Drought and herbivory as modulators of intraspecific differentiation in seedlings of a mountain tree

Paula Inés Marcora
Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad Nacional de Córdoba (UNC)

Sebastián Rodolfo Zeballos (sebazeba@hotmail.com)
Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad Nacional de Córdoba (UNC)

Ana Elisa Ferreras
Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad Nacional de Córdoba (UNC)

Gonzalo Arias
Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad Nacional de Córdoba (UNC)

Isabell Hensen
Martin-Luther University Halle-Wittenberg

Paula Andrea Tecco
Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad Nacional de Córdoba (UNC)

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Abstract

In mountain ecosystems, plant regeneration might be constrained by multiple factors that change along elevation gradients. Those factors might influence the expression of different traits among populations. Drought and herbivory are strong filters for seedling establishment and, therefore, important selective pressures. Populations of the tree *Maytenus boaria* support lower soil moisture and higher herbivory pressure at low elevations than at mid-elevations in Córdoba Mountains, central Argentina. Consequently, we expect that populations from the low elevation perform better in response to both factors than populations from mid-elevations.

Seedlings from the two elevation origins were exposed to two levels of simulated drought and herbivory in a greenhouse experiment. The selected elevations corresponded to the lowest edge of species distribution (with driest soils and highest herbivory pressure) and the central mid-elevation. Performance-related variables, biomass allocation patterns and several morphological and physiological traits were measured.

Seedlings from the low origin showed lower mortality, leaf number and water potential in response to drought than seedlings from the mid-origin. Herbivory affected the performance of seedlings and many plant traits, irrespective of the origin. The interaction between drought and herbivory increased the drought effects on some variables.

Seedlings of *M. boaria* from the low elevation, where conditions are drier and warmer, provide more drought-tolerant offspring than those from the cooler and moister mid-elevation.

Introduction

Natural gradients offer great opportunities to assess intraspecific differentiation of plant traits and predict how species may cope with environmental changes, especially those associated with climate change (Körner 2012; Ooi et al. 2012). In mountain ecosystems, plant regeneration might be constrained by multiple factors that change along elevation gradients (Körner 2012; Moreira et al. 2018). While the effects of temperature on seedling regeneration of woody species have been extensively studied (Harsch et al. 2009; Körner 2012), the effects of other stressors have received less attention (Moyes, Germino and Kueppers 2015). In this sense, drought and herbivory are among the main stressors for seedlings (Coley et al. 1996; Giménez-Benavides et al. 2007; Körner 2012), being important selective pressures (Agrawal 2002; Coley et al. 1985; López Goldar and Agrawal 2021; Muehleisen et al. 2020). Additionally, drought and herbivory are expected to change along elevation gradients. Herbivory often increases toward low elevations where temperatures and plant productivity are higher than in high elevations (Anderegg et al. 2015; Rodríguez-Castañeda et al. 2010; but see Moreira et al. 2018). In addition, in semiarid mountains, where precipitation increases with elevation, drought is expected to be the main hazard for seedling establishment at the warmer, lower edge of species' distribution (Engelbrecht et al. 2007; López Goldar and Agrawal 2021). Consequently, populations along those environmental gradients, which are continuously exposed to different levels of those stressors, might express differences in morphological or physiological traits to improve their performance under the environmental conditions of origin (Agrawal 2002; Halbritter et al. 2018; López Goldar and Agrawal 2021).

Survival and growth are important aspects of seedling performance during establishment, when plant size is a key determinant of competitive outcomes (Connolly and Wayne 1996). Indeed, their response to drought and herbivory has been extensively studied. Regarding functional traits, drought may promote physiological adaptations, such

as an increase in the minimum water potential (Bhaskar and Ackerly 2006) and a reduction in leaf number to maintain physiological activity (Gianoli and González-Teuber 2005; Liu et al. 2011). In terms of biomass allocation patterns, drought might promote higher investment in roots to increase plant water uptake (Barton et al. 2020; Taeger et al. 2014). Shifts in allocation from roots to shoots were found to confer tolerance to herbivory (Barton 2016; McNaughton 1983). After herbivory, the growth priority are new leaves, while root biomass decreases in damaged plants (Barton 2016; McNaughton 1983). Seedlings may also respond by increasing photosynthetic rate (Thomson et al. 2003). Finally, drought and herbivory may promote similar morphological changes, such as reduction in leaf number and area, and specific leaf area (Stevens et al. 2008; Thomson et al. 2003). Despite the large number of studies about drought and herbivory effects on adult plants, the interactive effects of both factors, especially on seedlings, have been scarcely studied (Barton 2013; 2016).

