



Meso-scale environmental heterogeneity drives plant trait distributions in fragmented dry grasslands

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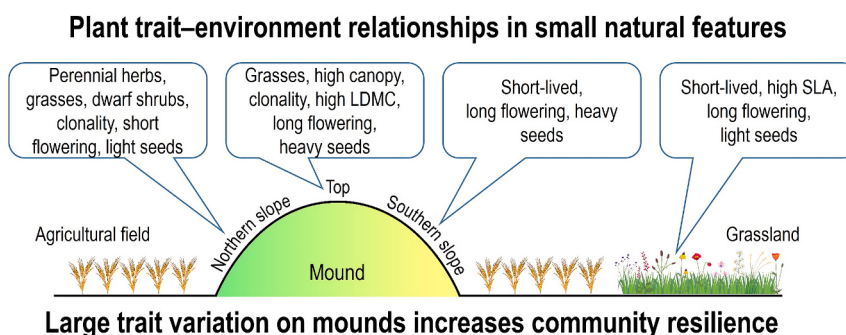
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HIGHLIGHTS

- Meso-scale environmental heterogeneity (EH) sustains high trait variability.
- Spatial and temporal components of EH affect plant traits on small natural features.
- Small natural features extend the trait spectrum of homogenous plain landscapes.
- Trait composition patterns driven by EH are consistent under various macroclimates.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental heterogeneity shapes the patterns of resources and limiting factors and therefore can be an important driver of plant community composition through the selection of the most adaptive functional traits. In this study, we explored plant trait–environment relationships in environmentally heterogeneous microsite complexes at the meso-scale (few meters), and used ancient Bulgarian and Hungarian burial mounds covered by dry grasslands as a model habitat. We assessed within-site trait variability typical of certain microsites with different combinations of environmental parameters (mound slopes with different aspects, mound tops, and surrounding plain grasslands) using a dataset of 480 vegetation plots. For this we calculated community-weighted means (CWMs) and abundance models. We found that despite their small size, the vegetation on mounds was characterized by different sets of functional traits (higher canopy, higher level of clonality, and heavier seeds) compared to the plain grasslands. North-facing slopes with mild environmental conditions were

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characterized by perennial species with light seeds, short flowering period, and a high proportion of dwarf shrubs sharply contrasted from the plain grasslands and from the south-facing slopes and mound tops with harsh environmental conditions. Patterns predicted by CWMs and abundance models differed in the case of certain traits (perenniality, canopy height, and leaf dry matter content), suggesting that environmental factors do not necessarily affect trait optima directly, but influence them indirectly through correlating traits. Due to the large relative differences in environmental parameters, contrasts in trait composition among microsites were mostly consistent and independent from the macroclimate. Mounds with high environmental heterogeneity can considerably increase variability in plant functional traits and ecological strategies at the site and landscape levels. The large trait variation on topographically heterogeneous landscape features can increase community resilience against climate change or stochastic disturbances, which underlines their conservation importance.

1. Introduction

Environmental heterogeneity is considered a major driver of species richness patterns from local to continental scales (Udy et al., 2021). Spatial variation of abiotic factors related to topography, climate, and soil can lead to the formation and coexistence of contrasting microsites that considerably increase the structural complexity of a habitat at multiple scales (Stein et al., 2014; Bátori et al., 2023). Complexity in the availability of resources and other limiting factors can increase the available niche space for partitioning, and, therefore, can facilitate the establishment, coexistence, and persistence of species and lead to enhanced biodiversity (Stein et al., 2014; Deák et al., 2021a).

As environmental factors affect the occurrences and abundances of plant species through their specific traits, trait-based approaches are feasible tools for understanding mechanisms that form species richness patterns related to environmental heterogeneity (Stark et al., 2017; Vernham et al., 2023). Species-specific traits related to germination, survival, growth, and reproduction represent different adaptation strategies to certain environmental conditions, and can considerably affect community-level trait compositions along environmental gradients, termed trait–environment relationships (Shipley et al., 2016). Morphological and physiological characteristics determine the establishment success of plants at a microsite characterized by a particular set of abiotic and biotic filters (Beck and Richards, 2023). The exclusion of a species can occur because it cannot survive under certain abiotic conditions (e.g., in harsh environments) or because of its weak competitive ability (e.g., in resource-rich environments) (Bergholz et al., 2017). By understanding this trait-mediated ecological sorting, we can gain a deeper understanding of the mechanisms affecting community assemblages and biodiversity in heterogeneous habitats, and we can predict which species from a regional species pool can establish and become abundant at a certain microsite (Janečková et al., 2017).

