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*Published Version:*
Pavanetto, N., Tordoni, E., Petruzzellis, F., Maccherini, S., Nardini, A., Beccari, E., et al. (2025). Effect of climate extremes and grazing on functional traits of a grassland community: insights from a 20-year experiment. ANNALS OF BOTANY, 136(1), 129-139 [10.1093/aob/mcaf059].

*Published:*
DOI: http://doi.org/10.1093/aob/mcaf059

**Annals of Botany**
Original Article

# Effect of climate extremes and grazing on functional traits of a grassland community: insights from a 20-year experiment

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## Running title

Changes in grassland functional traits over 20-years experiment

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## Abstract

**Background and Aims**: Climate change, particularly the increased frequency of extreme climatic events, poses significant challenges to the biodiversity and functionality of semi-natural grasslands. However, the response of plant functional traits of grassland communities to climate extremes is still an unresolved issue. Using data from a long-term experiment, we aimed to characterize the functional response of a grassland community to simultaneous long-term effects of grazing and climate extremes.

**Methods:** For over a 20-year period, we monitored the species composition of grazed and ungrazed grassland plots. We measured functional traits defining the Leaf Economics Spectrum (LES) and the Hydraulic Safety-Efficiency (HSE) trade-offs, and we identified the temporal dynamics of single traits at the community level as well as the changes in functional strategies among grazed and ungrazed communities. Then, we assessed the effect of climatic extremes in driving the changes in functional composition.

**Key Results**: Grazed plots, in the first few years, were dominated by fast-growing species with more acquisitive strategies compared to ungrazed plots. However, both communities showed a reorganization in the functional structure over time, pointing towards a selection of trait combinations favoring more conservative, stress-tolerant strategies. The joint effect of grazing and climate extremes significantly altered the functional composition of the grazed community, leading to a shift from species with grazing-tolerant traits to species with grazing-avoidant, and drought-tolerant, traits.

**Conclusions:** We found that grazing pressure generally promoted functional diversity but led to rapid shifts in community composition when combined with prolonged drought events. In contrast, the ungrazed community, dominated by species with conservative resource-use strategies, showed more stable functional richness and divergence, as well as a reduced sensitivity to climatic extremes. These results underscore the importance of carefully evaluating grazing in the context of climate change, particularly to guide restoration and conservation efforts.

**Keywords:** Calcareous grasslands, climate extreme events, community weighted means, drought, functional diversity, grazing, heatwaves, herbaceous plants, temporal dynamics, woody plants.

# Introduction

Climate change has widespread and pervasive impacts on ecosystem integrity and biodiversity (Pörtner *et al.* 2022). Global warming, which is usually associated with increased intensity and frequency of extreme climatic events (Myhre *et al.* 2019), is negatively affecting ecosystem functioning particularly in the Mediterranean region, where the warming rate is expected to be about 20% larger than globally (Peñuelas *et al.* 2017; Lionello and Scarascia 2018; Petruzzellis *et al.* 2022).

European semi-natural grasslands, i.e. grasslands of anthropogenic origin represent one of the most species-rich ecosystems on the continent and an essential source of ecosystem services (Bengtsson *et al.* 2019; Dengler *et al.* 2020; Prangel *et al.* 2024). Despite their ecological importance semi-natural grasslands have experienced a dramatic decline in both diversity and spatial extent in recent decades (Bardgett *et al.* 2021). This decline is mostly attributed to overexploitation (Rota *et al.* 2017; Dengler *et al.* 2020) and to the abandonment of grazing (Bengtsson *et al.* 2019), as well as extreme climatic events such as drought (Harrison *et al.* 2018; Luo *et al.* 2019) and heatwaves (Qu *et al.* 2020).

Generally, the interaction between grazing and extreme climate events (*e.g.* prolonged drought events and heatwaves) may favor synanthropic and generalist species (Evans *et al.* 2011; Smith 2011; Maccherini *et al.* 2018), which in turn is expected to alter both taxonomic and functional composition of grasslands (Carmona *et al.* 2012; Komac *et al.* 2015; Rota *et al.* 2017; Stampfli *et al.* 2018; Zhang *et al.* 2023; Lin *et al.* 2024). In particular, plant functional composition is assessed through measurements of plant functional traits, which are valuable proxies for determining plant adaptations to biotic and abiotic conditions (Westoby *et al.* 2002; Wright *et al.* 2004; Puglielli *et al.* 2022; Pavanetto, Niinemets, *et al.* 2024). Plant trait combinations can be summarized along major axes of variation (Díaz *et al.* 2016; Carmona *et al.* 2021; Puglielli *et al.* 2024), encapsulating strategies for resource allocation (Leaf Economic Spectrum - LES; Wright *et al.* 2004; Reich 2014) or water use (Hydraulic Safety-Efficiency - HSE, Petruzzellis *et al.* 2021). LES traits reflect the trade-offs among nutrient uptake, growth, and leaf lifespan (i.e. acquisitive vs conservative resource use strategies, Wright *et al.* 2004) and have been largely used to define grassland dynamics in response to climate variability (Sandel *et al.* 2010; Griffin-Nolan *et al.* 2019). LES traits received mixed support in relation to their capability to detect shifts in the functional strategies of grassland communities under prolonged drought or heatwaves (Hoover *et al.* 2014; Elst *et al.* 2017; Luo *et al.* 2019; Vitra *et al.* 2019), suggesting that these traits alone might not be enough to assess grassland communities' responses to climate extremes (Luo et al. 2019). The inclusion of hydraulic traits, therefore, could be effective in capturing overlooked key mechanisms driving community changes (Anderegg *et al.* 2016).