Different species coexisting along the same environmental gradient may show convergent suites of co-varying traits (i.e. plant syndromes; see Chapin et al. 1993). In the mountains of central Argentina, in response to the marked changes in environmental conditions across elevation, from the hot semi-arid climate of the lowlands to the cool humid highlands, several plant species, such as *Gymnocalycium monvillei*, *Polylepis australis*, *Escallonia cordobensis* and *Maytenus boaria*, show intraspecific trait variation along elevation gradients (Bauk et al.2017; Cáceres et al. 2021; Marcora et al. 2017, 2021; Schrieber et al. 2020). The South American tree *Maytenus boaria* is distributed along a broad elevation range, where populations show clinal variation in functional traits (Marcora et al. 2013). Indeed, seedlings of this species grown in a common garden have shown differentiation in their functional traits among four elevation origins, suggesting adaptations to local conditions. Performance-related traits (i.e. large leaves and high sapling height and biomass) that indicate high growth were mostly observed in saplings from lower or intermediate elevations, whereas the opposite was observed in saplings from higher elevations (Marcora et al. 2017). In these mountains, soil water content decreases towards low elevations, whereas air temperature increases; therefore, seedlings in lowlands are exposed to drought, even in the wet season (Tecco et al. 2016). Additionally, livestock load and arthropod diversity and abundance increase towards low elevations of these mountains (Marcora et al. 2013; Ramos 2018). Consequently, herbivory pressure increases toward lower elevations, where temperatures and productivity are generally higher (Anderegg et al. 2015; Rodríguez-Castañeda et al. 2010). Therefore, we postulate that contrasting levels of drought and herbivory will generate differential expression in plant functional traits and seedling recruitment between elevations. We selected populations from two elevations with contrasting conditions of herbivory and drought to assess intraspecific differences in their offspring. Since seedlings from the lower elevation come from individuals historically exposed to both stressors, we predict that the offspring of the population from the drier and warmer low elevation will be less affected by drought and herbivory than the offspring from the cooler and moister mid-elevation. Few studies have evaluated the interaction effects of drought and herbivory on plants, especially trees (reviewed by López Goldar and Agrawal 2021). Moreover, most predictions on species response to climate change are based on evidence from northern temperate regions (Chambers et al. 2017; Halbritter et al. 2018). Such predictions might not hold true in dry seasonal climates, which are widely represented in South America (Kuemmerle et al. 2017), as well as in other southern regions that remain poorly studied (Barton et al. 2020; Chambers et al. 2017; Halbritter et al. 2018).

Materials And Methods

Species and study area

Maytenus boaria Molina (Celastraeceae) grows in the mountains of central Argentina, hereafter Córdoba Mountains, along an elevation gradient from ~ 900 m a.s.l. to the highest elevation (about 2700 m a.s.l). Mean annual temperatures decrease from 15.7°C to 10.6°C (Marcora et al. 2008), while annual precipitation increases from 700 mm to 1000 mm (García 2013). Soil moisture increases with elevation (Tecco et al. 2016). In this study, we selected populations growing at two contrasting elevations to obtain the seeds for the experiment (32º 03' S; 64º 51' W). The two elevations were chosen to represent two contrasting situations: populations growing under maximum water stress and herbivory pressure (i.e., lowest edge of species distribution at 1200 m a.s.l.; low origin) and populations growing at the central elevation of this species in Córdoba mountains (1700 m a.s.l.), where maximal seedling survival was previously observed (i.e., mid-origin) (Marcora et al. 2013).

Experimental Design

Seeds were collected from 20 mature trees at each elevation in summer (February - March). After collection, seeds were set to germinate in germination chambers at 25/15°C (12/12 h light/dark photoperiod). Emerged seedlings were transplanted to 0.68-L plastic pots (one per seedling) containing a mixture of sand and soil in a 2:3 proportion. During the experiment, plants were grown in a greenhouse under an alternating photoperiod of about 12 h light and 12 h darkness (natural photoperiod of spring between 20/09 and 22/11). Mean air temperature and humidity in the greenhouse were 22.12 ºC (± 0.15 SD) and 65.36% (± 0.56 SD), respectively. Pots were automatically watered until the start of the experiment. To minimize the influence of initial height, seedlings were grouped in blocks of eight pots. Each block included seedlings from both origins in four treatment combinations: control, drought, simulated herbivory, and drought + simulated herbivory. A total of 15 blocks were established. An additional block was included, which contained only four pots with seedlings from the low origin. Blocks were rotated weekly. The complete experimental design consisted of 124 seedlings that were distributed in two drought treatments × two simulated herbivory treatments × two elevation origins × 16 or 15 replicates (for the low and mid-origin, respectively).

Pots were manually watered every 2–3 days, with half of them receiving 5 ml and the other half, 50 ml. The two water levels chosen were expected to mimic differences in soil moisture content measured at each elevation under field conditions (Tecco et al. 2016). Soil water content was 8.26% (± 4.11 SE) for the drought condition and of 26.99% (± 3.89 SE) for the well-watered condition (Moisture Probe Meter 160-B of ICT International Pty Ltd).

The simulated herbivory treatment was performed one week after the start of the differential watering treatment. We manually clipped with scissors half of the seedlings under each water regime level and removed all the biomass above cotyledons (Lorca et al. 2019). After the two months of experiment, all surviving seedlings were harvested.

