002**	1, 8	2.63	0.10	1, 180	0.06	0.80	1, 180
Plants with reproductive stalks	**13.68**	**0.006**	1, 8	0.24	0.62	1, 187	0.18	0.67	1, 187
No. of flowers	**6.28**	**0.012**	1, 8	0.29	0.59	1, 186	0.74	0.38	1, 186
No. of seeds	**4.55**	**0.032**	1, 8	0.24	0.62	1, 187	0.62	0.43	1, 187
Herbivore abundance									
Total sapsuckers	2.00	0.15	1, 8	0.04	0.84	1, 187	0.19	0.66	1, 187
Planthoppers	2.87	0.09	1, 8	0.58	0.44	1, 187	0.08	0.77	1, 187
Total chewers	0.19	0.66	1, 8	**4.34**	**0.037**	1, 187	**4.52**	**0.033**	1, 187

Notes: *F* is shown for aboveground biomass, and χ^2 value is shown for the rest of variables. Significant results ($P < 0.05$) are indicated in bold.

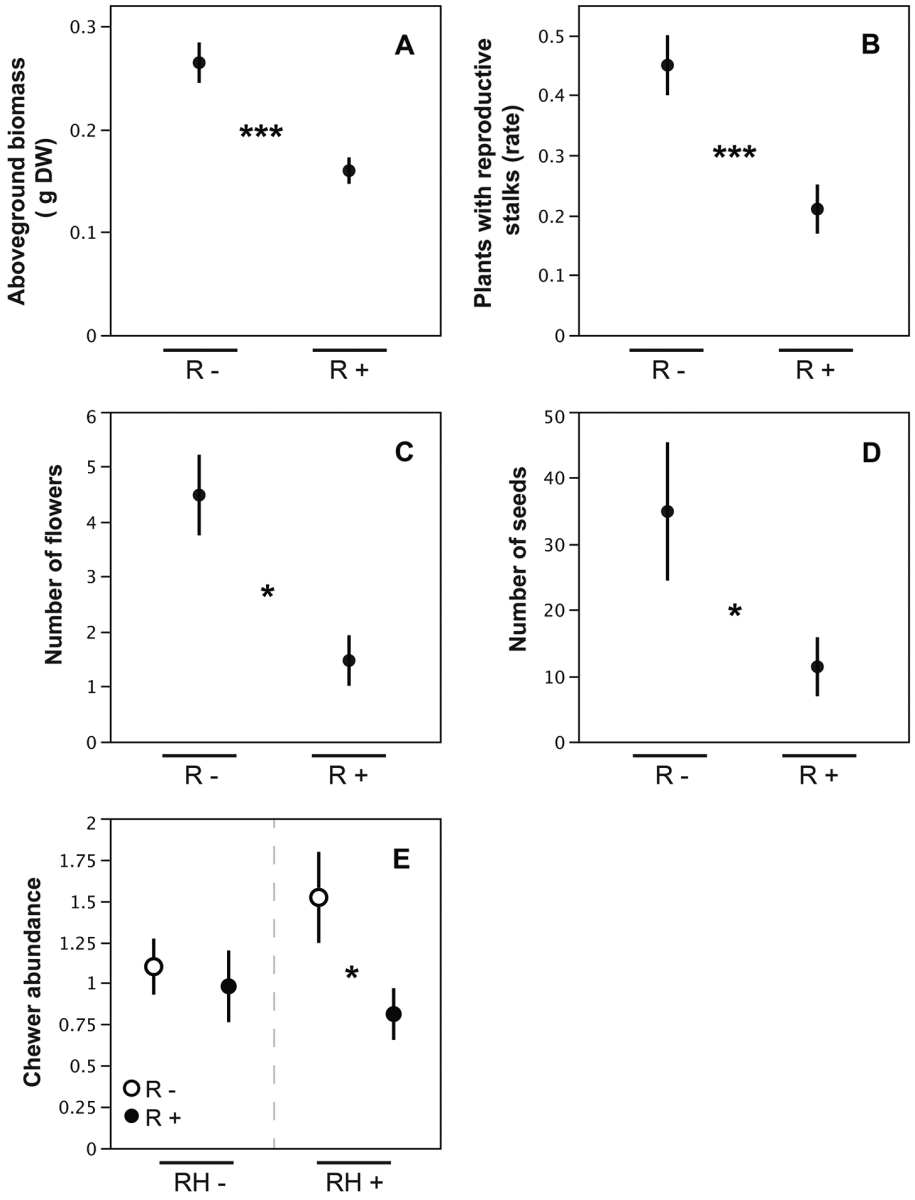

Fig. 1. Rainfall (R) effect on aboveground biomass (A), the rate of plants that produced reproductive stalks (B), the number of flowers (C), and the number of seeds (D) when considering all plants. Rainfall (R) and root herbivore (RH) interacting effect on chewer abundance (E) when considering all plants. ***$P < 0.001$, *$P < 0.05$. Mean ± SE are shown.

the rest of treatments (Appendix S5: Table S1, Fig. S1).