Within the HSE framework, leaf water potential inducing cell turgor loss ($\Psi_{tlp}$) is considered a proxy of drought resistance, while according to the "flux trait network" (Sack *et al.* 2013), leaf venation traits (i.e., vein length per unit leaf area, VLA) are mechanistically related to leaf hydraulic conductance ($K_{leaf}$) and could be considered as proxies of leaf water transport efficiency. The integration of these traits has elucidated patterns in woody plants and invasive species distributions and functions (Tordoni *et al.* 2019, 2020; Yan *et al.* 2020; Petruzzellis *et al.* 2021; Pavanetto, Carmona, *et al.* 2024), as well as grasslands' species response to drought (Sun *et al.* 2020; Kramp *et al.* 2022; Wang *et al.* 2024), Moreover, this integration has proven useful for understanding grasslands response to the combined effects of grazing and climate extreme events. Several studies found that $\Psi_{tlp}$ shifted toward higher values (i.e., lower drought resistance) in plots under drought stress (Griffin-Nolan *et al.* 2019; Kramp *et al.* 2022), suggesting that drought avoidant species display higher survival rates under drought in grassland ecosystems (Sun *et al.* 2020). However, we still lack an evaluation of the combined effect of climate extremes and grazing since most of the previous studies consisted of manipulative experiments under controlled conditions run for a relatively short period (4 years on average), not accounting for the natural variability of environmental conditions, which strongly affects plant functional composition (Vitra *et al.* 2019; Griffin-Nolan *et al.* 2021).

In this study, we assessed the changes in functional composition and diversity of a semi-natural grassland subjected to grazing pressure over a 20-year period. Over this time interval, we also assessed the impact of extreme climatic events such as prolonged droughts and heatwaves and occurrences of severe precipitations and frost days. Using a suite of traits derived from LES and HSE frameworks, we aimed at i) highlighting the variation of functional traits distribution over time between a grazed and an ungrazed plant community; ii) evaluating the changes in functional structure (i.e., the patterns of organization of species within the functional space, Mouillot *et al.* 2011) of the two communities, using a multivariate approach to highlight temporal shifts in the functional space; iii) identifying which climatic extremes had the highest impact on functional traits change rate. We expected the functional diversity of the communities to change over time with different extents between the grazed and ungrazed communities. We posited that climate extremes associated with water scarcity, such as droughts and heatwaves, would exert the strongest effect on driving changes in functional composition. Specifically, we expected that during years of reduced precipitation, species with a more conservative and drought tolerant strategy could better cope with reduced water availability, compared to species with a more acquisitive and drought avoidant strategy.

## Materials and Methods

*Study area and vegetation sampling*

The experimental area, located near Monte Labbro (42°49′13" N, 11°31′33″ E, WGS84) in Tuscany (Central Italy), lies in a semi-natural calcareous grassland that was subjected to restoration in the early spring of 2001 (see Detailed method S1 **[Supplementary Information]** for details). The experimental plots were located in a cleared overgrown pasture (before cutting, *Prunus spinosa* covered 80%), grazed by donkeys (0.5 $ha^{-1}$ $year^{-1}$), which were reintroduced into the area few years before the restoration; the site is occasionally grazed by sheep, cattle and hares. In 2001, sixteen plots were established with a randomized block design, with four blocks and four 3×5 m experimental plots in each block. Each block contained plots randomly assigned to one of four treatments: (1) no grazing or sowing, (2) sowing without grazing, (3) grazing without sowing, and (4) combined sowing and grazing. Ungrazed plots were fenced in spring 2002. Previous studies on the same plots indicated a negligible effect of sowing compared to grazing (Maccherini and Santi 2012). Consequently, this study focuses solely on the grazing factor. Vegetation surveys started in 2002 and were conducted annually in the second half of June, within 1 × 2 m subplots centered in each experimental plot. No vegetation surveys were done in 2005, 2017 and 2018. All vascular plants were identified during data collection, and their abundance was expressed as ground cover using a point quadrat method with a density of 100 pins $m^{-2}$. If shrubs were present in the plots, when their height exceeded 1 m, their percentage cover was visually estimated on a scale ranging from 0 to 100%. Additionally, each plot was visually inspected for species not detected by pins, recording them with a nominal ground cover of 0.05%.

### *Functional trait measurements*

In late June 2019, we collected 146 samples to measure the functional traits of the species contributing up to 70% of the cumulative ground cover of each year from 2002 to 2019 resulting in 31 sampled species. We decided to sample the most abundant (i.e. dominant, *sensu* Grime 1998) species in order to account for the species that are supposed to contribute the most to community and ecosystem processes (Sasaki and Lauenroth 2011; ; Pérez-Harguindeguy et al. 2013; Tordoni *et al.* 2019 but see also Avolio *et al.* 2019). We collected two to three whole individuals for each species and treatment (i.e., grazed or ungrazed). Following established methodologies  (Pérez-Harguindeguy *et al.* 2013, but see also Petruzzellis *et al.* 2019, 2021), we measured a set of plant functional traits usually associated with the LES, HSE, leaf vein architecture and water-use efficiency. Specifically, we measured the following functional traits: specific leaf area (SLA, mm2 mg-1); leaf dry matter content (LDMC, mg g-1); minor vein length per unit area (VLA, mm mm-2); water potential at turgor loss point ($\Psi_{tlp}$, MPa); carbon isotope composition (δ13C, ‰), leaf nitrogen content (LNC, %), and leaf carbon content (LCC, %) A detailed procedure of functional trait measurements is reported in Supplementary Methods S2 **[Supplementary Information]**. The main summary statistics for each trait for each species and each species' relative abundance for each year are reported in supplementary Table S1-Table S2 **[Supplementary Information]**.

In addition, to qualitatively assess changes in plant community composition and function, we categorized the sampled species into four functional groups ('grass', 'forbs', 'legumes' – including only the graminoids-competitor and nitrogen-fixer *Trifolium incarnatum* –,  and 'shrubs') following established methodologies (Lavorel *et al.* 2007; Liira *et al.* 2008). Details on this classification are provided in detailed methods S3 **[Supplementary Information]**, along with the relative ground cover values for the functional groups over the years (supplementary Figure S1 **[Supplementary Information]**).

