

RESEARCH ARTICLE

Diversity and mean specific leaf area of Mediterranean woody vegetation changes in response to summer drought across a double stress gradient: The role of phenotypic plasticity

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Abstract

Aim: Many aspects of vegetation response to increased drought remain uncertain but it is expected that phenotypic plasticity may be key to early adaptation of plants to environmental stress. In this work we observe the response of specific leaf area (SLA) of woody shrub vegetation to the summer drought typical of the Mediterranean climate. In addition, to observe the possible interaction between the impact of drought and the environmental characteristics of the ecosystems, communities from different edaphic and structural contexts distributed along the double stress gradient of the Mediterranean mountains (high temperature and low precipitation at low elevation; low temperature and high irradiation at high elevation) have been analysed.

Location: Central Mountain range of the Iberian Peninsula.

Methods: Along the entire altitudinal gradient, 33 shrub communities belonging to different habitat typologies (shrublands, rocky areas, hedgerows, understory) were sampled before and after the passage of summer, both in 2017 and 2019. A total of 1724 individuals and 15,516 leaves were collected and measured to estimate the mean values and diversity of SLA of each community.

Results: The community-weighted mean and functional divergence have inverse quadratic relationships with the environmental gradient. Shrub communities at both ends of the gradient have low mean SLA values and high functional divergence of this trait. Summer drought implies a generalised decrease in the mean SLA of the communities throughout the gradient, as well as an alteration in functional richness and uniformity. However, the effect of summer drought on the plant community is mediated by the microenvironmental characteristics of its habitat.

Conclusions: Drought acclimatisation of shrub communities through phenotypic plasticity leads to rapid changes in their functional leaf structure. In the long term, our results point to an increase in plant conservative strategies, reduced ecosystem productivity, slower nutrient recycling and the reduction of communities of specific habitats as drought increases.

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KEYWORDS

altitudinal gradient, community-weighted mean, functional diversity, Mediterranean shrublands, phenotypic plasticity, specific leaf area, stress gradient, summer drought

1 | INTRODUCTION

A progressive increase in global temperatures is predicted for the coming decades, which in the case of the Mediterranean climate will also be accompanied by a decrease in average precipitation (Ranasinghe et al., 2021). Increasing drought is expected to have a notable impact on Mediterranean vegetation, raising mortality rates or decreasing its biodiversity, among other effects (Peñuelas et al., 2017), but many aspects of plant adaptation and response to these changes remain uncertain. In this sense, functional traits have emerged as promising tools to study the response of plant communities to environmental changes (Guittar et al., 2016; Kimball et al., 2016). Many studies support the potential of functional traits as reliable predictors of plant–environment relationships, life strategies and ecosystem functioning (McGill et al., 2006; Violle et al., 2007; Lepš et al., 2011; Peralta et al., 2019); however, not all functional traits are equally informative and trait–environment and trait–fitness relationships are often context-dependent (Funk et al., 2017).

The functional characteristics of communities (or ‘functional structure’) can be defined through two components: composition and functional diversity (Ricotta & Moretti, 2011). Spatial and temporal variations in these two elements can result from the action of two distinct processes: on the one hand, the turnover of species with different functional traits; and, on the other hand, intra-specific variation in traits due to genetic variability or phenotypic plasticity (Kichenin et al., 2013; Pescador et al., 2015; Westerland et al., 2021). The latter has attracted much interest among researchers for its role in the early acclimatisation of vegetation to climate change (Nicotra et al., 2010). Phenotypic plasticity can act as a buffer to environmental changes (Valladares et al., 2014) as individuals try to display the optimal values of their traits in relation to new conditions (Hekneby et al., 2006; Scheepens et al., 2010; Freschet et al., 2015); however, such plasticity is subject to costs and limits and its relationship with fitness is complex to predict (Valladares et al., 2007).

The effect of drought or other environmental changes on the functional structure of vegetation can be addressed using a space-for-time substitution approach (Pescador et al., 2015; Guittar et al., 2016), where long-term changes are inferred from spatially distinct sites that are expected to represent certain stages in a temporal succession (Pickett, 1989). However, this approach has its limitations since the use of existing environments as proxies for possible future environments is not, in itself, sufficient to predict changes in vegetation (Sternberg et al., 2011) and also because it is not possible to distinguish phenotypic plasticity from spatial genotypic variability within a species. It is therefore much more informative to combine both a spatial and temporal perspective in

order to assess the response of different ecosystems to the same environmental change (Bongers et al., 2017; Bjorkman et al., 2018). In this respect, altitudinal gradients offer a wide range of different environmental conditions in a relatively small space (Körner, 2007), which is why they are commonly used in functional ecology studies (Milla et al., 2009; van de Weg et al., 2009; Schellenberger Costa et al., 2017). However, little research has been carried out in the Mediterranean mountains where high temperatures and low precipitation at low elevations and low temperatures and high irradiance at high elevations offer unique environmental variability and imply the presence of a double stress gradient for vegetation (Biase et al., 2021; Bricca et al., 2022). Assessing the functional response of vegetation to drought in a Mediterranean altitudinal gradient can provide us with relevant information to understand the possible impacts of climate change on the different plant communities characteristic of this climate, but also on multiple ecosystem processes as productivity or nutrient cycling (Diaz & Cabido, 2001).