In natural ecosystems, environmental heterogeneity is often associated with topographically heterogeneous structures, and many previous studies focused on this phenomenon at broad spatial scales. However, while most studies focused on large (Dainese et al., 2015; Stark et al., 2017) and small (Stark et al., 2017; Price et al., 2017) spatial scales, analyses aiming to reveal trait–environment associations at the meso-scale (ca. few meters) are still scarce and focused mainly on some specific habitat types such as inselbergs (de Paula et al., 2019; Chelli et al., 2019), bedrock glades (Beck and Givnish, 2021; Beck and Richards, 2023), and sand dunes (Conti et al., 2017). Although grasslands cover 26 % of the globe (Squires et al., 2018) and hold a considerable level of environmental heterogeneity at the meso-scale, they were rarely investigated in this regard. Studies at the meso-scale have primarily focused on patterns related to community weighted means of certain traits and functional diversity of plant communities. However, there is still a lack of studies that directly explore trait–environment relationships while taking into account the local species pools.

In our study, we aimed to explore trait–environment relationships in topographically heterogeneous landscape features (ancient burial mounds) that often maintain dry grassland fragments. Burial mounds are few meters high artificial earthen hills that were built for burial purposes during the Bronze and Iron ages. They are one of the most

widespread ancient man-made landscape features in the steppes (there are approximately 600,000 kurgans in Eurasia), and have a high potential to preserve species-rich dry grasslands even in fragmented agricultural landscapes (Deák et al., 2016; Dembicz et al., 2020; Apostolova et al., 2022; Gallé et al., 2022). We studied these landscape features, as they represent environmentally heterogeneous microsite complexes at the meso-scale by holding several contrasting microsites with a unique combination of various environmental conditions (Lisetskii et al., 2014; Deák et al., 2021a). In our previous study (Deák et al., 2021a), we revealed that mounds hold characteristic contrasting microsites (north-, east-, south-, and west-facing slopes, and the top) maintaining high variability regarding the level of abiotic stress ranging from harsh to mild (for details, please see Supplementary text 1, Table S1). Plant communities of these microsites are characterized by different species richness and composition; and these differences are driven by the unique combinations of environmental conditions typical of a certain microsite (Deák et al., 2021a).

Plant functional groups related to lifespan and growth forms together with traits of leaves, plant height, and seed mass can capture key aspects of ecological strategies that influence stress tolerance and persistence abilities in certain environments (Westoby, 1998). We predicted that species–environment associations are considerably influenced by trait-mediated ecological sorting. Short-lived species are expected to perform better in harsh environments, where due to their short life cycle, they can find the optimal time period for growth and reproduction, and they do not have to compete with perennial species with strong persistence abilities (like perennial grasses or forbs), which are generally typical to mild environments (Lhotsky et al., 2016). Plants with high specific leaf area (SLA) are generally fast-growing species with a good nutrient acquisition ability, and are expected to be characteristic of productive mild environments, while plants having high leaf dry matter content (LDMC) are expected to occur under harsh conditions characterized by drought stress (Backes et al., 2021). Short stature can be an adaptive strategy in harsh environments, where the proportion of successful competitors is low, and short species can fill the available gaps with optimal conditions. On the contrary, we expected tall-statured plants to be typical in resource-rich mild environments, where the limiting factor is the light (Janečková et al., 2017). Clonal growth is considered an important persistence trait that allows horizontal growth, space occupancy, and enhanced interspecific competition ability in resource rich environments (Heinken and Weber, 2013). Start and duration of flowering period are also expected to be influenced by environmental conditions, as in milder climate, the earlier flowering start and longer flowering period are typical (Dunne et al., 2003). Heavy seeds are expected to be a successful strategy to enhance seedling emergence under harsh conditions when resource availability is limited, but as a trade-off, increased seed weight decreases the spatial dispersal distance (Turnbull et al., 1999).