*Climatic extremes*

We obtained the climatic data from the nearest meteorological station (S. Fiora, Centro Funzionale Regionale of Tuscany, 687 m a.s.l., 5.56 km away from the study area). Following the guidelines of the Expert Team on Climate Change Detection and Indices (ETCCDI) (Zhang *et al.* 2011), we computed temperature and precipitation indices from 2002 to 2022. Specifically, we calculated the number of days with a minimum temperature ($T_{min}$) ≤ 0°C (FROST.DAYS) and the number of days with precipitation ≥ 20 mm (HEAVY.RAINS). Both indices were calculated for the period from the preceding December to June of each sampling year, aligning with the pre-growing season, which is crucial to assess the response of vegetation to such climatic drivers (Dukes *et al.* 2005). Additionally, to assess the impact of dry spells and heatwaves, we calculated the maximum number of consecutive days with precipitation <1 mm from January to June (DRY.DAYS) and the maximum number of consecutive days with temperatures > 30°C, calculated from July of the previous year to June of the current year (HEATWAVES). This period captures the full scope of seasonal temperature and precipitation fluctuations relevant to plant growth and survival (Teuling *et al.* 2010). A detailed representation of these climatic trends over the study period is provided in supplementary Figure S2 **[Supplementary Information]**.

### *Statistical Analysis*

### *Changes in trait composition over time*

To evaluate changes in the functional composition, we calculated the community-weighted mean values (CWMs) of each functional trait for each year and plot using abundance-weighted kernel density distributions (Maitner *et al.* 2023). Specifically, we used a non-parametric bootstrapping method to include intra-specific variability through statistical resampling, following the workflow proposed by Maitner *et al.* (2023). To calculate the trait distributions and CWM values, we used the *trait_np_bootstrap* function in the R (v4.4.1, R Core Team, 2024) package 'traitstrap' (Maitner *et al.* 2023; Telford *et al.* 2023), which utilises random sampling with replacement in proportion to species abundance to generate new distributions reflecting the variation in observed data. We did this separately for the grazed and ungrazed community. To ease the interpretability and visualization of the temporal patterns,  we grouped the trait distributions considering three temporal intervals (years 2002-2008, years 2009-2014, years 2015-2022); significant differences in the distribution of the values of each trait between the two communities were tested by performing a Kruskal-Wallis test followed by a post hoc Dunn's test with Holm-Bonferroni correction for multiple comparisons, using the function *dunn_test* in R package 'rstatix' (Kassambara 2023). Probability density distributions of each trait across all years for both ungrazed and grazed communities are reported in Figures S3-S4 **[Supplementary Information].**

### *Changes in the functional structure over time*

We evaluated the changes in the functional structure of the grazed and ungrazed community over the years using the Trait Probability Density framework (TPD - Carmona *et al.* 2016, 2019, 2024). First, we calculated a trait space by performing a Principal Component Analysis

(PCA) considering each species' average functional trait values (supplementary Figure S5 **[Supplementary Information]**). To evaluate the effect of phylogenetic relatedness in the position of the species within the trait space, we also performed a phylogenetically-corrected PCA (Revell 2009) which were later compared with the original PCA displaying a high degree of concordance (Procrustes correlation = 0.988, p = 0.001; see Supplementary Methods S4 **[Supplementary Information]** for further methodological details. We then retained the first two principal components, explaining 62.81% of the total variance, to estimate the TPD at the community level (i.e., for each plot) for each year and treatment, weighting the trait probability of each species by their relative abundances (supplementary Figure S6-S7 **[Supplementary Information]**). For each of the three temporal intervals delineated in the previous section, we derived the functional richness (FRic, the area occupied by the trait space) and functional divergence (FDiv, the tendency of most abundant trait values to be closer to the margins of the functional space in case of high divergence and vice versa) at the 99% quantile threshold. To highlight the portions of the functional space that underwent major changes across years, we took advantage of the grid-based nature of the TPD framework to calculate cell-wise temporal variation expressed in terms of standard deviations, and we averaged them as explained in the previous section to obtain three temporal intervals. We finally estimated overlap-based dissimilarity among temporal intervals to assess the portions of the functional space occupied by the grazed and ungrazed community (i.e., dissimilarity) and quantify potential shared portions in the functional space (i.e., nestedness, Carmona *et al.* 2016) at the 99% and 50% quantile thresholds, using the same procedure as Beccari *et al.* (2024a).

To break the well-known relationship between the functional metrics and species richness (Beccari *et al.* 2024b), we also computed a null model as follows: i) we first transformed abundance-data in presence-absence data and we created 499 randomizations of the community using the ‘Curveball algorithm’ (Strona *et al.* 2014), independently for grazed and ungrazed plots; ii) for each randomization we recalculated functional richness and divergence at the 99% of total probability; iii) we calculated standardized effect size as SES = observed value – mean (randomizations)/standard deviation (randomizations) and p-values by comparing the SES values with a cumulative normal distribution with mean = 0 and standard deviation = 1. For functional richness (sesFRic), negative SES values indicate that the species in each combination year-treatment tend to be more clustered in the functional space than expected by the same number of randomly selected species across the time-series in that treatment and vice versa. For functional divergence (sesFDiv), negative SES values would suggest a reduction in the prevalence of divergent/extreme values across the time series, with species converging toward the center of the functional space.

For temporal turnover, we used a slightly different approach to preserve the community structure across years while removing any directional temporal patterns, thus maintaining the ecological realism of the simulation process. Specifically, we used cycle-shift permutation, following the same approach described in Hallett *et al.* (2014, 2016), which preserves the temporal autocorrelation in the time series while breaking cross-correlations among species by reshuffling the starting time of each species along the time series in each permutation (Magurran *et al.* 2019). The same approach was used for dissimilarity metrics at 99% and 50% quantiles, and we calculated SES as explained above. In this case, a significant SES implies that the functional structure is changing faster than expected by chance alone.