In altitudinal gradients it is generally observed that average community trait values related to conservative and stress resistance strategies are favoured as altitude increases, to deal with low temperatures, UV-B radiation and frost (Sundqvist et al., 2013; Pescador et al., 2016). These strategies are also adaptive in the face of increasing aridity (Pérez-Ramos et al., 2017; Stanisci et al., 2020) but, in some cases, aridity can also promote drought ‘escape’ strategies and rapid resource acquisition (Carvajal et al., 2019; Griffin-Nolan et al., 2019). In fact, drought-tolerance and high-acquisition traits can coexist in the same plants (Bondi et al., 2023). Functional diversity has no clear relationship with altitude, and completely opposite results have been found (Pescador et al., 2015; Ding et al., 2019). On the other hand, drought usually leads to a reduction in functional diversity as the values of traits allowed by the narrow environmental filter converge (Pérez-Ramos et al., 2017; Luo et al., 2019; Stanisci et al., 2020; Zuo et al., 2021). In any case, the effect of altitudinal gradients and increased aridity on the functional structure of plant communities is highly dependent on the environmental context (Sundqvist et al., 2013; Carmona, Mason, et al., 2015; Wellstein et al., 2017), on the traits analysed (Pescador et al., 2015; Luo et al., 2019) and on the vegetation type studied (Zuo et al., 2021). In this regard, it should be noted that most research in this area has been conducted in grasslands and herbaceous communities (Carmona, Mason, et al., 2015; Griffin-Nolan et al., 2019; Luo et al., 2019; Stanisci et al., 2020), where species turnover can occur very rapidly and where functional behaviour is different from that of woody vegetation (Zuo et al., 2021). Very little research has focused on woody communities where species composition changes occur more slowly and where phenotypic plasticity may play a major role in acclimatisation to environmental changes.



The present research is based on a key functional trait, the specific leaf area (SLA) which is an indicator of several ecophysiological characteristics of plants such as relative growth rate or leaf longevity, and is strongly correlated with temperature, irradiance level and water or nutrient availability (Poorter et al., 2009; Scheepens et al., 2010). Low SLA values correspond to a higher tolerance to environmental extremes and to a more conservative strategy regarding the acquisition and residence time of nutrients and water in the plant, which also implies a lower productivity and growth rate (Lavorel & Garnier, 2002; Poorter et al., 2009). On the other hand, high SLA values correspond to a rapid resource acquisition strategy, and thus to a higher growth rate but lower tolerance to environmental stress, both due to aridity and low temperatures (Lavorel & Garnier, 2002; Pérez-Ramos et al., 2012). As for the relationship between this trait and ecosystem processes, the higher the SLA of vegetation, the higher the primary productivity of ecosystems, and the higher the rate of decomposition of matter and recycling of nutrients, especially carbon and nitrogen (Lavorel & Garnier, 2002; Poorter et al., 2009).

The aim of this work is to observe the response (mediated by phenotypic plasticity) of the functional structure of the SLA of woody shrub vegetation to the summer drought typical of the Mediterranean climate, providing a novel approach and relevant information to predict the impacts of climate change on Mediterranean vegetation and ecosystems, which are particularly sensitive to future climate scenarios (Peñuelas et al., 2017). Furthermore, in order to observe the possible interaction between the impact of drought and the environmental characteristics of ecosystems, communities from different climatic, edaphic and structural contexts distributed along the double stress gradient of the Mediterranean mountains have been analysed.

We expect to observe both spatial (along the environmental gradient) and seasonal (in response to summer drought) changes in the functional structure of the SLA of the studied communities, as well as an interaction between both changes. Specifically, we hypothesise that:

1. Due to the double stress gradient typical of Mediterranean mountain ranges (Biase et al., 2021) we expect similar strategies of plant communities at both ends of the gradient (Pérez-Ramos et al., 2017), observing a unimodal trend in functional variation. These more environmentally stressed communities should have the lowest mean values of SLA, as well as functional diversity, due to strong abiotic filtering.
2. With the passing of summer and its consequent drought, the vegetation will acclimatise (via phenotypic plasticity) with a generalised reduction in SLA. However, we expect that this plastic capacity will not be equal along the environmental gradient. There is a trade-off between phenotypic plasticity and stress resistance, due to the high physiological costs of this plasticity under unfavourable conditions (Valladares et al., 2007; Bongers et al., 2017). Thus, lower phenotypic plasticity is usually found in plants in more stressful environments (Stotz et al., 2021).

2 | MATERIALS AND METHODS

2.1 | Study area and vegetation

The study area in which this research has been carried out is the southeastern slope of the Sierra de Guadarrama mountain range (Madrid, Spain). The underlying geology is predominantly siliceous, with gneisses and outcrops of magmatic rocks, mainly granite, which are partially covered by tertiary sands and arkoses (García Cacho & Aparicio Yagüe, 1987). The climate is continental Mediterranean in character, with a strong summer drought, high seasonal fluctuations in temperature and moderate rainfall. There is a difference in altitude of about 1800 m a.s.l. from the foothills (approx. 600–700 m) to the summits (Peñalara Peak, 2428 m), which implies the presence of an important environmental gradient. Along this gradient the mean annual temperature varies from 15–16°C at the lowest altitudes to 4–5°C at some summits, while precipitation ranges from 400 to 500 mm in the foothills to 1000–1500 mm annually in the valleys with a more favourable orientation to the entry of humid air masses.

This research focuses on shrubby woody communities, specifically phanerophytes between 50 cm and 4 m in height. The study area presents a great diversity and variability of this type of community (Appendix S1), mainly dominated by Mediterranean acidophilous scrub such as rockrose (*Cistus* spp.), juniper (*Juniperus* spp.) and broom (*Cytisus* spp. and *Genista* spp.). It is also worth mentioning the presence of some Euro-Siberian elements that are of great relevance because they are at the limit of their area of distribution; this would be the case for the European spindle tree (*Euonymus europaeus* L.), or holly (*Ilex aquifolium* L.).

2.2 | Data collection

Sampling was carried out on 33 plots (Appendix S2) distributed along the entire altitudinal gradient of the southern slope of the Sierra de Guadarrama (Figure 1). These plots are selected in such a way that they are reasonably representative of the types of shrub vegetation characteristic of this slope of the Sierra. Thus, there are four main habitat typologies along the altitudinal gradient into which the plots can be classified: understory, shrubland, rocky and hedgerows (descriptions in the caption of Figure 2). These habitat typologies are not represented along the entire altitudinal gradient due to the climatic and land use history of the study area, but the sampled plots of each typology cover at least half of the gradient (Appendix S3).