Seedling measurements

Seedling performance was assessed in terms of mortality, final biomass and relative growth rate (RGR). The RGR was calculated as the difference between the final and initial height (log_{10} transformed) divided by time (two months) (Sánchez-Gómez et al. 2008). The initial height of seedlings was measured after applying the simulated herbivory treatment.

Leaf number (LN) was recorded and one leaf was taken from each seedling and scanned to calculate leaf area (LA; mm^2) using the ImageJ program (Schneider et al. 2012) and specific leaf area (SLA; $mm^2\ mg^{-1}$). Total leaf chlorophyll concentration was estimated using the SPAD502 chlorophyll meter (Konica Minolta). SPAD was

measured only in seedlings with at least one true leaf (n = 88), avoiding cotyledons, which always had higher SPAD values. Water potential (Wp_{shoot}; MPa) was measured in a subsample of 32 seedlings (4 replicates × 2 origins × 2 drought levels × 2 herbivory levels). This was performed within two minutes after harvest of each seedling using a pressure chamber (Scholander et al. 1965). Water potential measurements were taken inside the greenhouse at midday on sunny days (*ca.* from 12.00 to 14.00 h) using the principal shoot of each seedling. Harvested seedlings were separated into leaves, stem and roots, oven-dried at 70°C for 48 h and weighed. Final biomass, leaf, stem and root mass fraction (RMF; SMF; LMF, respectively), and root to shoot ratio (RM:SM) were calculated.

Data analysis

The following response variables were analyzed independently with a full linear mixed-effects model (function *lme*; package *lme4*): RGR, final biomass, RMF, SMF, LMF, RM:SM, LA, SLA, SPAD, and Wp_{shoot}. Final biomass, LMF, SMF, LA, SLA, and Wp_{shoot} were $\log_{10}$ transformed to meet assumptions of normality.

Seedling mortality was included as a binomial variable in a Generalized Linear Model (*glm* function, stats package) and p values were obtained using an Analysis of Deviance (*Anova* function, car package). In this case, overdispersion was checked (residual deviance < freedom degrees). Leaf number was analyzed using a Generalized Linear Mixed Model (*glmer* function, lme4 package) with a Poisson error distribution and overdispersion was checked following Zuur et al. (2009).

A systematic model simplification was applied (i.e., top-down model simplification beginning with a model with all the desired factors included) based on the AIC values. During this process, we compared all potential models with the null model and among them. The structure of the final models was obtained through the application of the likelihood ratio test (Zuur et al. 2009), using the anova function (Ferenc et al. 2021). The final model was analyzed including only the selected factors and the specific assumptions of each assessed model. All analyses and graphs were performed with RStudio ver. 1.1.463 (2018).

Results

At the end of the experiment, 100 seedlings (80.65%) were alive. Both drought and simulated herbivory had negative effects on most of the assessed variables (Table 1). For some variables, the effect of drought differed between elevation origins, whereas the effect of simulated herbivory was always independent of seedling elevation origin.

Table 1

Response of seedlings of the tree species *Maytenus boaria* from two elevation origins (O) to drought (D) and herbivory (H). Statistics and *p* values are provided only for variables included in the final models after backward model reduction process (see methods). RGR (relative growth rate), LMF (leaf mass fraction), SMF (shoot mass fraction), RMF (root mass fraction), LN (leaf number), LA (leaf area), SLA (specific leaf area), SPAD (estimation of chlorophyll content), Wp_{shoot} (water shoot potential).

Response variables		**(intercept)**	**Drought (D)**	**Herbivory (H)**	**Origin (O)**	**D*H**	**D*O**	**H*O**	**H*O*D**
Performance	Mortality		χ^2 = 41.14 **p < 0.001**	χ2 = 2.71 p = 0.10	χ2 = 5.55 **p = 0.02**				
	RGR	F = 51.81 p < 0.0001		F = 4.40 **p = 0.03**					
	Final biomass	χ^2 = 233.63 p < 0.0001	χ^2 = 0.99 p = 0.32	χ^2 = 18.07 **p < 0.0001**		χ^2 = 0.15 p = 0.69			
Biomass allocation	LMF	χ^2 = 395.52 p < 0.0001	χ^2 = 0.6.93 **p < 0.01**	χ^2 = 1.49 **p < 0.01**		χ^2 = 4.36 **p = 0.04**			
	SMF	χ^2 = 35.85 p < 0.0001	χ^2 = 1.83 p = 0.17	χ^2 = 0.21 p = 0.64	χ^2 = 0.35 p = 0.55	χ^2 = 0.51 p = 0.47	χ^2 = 1.29 p = 0.25	χ^2 = 0.14 p = 0.7	χ^2 = 0.7 p = 0.47
	RMF	χ^2 = 393.66 p < 0.0001	χ^2 = 9.76 **p < 0.001**	χ^2 = 2.65 p = 0.10		χ^2 = 1.61 p = 0.20			
	RM:SM	F = 137.77 p < 0.0001	F = 7.31 **p < 0.01**	χ^2 = 2.67 p = 0.10		χ^2 = 5.24 **p = 0.02**			
Morphological traits	LN	χ^2 = 77.89 p < 0.0001	χ^2 = 20.74 **p < 0.0001**	χ^2 = 2.49 p = 0.11	χ^2 = 0.77 p = 0.38	χ^2 = 0.57 p = 0.45	χ^2 = 19.63 **p < 0.0001**	χ^2 = 0.5 p = 0.47	χ^2 = 1.6 p = 0.21
	LA	F = 182.06 p < 0.0001	F = 9.35 **p < 0.01**	F = 0.09 p = 0.76		F = 2.63 p = 0.10			