### *Relationships between climatic extremes and changes in functional composition*

To assess the potential effect of the climatic extremes on functional composition, we first calculated the annual rate of change of CWMs (i.e., the trait velocity, Trugman *et al.* 2020) for each trait as the difference of CWM values between one sampling year and the successive one, divided by the length of the sampling period. This approach allowed us to account for the gaps in the vegetation surveys and, since the climatic variables were calculated over the periods preceding each sampling year, permitted direct comparisons between the trait's annual rate of change and climatic extremes. After that, we fitted linear mixed models (LMMs), separately for the grazed and ungrazed community, setting trait velocities as the response variable and climatic extremes and plots as fixed and random effects, respectively. Plots were included as a random effect to control for the repeated measurement per plot (Martínez-Villa *et al.* 2024). Prior to the modelling procedure, we checked multicollinearity between climate predictors using Pearson's correlation coefficient (see supplementary Table S3 **[Supplementary Information]).** Each model retained all the climate extremes, as their correlations were lower than |0.7| (Dormann *et al.* 2013). Each predictor was standardized to zero mean and unit variance to obtain comparable coefficients. To account for second-degree non-linear relationship between the predictors and the response variables, we included a second-degree (quadratic) orthogonal polynomial term for each climate extreme. We checked whether to include or not the quadratic terms by performing multiple Likelihood Ratio Test (LRT) between models with and without quadratic terms by using the base R function 'anova'. (p-value always < 0.001 when including quadratic terms). All LMMs were performed using the REML method for the estimation.

## Results

### *Changes in trait composition over time*

The relative cover of the functional groups followed different temporal trends in grazed and ungrazed plots (supplementary Fig S1 **[Supplementary Information]**), which was reflected in the different community-weighted trait distributions. Indeed, trait distribution was significantly different between grazed and ungrazed communities across all temporal intervals (Dunn's test always significant with $p < 0.001$), except for LCC in the first two temporal intervals (**Fig. 1**). Grazed plots consistently showed higher values of SLA, VLA, $\Psi_{tlp}$ and LNC compared to ungrazed plots (**Fig. 1b-d,g**). Conversely, LDMC and $\delta^{13}C$ values were higher in ungrazed communities (**Fig. 1a, e**). Over the study period, both community types experienced a shift towards higher LDMC and lower SLA, $\Psi_{tlp}$, and LNC, particularly marked in the final period (years 2015-2022). VLA values increased over time in the ungrazed community, while showing a slight decline in the grazed one. Both $\delta^{13}C$ and LCC slightly increased in the last temporal interval in both grazed and ungrazed plots.

*Temporal variation of the functional structure*

The first axis (PC1) of the functional trait space (46.31% of total variance) was mainly related to SLA and LNC, and to LDMC and $\delta^{13}C$, representing a gradient between acquisitive and conservative resource-use strategies **(Fig. 2**, supplementary Table S4 **[Supplementary Information]**). The second axis (PC2), accounting for 16.5% of the total variance, was primarily associated with VLA, $\Psi_{tlp}$, and LCC (**Fig. 2**), which further differentiated species based on drought tolerance and photosynthetic efficiency. Grazed plots consistently exhibited higher functional diversity (**Fig. 2 a,d,g**)  than ungrazed ones (**Fig. 2 c,f,i**), with mean sesFRic of 8.05 (range 1.68 — 17.52) and 0.01 (-1.94 — 3.52), respectively (supplementary Table S5 **[Supplementary Information]**). Notably, we detected a steady increase in functional richness especially for ungrazed plots that shifted from negative to positive values of SES, suggesting an introduction of new functional strategies that expanded the functional diversity of these communities (supplementary Figure S6 **[Supplementary Information]**). In contrast, we observed a strong significant decoupling for sesFDiv between grazed (clustering) and ungrazed (dispersion) plots in the years 2015-2022, a pattern absent in earlier intervals**(**supplementary Table S6 **[Supplementary Information])**.

Over time, species in both grazed and ungrazed plots strongly reorganized in the functional space (**Fig. 2**). Both communities showed a consistent shift towards more conservative strategies, as highlighted by the patterns of temporal variation showing changes in the functional structure towards higher values of LDMC, $\delta^{13}C$ and LCC, despite some nuances between treatments (**Fig. 2j,k)**. Indeed, the temporal variability of ungrazed plots (**Fig. 2k)** is clustered in a smaller, central portion of the trait space suggesting minimal but consistent temporal changes promoting a higher stability over time, with limited shifts in trait composition. In contrast, grazed plots displayed higher standard deviations across the trait space, indicating substantial temporal fluctuations in functional composition (**Fig. 2j)**.

Dissimilarities between the two treatments were significant from the seventh year for the whole functional space, whereas the high-density regions within the trait space (i.e., the ones in the 0.50 quantile) were more dissimilar than expected by chance in all comparisons (**[Supplementary Information]** Table S7**)**. Moreover, negative values of nestedness indicate that trait combinations between treatments are minimally shared, further indicating a strong shift of the hotspots (i.e., high-density regions) within the trait space. This aspect can be visually appreciated by looking at the trait probability distributions across all years for both ungrazed and grazed communities in supplementary Fig. S6 – Fig. S7 **[Supplementary Information]**.

### *Relationships between Traits and Extreme Climate Variables*

The annual rate of change of the CWM of each trait was significantly associated with the climate variables (supplementary Table S8 **[Supplementary Information]**). In general, both communities showed a similar relationship between CWM's annual rates of change and climatic extremes. Specifically, SLA and LNC showed negative rates of change, while LDMC, $\delta^{13}C$ and LCC exhibited positive rates of change in response to increasing dry spell duration during spring (DRY.DAYS) in both grazed and ungrazed plots (**Fig. 3a-b,e-g**). Notably, $\Psi_{tlp}$ and VLA values remained stable with low to moderate DRY.DAYS but decreased during prolonged dry periods for both community types (**Fig. 3c-d)**. Years with higher heatwaves duration (HEATWAVES) were generally associated with shifts in community trait towards higher values of SLA, $\Psi_{tlp}$, VLA and LNC and lower values of LDMC and $\delta^{13}$C (**Fig. 3h-l, n)**. Years associated with a higher number of heavy precipitation days (HEAVY.RAINS) showed little changes in community trait values for almost all trait considered; conversely, years with lower HEAVY.RAINS shifted towards lower values of LDMC and LCC and higher values of SLA, $\Psi_{tlp}$, and LNC (supplementary Fig. S8 **[Supplementary Information])** in both community types. Model's coefficients for the grazed community were always higher than ungrazed plots, underscoring higher variability in trait response. Significant relationships for the number of frost days (FROST.DAYS) were found for a few traits only in the grazed community. However, they held negligible influence in traits' rates of change (supplementary Fig. S8 **[Supplementary Information])**.