To test the above-mentioned trait–environment relationships, we studied which plant traits determine community assemblages at different mound microsites and in the surrounding plain grasslands. For this purpose, we used two approaches that complement each other. We used community-weighted means (or proportions, for categorical traits) for describing community-level trait patterns focusing on the effect of the

environment on the optimal trait value. Additionally, we also fitted CATS (community assembly via trait selection) models (Shiple et al., 2007; Warton et al., 2015), which reveal whether there is an environmental selection affecting specific traits. We studied mounds located in different geographical regions representing subhumid (Hungary) and semiarid (Bulgaria) macroclimates. We aimed to answer the following questions: (i) Is there a community-level within-mound variability in the weighted mean of plant traits associated with the mound microsites? (ii) Do the trait combinations typical of mound microsites differ from the trait values of nearby plain grasslands? (iii) Do certain microsites differ only in their optimal trait values, or also in the strength of environmental filtering?

2. Methods

2.1. Study regions

Our two study regions are located in the lowlands of East Hungary and South Bulgaria (Fig. S1). The Hungarian study region is situated in the Great Hungarian Plain that is characterized by a dry sub-humid continental macroclimate (aridity index 0.53; Zomer et al., 2022). The mean annual precipitation is 545 mm, and the mean annual temperature is 10.2°C. The Bulgarian study region is located in the valley of the Maritsa River and has a semi-arid continental climate with a sub-Mediterranean influence (aridity index 0.44). The mean annual precipitation is 519 mm, and the mean annual temperature is 12.2 °C (Fick and Hijmans, 2017). In historical times, both lowland regions held considerable amounts of dry grasslands typical of the forest-steppe biome (Deák et al., 2021b). The intensification of land use over the past millennia has led to a significant loss of dry grasslands, thus, currently the study regions are characterized by a high proportion of agricultural fields. Larger stands of dry grasslands are mostly restricted to protected areas in both regions, and in general, only small fragments of plain grasslands remained in the non-protected agricultural landscapes.

2.2. Site selection

In both regions, we designated eight sites, each consisting of a mound covered by dry grassland vegetation (Fig. S2) and the closest plain dry grassland patch that is representative of the study area. For searching the nearest plain grassland patches we used our former field experiences in the area, and orthorectified satellite images provided by Google Maps (Google Maps, 2023). The mean distance (the smallest distance between the edge of the habitat patches) between mounds and plain grasslands were 1033 ± 1263 m (mean $\pm$ SD) in Hungary and 743 ± 782 m in Bulgaria. For this study, we selected isolated mounds (i.e., mounds that were surrounded by non-grassland habitats), as in these regions (as well as other regions in the European forest-steppe biome), mounds typically act as habitat islands embedded in anthropogenic habitats (Deák et al., 2016; Dembicz et al., 2020). To reduce the possible effects of habitat area (i.e., mound size) and differences in topography, we selected mounds with similar area, relative height, and slope inclination. The mean area of the mounds was 3777 ± 1665 m² (mean $\pm$ SD) in Hungary and 3196 ± 2138 m² in Bulgaria. They were 5.1 ± 2.0 m high in Hungary and 6.5 ± 3.5 m in Bulgaria. The mean slope angle was $12.0 \pm 2.9^\circ$ in Hungary and $16.4 \pm 4.4^\circ$ in Bulgaria. All mounds were unmanaged and were not affected by any recent anthropogenic disturbances (e.g., construction works and trampling) or the encroachment of invasive species.

2.3. Field survey

2.3.1. Vegetation

At each site, we surveyed the vegetation of the five mound microsites (north-, east-, south-, west-facing slopes, and top) and the surrounding

plain grassland. The vegetation was surveyed at the peak of the growing season (May–June 2019) to be able to detect the highest number of species, including spring short-lived and perennial species. At each microsite and in plain grasslands, the vegetation was surveyed in five 1 m $\times$ 1 m plots (30 plots per site). On the mound slopes the plots were placed on the middle of the slopes. On mound tops and in plain grasslands we placed the plots randomly. As plain grasslands had larger size compared to mound microsites, random plots were placed within an area comparable to a mound microsite (approximately 700 m²). Altogether, we sampled 480 plots in total (2 regions $\times$ 8 sites $\times$ 30 plots). In each plot, we visually estimated the abundance (as percentage cover on a continuous scale) for all vascular plant species. We estimated absolute species abundances; thus, individual abundance values were estimated independently of the total abundance of all species.

