



Three years of warming and rainfall reduction alter leaf physiology but not relative abundance of an annual species in a Mediterranean savanna

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ABSTRACT

Increasing air temperatures and decreasing rainfall can alter Mediterranean ecosystems, where summer heat and drought already limit plant regeneration. Manipulative field studies can help to understand and anticipate community responses to climate changes. In a Mediterranean oak wooded pasture, we have investigated the effects of warming (W, via open-top chambers increasing 1.4 °C mean air temperature), reduced rainfall (D, via gutters removing 33% of rainfall) and the combination of both factors (WD) on the winter-annual *Geranium dissectum* L. We measured reproductive phenology and output, leaf physiology during the reproductive phase, and plant relative abundance. Warming had a positive effect on plant height and little effects on leaf physiology. Rainfall reduction enhanced leaf water use efficiency. However, the most noticeable effects occurred in WD plants, which exhibited lower leaf predawn water potential and earlier flowering phenology in the first year of treatment, and a higher ratio of leaf dark respiration (R) to net CO₂ assimilation (P_n) at comparable temperatures in the third year, compared to control plants. Leaf R at ambient temperature was similar across climatic treatments. The relative abundance of *G. dissectum* decreased by 23% over three years, but similarly across treatments. A short life cycle helps *G. dissectum* to escape severe late-spring heat and drought stress. Moreover, stomata closure and thermal acclimation of R can attenuate plant stress impact on reproduction. Adaptability of the short-lived annual *G. dissectum* could mitigate climate change impact on community composition over short periods (e.g. three years); however, a reduction in net carbon gain could eventually affect its reproductive success and persistence in the community.

1. Introduction

Ecosystems are facing an unprecedented change in temperature. At a global scale, every decade has been warmer than the previous one since 1970, with northern temperate regions having experienced a 0.2 °C increase in temperature per decade over this time (Dillon et al., 2010; Lenssen et al., 2019; GISTEMP Team, 2021). In some regions, a reduction in precipitation will likely accompany the increase in temperature projected for this century. One such region is the Mediterranean basin (García-Ruiz et al., 2011), where plants already face high temperatures and low precipitation during summer. While plants are adapted to wide

changes in temperature and rainfall between years, warmer and drier conditions can enhance stress, reduce growth, reproductive success and survival (i.e. plant fitness), and alter plant communities. Over few years, e.g. in less than 5 years, sustained climate alterations can increase seedling mortality, reduce seed production or preclude seed germination, with these changes resulting in a new community of individuals better adapted to the new climatic conditions (Klein et al., 2004, 2008; Walker et al., 2006; Zeiter et al., 2016). Microevolution and phenotypic plasticity underpin rapid responses of plant communities to environmental changes. Thus, assessing plant functional traits related with plant phenology, morphology and physiology of dominant species in response

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to long-term changes in temperature and/or precipitation can provide valuable insights into plant fitness (Pérez-Ramos et al., 2019), and help to predict and ultimately mitigate future changes in community composition (Violle et al., 2007). This can be especially important in agroforestry systems, where high and rapid changes in the abundance of forage species in response to climate change can have important socio-economic consequences (Klein et al., 2008; IPCC et al., 2014; Bishaw et al., 2022).

The responses of plants to environmental changes integrate multiple functional axes. Changes in plant phenology, growth and reproductive outcome have been observed in experimental field studies simulating future climate conditions. Warming generally advances plant phenology, so that leaves and flowers emerge earlier (Whittington et al., 2015; Valencia et al., 2016; Rice et al., 2018); in some cases leaves senesce earlier too (Jochum et al., 2007), but in others, senescence remains unaltered or is delayed by warming, which results in an extended growing season length (Rice et al., 2018). Partly due to these phenological changes, pollination success can decline because of a mismatch between flowering time and pollinator development (e.g. Solga et al., 2014). Moreover, fruit development, fruit predation and vegetative size at reproduction time can also change, which may result in negative warming effects on reproductive success across species (Hovenden et al., 2008a, 2008b; Springate and Kover, 2014; Valencia et al., 2016). Drought stress, often enhanced by warming, has variable effects on leaf-out and reproductive phenology, but it tends to accelerate leaf senescence (Kramer and Boyer, 1995; Munné-Bosch and Alegre, 2004; Feller, 2016) and reduce reproductive success (Peñuelas et al., 2004a; Hovenden et al., 2008a, 2008b; Valencia et al., 2016; Zeiter et al., 2016).

The above responses depend on the effects of temperature and water availability on plant metabolism. The temperature optimum of leaf photosynthesis is lower than the temperature at which leaf respiration (R) peaks (Scafarò et al., 2021). Thus, warming can reduce leaf net CO₂ assimilation (P_n), and the carbon pool available for storage, growth and reproduction. However, acclimation of photosynthetic and respiratory metabolism to sustained increases in temperature can avoid heat stress. Thermal acclimation of R to long-term warming reduces the slope and/or intercept of short-term R-temperature response curves, as compared to less warm periods or treatments (Atkin and Tjoelker, 2003; Slot and Kitajima, 2015; Slot and Winter 2018; Scafarò et al., 2021). Because of thermal acclimation of R, plants growing at different temperatures can exhibit similar R rates, and maintain a similar carbon balance. A similar reduction in R and the sensitivity of R to temperature can occur in response to soil water deficit (Atkin and Macherel, 2009; Rodríguez-Calcerrada et al., 2011). Saving carbon in maintenance processes during stress allows for more carbon to be available for defense, reproduction, storage, or future growth in the case of perennial species. In contrast, annual species do not store reserves in vegetative organs and may respond to stress by increasing leaf turnover rates and nutrient remobilization to reproductive organs in order to enhance reproductive output rather than vegetative persistence (Bazzaz et al., 1987). This can increase leaf R in non-senescent leaves (Bunce, 2007). In fact, leaf metabolic responses to stress can be intimately linked to changes in leaf life span and reproductive output in annuals. Some winter annuals exhibit rapid photosynthetic acclimation to seasonally changing temperatures, which can provide them with a competitive advantage over summer annuals (Regehr and Bazzaz, 1976; Cohu et al., 2014), for example by increasing vegetative size (and flower and fruit production) at reproduction time (Valencia et al., 2016). There are few studies on thermal acclimation of leaf R in annuals, with these studies providing contrasting results on the extent of acclimation (Talts et al., 2004; Bunce, 2007). Overall, there is a lack of studies on how long-term metabolic acclimation to simultaneous stresses affects plant fitness.