Effects of the experimental treatments on aboveground herbivorous insects

During the study period, several species of chewing and sap-sucking insects visited and fed on the experimental plants (Appendix S2). Most chewers (93%) were caterpillars of specialist species; the pierid butterflies *Pieris rapae* L., *Pontia daplidice* L., and *Euchloe crameri* Batler L.; the diamondback moth (*Plutella xylostella* L., Plutellidae); and an unidentified gelechiid moth species. Generalist chewers belonged to three species of

Table 3. LMM and GLMM results for the effect of rainfall (R), root herbivores (RH), and their interaction (R × RH) on plant traits and herbivore abundance for flowering plants only.

	R			RH			R × RH		
Metric	F/χ^2	*P*	df	F/χ^2	*P*	Df	F/χ^2	*P*	df
Plant traits									
Flowering days	0.11	0.75	1, 6	**4.26**	**0.045**	1, 43	1.40	0.24	1, 43
No. of flowers	3.19	0.07	1, 8	0.13	0.71	1, 52	0.10	0.75	1, 52
No. of seeds	0.51	0.47	1, 8	0.31	0.57	1, 53	0.00	0.99	1, 53
Herbivore abundance									
Total sapsuckers	1.67	0.19	1, 8	0.12	0.72	1, 54	0.19	0.66	1, 54
Planthoppers	**3.91**	**0.048**	1, 8	0.26	0.61	1, 54	0.00	0.99	1, 54
Total chewers	0.15	0.69	1, 8	**5.21**	**0.022**	1, 54	1.40	0.23	1, 54

Notes: *F* is shown for flowering days, and χ^2 value is shown for the rest of variables. Significant results ($P < 0.05$) are indicated in bold.

beetles, *Galeruca angusta* (Kuster), *Mylabris quadripunctata* L., and *Mylabris hieracii* (Graells) as well as some unidentified species of Orthoptera. Several sapsuckers were recorded feeding predominantly on stems and reproductive stalks; they were represented mainly by phloem-feeding generalist planthopper species (80% of all sapsuckers; *Agalmatium bilobum* Fieber Hemiptera, Issidae, and an unidentified cicadelid), aphids (*Myzus persicae* Sulzer, *Aphis fabae* Scopoli, *Brevicoryne brassicae* L., and *Lipaphis erysimi* Kaltenbach), and shield bugs (such as *Ventocoris* spp.).

For all plants, there was a significant interaction between treatments (root herbivores and rainfall) on the abundance of chewers (Table 2), with enhanced rainfall reducing the abundance of chewers but only in the presence of root herbivores (Fig. 1E). No effect of the experimental treatments was observed for all sapsuckers or planthoppers only (Table 2).

For flowering plants only, enhanced rainfall reduced by nearly 50% the abundance of planthoppers (Fig. 2D; Table 3), but there was no effect on all sapsuckers (Table 3). Chewers were positively affected by root herbivores (Fig. 2E; Table 3), with no significant effects of rainfall or the interaction between the two treatments (Appendix S4).

Discussion

Effects of rainfall and root herbivores on plants

One of the most important results found in this study is that enhanced rainfall severely affected *M. moricandioides*, reducing by half the number of plants that produced reproductive tissue. Multiple environmental and endogenous signals (e.g., photoperiod, temperature, and resource availability) are known to induce and regulate plant flowering (Simpson and Dean 2002, Putterill et al. 2004). As a result, plants may adjust flowering to changing environmental conditions (Putterill et al. 2004). In Mediterranean dryland ecosystems, drought and enhanced rainfall have been shown to affect flowering of several short-lived species by decreasing plant biomass (Hänel and Tielbörger 2015, Nielsen and Ball 2015). In a similar way, enhanced rainfall affected *M. moricandioides* by reducing biomass and the number of reproductive stalks in our experiment. *Moricandia moricandioides* is probably adapted to flower during hot and dry conditions in the study area, so unexpected precipitation could have triggered physiological changes resulting in reduced plant growth. In this way, not only the amount of precipitation but also its timing and intensity would have determined the performance of this semiarid herb (Lázaro et al. 2001). In our experiment, the combination of high temperatures during the raining period with extreme rainfall events probably diminished soil water holding, reducing water availability for the plants. A reduction in size can result in lower survival, competitiveness, and reproductive output in plants (Kozłowski 1992). In the case of *M. moricandioides* in the study area, plants with insufficient biomass unable to reproduce may alternatively reallocate resources to roots and favor next season resprouting. However, only