2.3.2. Environmental parameters

In our former study (Deák et al., 2021a) based on detailed field measurements of environmental variables, we characterized the mound microsites based on microclimate variables (air temperature and relative humidity; one-minute intervals for 24 h) and the volumetric water content of the soil (in the upper 12 cm of the soil) (Supplementary text 1, Table S1). Based on the combinations of the measured environmental parameters north-facing slopes were considered as “constantly mild” microsites, the south-facing slopes and the top as “constantly harsh” ones. The east- and west-facing slopes were characterized by varying environmental conditions, the east-facing slope being a harsher habitat than the west-facing slope. These types of microhabitats can also be found in similar sized and shaped landscape structures such as inselbergs, bedrock glades or sand dunes (de Paula et al., 2019; Beck and Richards, 2023; Conti et al., 2017). In order to reveal the general patterns in trait abundances among microsites with contrasting combination of environmental conditions (harsh versus mild, stable versus varying), instead of studying the individual effects of certain environmental parameters we focused on the patterns on the microsites level.

2.4. Plant traits and functional groups

In the present study, we used a dataset that describes the abundance of 301 vascular plant species (Deák et al., 2021a) and nine plant traits (Table 1). To represent the characteristic trait values in the study region, trait data were preferably collected from regional Hungarian and Bulgarian data sources (for these please refer to Table 1), and we also used our own trait measurements from the study regions (for in total eight species). If regional data were not available, we used international databases (LEDA – Kleyer et al., 2008 and CLO-PLA – Klimešová et al., 2017) to complete the dataset. If multiple records were available in a database for a certain trait, we calculated the mean of the values.

For 29 of 301 species, representing 7.63 % of the total abundance, some of the trait values were not available (the proportion of species with missing data for each trait is provided in Table S2). To explore the potential correlation between the traits studied, we used Spearman correlation.

2.5. Data analysis

2.5.1. Community weighted proportions and means

Community-weighted means or proportions of traits are widely applied to describe community-level trait patterns (Violle et al., 2007). This method is proper for exploring optimal trait values in microsites characterized by different environmental conditions. However, it does not count for that the strength of environmental selection (the rate of decline of abundances away from the optimal trait value) may change along environmental gradients. Furthermore, CWM is influenced not only by the actual selection (sensu Vellend, 2010) for species pool, but also by the composition of the species pool formed by evolutionary and ecological processes in the past.

Table 1

Plant traits used in the analyses: trait names and units, ecological importance, scale type, range of values in our study and sources of trait data. Databases containing regionally collected data are marked with an asterisk.

Trait	Ecological importance	Scale type	Range of values	Source
Life span	On-spot persistence; space occupancy	Categorical	[short-lived; perennial]	Delipavlov et al., 2003*; Király, 2009*
Morphological group	Resource economy, stress- and disturbance tolerance	Categorical	[grass; forb; dwarf shrub]	Delipavlov et al., 2003*; Király, 2009*
Specific leaf area (SLA) (mm ² mg ⁻¹)	Resource economy; stress- and disturbance resistance	Numerical (continuous)	4.75–108.12	Kleyer et al., 2008; E.-Vojtkó et al., 2020*
Leaf dry matter content (LDMC) (mg g ⁻¹)	Stress- resistance	Numerical (continuous)	10.30–755.73	Kleyer et al., 2008; E.-Vojtkó et al., 2020*
Canopy height (cm)	Resource economy; space occupancy	Numerical (continuous)	3–130	Delipavlov et al., 2003*; Király, 2009*
Clonal index	On-spot persistence; reproduction; space occupancy	Numerical (ordinal)	0–6	Klímešová et al., 2017
Start of flowering period (month)	Reproduction strategy; stress- and disturbance avoidance	Numerical (discrete)	1–8	Delipavlov et al., 2003*; Király, 2009*
Duration of flowering period (month)	Reproduction strategy; stress- and disturbance avoidance	Numerical (discrete)	1–12	Delipavlov et al., 2003*; Király, 2009*
Seed mass (g/ thousand seeds)	Dispersal ability; on-spot persistence	Numerical (continuous)	0.02–104	Török et al., 2013*, 2016*; SID, 2022

Community weighted proportions and mean trait values (CWMs) were calculated using the percentage cover of species. For categorical traits (Table 1), the relative abundance (i.e., the coverage proportion of species characterized by the given trait level in the total coverage) of each trait level (e.g., perennial, short-lived) was calculated. The microsite-level differences in the CWMs were tested using generalized linear mixed-effects models (GLMMs). Relative abundances of categorical traits were analyzed with models using the ordered Beta model family with logit link function. For continuous traits, we used Gamma model family with log-link function, because this model family handles well skewed positive continuous variables. Model assumptions were checked by visual inspection of diagnostic plots.