Response variables		(intercept)	Drought (D)	Herbivory (H)	Origin (O)	D*H	D*O	H*O	H*O*D
	SLA	χ^2 = 4592.197 p < 0.0001		χ^2 = 14.169 **p < 0.0001**					
Physiological traits	SPAD	F = 303.11 p < 0.0001	F = 4.38 **p = 0.04**	F = 2.53 p = 0.11		F = 7.96 **p = 0.01**			
	Wp_{shoot}	F = 0.02 p < 0.88	F = 9.86 **p < 0.01**		F = 1.32 p = 0.25		F = 5.23 **p = 0.03**		

Performance and biomass allocation patterns

Mortality of *M. boaria* seedlings differed between drought levels and between elevation origins, whereas there was no effect of simulated herbivory on mortality (Table 1). Drought increased seedling mortality, and this effect was stronger on seedlings from mid-origin (Fig. 1a). Simulated herbivory had a positive effect on RGR, whereas simulated herbivory and drought interaction showed a tendency to a higher RGR at mid-origin (Fig. 1b). Simulated herbivory negatively affected the final biomass of seedlings (Fig. 1c; Table 1).

None of the biomass allocation components were affected by origin. LMF showed a pronounced reduction by the joint effects of drought and simulated herbivory (Fig. 2a). SMF was not affected by any treatment (Fig. 2b), whereas RMF increased under drought (Fig. 2c). Seedling RM:SM increased under the joint effects of drought and simulated herbivory (Fig. 2d).

Seedling traits

Most morphological and physiological traits were affected by drought, and some of them were affected by the interaction between drought and simulated herbivory or elevation origin (Table 1). LN was reduced by the effect of drought and origin interaction (Fig. 3a, b). Seedlings from the low origin showed fewer leaves than those from the mid-origin under drought alone and by the interaction of drought and simulated herbivory (Fig. 3b). LA was reduced by drought, whereas SLA was increased by herbivory (Fig. 3c).

Seedling SPAD increased under the effects of the drought and simulated herbivory interaction (Fig. 4a). Finally, Wp_{shoot} was affected by the interaction between drought and elevation origin, showing a decrease in response to drought that was significantly lower in seedlings from the low-origin (Fig. 4b).

Discussion

Mortality patterns and most of the morphological and physiological variables of *Maytenus boaria* at the regeneration stage showed that drought is a stressful factor that promotes intraspecific trait differentiation

between populations from elevations with contrasting water conditions. In line with our prediction, we found interactions between elevation origins and drought for some variables, suggesting that *M. boaria* populations from low elevations under drier and warmer conditions provide better-performing offspring under drought than populations from cooler and moister elevations. Additionally, seedlings from both origins exposed to drought were able to counterbalance the effect on biomass through a higher root investment. Simulated herbivory had effects on seedling performance, increasing RGR but reducing the final biomass; however, contrary to our predictions, the effect was similar in seedlings from both elevation origins. Additionally, seedlings showed differences in RGR between populations from different elevations. Our results are in line with a previous study involving *M. boaria*, which reported intraspecific differentiation in response to temperature among populations from different elevations (Marcora et al. 2017). In that study, using a reciprocal experiment in germination chambers, four populations of this species from different elevation origins showed differences in their optimum germination temperature. Altogether, our results highlight the presence of intraspecific differentiation between populations of *M. boaria* in response to different factors that change along the elevation range of distribution.

Drought effects

Among the predictors of seedling performance, mortality showed intraspecific differentiation between elevation origins in response to drought. The low-origin population showed a better response to drought, a common condition found in the low edge of this mountain gradient (Tecco et al. 2016). We infer that the higher survival of seedlings from the low than from the mid-origin is due to changes in morphological and physiological traits in response to the selective pressure of drought at this edge of the gradient (Taeger et al. 2015). Indeed, among the assessed morphological traits, seedlings from the low origin showed a reduction in the number of leaves in response to drought compared to the well-watered seedlings. This response is a well-recognized desiccation-avoidance adaptation of woody plants (Gurevitch et al. 1986; Yin et al. 2005). Additionally, while seedlings from the cool and moist elevation (mid-origin) showed small differences in Wp_{shoot} between watering levels, those from the low origin had more negative values in response to drought, i.e. three times lower than well-watered seedlings (Table 1; Fig. 3e). These lower values indicate a higher degree of tolerance to physiological drought in plants from the low than those from the mid-origin. The minimum Wp_{shoot} value reached by a plant has been related to high capacity to cope with water shortage (Bhaskar and Ackerly 2006; Soliani et al. 2021). Indeed, the Wp_{shoot} indicate the maximum water deficit that leaves and xylem can tolerate to maintain physiological activity (Bhaskar and Ackerly 2006). Drought is one of the most pervasive climate change threats affecting plants worldwide (IPCC 2014). Differentiation at the population level in response to drought revealed stronger effects on species contraction than predicted (Barton et al. 2020). Considering our results, the predicted increase in drought towards high elevations in the study area (García 2013) due to climate change may strongly affect the offspring and possibly other ontogenetic stages of this mountain tree (Cotado and Munné-Bosch 2020).