## Discussion

Grazing disturbances tended to favor species characterized by more acquisitive and fast-growing strategies, contrasting with the conservative, slow-growing strategies predominant among species in ungrazed plots. Over time, however, both the grazed and ungrazed communities experienced a significant reorganization in their functional structures, suggesting a combined influence of grazing and climate extremes in shaping their long-term structural and functional dynamics.

### *Grazing and climate extremes drive changes in functional composition and diversity*

Perennial and annual herbs, including the annual nitrogen-fixer *Trifolium incarnatum*, were the dominant species in grazed plots. These species generally thrive only under grazing conditions and compete with perennial grasses for land cover (supplementary Fig. S1 **[Supplementary Information]**) (Hayes and Holl 2003). These species aligned with the acquisitive side of the LES (**Fig. 1a,b, Fig. 2**), minimizing leaf construction and maintenance costs while maximizing the ability to acquire resources and reproduce rapidly (Wright *et al.* 2004; Reich 2014; Díaz *et al.* 2016). Furthermore, species in grazed plots tended to adopt strategies maximizing water transport efficiency  at the expense of lower drought resistance compared to ungrazed plot (Nardini and Luglio 2014; Zhu *et al.* 2018). Consistently, grazed plots had lower $\delta^{13}$C and higher LNC CWM values (**Fig. 1e,g, Fig. 2**), indicating potential higher gas exchange and photosynthetic rates (Farquhar *et al.* 1989; Wright *et al.* 2004; Sack *et al.* 2013) than in ungrazed plots. This grazing-tolerance strategy reflects a competitive edge for species in grazed plots, characterized by rapid nutrient acquisition to compensate tissue loss through regrowth  (Díaz *et al.* 2007; Kurze *et al.* 2021). Conversely, ungrazed plots were dominated by more conservative species such as *Bromus erectus*, which is a tall-growing grass well-adapted to drought that produces dense litter layers when abundant, potentially inhibiting other species' growth (Poniatowski *et al.* 2018). These species showed a more conservative growth strategy, developing durable leaves (higher LDMC, **Fig. 1a**) with higher drought stress resistance (lower $\Psi_{tlp}$, **Fig. 1c**) at the cost of reduced water transport efficiency (lower VLA, **Fig. 1d**), photosynthetic capacity (lower LNC, **Fig. 1g**) and carbon fixation rates (higher $\delta^{13}$C, **Fig. 1e**).

However, the temporal variations of the functional structure highlighted that both grazed and ungrazed communities have undergone a consistent shift towards more conservative strategies, with a general increase in the diversity of strategy present (**Fig. 2**, supplementary Fig. S5 **[Supplementary Information]**). Specifically, the ungrazed community exhibited increased functional richness and functional diversity over time, suggesting an expansion in functional strategies. Species in the functional space were initially clustered towards the center (supplementary Fig. S6 **[Supplementary Information]**), but over time they shifted into two different hotspots towards regions of the functional space associated with higher LDMC, VLA and LCC. This shift suggests the introduction of new specialized, more conservative strategies, thus leading to an increase in functional divergence and richness. This pattern was likely driven by the gradual encroachment of woody shrubs (e.g., *Acer monspessulanum, Prunus spinosa*, supplementary Fig. S1 **[Supplementary Information]**), whose trait suites and growth strategies significantly differ from those of herbaceous plants (Šímová *et al.* 2018), and occupy a different region in the global spectrum of plant form and function (Díaz *et al.* 2016). On the contrary we observed a gradual reduction in sesFDiv over time in grazed plots, where species positioning in the functional space has shifted from regions associated with grazing-tolerance strategies (higher SLA, LNC and $\Psi_{tlp}$) (Díaz *et al.* 2007) towards regions associated with grazing-avoidance. Given that grazing pressure has remained consistent throughout all years, these patterns suggest a combined effect of climate factors in driving these changes, rather than grazing alone.

### *Effect of climatic extremes on communities' functional composition*

Results from the models showed that the occurrence of prolonged climate extremes had a strong effect on the CWM rate of change of most of the traits considered, with grazed communities showing higher sensitivity, as indicated by the model's slope coefficients (supplementary Table S8 **[Supplementary Information]**). Climate extremes related to precipitation alteration tended to have the highest impact on trait change. Indeed, prolonged spring drought events (DRY.DAYS) promoted a shift towards a more drought-tolerant community in both grazed and ungrazed plots. When considering heatwaves duration (HEATWAVES), we found the opposite pattern in both communities, with species with an acquisitive strategy being favored against species with a more conservative strategy in years with higher HEATWAVES values. Since we initially considered both DRY.DAYS and

HEATWAVES as proxies of water shortage conditions, we expected to find a similar response in trait CWM annual change rate as a response to high DRY.DAYS and HEATWAVES values. On the contrary, our results suggest that grasslands may respond differently to prolonged dry spells and the occurrence of heatwaves. In our study, DRY.DAYS tended to be negatively correlated with HEATWAVES (supplementary Table S3 **[Supplementary Information]**), indicating that the years with higher heatwaves were not necessarily the ones with reduced precipitation. We hypothesized that in years with reduced precipitation (i.e., the ones with higher DRY.DAYS), species with a more conservative and drought tolerant strategy  could better cope with reduced water availability, compared to species with a more acquisitive and drought avoidant strategy  (French *et al.* 2019). This strategy seemed successful in years with higher HEATWAVES, when a higher water transport efficiency (i.e. high VLA - Scoffoni *et al.* 2016) could have helped to sustain the transpiration rates determined by high air temperature and VPD, but not in years with limited water availability, like the ones with higher DRY.DAYS. To support this hypothesis, forb species, which tended to have higher VLA than the other species (supplementary Table S1 **[Supplementary Information]**), seemed favored in years with higher HEATWAVES, confirming their ability to grow under sufficient soil moisture even when moderate heatwaves occur (De Boeck *et al.* 2016; Corona-Lozada *et al.* 2019). In addition, the shift toward a more drought-tolerant community in years with higher DRY.DAYS was likely due to the decrease in annual forbs and N-fixers like *T. incarnatum*, coupled with an increase in perennial drought tolerant grasses (supplementary Fig. S1 - Fig. S2 **[Supplementary Information]**) (Gargallo-Garriga *et al.* 2014).