The plots are approximately 1 ha in size and their shape depends on the habitat typology. Thus, the 'hedgerow' type plots are necessarily elongated and the rest of the plots are circular (Figure 2). The mean annual temperature, annual precipitation and altitude of the plots have been obtained from the digital elevation model (DEM) and the Digital Climate Atlas of the Iberian Peninsula (Ninyerola et al., 2005).

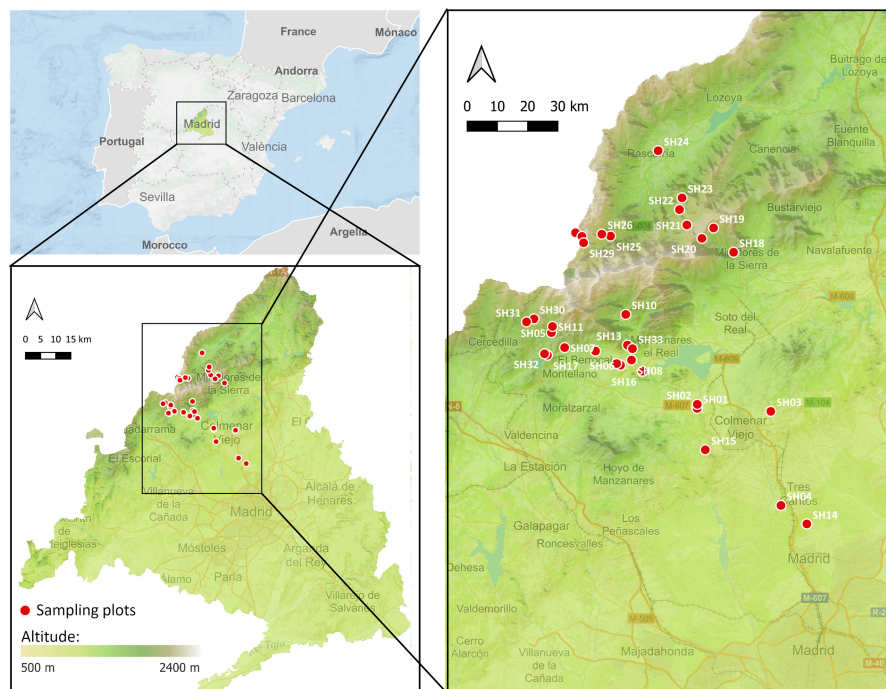


FIGURE 1 Location of the study area and sample plots (red dots) on the digital elevation model of the Community of Madrid.

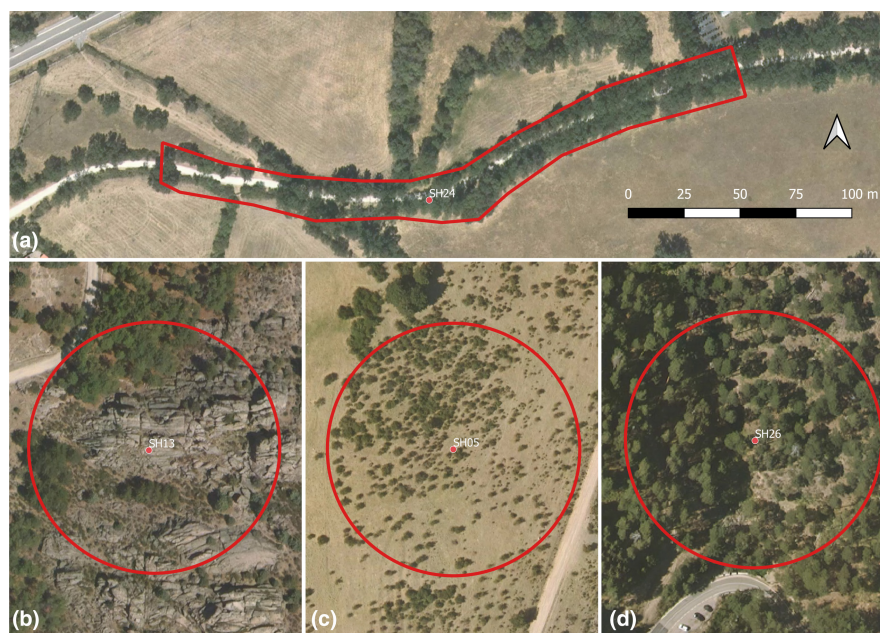


FIGURE 2 Aerial image and delimitation of four sampling plots reflecting the four habitat typologies of the study area. (a) A hedgerow, which are valley floor communities on deeper soils. Due to the current structure of the landscape in the Guadarrama Mountains, these hedgerows are almost always associated with livestock tracks, fences, pastures and woody pastures of ash or oak used for livestock. (b) A rocky plot, characterised by a high proportion of exposed rocks on which the shrub community settles, in many cases taking advantage of the crevices to develop. (c) Shrubland, that is plots dominated by shrub vegetation, where the presence of trees is low or non-existent. These are generally areas associated with medium and steep slopes or scrubby pastures. (d) An understory, which are shrub communities associated with forest stands, in which the tree canopy significantly limits the entry of light to the lower strata.