In the models, CWMs were used as response variables (in separate models for each trait, or in the case of categorical traits, for each trait level), while microsite, region and their interaction were predictor variables. Statistical significance of partial effect of individual predictors and their interaction term were assessed by ML-ratio tests. Microsite nested in mound ID was used as random factor to control for the non-independence of data within mounds and for the multiple samples within microsites. Multiple comparisons (microsites within the regions and among regions) followed Tukey's procedure with adjusted *p*-values.

2.5.2. Abundance models

CATS models using species abundances can effectively complement CWM models as they account for the probable environmental selection acting at the microsites (Shipley et al., 2007). Significant trait–environment interaction in CATS models indicates that environmental selection on the traits depends on the environmental conditions. The environment may influence the optimal trait value and/or the strength of selection, and the two effects can be separated by inspecting the parameters of the fitted model.

In CATS regressions, the abundance of species was considered as a response variable. The fixed part of the model consisted of the environment, trait, and trait:environment interaction as predictors. To avoid fitting too complex models, separate regressions were fitted for each trait. In the case of stabilizing selection on continuous traits (i.e., when intermediate trait value is optimal), second-order polynomials should be fitted (Botta-Dukát, 2022) (Fig. S3). It can be done by including the trait, its square, and their interaction with the environment as independent variables. In our case, the environment was characterized by two categorical variables (region and microsite) and their interaction.

Abundances of the same species in different plots are not independent. Moreover, abundances of species in the same plot may depend on each other (e.g., due to interactions between species). To handle these non-independences, random-slope mixed-effects models were fitted (ter Braak, 2019). The applied model structure was the following:

$$\text{abundance} \sim \text{region} * \text{microsite} * (\text{trait} + \text{squared.trait}) + (1 + \text{region} + \text{microsite} + \text{region} : \text{microsite} | \text{species.ID}) + (1 + \text{trait} + \text{squared.trait} | \text{plot.ID})$$

Note that squared trait values were included only for continuous traits. To reduce the correlation between independent variables, continuous traits were centered and standardized prior to the analysis. Before the analyses, the estimated cover values were divided by the observed lowest non-zero value. This resulted in count-type variables that could be modelled by GLMMs using Poisson distribution and log link.

For continuous traits, the fixed part of the model could be written in the following form:

$$\log(\widehat{Y}_{ij}(T)) = \beta_{0ij} + (\beta_1 + \beta_{2i} + \beta_{3j} + \beta_{4ij})T + (\beta_5 + \beta_{6i} + \beta_{7j} + \beta_{8ij})T^2$$

where $\widehat{Y}_{ij}(T)$ is the expected abundance of a species with trait value *T* in region *i* and microsite *j* and betas are the estimated parameters. Note that since we focused on trait–environment interactions, some parameters are summed into β_{0ij} .

After the following re-parametrization

$$s_{ij} = \frac{1}{2(\beta_5 + \beta_{6i} + \beta_{7j} + \beta_{8ij})}$$

$$o_{ij} = -\frac{\beta_1 + \beta_{2i} + \beta_{3j} + \beta_{4ij}}{2(\beta_5 + \beta_{6i} + \beta_{7j} + \beta_{8ij})}$$

$$A_{ij} = \exp\left(\beta_{0ij} - \frac{\beta_1 + \beta_{2i} + \beta_{3j} + \beta_{4ij}}{2}\right),$$

the equation can be written as

$$\widehat{Y}_{ij}(T) = A_{ij} \exp\left(\frac{(o_{ij} - T)^2}{s_{ij}}\right).$$

If $s_{ij} < 0$, it is an equation of a bell-shaped (Gaussian) curve with maximum at o_{ij} . Thus, o_{ij} is the optimal trait value at microsite (*M*) *j* in region (*R*) *i*; A_{ij} is the expected highest abundance, and s_{ij} determines the width of a bell-shaped curve: lower parameter value means a narrower curve.