Finally, a suite of traits related to a stress resistance syndrome (e.g., SPAD, RMF; *sensu* Chapin et al. 1993) also indicated that the species has certain ability to respond to drought at both elevations. Thus, SPAD measures increased in seedlings of both origins exposed to drought (Table 1). The variation in SPAD values has been found to be positively correlated to chlorophyll content, photosynthetic rate, absorption of photosynthetic active radiation, and carbohydrate content in leaves (Fotovat et al. 2007; He and Sun 2016). Additionally, the increase in RMF was also common in both origins (Fig. 2c). Biomass allocation to roots enhances the root surface area available for water uptake. In this sense, a study involving 107 woody species found that high SPAD values were related to a larger root surface area, which promoted higher tolerance to multiple stresses (He and Sun 2016).

Consequently, the increase in SPAD and RMF in response to drought is expected to improve seedling survival and growth (Khurana and Singh 2001). This tolerance response has been found in species adapted to moisture-deficit conditions (Chapin et al. 1993; Wright et al. 1992). Overall, the responses to drought shared between the *M. boaria* populations from both contrasting elevations do not prevent the two-fold higher mortality driven by water stress in the offspring of the mid-origin populations.

Simulated herbivory effects

Unlike predicted, simulated herbivory did not drive differential responses between seedlings of contrasting elevation origins. This could be driven by the absence of intraspecific differences in the response to this factor between populations, although complemented simulation approaches are needed to confirm this inference (see below). The increase in RGR and the decrease in final biomass were responses shared by seedlings exposed to simulated herbivory from both elevation origins. Under simulated herbivory, seedlings showed rapid vertical growth; this response can allow them to escape apical meristem damage by mammalian herbivores, often resulting in greater survival and reproduction (Keefover-Ring et al. 2016). After simulated herbivory, seedlings showed a lack of full tolerance (until the end of the study), since they did not reach the same final biomass as the uncut control (Fig. 1c). It is possible that seedlings need more time to achieve full tolerance. In fact, two months after biomass was clipped, despite the lost biomass, seedlings reached heights similar to those prior to cutting (data not shown). This response highlights the ability of this species to resprout after herbivory. However, their resprouting ability could then decrease after repeated browsing events, threatening their survival, as was found under field conditions across its distribution range (Marcora et al. 2013; Svriz et al. 2013). Further studies are needed to assess if the species showed intra-specific trait differentiation in its capacity to compensate biomass loss by hervibory under other browsing intensities or as an induced response to herbivore, for example as a response to saliva (Keefover-Ring et al. 2016; Rooke 2004). Additionally, other traits related to plant defenses in response to herbivory should be measured to better understand the mechanisms of defense and tolerance of this species (Keefover-Ring et al. 2016; Rooke 2004).

-*Joint effects of drought and simulated herbivory*

Our results suggest that the response of different variables to the combined effects of both stress factors is mainly driven by drought. Accordingly, under the combined effects of both stressors, LMF, RMF, R:S ratio and chlorophyll content (SPAD) followed the expected trend in response to drought (He and Sun 2016; Keefover-Ring et al. 2016) and was in contrast to the expected response to simulated herbivory (Gassmann 2004; Thomson et al. 2003). By contrast, RGR increased by the combination of both factors and showed different tendencies between populations; however, this triple interaction was not statistically significant. Under the combination of both stress factors, the low populations seem to respond to the combination of both factors with a lower RGR than the mid-population (Fig. 1b). In this case, the low origin showed a more conservative strategy in the use of resources (Chapin 1993) under the interaction of both stress factors than the mid-origin; this response would be driven by drought and might reduce damage by water stress, which is more frequent at the lower edge of distribution. In turn, the mid-population showed a tendency to higher RGR, which would be driven mainly by simulated herbivory instead of drought.

Final remarks

In many regions of the world, the frequency and intensity of droughts is increasing due to global climate change (IPCC 2014), with a significant influence on forest regeneration (Anderegg et al. 2015). Similarly, the increase in

rainfall heterogeneity is promoting local drought in Córdoba Mountains (García 2013). Our results suggest that *M. boaria* seedlings of the population from the lowest elevation edge of distribution are more tolerant to drought, which might be advantageous to withstand the predicted drought increases. Thus, the ongoing climate change might negatively affect the regeneration of *M. boaria* more critically at the central and probably at the upper edge of its distribution. However, to assess local adaptations in *M. boaria* populations, experiments of reciprocal transplants under field conditions will be needed. Additionally, it would be important to discriminate whether the maternal effects associated with different levels of herbivory and water stress at each elevation could mediate the adaptive response found in *M. boaria* seedlings from each origin (Agrawal 2002). Finally, this study shows the importance of considering the intraspecific differentiation of the native species to understand consequences for populations in the present global change scenario (Taeger et al. 2015).