For community sampling, we identified all shrub species present (including those capable of reaching tree-like size at maturity) and estimated visually, and with the support of aerial photographs, the percentage of cover they occupied in each plot. We collected leaves for all species in each plot following an abundance-weighted

trait sampling scheme (Carmona, Rota et al., 2015). At least one individual per species was sampled, and up to three or four individuals for the most abundant species in all the plot. Less than 10 individuals per plot were never sampled even in the simplest communities. For the random selection of the sampled individuals,



four random points were generated (with GIS) within the plot and individuals of each species closest to these points were sampled. As the SLA also varies in different parts of the same plant (Hulshof & Swenson, 2010), nine mature leaves in good condition from one year were taken from each individual: three leaves from the sunniest surface (south side of the plant), three from the least sunny surface (facing north) and the last three from the shadiest area inside the plant. In order to observe the influence of seasonality on SLA, the reported sampling was carried out in two different seasons during 2017. Firstly, sampling was carried out in June to obtain SLA data from the communities before the summer and the following sampling took place in September, so that changes in SLA values could be observed after the passage of summer and the associated water stress. These same samples were replicated in 2019, when climatic conditions were different from those of 2017. As the sampling of individuals was random, the same individuals of the species were not always sampled at each of these four sampling moments. Once all samples were collected in the field, they were processed in the laboratory. The leaf dry mass and leaf area of each sample was measured following standardised methodology (Perez-Harguindeguy et al., 2016) for calculating the SLA value of each individual. In the case of species with photosynthetic stems (brooms of tribe Genisteae R.Br.), we had to adapt the protocol (Appendix S4). A total of 1724 individuals (15,516 leaves) were measured.

2.3 | Calculation of functional structure

A common index for assessing mean trait responses to environmental variation is the community-weighted mean (CWM) (Ricotta & Moretti, 2011). On the other hand, functional diversity can be decomposed into three independent elements, proposed by Mason et al. (2005), which relate to different community properties: functional richness, which indicates the amount of functional space occupied by species in a community; functional evenness, which measures the degree of regularity in the distribution of species abundances in the functional space; and functional divergence, which is a measure of functional dissimilarity between species in the community. Thus, to estimate the different elements of the SLA functional structure we calculated: the CWM, which reflects the most common SLA value in a community, the Feve index (Villéger et al., 2008) as an estimate of functional evenness, and the RaoQ index (Botta-Dukát, 2005), which is a combination of both functional richness and functional divergence. These indices have been obtained using the *FDiversity* package of R (Casanoves et al., 2011) from our matrix of species abundances in each plot and the matrix of SLA values for each of those species in each of the plots, in each year and in each month. As the SLA is a continuous trait, Euclidean distances have been used to calculate the indices. The RaoQ index can be influenced by species richness (Mason et al., 2013), which is not the case for Feve. To obtain values of richness and functional divergence independent of the number of species, Mason et al. (2013)

propose to calculate the Rao index standardised effect size in two different ways:

1. Functional richness (Fric) can be estimated by comparing the observed RaoQ index, considering only the presence of species in the plots ($RaoQ_{pr}$), with the values generated by a null matrix swap model based on the species presence/absence matrix.
2. Functional divergence (Fdiv) can be assessed by comparing the observed Rao index, taking into account species abundances ($RaoQ_{ab}$), with the null values generated by randomising abundances across species but within plots (Hardy, 2008).

Based on this approach we constructed the two null models (for Fric and Fdiv) using the R package *picante* (Kembel et al., 2010); 10,000 randomisations of each null model were performed. As the null values of the RaoQ index did not have the normal distribution necessary to calculate the standardised effect sizes, we followed the recommendations of Botta-Dukát (2018) and calculated the Effect Sizes (ES) as the probit-transformed *p*-values of the differences in RaoQ values between the observed and null communities (see Appendix S5 for more details). This calculation was performed using the *BAT* package (Cardoso et al., 2015). This method has been used previously in field studies (Mason et al., 2012; Carmona, Mason, et al., 2015; Lhotsky et al., 2016), demonstrating its reliability in detecting changes in functional diversity along gradients and over time.

2.4 | Statistical analysis

To characterise our environmental gradient more precisely, we performed a Principal component analysis (PCA) with three major climatic descriptors that strongly influence vegetation: mean annual temperature, annual precipitation and irradiance level (calculated using DEM). We also added summer temperature and precipitation to control the fact that summer itself could have slightly different characteristics between plots. The first axis of the PCA, which absorbs 70% of the variance of the data, is the explanatory variable that has subsequently been used as the descriptor of our environmental gradient. The values of this descriptor increase as temperature increases and decrease as precipitation increases (Appendix S6) and are highly correlated with altitude ($R^2 = 0.828$).

We have analysed the data using linear mixed models with the plots as random factors to avoid temporal pseudoreplication. The fixed factors included in the models were year, month, habitat typology (understorey, shrubland, rocky and hedgerow), environmental gradient descriptor and interactions. Different mixed models have been fitted for each response variables: the CWM, the functional evenness (Feve) and the effect sizes of functional richness (ESFric) and functional divergence (ESFdiv) of SLA. Since a non-linear relationship between the environmental gradient and the response variables might be expected, the environmental gradient factor has also been taken into account in a quadratic form in the model's fit. We have built these models in R using the *nlme* package (Pinheiro