Warming and drought often have interactive effects. Studies on experimental warming and drought have shown that the combination of both factors sometimes result in a unique molecular and physiological response that is not predictable from the response to warming and

drought alone (Jiang and Huang, 2000; Hussain et al., 2019; Chano et al., 2021). Warming increases soil evapotranspiration and can enhance the reduction in soil water availability that occurs during summer. On the other hand, increased drought can exacerbate warming effects on leaf temperature by reducing stomatal conductance and transpirational cooling (Zhou et al., 2017), which can have synergistic effects on P_n and R (Jiang and Huang, 2000; Smith et al., 2016; Hussain et al., 2019; but see Zhou et al., 2017). In other cases, phenotypic changes in response to one factor can limit an effective response to another. For example, increases in specific leaf area and shoot biomass in response to warming (Poorter et al., 2009; Chano et al., 2021) could compromise an effective acclimation of the plant to drought by enhancing transpiration.

In this work, we evaluated the physiological, phenological and reproductive responses of *Geranium dissectum* L. to three years of experimental warming (W) and rainfall reduction (D) – applied separately and in combination – in a field setting in southwestern Spain. *G. dissectum* is an autogamous winter annual herb of ample distribution in Europe and central Asia (Aedo et al., 2005); common in Mediterranean grasslands and wooded pastures, where it is important for livestock due to its advanced spring phenology and fast growth. The overarching goal of the work is to understand the mechanisms that can underpin future responses of annual plant communities to climate change, an important information to adapt current management strategies to the new climate conditions forecasted by climate change models. We hypothesized that i) experimental warming and drought would affect leaf physiology, in particular the response of leaf R to short-term changes in temperature and the balance between P_n and R at a common temperature; ii) warming and drought would have interactive effects; and iii) these climate-induced changes in leaf physiology, and plant reproductive effort and phenology would result in relevant shifts in the relative abundance of *G. dissectum* compared with other plant species of the community.

2. Material and methods

2.1. Location

The study was conducted in a wooded pasture (i.e. *dehesa* in Spanish) located in *Los Pedroches* valley, Andalusia, Spain (38° 20' 49.12"N, 4° 48' 58.54"W). The selected site was at 695 m above sea level, in a flat terrain. Sparse adult trees of *Quercus ilex* L. form an open canopy that allows grassland vegetation to cover almost 100% of the soil. Sheep grazing and swine acorn feeding prevent shrub and tree encroachment. This agroforestry system dominates the landscape of the region and provides a sustainable way of life for rural population.

The climate is Mediterranean. Mean annual temperature is 15.2 °C and mean annual precipitation 479 mm. During summer, mean temperatures are high (25.2 °C) and rainfall is less than 35 mm. Winters are mild, with a mean temperature of 7.2 °C (2007–2019 data from *Hinojosa del Duque* meteorological station, located at 543 m a.s.l., 35 km away from the experimental site). The soil is frank-sandy, acidic and poor in some macronutrients such as phosphorus.

2.2. Experimental design

Six plots of 4 × 6 m size were permanently instrumented to simulate the increases in warming and rainfall reduction projected for the Mediterranean region for the next decades (Spinoni et al., 2018). The plots were fenced to avoid instrumental damages by large herbivores. The treatments were: warming (W), rainfall reduction (D), warming plus rainfall reduction (WD) and control (C). *In-situ* rainfall reduction was achieved by means of transparent methacrylate gutters, suspended at approximately 1.5 m height in one end and inclined 20° to move the rain outside the plots. The gutters, covering half of each replicate plot, were spaced to reproduce a reduction in rainfall of 33%. On each side of the

plot, *In-situ* warming was achieved by using Open Top Chambers (OTCs), built up with six transparent methacrylate panels (with 93.7% irradiance transmittance in the range 280–750 nm wavelength), inclined 45° to form a six-sided truncated pyramid of 0.65 m² base and 40 cm height. Thus, on each replicate plot, there were eight subplots, two per climatic treatment.

We finished the installation of the experiment by September 2016, before the beginning of autumn rains and the germination of most annual plants and the target species *G. dissectum*. We monitored climatic variables on each treatment during the following three growing seasons, from November through April (see below). Early germination in late autumn allows seedlings of *G. dissectum* to develop a small root system and some few basal leaves over first winter months. Thereafter, in late winter or early spring, growth increases markedly. In the study site, this species completes its life cycle by the end of April or beginning of May. On 4–6 April 2017 and 1–3 April 2019, when plants had reached the reproductive stage, we selected six plants of *G. dissectum* per treatment for measuring leaf gas exchange and water potential (see below). The plants had no apparent symptoms of disease or necrotic leaves. Three plants sampled in 2019 had a few bottom leaves that were yellowing – none in 2017. In addition, we evaluated the change in plant community composition since April–May 2017 to April–May 2019 (see below).

2.3. Environmental variables

We measured volumetric soil water content in three sub-plots per treatment and air temperature in one sub-plot per treatment. Soil water content at 10-, 20-, 30- and 40-cm depth was measured during the growing season, approximately every 7–14 days during spring. We inserted a soil moisture probe (PR2/4 Profile Probe, Delta-T Devices, Cambridge, UK) into 40-cm long tubes permanently installed on the soil. Air temperature near the soil surface was measured every hour with automated temperature data loggers (HOBO Pendant UA-001-64, Onset, Bourne, MA).

We did not monitor rainfall in the experimental site. Annual rainfall from the *Hinojosa del Duque* meteorological station, the nearest station to our study site of comparable physiographic characteristics, was 275, 534 and 228 mm in 2017, 2018 and 2019, respectively (223, 399 and 167 mm during each respective growing season).

2.4. Leaf physiology

Two healthy leaves per plant were harvested, one before dawn and the other at midday, to measure leaf predawn (Ψ_{pd}) and midday (Ψ_{md}) water potential using a custom-made pressure chamber, with ± 0.5 bar precision (Manofrigido, Lisbon, Portugal).

Gas exchange was measured on one healthy, intact leaf per plant, using a portable photosynthesis system (Li-6400; Li-Cor Inc.) coupled to a 6-cm² cuvette (6400-02B LED Light Source). We excluded from sampling small leaves in the upper part of the stem and old leaves when they were clearly chlorotic. Tape was loosely fit around the stem or the petiole to identify the leaf being measured. Leaves were harvested at the end to measure the area and dry mass of the leaf portion fitting into the cuvette to calculate leaf mass per area (LMA) and recalculate leaf gas exchange rates.