We can formulate six null-hypotheses related to trait–environment

interactions:

- (1) $H_0(R:T): \beta_{2i} = 0 \forall i$
- (2) $H_0(M:T): \beta_{3j} = 0 \forall j$
- (3) $H_0(R:M:T): \beta_{4ij} = 0 \forall i, j$
- (4) $H_0(R:T^2): \beta_{6i} = 0 \forall i$
- (5) $H_0(M:T^2): \beta_{7j} = 0 \forall j$
- (6) $H_0(R:M:T^2): \beta_{8ij} = 0 \forall i, j$

If $H_0(R:M:T^2)$ is true, there is no interactive effect of region and microsite on the width of the bell-shaped curve. If both $H_0(R:M:T)$ and $H_0(R:M:T^2)$ are true, there is no interactive effect of region and microsite on the optimal trait value. The interpretation of the other null-hypotheses follows the same logic (Fig. S4). Hypotheses were tested by ML-ratio tests.

The estimated β -s is approximately normally distributed, but it was not true for the estimated optima. Therefore, if we reject the null-hypothesis of no difference between optima, the 95 % confidence interval of optima and their differences should be estimated by parametric bootstrap. During this procedure, we took 1000 samples of the multivariate normal distribution of β -s using the estimated mean and covariance matrix. Then, we calculated the optima and differences between them from these random β values and finally, we estimated the threshold of the 95 % confidence interval of the calculated estimates.

For categorical traits, the fixed part of the model is simpler because there is no quadratic part:

$$\log(\widehat{Y}_{ij}(T)) = \beta_{0ij} + (\beta_1 + \beta_{2i} + \beta_{3j} + \beta_{4ij})T$$

For binary traits, the possible values of T are 0 and 1. Therefore,

$$\begin{aligned} \log\left(\frac{\widehat{Y}_{ij}(T=1)}{\widehat{Y}_{ij}(T=0)}\right) &= \log(\widehat{Y}_{ij}(T=1)) - \log(\widehat{Y}_{ij}(T=0)) \\ &= \beta_1 + \beta_{2i} + \beta_{3j} + \beta_{4ij} \end{aligned}$$

Hereafter, this log ratio of expected abundances will be referred to as the ‘advantage’ of the category coded by 1 over the other (reference) category. Since the advantages are linear combinations of estimated parameters, their confidence interval can be easily calculated by standard methods.

Note that CWM for categorical traits is the sum of abundances of species belonging to the given category. This sum of abundances equals the product of the expected abundance of a species belonging to a given category and the number of such species in the species pool. Therefore, differences in CWMs may be caused by differences in the composition of the species pool that formed evolutionary and ecological processes in the past. On the other hand, the advantage of a category over the other defined here mirrors only the actual selection from the species pool.

2.5.3. Data handling and packages used

All data handling and statistical data analyses were carried out in R (version 4.1.2, R Core Team, R, 2013). The models were fitted using the ‘glmmTMB’ (Brooks et al., 2017) package. The ‘DHARMA’ (Hartig, 2022) package was used for model diagnostics. Estimated marginal mean values and parameter contrasts were extracted using the ‘emmeans’ package (Lenth, 2019). In the parametric bootstrap, random numbers with multivariate normal distribution are generated using the ‘mnormt’ package (Azzalini and Genz, 2022). Figures were created using the ‘ggplot2’ (Wickham, 2016), and ‘cowplot’ (Wilke, 2020) packages.

3. Results

3.1. Community weighted proportions and means

We found that community-weighted proportion of perennials was higher in Hungary compared to Bulgaria (Table 2, Fig. 1A, Table S3). In

Bulgaria, the proportion of perennials was higher on north-, south-, and west-facing slopes and mound tops compared to plain grasslands. It was also higher on west-facing compared to east-facing slopes. Short-lived species showed the opposite pattern. The proportion of grasses was higher in Hungary, while the proportion of herbs was higher in Bulgaria (Table 2). In Bulgaria, the proportion of grass species was higher on north-facing slopes and mound tops compared to the east- and south-facing slopes (Fig. 1B). In Hungary, the proportion of grasses was higher on mound tops compared to west-facing slopes and plain grasslands. In Bulgaria, the proportion of forb species was lower on north-facing slopes and top compared to south-, and east-facing slopes.