Our findings suggest a joint effect of grazing and climate extremes in shaping the functional composition of the grassland community. Specifically, selective pressure from grazing seems to favor grazing-tolerant species in years with ample water availability throughout the vegetative season (Zhang *et al.* 2023) while, on the other hand, an increase in drought events in recent years has promoted a change in community composition towards species with grazing-avoidance strategies. Indeed, the trait suites of grazing-avoidant species not only provide an advantage in the context of grazing but also offer enhanced drought tolerance (de Bello *et al.* 2006), underscoring the convergent selective pressures of grazing and aridity (Carmona *et al.* 2012). These results also suggest that the changes in functional composition will likely affect ecosystem processes due to the combined effect of grazing and climate extremes. In particular, carbon and nutrient cycling are strongly correlated to LES traits such as LMA and LNC (Cornwell *et al.* 2008), and our results suggest that the interplay between grazing alteration and prolonged drought could favor species with slower decomposition rate, thus decreasing soil C and N availability (Luo *et al.* 2022). However, nutrient cycling in grasslands is a complex mechanism (Luo *et al.* 2022; Vilonen *et al.* 2022), and future studies are needed to improve our ability to predict its future dynamics.

## Conclusions

Our findings underscore the intertwined roles of grazing and climate extremes as driving forces in the long-term functional composition of the grassland community. Grazing pressure promoted species that adopt more acquisitive and fast-growing strategies, generally contributing to a functionally diverse community. However, when coupled with prolonged drought events, we found a noticeable shift in community composition towards species with more conservative strategies, showing both drought-tolerant and grazing-avoidant strategies. Conversely, the ungrazed community, characterized by a dominance of species with more conservative resource acquisition strategies exhibited greater stability in functional richness and divergence, alongside reduced sensitivity to climatic extremes.

Although our results support a generalizable understanding of plant community response to grazing and climate extremes based on differences in functional traits, we acknowledge that our dataset has potential limitations. Specifically, traits were measured on few individuals per species in a single year, due to technical and logistical constraints. While similar trait-base approaches have been widely utilized in assessing functional composition over time (e.g., Vandewalle et al. 2014), future studies would benefit from repeated functional traits measurements across multiple years and larger number of individuals per species.  Such longitudinal sampling would better capture intraspecific variability and phenotypic plasticity (Mudrák *et al.* 2019), thereby proving a more comprehensive view on the dynamics of functional composition changes in grasslands and their implications for community resilience.

## Funding

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B63C22000650007", Project title "National Biodiversity Future Center - NBFC". ET is currently supported by Estonian Research Council grants (MOBERC100 and PRG2142). FP is currently supported by the funding PON Ricerca e Innovazione D.M. 1062/21– Contratti di ricerca, from the Italian Ministry of University (MUR).

## Acknowledgments

NP, ET, FP and GB conceptualized the study and planned the data analysis. NP, ET, FP, GB, SM and EB measured the functional traits. SM, GB and EF provided the vegetation data. NP and ET performed the data analysis. NP, ET and FP wrote the first draft of the manuscript. EB, SM, AN, EF, CR, DR, LC and MC contributed to the critical revision of the manuscript to produce the final version.

## Conflict of Interest Statement

The authors declare no competing interests.

## Data availability

The datasets along with the R material needed to replicate the main analysis and figures in this manuscript  are available in 'figshare' under embargo (private link: https://figshare.com/s/63166078e4776aebe8e8 )