Net CO₂ assimilation (P_n) and stomatal conductance to water vapor (g_s) were measured once in 2017 and once in 2019, around midday (from 13.00 to 15.00 local time), at 400 ppm air CO₂ concentration, ambient temperature (19–23 °C), ambient relative humidity (40–75%) and saturating light (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Respiration (R) was measured in darkness, repeatedly in the same leaf over three sampling days of 2017 and 2019. Measurements were made at ambient temperature (2–29°C) and ambient relative humidity (35–95%) during nighttime and daytime to have a range of temperatures as wide as possible. During the day, the leaves were darkened with aluminum foil for 25–35 min before measuring R to ensure darkness acclimation (see [Atkin et al., 2000](#) or

[Rodríguez-Calcerrada et al., 2010](#) for similar measurement protocols).

2.5. Plant reproductive phenology and reproductive effort

In 2017 (not in 2019), we monitored weekly the reproductive phenology of all *G. dissectum* plants present in six replicate sub-plots per treatment. We annotated the beginning, peak and end of flowering and fruit production. We defined the beginning of flowering and fruit production as the day of year (DOY) in which we observed the first flower and fruit, respectively, in at least one plant. The peak of flowering and fruit production was the DOY in which the number of flowers and fruits, respectively, was the highest. The end of flowering and fruit production was the DOY in which the last flower wilted and the last fruit ripened, respectively. Finally, the duration of the flowering and fruit production periods was computed as the difference between the end and the beginning of flowering and fruit production, respectively.

In 2019 (not in 2017), we counted the number of fruits produced by the plants sampled for leaf physiology; we also measured the plant height using a rule. As a coarse proxy of the reproductive effort, we divided the number of fruits by the plant height.

2.6. Plant community composition

In April–May of 2017 and 2019, we identified all vascular plant species in the six replicate sub-plots per treatment. We placed four 21 × 21 cm quadrats (formed by 9 squares of 7 × 7 cm) to quantify species relative abundance as the number of squares where each species was present divided by the number of squares. We expressed the data in percent units.

2.7. Data analyses

To test if the relationship between leaf temperature and R was significantly different among climatic treatments, we used a linear model to test the interaction effect between leaf temperature and the factor “treatment” on the natural logarithm of R (separately for 2017 and 2019). Moreover, for each plant, we calculated the temperature sensitivity of R by means of the Q_{10} index; i.e. the proportional change in R for a 10 °C change in temperature, as:

$$Q_{10} = e^{10k},$$

where k is the slope of the linear relationship between leaf temperature and the natural logarithm of R. Then, we calculated R at the mean ambient temperature registered over the 10 days preceding the last day of sampling in each treatment (R_{amb}), and at a standardized temperature of 20 °C (R_{20}) to compare all treatments at the same temperature. To do this we used the following equation:

$$R_i = R_0 Q_{10}^{\frac{T_i}{10}},$$

where R_0 is a reference R at 0 °C, resulting from the linear relationship between leaf temperature and the natural logarithm of R, and the subscript i for R_i and T_i refers to either ambient or standardized R and T, respectively.

Means of Q_{10} , R_{amb} and R_{20} as well as all other measured or calculated variables related with plant phenology and reproduction were compared between years and treatments via analysis of variance (ANOVA). The main and interaction effects of factors “year” and “treatment” were tested via factorial ANOVA. Independently of the interaction effect, means were compared among the four treatments separately by years via one-way ANOVA. We checked the normality of residuals with residual plots and the homogeneity of variances among treatments with the Levene test. Leaf gas exchange rates of one plant in 2017 were abnormally high and were deleted from the dataset. Rates of P_n and g_s of three plants were missing in 2017. The final number of

Table 1

Abiotic variables in the two growing seasons of sampling for leaf physiology (i.e. 2017 and 2019) and averaged over all three growing seasons (2017–2019) since the set-up of the experiment in the following treatments: warming (W), rainfall reduction (D), warming plus rainfall reduction (WD) and control with no experimental manipulation (C). Variables are: T_{mean} (°C), mean daily air temperature averaged from November through April; T_{max} (°C), maximum daily air temperature averaged from November through April; T_{min} (°C), minimum daily air temperature averaged from November through April; $T_{sampling}$ (°C), mean daily air temperature averaged over the 10 days preceding the last day of sampling (6 April 2017 and 3 April 2019); SWC (%), volumetric soil water content averaged from November through April; and $SWC_{sampling}$ (%), volumetric soil water content in the last day of sampling. Air temperature was measured in one replicate sub-plot per treatment and SWC in three sub-plots per treatment; SWC data are means \pm standard error.

	Treatment	T_{mean}	T_{max}	T_{min}	$T_{sampling}$	SWC	$SWC_{sampling}$
2017	C	9.4	18.1	4.5	11.6	27.8 \pm 4.4	26.5 \pm 4.8
	D	10.0	20.0	4.5	13.1	21.4 \pm 4.2	19.3 \pm 4.8
	W	10.1	20.1	5.1	12.2	26.3 \pm 2.8	24.2 \pm 3.1
	WD	10.5	20.1	5.4	12.7	21.9 \pm 4.0	18.6 \pm 4.6
2019	C	7.7	15.2	2.9	11.3	23.1 \pm 3.8	16.1 \pm 2.6
	D	8.9	21.9	2.8	13.3	19 \pm 4.1	11.2 \pm 2.9
	W	9.3	20.9	3.5	13.5	22.1 \pm 2.4	14.7 \pm 2.0
	WD	9.7	17.5	5.2	14.1	20.2 \pm 4.0	12.7 \pm 3.7
2017–2019	C	8.5	17.3	3.3	12.4	24.4 \pm 3.9	17.8 \pm 3.2
	D	9.5	20.8	3.8	13.1	19.6 \pm 4.2	12.5 \pm 3.1
	W	9.8	21.4	4.2	12.8	23.5 \pm 2.7	15.4 \pm 2.8
	WD	10.0	19.1	5.1	13.2	20.6 \pm 3.9	13.2 \pm 3.4

replicates per treatments was six in 2019 and 4–6 in 2017. When assumptions of parametric analysis were not met, as was the case for the ratio of R_{20} to P_{n20} , the data were log-transformed to improve homoscedasticity. When means were significantly different at $P < 0.05$, post-hoc comparisons (Tukey’s HSD tests) were applied to discern which treatments were significantly different from each other. We used Statistica 8.0 (StatSoft, Inc) to do the analyses.