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Paula Marcora and Sebastián Zeballos contributed to the study conception and design. Material preparation and data collection were performed by Sebastián Zeballos, Gonzalo Arias and Paula Marcora. The analysis data were performed by Ana Ferreras, Sebastián Zeballos and Paula Marcora. The first draft of the manuscript was written by Paula Marcora and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

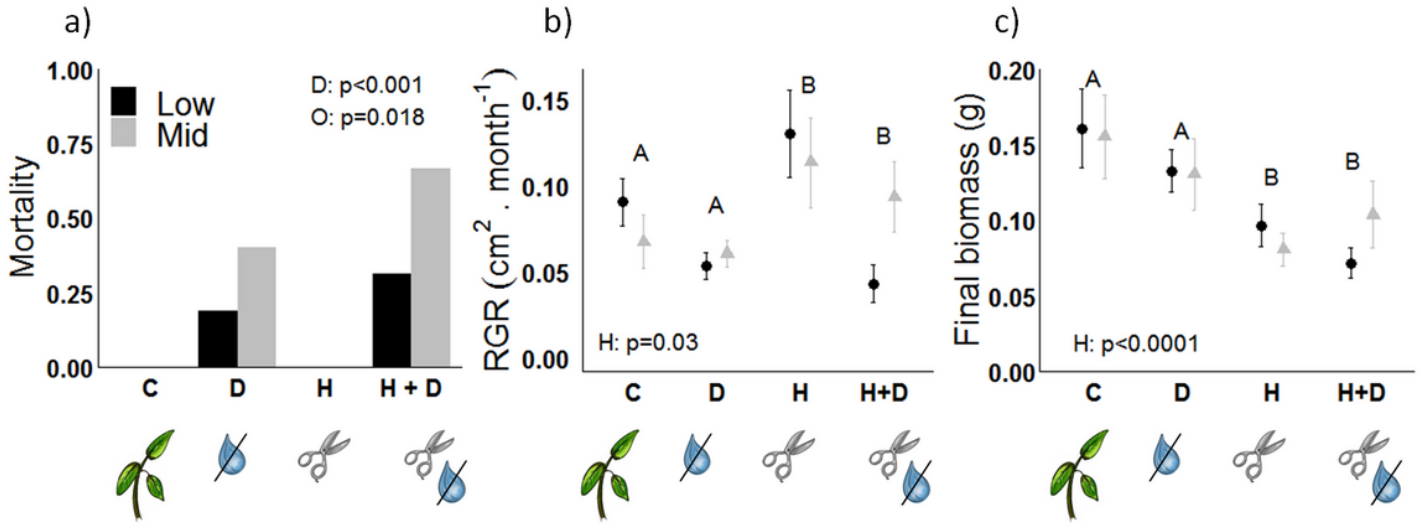

Figure 1

Predictors of performance of *Maytenus boaria* seedlings of low (black bars and points) and mid- (white bars and points) elevation origins in response to drought and herbivory. a) Seedling mortality (proportion); b) relative growth rate (RGR; cm/month); c) final biomass (g). Four treatments were applied to seedlings: control (C), drought (D), simulated herbivory (H), drought and simulated herbivory (DH). Points represent the mean ± SE. Different letters indicate differences among levels of factors when the interaction between two factors was significant (see Table 1).