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# Figures

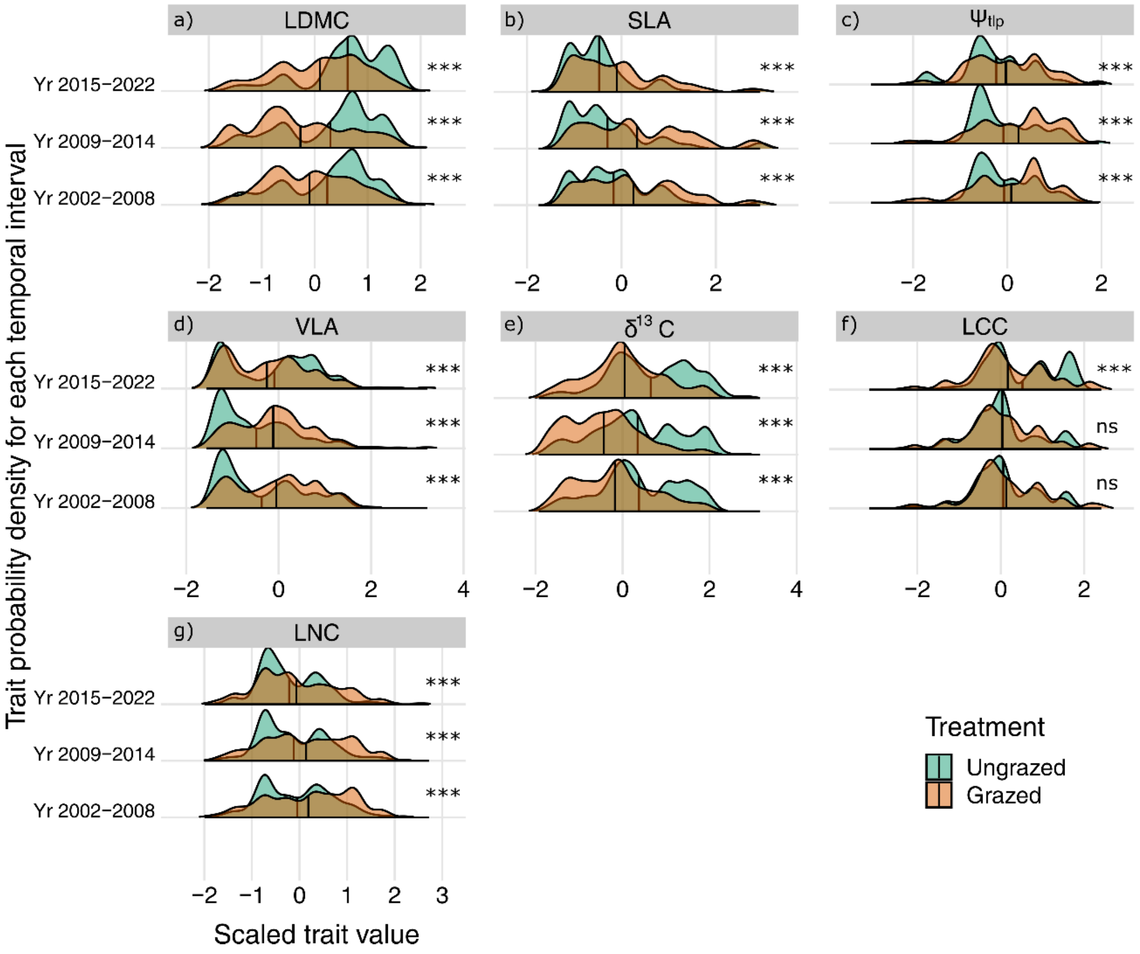

**Fig. 1.** Probability density distributions of each trait in grazed (orange) and ungrazed (green) community for each temporal interval, obtained after the non-parametric bootstrapping of trait data. Trait probability densities were estimated separately for each treatment. The vertical black lines within the density plots represent the mean value of the distribution. (a) leaf dry matter content (LDMC), (b) specific leaf area (SLA), (c) water potential at turgor loss point ($\Psi_{tlp}$), (d) minor vein length per unit area (VLA), (e) carbon stable isotope composition ($\delta^{13}C$), (f) leaf carbon content (LCC), (g) leaf nitrogen content (LNC). P-values of Dunn's test with Holm-Bonferroni correction for multiple comparison testing for the differences in the distribution of each trait values between grazed and ungrazed community for each temporal interval are reported (ns: not significant; **: $p < 0.01$; ***: $p < 0.001$).

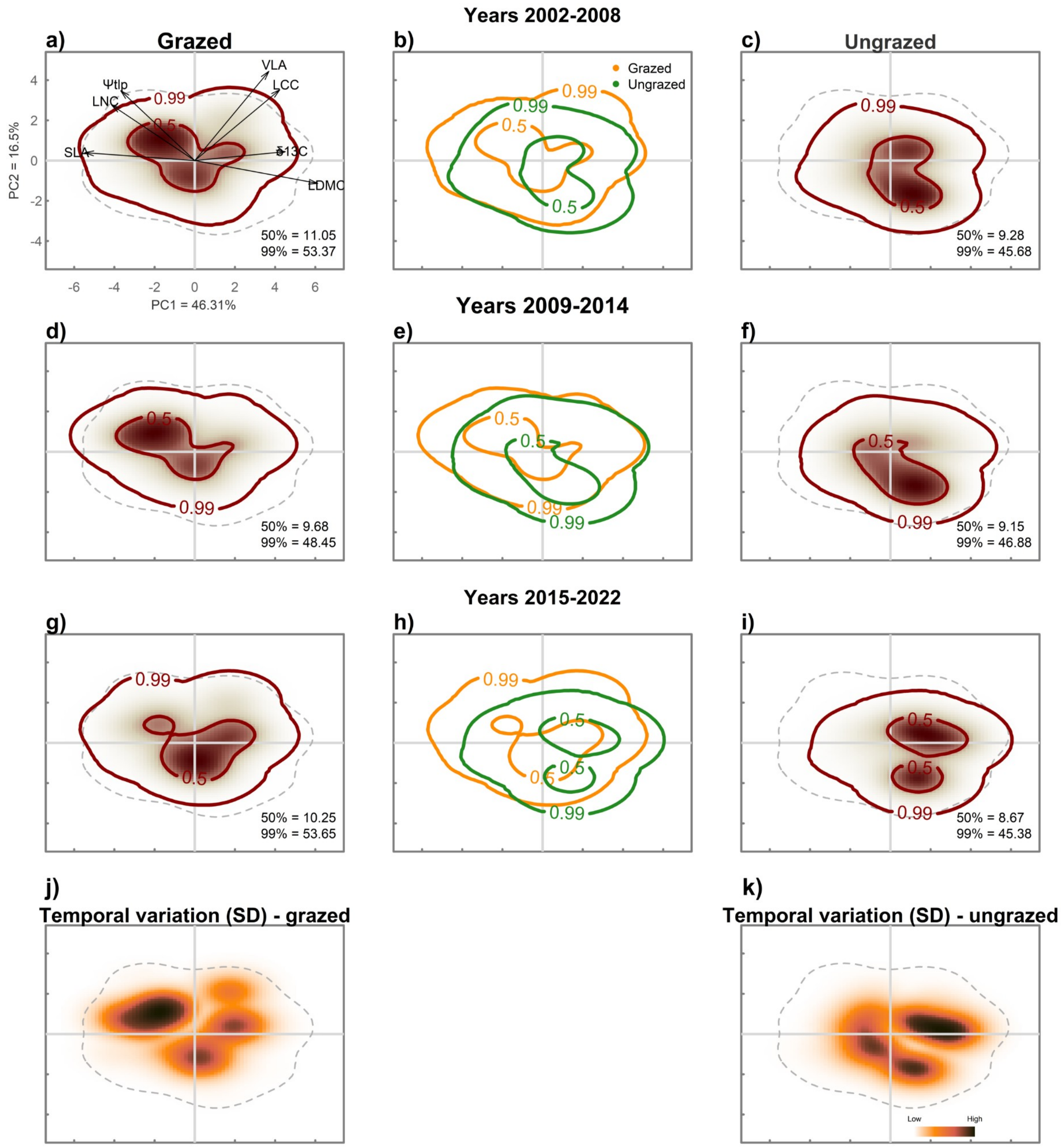

**Fig. 2.** Probabilistic distribution of trait combinations in the functional trait space defined by the PCA in the grazed (panels a,d,g) and ungrazed panels c, f and i) community for each temporal interval. The colours indicate the probability densities, from high (dark brown) to low (light brown) probability. Contour lines indicate the 0.99, 0.50 and 0.25 quantile of the total probability distribution. Functional richness (FRic) values estimated considering 0.50 and 0.99 quantile for each community and temporal interval are reported. Central panels (b, e,