3. Results

3.1. Changes in air temperature and soil water content

Air temperatures were higher in W and WD treatments. Averaged over the three growing seasons, mean, maximum and minimum daily temperatures were 1.4, 3.0 and 1.4 °C higher in W and WD than in C,

respectively (Table 1). Averaged over the 10 days preceding the last day of sampling, mean daily temperatures were 0.8 °C higher in WD and W than in C in 2017 and 2.5 °C higher in WD and W than in C in 2019. Minimum daily temperatures in D were more similar to those in C, whereas mean and maximum daily temperatures in the treatment D were, unexpectedly, more similar to those in W and WD than to those in C, probably by an effect of microtopography of the sub-plots.

Volumetric soil water content (SWC) averaged over the three growing seasons was 4% units lower in D and WD (20.1%) than in C and W (24.0%; Table 1). Differences among treatments were consistent in 2017 and 2019. During sampling for leaf physiology, on the first week of April, SWC was 6.4% units lower in D and WD (19.0%) than in C and W (25.4%) in 2017 and 3.4% units lower in D and WD (12.0%) than in C and W (15.4%) in 2019.

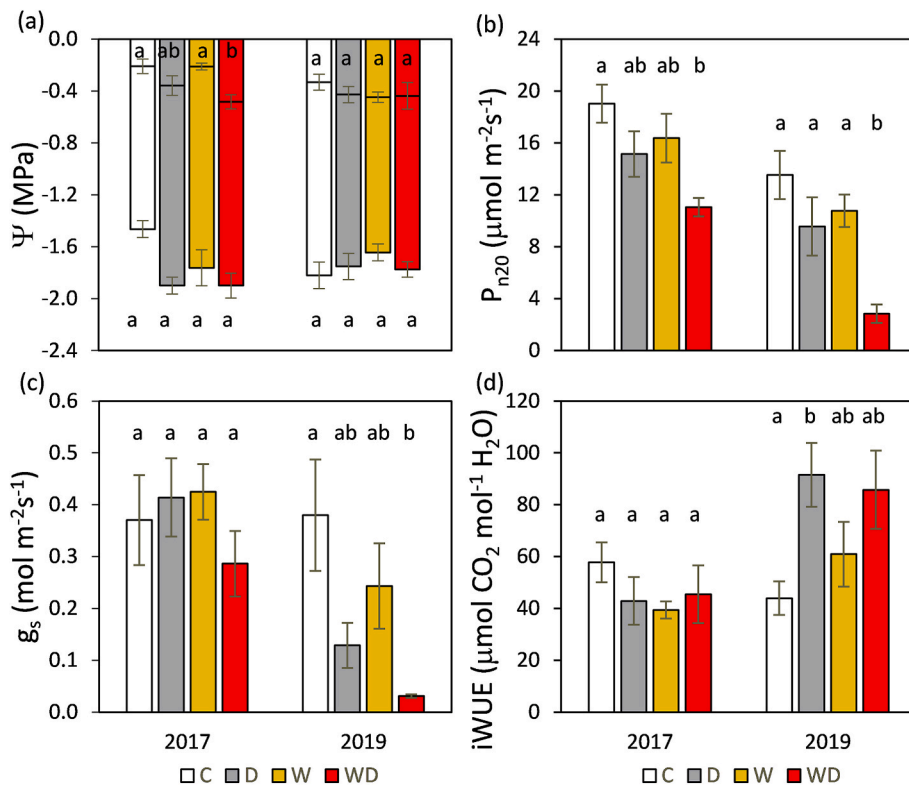


Fig. 1. Means (\pm SE) of leaf predawn (upper bars) and midday (lower bars) water potential (Ψ , a), leaf net CO_2 assimilation at ambient temperature (i.e. 20 °C, P_{n20} , b), leaf stomatal conductance to water vapor (g_s , c) and intrinsic water use efficiency (iwUE, d) in plants from the climatic treatments warming (W), rainfall reduction (D), warming plus rainfall reduction (WD) and control (C). Different letters separate significantly different means according to Tukey’s HSD tests performed separately for 2017 and 2019.

3.2. Leaf water potential and leaf photosynthetic variables

Leaf predawn water potential (Ψ_{pd}), P_{n20} , g_s and intrinsic water use efficiency (i.e. the ratio of P_{n20} to g_s ; iWUE) varied significantly between years; all these variables except iWUE were lower in 2019 (Fig. 1). The differences between years were similar in all climatic treatments except for Ψ_{md} and iWUE, for which the interaction effect between year and treatment was significant at $P < 0.05$. Midday water potential (Ψ_{md}) was 0.2 MPa lower in 2019 than 2017 in control (C) plants, but it was between 0.1 and 0.4 MPa higher in 2019 than 2017 in all the climatic treatments (i.e. W, D, and WD). Similarly, interannual changes in iWUE were different in C and the climatic treatments: iWUE was $14 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ lower in 2019 in C plants, but it was 49, 22 and $40 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ higher in 2019 in D, W and WD, respectively. Although the year by treatment effect was not significant at $P < 0.05$ for g_s , it is worth noting that this variable did not change between years in C plants but decreased by 0.29, 0.18 and $0.26 \text{ mol m}^{-2} \text{ s}^{-1}$ in D, W and WD, respectively (Fig. 1).

Differences in leaf water potential and leaf photosynthetic variables among treatments were not the same in 2017 and 2019. In 2017, Ψ_{pd} was significantly lower in WD (-0.48 MPa) than in the treatments W and C (-0.21 MPa), and intermediate in D (-0.36 MPa ; P -value ANOVA: 0.006; Fig. 1a). Similar results were found for P_{n20} ; it was significantly lower in WD ($11.0 \mu\text{mol m}^{-2} \text{ s}^{-1}$) than in C ($19.0 \mu\text{mol m}^{-2} \text{ s}^{-1}$), and intermediate in W and D (P -value ANOVA: 0.036; Fig. 1b). The other variables – Ψ_{md} , g_s and iWUE – did not change significantly among treatments in 2017. In 2019, leaf water potential did not change among treatments, neither before dawn nor at midday, but leaf photosynthetic variables did significantly change. The combination of W and D resulted in 5-times lower P_{n20} and 12-times lower g_s in WD plants than in C plants (P -values ANOVA: 0.001 for P_{n20} and 0.015 for g_s ; Fig. 1 b, c). Similarly, D caused a higher reduction in g_s than in P_{n20} , although such reductions were of lower magnitude and not statistically significant. Thus, iWUE was double in WD ($85.7 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) and D ($91.6 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) than in C plants ($44.0 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$; P -value ANOVA: 0.038; Fig. 1d).