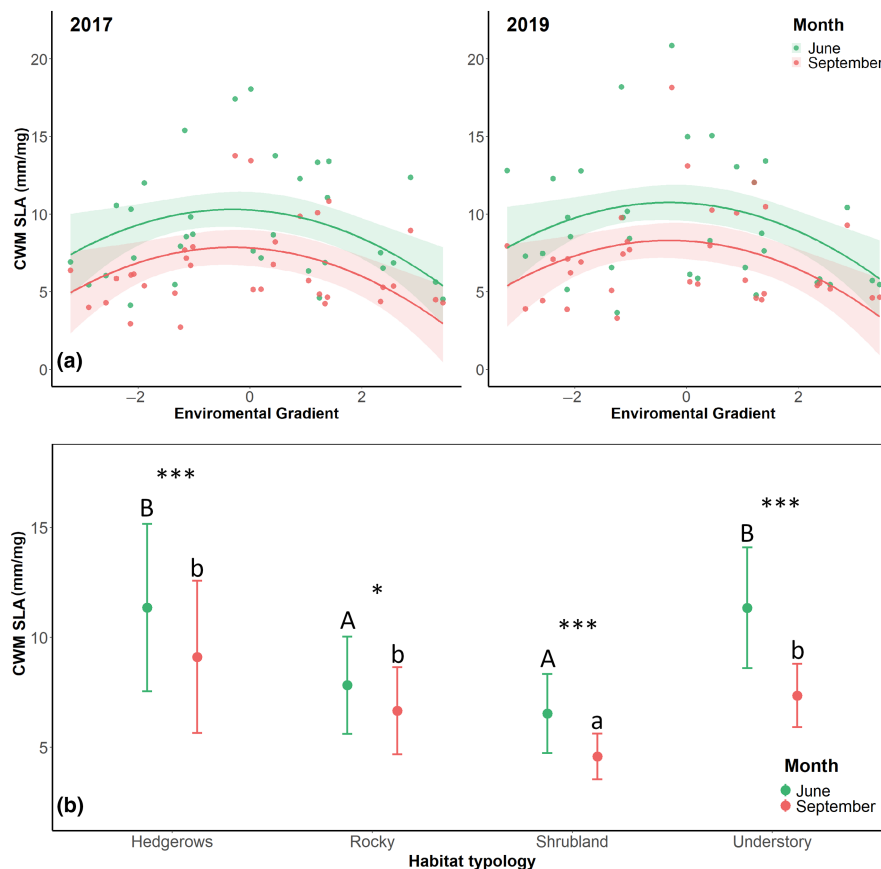


FIGURE 3 Relationship of the community-weighted mean (CWM) of the SLA with the explanatory variables of the study. (a) The variation of CWM in the environmental gradient in the two years of study, as well as the function and confidence intervals (colour bands) estimated by the model. (b) The CWM specific leaf area (SLA) mean (points) and standard deviation (ranges) in the different habitat typologies before and after the summer (for the two years as a whole). Different letters indicate significant differences between the levels of the 'habitat typology' factor. Asterisks and uppercase in the plot indicate significant differences between months within each habitat type. *, p -value < 0.05; **, p -value < 0.01; ***, p -value < 0.001.

et al., 2017) and we have fitted them following Zuur et al. (2009), choosing for each response variable the model with the lowest Akaike information criterion (AIC) value after adjusting of random and fixed factors. Analysis of the residuals and verification of the model assumptions have been carried out in each case. To analyse the origin of possible temporal changes in SLA two mixed models were also constructed for leaf area and leaf dry mass CWM. As these last variables do not follow a parametric distribution, they were analysed using generalised linear mixed models (gamma family), built with the R package *glmmTMB* (Magnusson et al., 2017) and adjusted with the same procedure as described above. Post-hoc analyses and pairwise comparisons of levels were performed using the *eemmeans* and *multcomp* packages of R (Hothorn et al., 2016; Lenth & Lenth, 2018). The marginal R^2 (variance explained by the fixed factors) and the conditional R^2 (variance explained by the random factors) were calculated using the *MuMin* package of R (Barton & Barton, 2015).

3 | RESULTS

The environmental gradient has a significant concave quadratic relationship for the SLA CWM (Figure 3a) and ESFric (only in September for this last one; Figure 4b) and a convex quadratic relationship with ESFdiv (Figure 4d). The CWM of the SLA varies significantly between the different typologies (Table 1a); shrublands and rocky areas have a lower CWM than hedgerows and understorey, although this relationship changes slightly in September (Figure 3b). In ESFric, plot typology also has a significant effect (Table 2b), with understorey having a significantly higher functional richness than the other typologies (Figure 4c).

The passage of summer ('Month') has a significant effect on all response variables except ESFdiv. In September we found a lower CWM of the SLA along the entire gradient; however, this reduction is not equal among plot typologies (Table 1a); the post-hoc tests of

FIGURE 4 Relationship of the different functional diversity indices with significant fixed factors of their models. (a, c) Mean (points) and standard deviation (ranges). Different letters indicate significant differences between the levels of the factor 'habitat typology'. (b, d) Functions (line) and confidence intervals (bands) estimated by the models. Asterisks and uppercase in the plot indicate significant differences between months within each plot type. *, p -value < 0.05; **, p -value < 0.01; ***, p -value < 0.001.