h) show the overlap between the two communities in each temporal interval (orange: grazed community; green: ungrazed community). Bottom panels (j, k) shows the total temporal variation of functional structure (expressed as standard deviation) for the grazed (i) and ungrazed (k) community, from higher (dark brown) to lower (light brown) values of temporal variation. Variables are: leaf dry matter content (LDMC), specific leaf area (SLA), water potential at turgor loss point (Ψtlp), minor vein length per unit area (VLA), carbon stable isotope composition (δ13C), leaf carbon content (LCC), leaf nitrogen content (LNC).

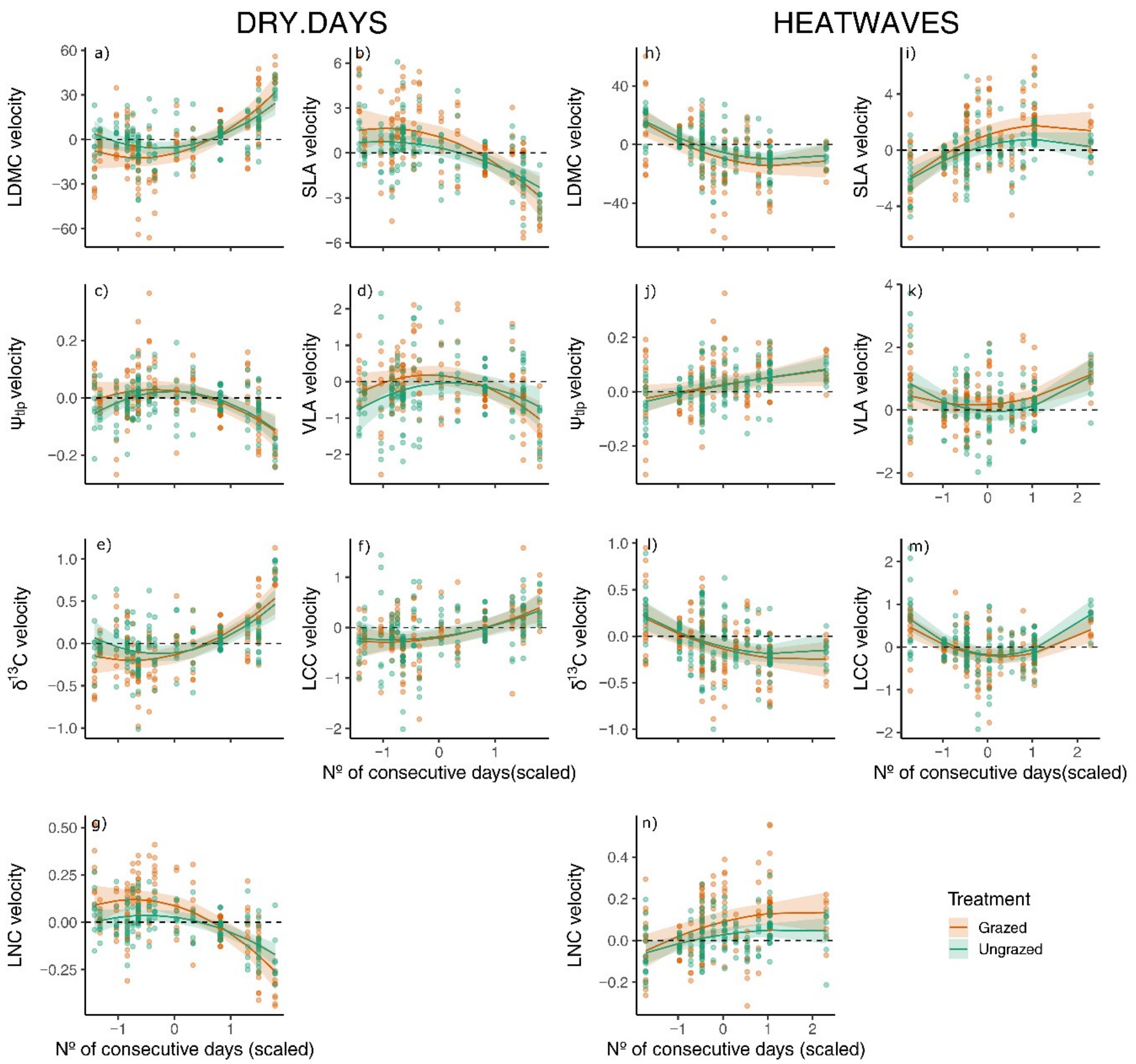

**Fig. 3.** Relationships between prolonged periods of drought and heat and annual rate of change (i.e. velocity) of (community weighted) trait means for grazed and ungrazed community. Trait velocity of zero represent no change over time, while positive and negative values represent an increase vs decrease in trait community value respectively. Linear mixed models were fitted separately for the two treatments. Only significant relationships are reported (see Table S8 [Supporting Information] for models' coefficients and statistics). Each point represents the trait velocity for each plot in a given year, for grazed (orange) and ungrazed (green) community. Ribbons represent the 95% confidence intervals. Climatic variables were scaled to mean zero and variance one.(a,h) leaf dry matter content (LDMC),

(b,i) specific leaf area (SLA), (c,j) water potential at turgor loss point ($\Psi_{tlp}$), (d,k) minor vein length per unit area (VLA), (e,l) carbon stable isotope composition ($\delta^{13}C$), (f,m) leaf carbon content (LCC), (g,n) leaf nitrogen content (LNC). Climate variables are: Maximum consecutive number of consecutive days with precipitation <1 mm in a given year (DRY.DAYS) and maximum consecutive number of annual days with T > 30° C in a given year (HEATWAVES).