3.3. Leaf respiratory variables and comparison with photosynthetic rates

Leaf dark respiration (R) increased exponentially with measurement temperature (Fig. 2). The Q_{10} , an index of the temperature sensitivity of R, was similar in both years (Fig. 3a). However, rates of R (computed at ambient temperature or 20°C) were significantly lower in 2019 than 2017 (Fig. 3 b-e). The ratio of leaf respiration to net CO_2 assimilation at 20°C (R_{20}/P_{n20} ; Fig. 3f), tended to be higher in 2019, especially in D and WD treatments, although the interaction effect between year and

treatment was not significant for any variable.

Differences in leaf respiratory variables among treatments were higher in 2019 than in 2017. In 2017, there was a trend for area- (R_{20}) and mass-based ($R_{20,m}$) respiration at 20°C to be lower in plants exposed to climatic treatments (Fig. 3 b, c), but none of these differences were significant at $P < 0.05$. In 2019, however, R_{20} was significantly lower in WD than in C (0.52 vs $0.82 \mu\text{mol m}^{-2} \text{ s}^{-1}$, respectively), and intermediate in W ($0.73 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and D ($0.77 \mu\text{mol m}^{-2} \text{ s}^{-1}$; P -value ANOVA: 0.029; Fig. 3b); and the same was true for $R_{20,m}$ (P -value ANOVA: 0.017; Fig. 3c), given non-significant differences between treatments in LMA (Fig. 3d). Because of this, R rates measured at the ambient temperature of each treatment (over the 10 days preceding the end of the sampling; R_{amb}) were not significantly different among treatments (Fig. 3e), despite mean air temperatures differed by almost 3°C among them during this time (Table 1). The higher decline in P_{n20} than in R_{20} resulted in higher R_{20}/P_{n20} in D and more clearly in WD than in C (P -value ANOVA: 0.018; Fig. 3f).

3.4. Plant reproductive phenology and output

In 2017, the peak of flowering was approximately two weeks earlier in the WD treatment than in treatments C and D ($P = 0.011$; Fig. 4a). There was no other significant difference in reproductive phenology at $P < 0.05$.

In 2019, we counted the number of fruits produced by plants sampled for physiology. The mean number of fruits per plant tended to decrease in WD plants relative to the other climatic treatments (Fig. 4b). The number of fruits per unit shoot length also tended to be lower in WD plants (Fig. 4c), given the higher height of warmed plants (Fig. 4d). However, differences in fruit production were not significantly different among treatments at $P < 0.05$.

3.5. Changes in plant community composition

The three most abundant species in the first inventory exhibited important changes in their relative abundance over time: *G. dissectum* and *Erodium moschatum* were less abundant in 2019 than in 2017, while *Hordeum murinum* subsp *leporinum* increased its abundance in 2019 (Fig. 5). However, the climatic treatments did not affect the variation in relative abundance of any of the above-mentioned species.

4. Discussion

The first hypothesis of this work posing that experimental warming and rainfall reduction would affect leaf physiology received partial support. Leaf physiology changed little in response to a sustained 33%

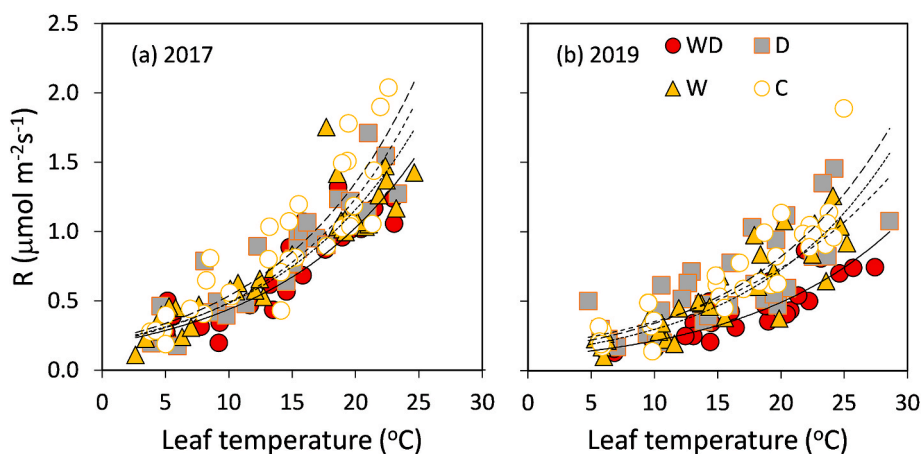


Fig. 2. Diurnal evolution of leaf dark respiration (R) with increasing temperature in 2017 (a) and 2019 (b) in plants from the climatic treatments warming (W), rainfall reduction (D), warming plus rainfall reduction (WD) and control (C).