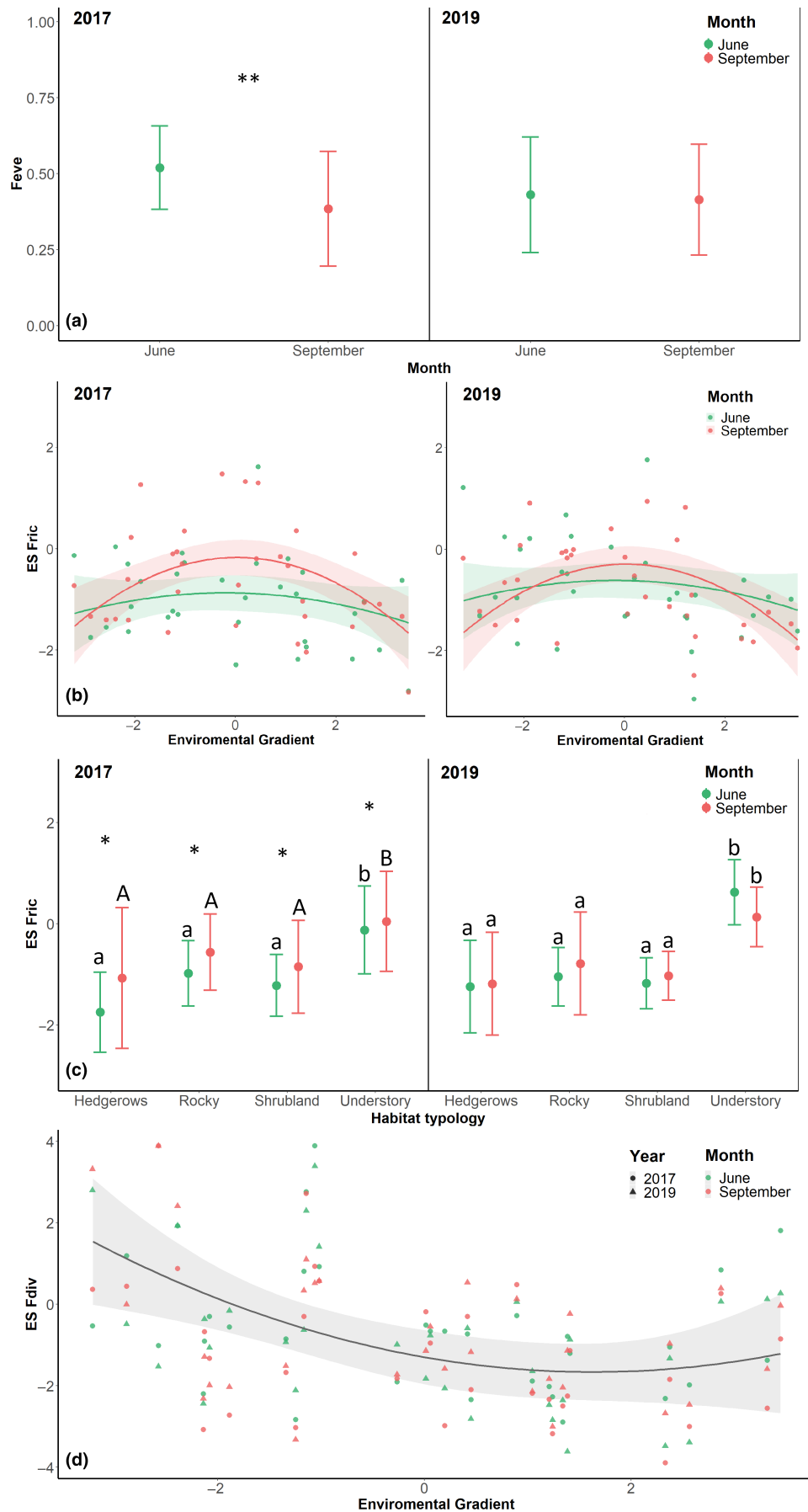




TABLE 1 Analysis-of-variance and coefficients tables [with beta coefficients (*B*) and standard errors (*SE*)] of the fitted linear mixed model for the CWM of SLA, and the fitted generalised linear mixed models (glmm) for the CWM of leaf dry mass and the leaf area.

(a) CWM SLA	<i>df</i>	<i>F</i> -value	<i>p</i> -value	R ² marginal	R ² conditional
Year	1	4.524	0.036	0.577	0.898
Month	1	149.348	<0.001		
Enviromental gradient (poly)	2	5.475	0.010		
Habitat typology	3	10.466	<0.001		
Month: Habitat typology	3	10.836	<0.001		
Coefficient table		<i>B</i>		<i>SE</i>	
Intercept (Hedgerows, June, 2017)		13.825		1.003	
Year 2019		0.445		0.209	
September		-2.753		0.454	
Enviromental gradient, 1		-0.218		0.258	
Enviromental gradient, 2		-0.348		0.118	
Rocky		-5.127		1.171	
Shrubland		-6.232		1.236	
Understorey		-1.246		1.457	
September: Rocky		1.594		0.605	
September: Hedgerows		0.798		0.592	
September: Understorey		-1.725		0.642	
(b) CWM leaf dry mass	<i>df</i>	χ^2	<i>p</i> -value	R ² marginal	R ² conditional
Year	1	1.683	0.194	0.420	0.946
Month	1	19.133	<0.001		
Enviromental gradient (poly)	2	6.109	0.047		
Habitat typology	3	8.955	0.030		
(c) CWM leaf area	<i>df</i>	χ^2	<i>p</i> -values	R ² marginal	R ² conditional
Year	1	9.386	0.002	0.431	0.967
Month	1	7.760	0.005		
Enviromental gradient (poly)	2	7.879	0.019		
Habitat typology	3	12.778	0.005		
Month: Habitat typology	3	11.648	0.009		
Coefficient table		<i>B</i>		<i>SE</i>	
Intercept (Hedgerows, June, 2017)		7.091		0.390	
Year 2019		0.116		0.038	
September		-0.229		0.082	
Enviromental gradient, 1		0.013		0.103	
Enviromental gradient, 2		-0.130		0.047	
Rocky		-0.82		0.455	
Shrubland		-1.70		0.482	
Understorey		-1.410		0.570	
September: Rocky		0.221		0.110	
September: Shrubland		0.317		0.107	
September: Understorey		0.043		0.117	

Note: In the latter, as glmm uses a logarithmic link function, the coefficients shown are the Naperian logarithmic transformation of the real values of leaf area and leaf dry mass. The environmental gradients 1 and 2 refer to the first and second degrees of the quadratic function. *p*-Values below 0.05 are highlighted in bold.



TABLE 2 Analysis-of-variance and coefficients tables [with beta coefficients (*B*) and standard errors (*SE*)] of the fitted linear mixed model for the SLA.