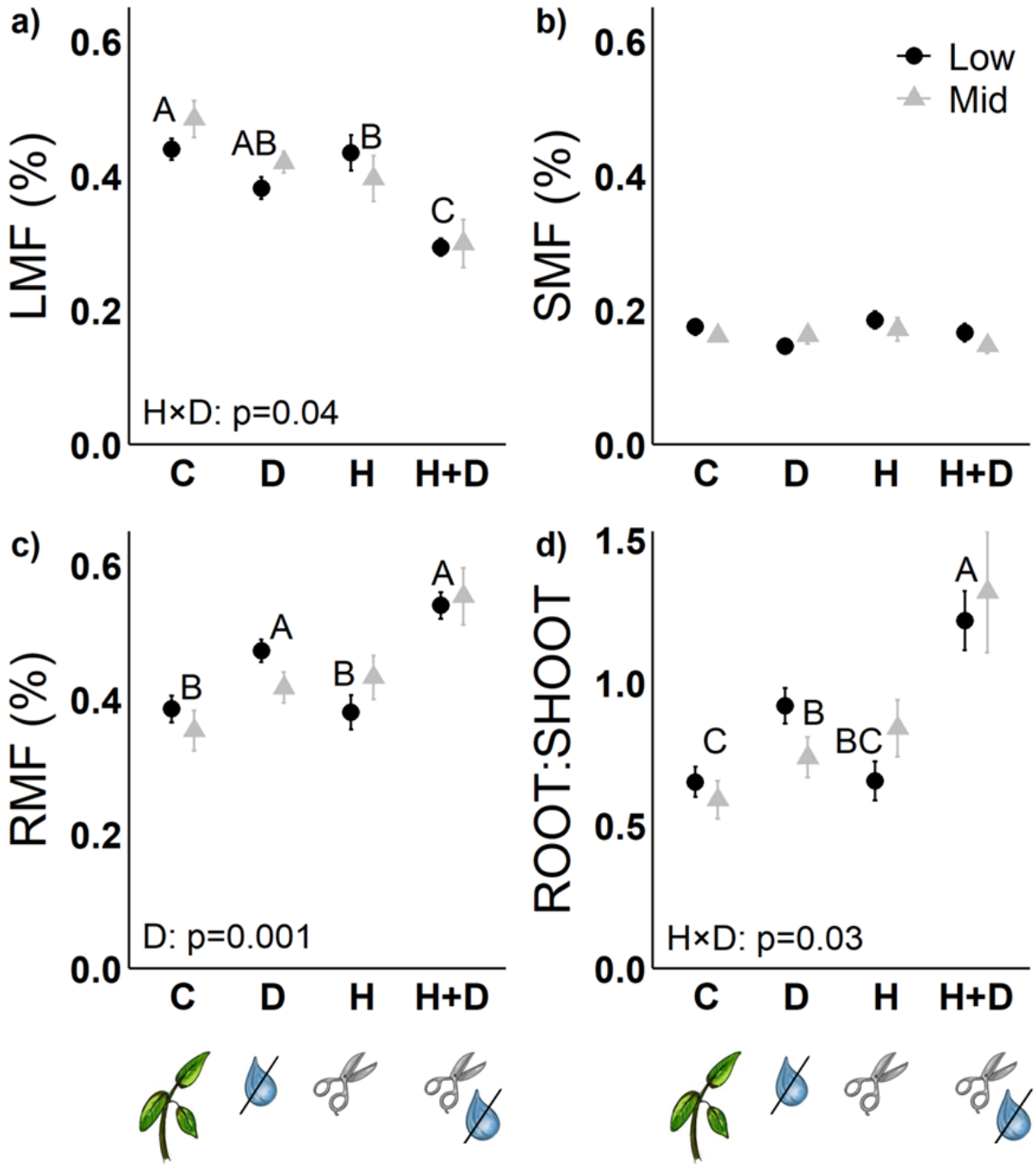

Figure 2

Biomass allocation patterns of *Maytenus boaria* seedlings from low (black points) and mid- (white points) elevation origins in response to drought and simulated herbivory. a) Leaf mass fraction (LMF); b) Stem mass fraction (SMF); c) Root mass fraction; and d) Root to shoot ratio (RM:SM). Points represent mean ± SE.