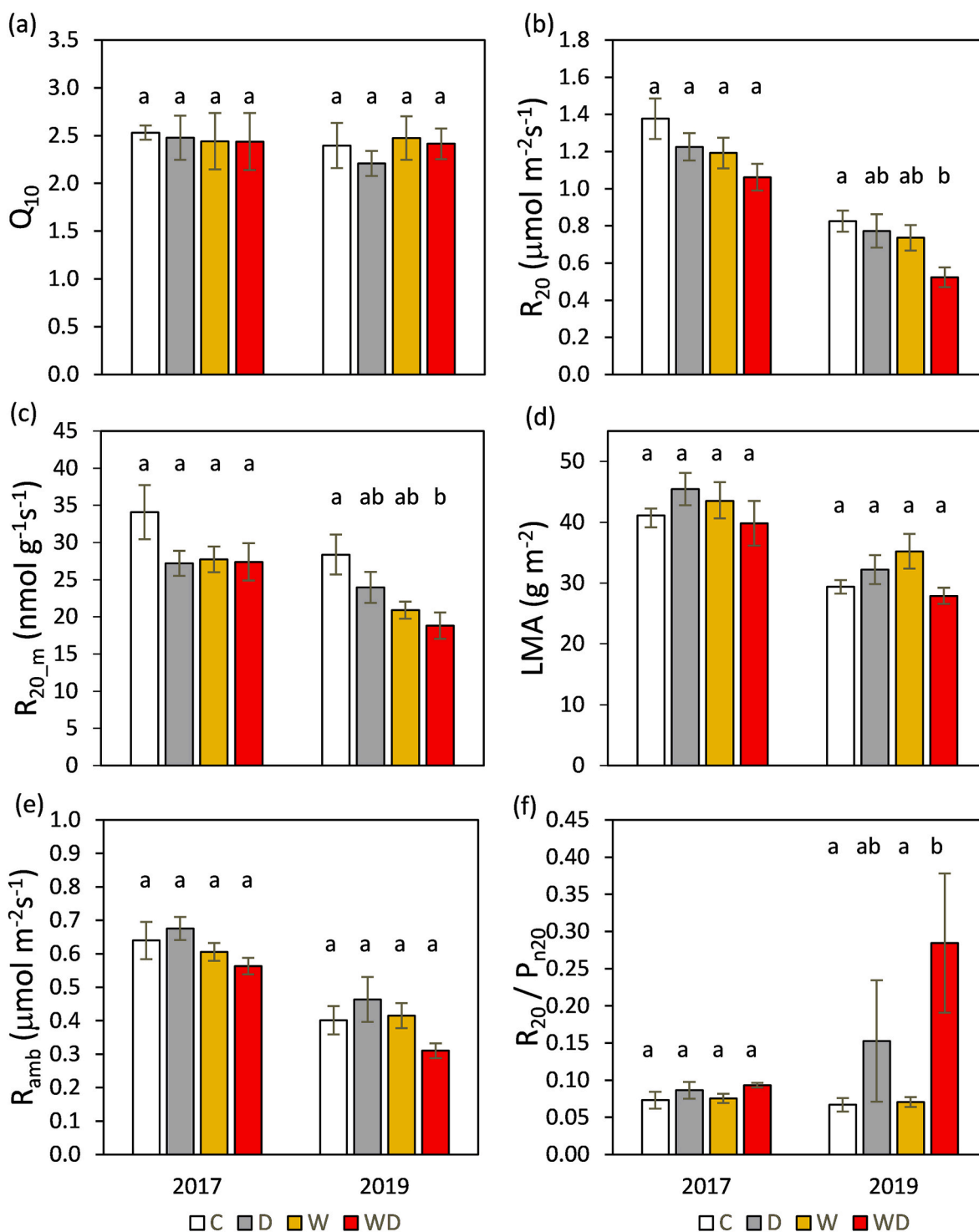


Fig. 3. Means (\pm SE) of leaf dark respiration sensitivity to diurnal changes in temperature (Q_{10} , a), leaf dark respiration at 20 °C (R_{20} , b), leaf dark respiration at 20 °C on a per-dry-mass basis (R_{20_m} , c), leaf mass per area (LMA, d), leaf dark respiration at ambient temperature (R_{amb} , e) and the ratio of R_{20} to P_{n20} (f) in plants from the climatic treatments warming (W), rainfall reduction (D), warming plus rainfall reduction (WD) and control (C). Different letters separate significantly different means according to Tukey's HSD tests performed separately for 2017 and 2019. R_{amb} was calculated at the prevailing air temperature in each climatic treatment over the 10 days preceding the last day of sampling.

reduction in rainfall or a 1.4 °C increase in mean air temperature during the vegetative period. However, remarkable changes were observed in response to the combined application of experimental warming and rainfall reduction (WD), with plants exhibiting a significant decline in photosynthesis (P_{n20}) and respiration (R_{20}), and a significant rise in the ratio R_{20} to P_{n20} as compared to C plants. Warming and rainfall reduction had synergistic effects on leaf physiology and flowering peak time,

which occurred two weeks earlier in WD plants than in C plants. This is in line with previous works where drought stress exacerbates heat stress effects on photochemical efficiency, plant desiccation and epigenetic regulation (Jiang and Huang, 2000; Chano et al., 2021). However, at odds with our third hypothesis, physiological changes did not have a significant impact on reproductive output or relative abundance of *G. dissectum* after three years of treatment application. We discuss

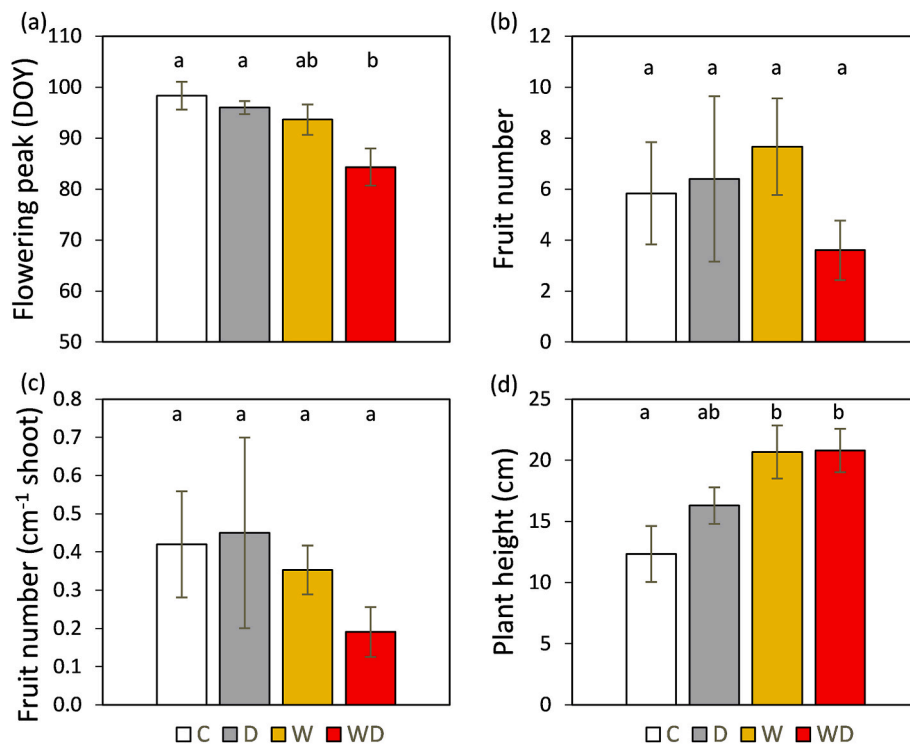


Fig. 4. Means (±SE) of flowering peak time (a), fruit number per plant (b), fruit number relative to plant height (c), and plant height (d) in plants from the climatic treatments warming (W), rainfall reduction (D), warming plus rainfall reduction (WD) and control (C). Different letters separate significantly different means according to Tukey's HSD tests. Flowering peak time is for 2017. The rest of variables are for 2019.