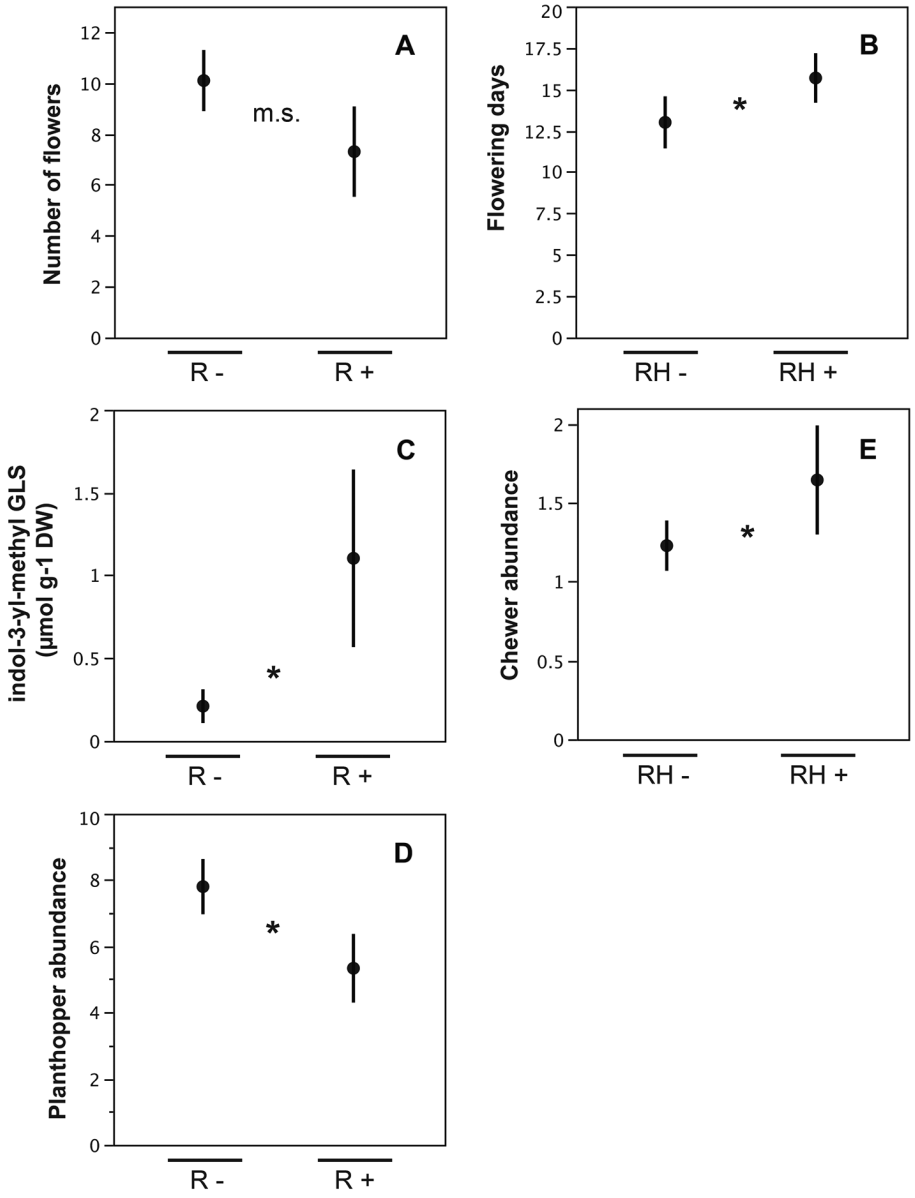

Fig. 2. Rainfall (R) effect on the number of flowers (A), indol-3-yl-methyl GLS concentration (C), and planthopper abundance (D) when considering only the flowering plants. Root herbivore (RH) effect on the number of flowering days (B), and chewer abundance (E) when considering only the flowering plants. *$P < 0.05$, $^{ms}P = 0.07$. Mean ± SE are shown.