(a) Feve	<i>df</i>	<i>F</i> value	<i>p</i> -value	R ² marginal	R ² conditional
Year	1	1.223	0.271	0.078	0.275
Month	1	8.014	0.006		
Year: Month	1	4.931	0.029		
Coefficient table			<i>B</i>	<i>SE</i>	
Intercept (june, 2017)			0.519	0.030	
Year 2019			−0.088	0.038	
September			−0.135	0.038	
2019: September			0.118	0.054	
(b) ESFric	<i>df</i>	<i>F</i> value	<i>p</i> -value	R ² marginal	R ² conditional
Year	1	0.530	0.468	0.418	0.693
Month	1	5.065	0.027		
Enviromental gradient (poly)	2	4.097	0.028		
Habitat typology	3	6.873	0.001		
Year: Month	1	3.988	0.014		
Month: Enviromental gradient (poly)	2	4.467	0.049		
Coefficient table			<i>B</i>	<i>SE</i>	
Intercept (Hedgerows, June, 2017)			−1.383	0.274	
Year 2019			0.259	0.135	
September			0.701	0.167	
Enviromental gradient, 1			−0.017	0.073	
Enviromental gradient, 2			−0.044	0.034	
Rocky			0.394	0.298	
Shrubland			0.274	0.316	
Understorey			1.491	0.374	
Year 2019: September			−0.380	0.190	
September: Enviromental gradient, 1			0.026	0.051	
September: Enviromental gradient, 2			−0.084	0.028	
(c) ESFdiv	<i>df</i>	<i>F</i> value	<i>p</i> -value	R ² marginal	R ² conditional
Year	1	0.151	0.699	0.267	0.732
Month	1	1.167	0.283		
Enviromental gradient (poly)	2	5.221	0.012		
Habitat typology	3	2.020	0.133		
Coefficient table			<i>B</i>	<i>SE</i>	
Intercept (Hedgerows, June, 2017)			−0.456	0.587	
Year 2019			0.063	0.162	
September			−0.175	0.162	
Enviromental gradient, 1			−0.446	0.153	
Enviromental gradient, 2			0.137	0.070	
Rocky			−0.612	0.671	
Shrubland			−1.619	0.711	
Understorey			−0.604	0.842	

Note: The environmental gradients 1 and 2 refer to the first and second degrees of the quadratic function. *p*-Values below 0.05 are highlighted in bold.

the interaction term reflect that the reduction of the SLA CWM in September is significantly higher in hedgerows and understorey than in rocky and shrubland plots (p -value < 0.001). The seasonal variation in SLA is mainly due to a significant increase in leaf dry mass (Table 1b). Leaf area (Table 1c) only decreases significantly in hedgerows (p -value = 0.029). The passage of summer also leads to a decrease in the functional evenness (FEve) of the communities, but only in 2017 (Table 2a). On the other hand, between June and September 2017 a significant increase in ESFric can be observed in the middle zones of the environmental gradient. In 2019, by contrast, no significant variation is observed after the summer. In 2019 the baseline CWM of the communities was significantly higher than in 2017.

4 | DISCUSSION

As we hypothesised, the passage of summer and its consequent summer drought has a significant effect on the SLA functional structure of shrub communities, and this effect also varies according to the environmental characteristics of the study plots. We also observed changes along the gradient and between plot typologies. While the variation in SLA across the environmental gradient and habitat types may be due to the joint action of phenotypic plasticity, genetic differences and species turnover, temporal variation is only possible due to the phenotypic plasticity of the shrub communities, as they have not changed their taxonomic structure (species and abundances) in the short time spans observed.

The observed quadratic relationship between SLA CWM and environmental gradient supports the evidence of a double stress gradient in Mediterranean mountain systems (Schöb et al., 2013; Granda et al., 2014; Biase et al., 2021). Communities at lower elevations are exposed to higher temperatures and lower water availability, so that sclerophily (lower SLA) seems to be the dominant strategy to avoid tissue damage and limit evapotranspiration (Costa-Saura et al., 2016). In the middle zones, where water stress decreases and temperatures are moderate, we found the highest SLA values for the plant community as a whole. At higher altitudes, the SLA of the communities falls back to values similar to those found in the foothills, which may be due to the combined action of many other factors that are limiting for plant development. At higher altitudes, the content of organic matter and nutrients in the soil generally decreases (Schöb et al., 2013), which leads to more conservative strategies in the acquisition of resources. UV-B radiation and the proportion of frost are also more intense at higher altitudes, so plants need to generate non-photosynthetic protective tissues, which leads to a decrease in SLA (Pescador et al., 2015). There are very few precedents that have found the unimodal trend described here (Biase et al., 2021; Bricca et al., 2022), where the foliar strategies of plants are similar at the two extremes of the environmental gradient (Pérez-Ramos et al., 2017).

On the other hand, the results for the functional divergence contradict our initial hypothesis. We expected to find a lower functional

divergence at the extremes of the gradient, due to a strong environmental filtering that converged the SLA values of the main species (Carmona, Mason, et al., 2015; Schellenberger Costa et al., 2017; Luo et al., 2019). One explanation for the higher functional divergence observed at the extremes of the environmental gradient (especially at maximum altitudes) can be found in facilitation processes (Bricca et al., 2019; Wang et al., 2021), that tend to become more important as abiotic stress increases (He et al., 2013) and in which the presence of facilitator and facilitated species with different traits (Schöb et al., 2012) increases that divergence. Despite the quadratic trend observed, functional divergence is lower in the drier lowlands than at higher elevations. This may be because the facilitation processes are not as strong in these low areas and the study lacks an even more arid extreme of the gradient, or perhaps divergence is occurring in other traits related to water resources that we have not measured, such as root traits (Fry et al., 2018). The lower values of functional divergence in the middle zones of the gradient, which are more environmentally favourable and where we found the highest species richness (Appendix S6) and CWM SLA, may be reflecting a process of 'weaker competitor exclusion' (Mayfield & Levine, 2010; Bricca et al., 2019), where a few species with more competitive traits dominate the community.

The summer drought has implied a generalised decrease in SLA CWM across the gradient, a result that we expected to find and that is consistent with the results of previous research where a decrease in SLA is observed in dry months (Garnier et al., 2001; Rossatto et al., 2013) or over several decades of increased aridity (Magaña Ugarte et al., 2020). The decrease in SLA in our study is mainly due to an increase in leaf weight that may come from two distinct but complementary acclimation processes: some shrubs lose part of their leaves before the summer drought (Simões et al., 2008) and could be expected to lose the leaves less adapted to summer conditions (intracohort selection), that is those with higher SLA. Hardening of existing leaves could also occur due to wax accumulation, cutinisation of epidermal walls or an increase in the number of cells per unit leaf volume (Scheepens et al., 2010; Jung et al., 2014). On the other hand, the results do not reflect our predictions that the size of SLA variation between June and September, that is, the degree of phenotypic plasticity, would be smaller in the more stressed areas (higher and lower elevations) than in the more favourable areas for plant development, following the 'hypothesis of reduced plasticity' (Valladares et al., 2007). The reason for not finding differences in phenotypic plasticity could be that in the middle zones, with favourable climate and high species richness (Appendix S6), high interspecific competition could also reduce the degree of intraspecific variability and plasticity due to species niche packaging (Violle et al., 2012; Siefert et al., 2015).