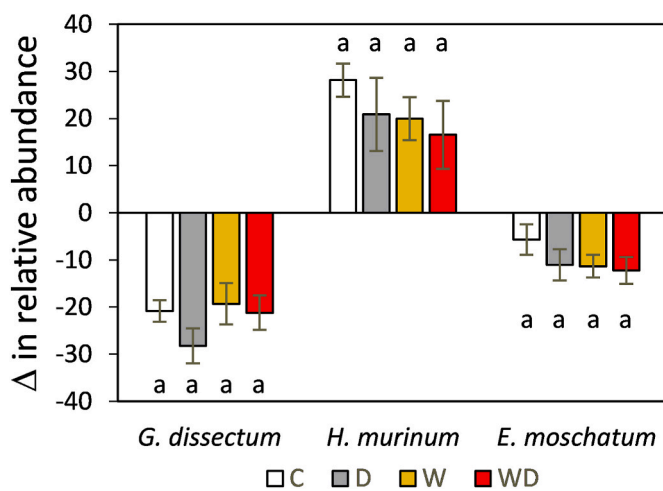


Fig. 5. Mean (±SE) change in species relative abundance (in terms of plant cover) in the climatic treatments warming (W), rainfall reduction (D), warming plus rainfall reduction (WD) and control (C). Different letters separate significantly different means according to Tukey's HSD tests. The species (*Geranium dissectum*, *Hordeum murinum* subsp. *leporinum* and *Erodium moschatum*) were the most abundant in the first year of the study.

whether leaf physiological changes reflected an advancement of the life cycle induced by climatic alterations, which would allow plants to grow and reproduce before severe stress, or whether they reflected a response to heat and drought stress that might eventually influence the reproductive output.

Warming had a positive effect on plant height, as observed in other short-lived annuals (Chaves et al., 2003; Heschel and Riginos, 2005). Higher plant height in W is not surprising because plant growth increases with temperature in species with high metabolic temperature

optima that are not limited by other stresses (Loik et al., 2000; Rustad et al., 2001; Peñuelas et al., 2004b). The fact that drought did not override the positive effect of warming indicates that warming effects on plant growth occurred before soil water content reductions caused by summer drought and rainfall exclusion were severe enough to reduce soil water potential, soil water and nutrient uptake, and cell turgor. This is consistent with earlier flowering peak in WD and (non-significantly) in W, and with similar height of C and D plants, which would complete their final size early in the growing season, to escape severe soil desiccation. Thus, observed changes in leaf physiology were irrelevant for growth, as plants had reached their final size at the time of sampling for leaf physiology. In the same study set-up, another herbaceous species (*Hordeum murinum* subsp. *leporinum*) exhibited earlier flowering onset in W and WD treatments (Chano et al., 2021). Valencia et al. (2016) discussed that high advancement of flowering phenology induced by warming, in comparison with other reproductive traits, was related to the absence of water-limiting effects associated to warming early in the growing season in a semi-arid Mediterranean grassland. Studies in humid sites where warming does not likely increase water stress have also reported advanced flowering phenology (e.g. Rice et al., 2018). Here, however, warming effects on advancing flowering peak time – not flowering onset – were only significant when combined with reduced rainfall, which suggests that early, mild water stress and warming triggered an abrupt increase in flowering. Hormonal signals or even transient increases in some soluble sugars (e.g. glucose) at mild water stress can prompt a vegetative phase change (Chaves et al., 2003; Yang et al., 2013; Rankenberg et al., 2021).

Under the rationale of an advance in plant's life cycle, leaf physiological changes in WD plants would reflect faster leaf ageing. Leaf heating caused by warming and drought (via stomata closure and reduced transpiration cooling) accelerates leaf ageing, which feeds forward on g_s and P_n declines brought about by stress (Chaves et al., 2016; Feller, 2016; Sade et al., 2018; Berwaers et al., 2019; Rankenberg et al., 2021). Thus, at the time of sampling, leaves of WD plants would have

remobilized a larger amount of carbohydrates and mineral nutrients to reproduction than those of C plants. This could affect leaf R by reducing both carbohydrate substrates (and mitochondrial activity) and nitrogen available for respiratory enzymes (and respiratory capacity). The similar sensitivity of R to short-term changes in temperature (Q_{10}) across climatic treatments suggests that carbohydrate substrates were not limiting for R, and that lower respiratory capacity underpinned lower R of WD leaves (Atkin and Tjoelker, 2003; Rodríguez-Calcerrada et al., 2010). The higher value of R_{20}/P_{n20} in WD than in W and C plants is consistent with earlier effects of stress-induced leaf senescence on photosynthesis than respiration (Munné-Bosch and Alegre, 2004; Feller, 2016). This is explained by the need of energy for catabolic processes and nutrient export during senescence; even if in some annual species, dry matter remobilization rates from leaves are not related with R rates (Bunce, 2007). However, contrary to a hypothetical stress effect in accelerating leaf ageing and the plant's life cycle, the increase in iWUE (i.e. P_n/g_s) in WD plants suggests that the main factor limiting P_n was low g_s , rather than the reduced carboxylation efficiency or photochemical efficiency (and P_n) that would be associated to leaf nutrient resorption. Moreover, leaves were not apparently yellowing during sampling and, although peak flowering occurred earlier in WD, fruit phenology was similar across treatments. An unequivocal explanation to lower leaf gas exchange in WD plants is not possible without data on foliar nutrient concentrations.

Reduced rainfall, alone or in combination with W, provoked higher stomata closure in 2019 than in 2017. Stomata closure is a typical drought response of land plants that reduces leaf desiccation and the risk of xylem embolism (Kramer and Boyer, 1995; Chaves et al., 2003). In fact, differences in Ψ between climatic treatments were lower in 2019 than in the less dry 2017. The decline in g_s limits CO_2 diffusion to the chloroplasts but increases iWUE. The effects of iWUE on plant fitness are not straightforward. Positive and negative effects have been reported for annual species, for example in relation to drought timing. Low g_s and high iWUE were positively related with reproductive fitness in the annual plant *Impatiens capensis* when drought occurred late in the growing season (Heschel et al., 2002), whereas they were negatively related with reproductive fitness when drought occurred early (Heschel and Riginos, 2005). *G. dissectum* is a short-lived winter annual that has usually completed its life cycle by the end of April in the study area (well before summer drought). Preventing water loss and xylem embolism via stomata closure would enable an adequate provision of water to developing fruits (Dudley, 1996). However, a conservative water use may be irrelevant or detrimental to fitness as it comes at the cost of carbon gain and availability of photoassimilates for reproduction (Geber and Dawson, 1990; Chaves et al., 2003; Heschel and Riginos, 2005; Ruan et al., 2012; Sade et al., 2018). The positive relationship between reproduction output and carbon assimilation explains why osmotic adjustment, an effective mechanism to keep turgor and P_n , favors grain yield in water-stressed environments (Santamaría et al., 1990). The reduction in g_s , however, suggests a decrease in leaf turgor that might affect other plant organs and, in any case, it reduces P_n .