~6% of nonflowering *M. moricandioides* plants resprouted in the next season, indicating that resource reallocation to roots may have been limited. This idea is reinforced because there was no effect of enhanced rainfall on C/N content in leaves, what would have been expected, if nutrient reallocation to roots had occurred.

Enhanced rainfall also reduced the number of flowers in the subset of plants that produced reproductive tissue. Previous experiments on the system with a similar rainfall simulation (González-Megías and Menéndez 2012) showed a negative effect of enhanced rainfall on the number of flowers but not on plant size or on the

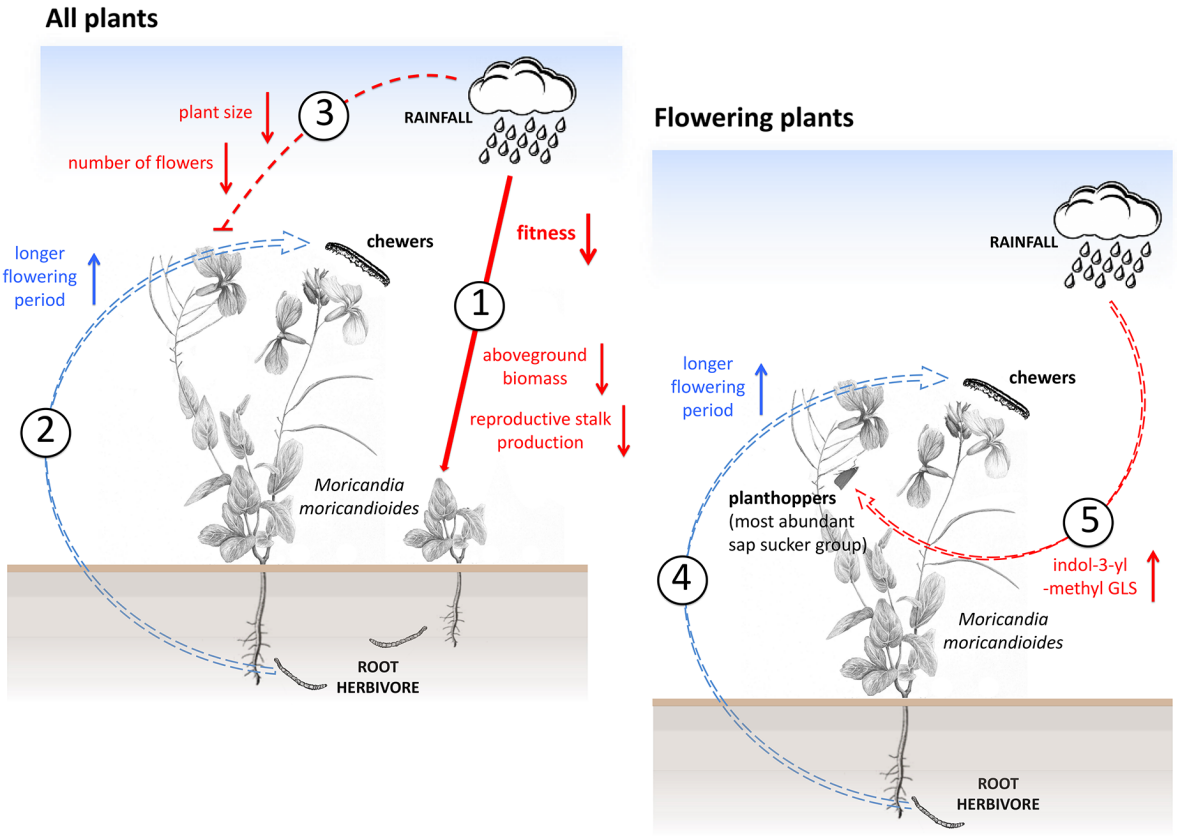

Fig. 3. Illustration of the main effects of rainfall (red) and root herbivores (blue) on *M. moricandioides* and indirectly on aboveground herbivorous insects. For all plants, (1) enhanced rainfall negatively affected aboveground biomass as well as the number of reproducing plants and the number of seeds. (2) Plants facing root herbivory flowered for a longer period, elongating feeding time for chewers on reproductive tissue, and thus probably increasing their survival and abundance. (3) Root herbivore effect on chewers was disrupted by enhanced rainfall, likely because plants were smaller and produced less flowers. For flowering plants, (4) there was a positive effect of root herbivores on chewers with no influence of rainfall, supporting the assumption that enhanced rainfall disrupted the interaction through its effect on plant performance. (5) Enhanced rainfall reduced the abundance of the main sapsucker guild, planthoppers. This effect likely occurred because enhanced rainfall increased the concentration of indolic GLSs. These chemical defenses are usually detrimental to nonspecialist suckers.