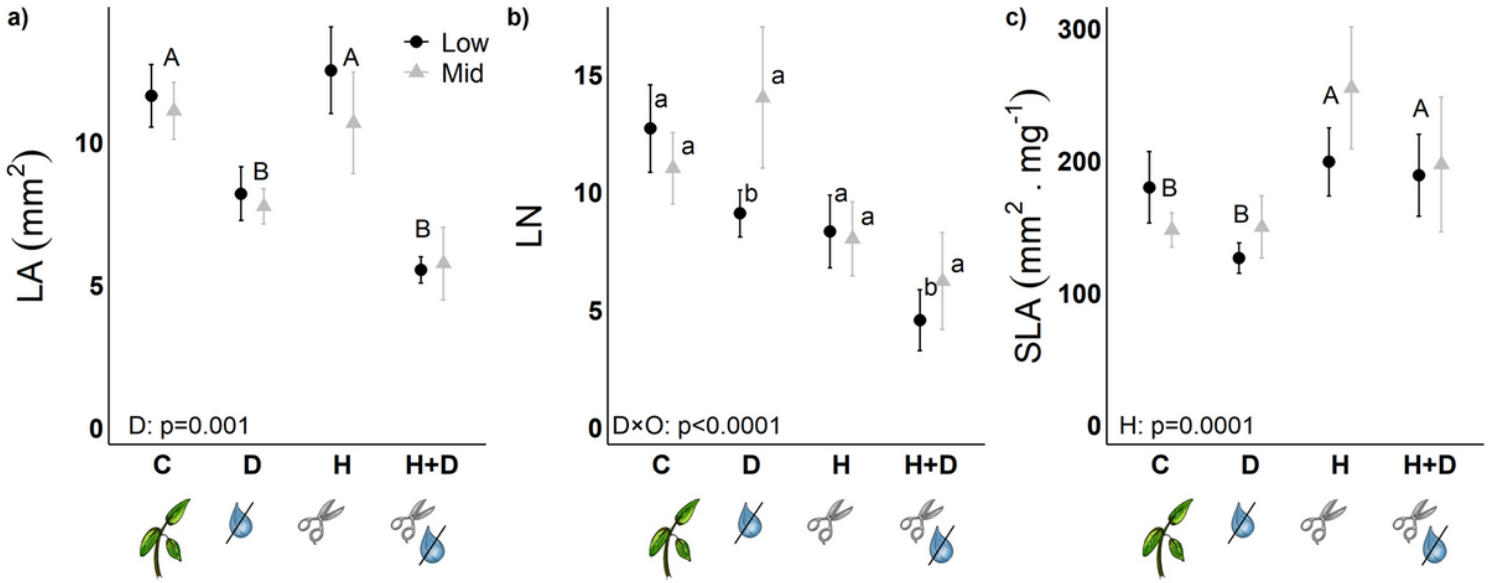

Figure 3

Morphological traits of *Maytenus boaria* seedlings of low (black points) and mid- (white points) elevation origins in response to drought and simulated herbivory. a) Leaf area (LA; cm^2); b) Leaf number (LN); c) Specific leaf area (SLA; cm^2/g). Points represent mean ± SE. Capital letters indicate statistically significant differences of the main effect factors and interactions between factors by small letters.

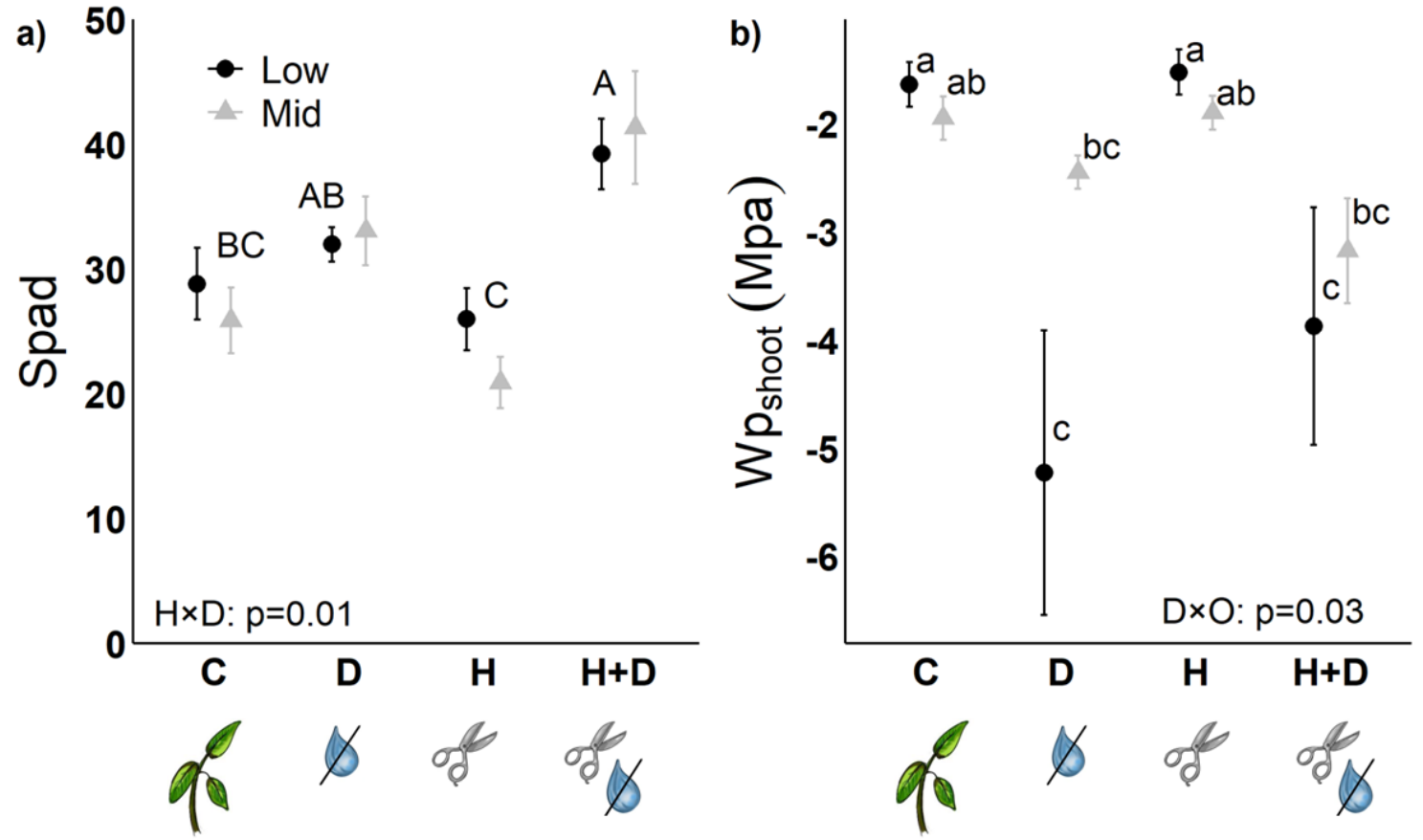

Figure 4

Physiological traits of *Maytenus boaria* seedlings of low (black points) and mid- (white points) elevation origins in response to drought and simulated herbivory. a) SPAD; b) Water seedling potential of shoots (Wp_{shoot}; MPa). Points represent mean ± SE.