Leaf metabolic acclimation to elevated temperatures contributes to save carbon, as it results in near homeostatic respiration to photosynthesis ratios across ambient temperatures (e.g. Bronson and Gower, 2010; Dusenège et al., 2019). However, this is rarely the case in response to elevated water stress (Galmés et al., 2007; Atkin and Macherel, 2009; Rodríguez-Calcerrada et al., 2011). Here, although the decrease in basal R resulted in similar R rates across treatments (i.e. similar R_{amb} despite higher temperature in the OTCs) and attenuated the decline in net carbon gain caused by photosynthetic limitations, the R_{20} to P_{n20} ratio reached almost 30% on average in WD plants (vs 7% in control plants). This did not seem to affect carbon available to form flowers or fruits (Fig. 4b and c), which can appear before severe water stress and photosynthetic limitations occur (Yang et al., 2013; Rankenberget al., 2021). However, it might eventually affect seed maturation (e.g. Schmidt et al., 2020), a feature not evaluated in this study, which can

further reduce seed viability or seedling vigor (Hovenden et al., 2008b). The effect of reduced net carbon gain on reproductive output varies across species and years of different reproductive phenology (Geber and Dawson, 1990; Lambrecht et al., 2007) and deserves further study in the case of *G. dissectum*.

In spite of the advantages of *in situ* climate manipulation for evaluating plant responses to projected climate changes, there are undesired side effects of the use of gutters and OTCs to reproduce reduce rainfall and increase temperature, respectively. The question of experimental warming passively via the greenhouse effect of the OTCs is especially controversial for their potential disruption of wind, rainfall, irradiance or air relative humidity, among other drawbacks (see e.g. De Frenne, 2015). An increase in temperature by OTCs can lower the air relative humidity compared to control plots (as warmer air saturates with more water vapor than cooler air), but can also increase it (Ivanova et al., 2016; Frei et al., 2020). We acknowledge that a change in vapor pressure deficit (VPD) caused by the OTCs, or less likely the methacrylate gutters, placed well above the vegetation canopy top to reduce rainfall, could affect leaf physiology. Transpiration and water use efficiency are very sensitive to VPD (e.g. Rawson et al., 1977; Grossiord et al., 2020), but also g_s and iWUE (e.g. Grossiord et al., 2020), which were affected by W, D and WD (Fig. 1).

Changes in leaf physiology did not scale up to the community level. The relative abundance of *G. dissectum* decreased by 20–30% over three years, similarly across climatic treatments, in parallel to a 20–30% increase of the co-dominant grass *Hordeum murinum* subsp. *leporinum*. This is consistent with the non-significant impact of treatments on *G. dissectum* reproductive effort, at least in fruit counts, and with the fact that interannual variations in temperature, rainfall and soil water content exceeded climatic alterations imposed by our experimental system (see Material and Methods and Table 1). In Mediterranean-type ecosystems, interannual climate variability is high and partly responsible of high interannual variability in species turnover, particularly in dry grasslands dominated by annual species (Cleland et al., 2013; Pérez-Ramos et al., 2019). This is related with sensitivity of seed production and germination to climatic fluctuations, which can also affect competitive interactions with other species and speed up rapid changes in community composition (Lloret et al., 2004; Hovenden et al., 2008a). Moreover, stochastic processes not necessarily related to climate fluctuations contribute to interannual changes in vegetation in annual grasslands with persistent seed banks (Lavorel and Lebreton, 1992). Complex interactions between the seed pool and vegetation recruitment make predictions of plant community dynamics equally complex (Peñuelas et al., 2004b; Hamann et al., 2021). The role of reserves in buffering plant stress in perennial species may lead to think that changes in community composition can be especially fast in grasslands dominated by annual species (Hovenden et al., 2008a). However, short life cycles and high reproductive effort of annuals enhance adaptability, and would tend to mitigate community fluctuations in response to climate changes (Springate and Kover, 2014). Finally, it is unlikely that the seed bank of *G. dissectum* was persistent enough to provide new seedlings over the course of the experiment, given the thin seed coat and high seed annual mortality rate in the soil of this species (Gardarin et al., 2010). However, it cannot be ruled out that seeds from plants outside the experimental plots germinated inside them.

4.1. Concluding statements

Climate models forecast an increase in temperature and a reduction in precipitation for southwestern Europe during the XXI century (IPCC et al., 2014; 2018; Spinoni et al., 2018). Experimental warming and rainfall reduction applied here to simulate the climate changes forecasted for the region before the end of this century had a significant impact on leaf physiology of the annual species *G. dissectum*. Contrary to the hypothesis that leaf stress induced by warming and rainfall reduction would be detrimental to the species, our data suggest that,

potentially more stressful conditions in WD plots – reduced leaf gas exchange and leaf respiration – were not enough to affect the reproductive output and relative abundance of *G. dissectum*. An alternative hypothesis to explain these results is that improved water-use efficiency and decreased metabolic activity in 2019 constituted an adaptive response to cope with, and maintained the reproductive output after three years of increased heat and drought. Even if the physiological responses were the result of an advanced leaf senescence, they could still reflect an adaptive response to complete the life cycle before heat and drought stress are too high. The continuation of observations in this experimental set-up, or the use of seeds collected from each climatic treatment in controlled studies will elucidate whether observed responses in leaf physiology and flowering phenology reflect selection or phenotypic plasticity in response to interannual climate changes.

CRedit authorship contribution statement

Jesús Rodríguez-Calcerrada: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft. **Víctor Chano:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Luis Matías:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing – review & editing. **María Dolores Hidalgo-Galvez:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Jesús Cambrollé:** Data curation, Investigation, Methodology, Writing – review & editing. **Ignacio Manuel Pérez-Ramos:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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