overall reproductive output. However, in the present study the enhanced rainfall simulation reduced plant fitness, measured as the number of seeds produced by the plants. Hence, the effects of rainfall shifts on plants such as *M. moricandioides* may be year-dependent and vary in intensity in environments with severe and fluctuating conditions such as arid and semiarid ecosystems, in which plant growing seasons are short and unpredictable (Hänel and Tielbörger 2015 and references therein). In this case, the change in rainfall also provoked the induction of some GLSs in the plants. These results could indicate that plants were investing in chemical defense although GLS concentration is also known to respond to different water regimes in other Brassicaceae (for a review see Metz et al. 2014).

The root herbivore treatment only affected *M. moricandioides* flowering pattern by influencing flowering duration. Belowground herbivory may have provoked nutrient flows from roots to the aboveground tissues, which often occur in short-

lived plants when root defenses are ineffective (Moore and Johnson 2017). This reallocation may have led to a prolonged flowering duration at the expense of a less intense flowering. Despite the effect of root herbivores on flowering pattern, there was no effect on plant reproductive output, what could also be due to a higher abundance of chewers on these plants. Neutral effects of root herbivores on plant reproductive success have been reported previously for our system (González-Megías 2016) and in other Brassicaceae species (Poveda et al. 2005), in contrast to most plant species that cannot fully compensate after root herbivore attack (Zvereva and Kozlov 2012).

Effects of rainfall and root herbivores on herbivorous insects

On flowering plants, chewers, mainly pierid caterpillars, were positively affected by root herbivores. This positive effect can be related to the longer flowering duration triggered by root herbivores that could increase the feeding time for chewers in reproductive tissue. Most pierid species prefer to feed on reproductive tissues, which increase their survival (Lucas-Barbosa et al. 2014). Nevertheless, when considering all experimental plants, enhanced rainfall disrupted the positive effect of root herbivores on chewers. The enhanced rainfall effect was likely the result of a density-mediated indirect interaction, that is, a reduction of resources (smaller plants with less flowers). A change in plant architecture is well known to affect the abundance of insect herbivores associated with a particular plant (Haysom and Coulson 1998).

Planthoppers were negatively affected by enhanced rainfall, although this effect was only detectable in plants that have produced flowering stalks. Reduction in flower number in plants exposed to enhanced rainfall may have been one of the causes of reduced planthoppers abundance, as these herbivores seem to prefer sucking on reproductive tissues (Poveda et al. 2005, González-Megías and Müller 2010). However, the main mechanism is likely to be the induction of plant chemical defenses (GLSs) in plants under the enhanced rainfall treatment, which are transported by the phloem (Chen and Andreasson 2001). Plant defenses are usually more effective against generalist than specialist herbivores (Núñez-Farfán et al. 2007, Hopkins et al. 2009). In particular, indolic GLSs are often detrimental to nonspecialist sapsuckers (Pfalz et al. 2009). In semiarid environments, where there is low potential for plants to compensate for the loss of biomass to herbivory, any plant trait that reduces herbivore damage is fundamental (Herms and Mattson 1992).

Conclusions

Our results highlight that a likely change in precipitation patterns, with a rainfall shift from spring to summer due to climate change, could strongly affect *M. moricandioides* flowering process and reproductive output, for which prior biomass acquisition may be determinant. This study thus supports the hypothesis that short-lived plants are highly sensitive to changing climatic conditions (Voigt et al. 2003, Morris et al. 2008, Jamieson et al. 2012). The present study also provides robust evidence that change in rainfall patterns could affect insect groups in different ways, causing changes in the strength and/or the sign of insect interactions. Further experiments are necessary to determine how belowground herbivores will be affected by changes in rainfall patterns. A key result of our study is that several mechanisms are involved in those responses, likely acting at different life stages of the plant (vegetative and flowering stage, see Fig. 3). One mechanism is related to biomass acquisition by plants, which directly affected plant fitness and herbivore abundance. Another mechanism is related to the induction of chemical defenses, which affected generalist herbivores and was evident in plants that managed to reproduce. Therefore, a future climate change scenario in which plant reproductive phenology could be severely constrained by changes in precipitation may disrupt plant–insect synchrony and trigger trophic cascade effects.

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