In terms of habitat typology, the fact that hedgerows and understorey have the highest SLA values could be explained by some of their properties. In hedgerows, stone walls and associated woody vegetation limit wind speed and favour the retention and accumulation of sediment, leaves and other organic matter, thus increasing soil fertility, soil depth and water concentration



(Sanchez & McCollin, 2015), which are positively correlated with SLA (Pérez-Ramos et al., 2012). In the understorey, high SLA CWM values could be due to the shading provided by the tree canopy. Understorey shrubs must generate larger leaves to compensate for the light filtered by the tree canopy (Valladares et al., 2016) so that plants under tree cover tend to have higher SLA than those located in forest clearings (Carlucci et al., 2015). As they have large leaves (with a high evapotranspiration potential), understorey shrubs would be particularly sensitive to summer drought (Valladares & Percy, 2002; Valladares et al., 2016). Such negative correlation between shade tolerance and drought tolerance (Niinemets & Valladares, 2006) could push the understorey vegetation to strongly acclimatise its foliar traits. This could explain why understorey, but also hedgerow (which has the highest CMW of leaf area), experienced the greatest reduction in SLA due to phenotypic plasticity in the face of summer. In addition, tree cover can be a disadvantage for shrubs during summer by increasing competition for the few water resources remaining in the substrate (Riegel et al., 1992; Valladares et al., 2016). The highest functional richness of the community is also observed in the understorey, possibly because, at the fine scale, there is high light heterogeneity (Carlucci et al., 2015). Thus, in the very shaded areas we find species with a very high SLA, while in the clearings left by the trees we find species with a lower SLA, which increases the range of existing values for this trait (Qi et al., 2015).

Rocky and shrublands are habitats highly exposed to solar radiation, and a low SLA is adaptive, but also they are the habitat with the lowest decrease in CWM SLA of their vegetation with the passage of summer. These communities already have their foliar traits adapted to drought conditions and may have less need for acclimatisation during the summer, or perhaps they would reach a physiological limit to further reducing their SLA, that is, the 'hypothesis of reduced plasticity' (Valladares et al., 2007). Other microclimatic factors may be influencing the lower SLA reduction observed for these habitats. For example, in rocky habitats, the roots of woody plants grow through rock crevices in search of water reservoirs that play a key role in dry seasons when soil water has been depleted (Schwinning, 2010), this could reduce the water stress to which these communities are subjected during the summer.

Although the relationship between fitness and phenotypic plasticity is unclear, Bongers et al. (2017) found that lower plasticity in the SLA of Mediterranean shrub species was related to higher drought survival, while high plasticity correlated with better recovery after drought. Based on these findings and our results, we would expect that in the long term, hedgerow and understorey communities with high phenotypic plasticity will be more threatened than rocky and shrubland communities by the expected increase in drought.

Functional richness and evenness of the communities are also affected by the summer drought. Functional richness increased in the middle zones of the environmental gradient with the passage of the 2017 summer, which is contradictory to our initial hypothesis of a reduction in functional space and possible foliar strategies with increasing water stress. This may stem from the fact that in

these middle zones, with more favourable conditions, some areas within the plots, where the higher-SLA species develop, still retain high concentrations of water for topographic or edaphic reasons. These high-SLA species could spend the summer without modifying their foliar traits as they are not suffering from the summer drought (or at least not in such a pronounced way). Thus, if the high-SLA species maintain their SLA values, but the rest of the species suffer a decrease in SLA, the range of values widens and therefore the functional richness is increased. This interpretation could also explain the decrease in functional evenness after the summer of 2017. In this differential response of plants to summer drought, the parts of the functional space between plants that maintain their SLA and those that reduce it would be empty, thus decreasing the Feve index. The increase in functional richness may also be due to the fact that, in the middle zones, where there is a greater number of species, a higher number of different strategies to drought also coexist, that is, species that maintain their SLA values (drought-tolerant) and species with high phenotypic plasticity (drought avoiders).

As the effect of month on functional diversity varies from year to year, we can assume that differences in the starting conditions of each year and in how each summer develops have a large influence on the seasonal variation of the measured indices. The year 2019 had a much wetter spring than 2017, which explains the lower CWM of the starting SLA in the latter. The course of the summer, however, was much drier in 2019, which may have conditioned the functional reaction of all plants to be very similar, decreasing the SLA in all of them but without altering the distributions in the functional space.

These results provide relevant information for understanding the functional behaviour of Mediterranean vegetation, its early response to drought and the possible implications of these long-term changes in the context of global warming. It can be expected that continued increases in drought may promote a conservative foliar strategy in woody vegetation, thus altering the productivity and nutrient cycling of Mediterranean ecosystems.

AUTHOR CONTRIBUTIONS

Francisco M. Azcárate conceived the idea and designed the hypotheses and sampling scheme; all authors participated in the sampling; Alejandro Carrascosa and Laura Morgado measured the functional traits; Alejandro Carrascosa and Mariola Silvestre analysed the data; Alejandro Carrascosa led the writing of the manuscript. All authors contributed critically to drafts and gave final approval for publication.

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DATA AVAILABILITY STATEMENT

All data are available in the [Supporting Information](#).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Table of shrub species found on the study plots in the 2017 and 2019 surveys.

Appendix S2. Table of coordinates and altitude of the 33 plots sampled.

Appendix S3. Specification of habitat typologies and relation with the environmental gradient.

Appendix S4. Special protocol for SLA measurements of broom species.

Appendix S5. Functional richness and divergence calculation.

Appendix S6. PCA for the construction of the environmental gradient and relationship with other variables.

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