In Hungary, the proportion of forbs was lower on east-facing slopes and tops compared to plain grasslands. In both regions, the community-weighted proportion of dwarf shrubs was higher on north-facing slopes compared to south-facing slopes (Table 2, Fig. 2A). The CWM of SLA was higher in Hungary compared to Bulgaria. As a general pattern, plant communities on east-facing slopes were characterized by larger SLA than communities on tops (Table 2, Fig. 2B). The CWM of LDMC was higher in Hungary, but we did not find any significant differences between microsites (Table 2). The canopy height was larger on south-facing slope and on the mound top compared to plain grasslands (Table 2, Fig. 2C). The CWM of clonal plants was overall higher on the mounds than in plain grasslands (Table 2, Fig. 2D). Flowering started earlier on mounds situated in Bulgaria. In both regions, flowering started the earliest on east-facing slopes and was the latest on west-facing slopes (Table 2, Fig. 2E). Flowering lasted longer in Bulgaria. In both regions the duration of flowering period was shorter on north-facing slopes compared to south-facing slopes, and plain grasslands (Table 2, Fig. 2F). The CWM of seed mass was larger on east-, south-, and west-facing slopes, and on tops compared to plain grasslands (Table 2, Fig. 2G). Also, it was larger in south-facing slopes and tops than in north-facing slopes.

3.2. Abundance models

In all regions, perennial species had a higher advantage over short-lived ones on north-facing slopes compared to south-facing slopes (Table 3, Fig. 3A). The optimal trait values for LDMC were lower in Bulgaria than in Hungary. We found that in Bulgaria, the optimal value of LDMC was highest on north-facing slopes, and lowest on south-facing slopes and in plain grasslands (Fig. 3B). The optimal value of LDMC was lower in west-facing slopes than in north-facing slopes but was higher than on south-facing slopes and in plain grasslands. The optimal canopy height was higher in Hungary compared to Bulgaria. In Hungary, the optimal height was lower in plain grasslands compared to north-, and east-facing slopes, and top of mounds (Fig. 3C). The optimal date for the start of flowering was earlier in Bulgaria than in Hungary, but the width of the bell-shaped curve did not differ between regions. The effect of the morphological group, SLA, clonality, duration of flowering, and seed mass on the expected abundance did not vary between regions and microsites.

3.3. Trait correlations

The perennial species were characterized by low SLA and high clonality (Fig. S4). Short-lived species showed the opposite. The trait correlations show that the forb species flowered early in the growing season, their flowering lasted long, and they were characterized by low clonality and low LDMC values. The grass species assessed were late flowering and characterized by high clonality and high LDMC values. The dwarf shrubs started flowering late. Species with large SLA started flowering earlier, were shorter, and had a smaller seed mass. Species with large LDMC had a short flowering duration and high clonality. Tall species had heavy seeds. Clonal species had short flowering duration.

Table 2

Analysis of deviance tables for the final reduced GLMMs fitted on CWMs of selected plant traits ($n = 12$) as a function of region ($n = 2$; Hungary and Bulgaria) and microsites ($n = 6$; north-, south-, west- and east-facing mound slopes, mound tops, and nearby plain grasslands). Significant effects are marked with boldface. Partial effect of two main factors was tested after excluding the interaction from the model.

Response variable	Region			Microsite			Microsite: region		
	Chi-square	df	p	Chi-square	df	p	Chi-square	df	p
Life span (perennial)	0.867	1	0.352	17.804	5	0.003	16.939	5	0.005
Life span (short-lived)	0.867	1	0.352	17.804	5	0.003	16.939	5	0.005
Morphological group (grass)	5.218	1	0.022	16.728	5	0.005	19.475	5	0.002
Morphological group (forb)	7.573	1	0.006	17.951	5	0.003	23.280	5	<0.001
Morphological group (dwarf shrub)	2.070	1	0.150	13.228	5	0.021	3.297	5	0.654
Specific leaf area (SLA)	5.256	1	0.022	11.200	5	0.048	4.298	5	0.507
Leaf dry matter content (LDMC)	18.582	1	<0.001	2.921	5	0.712	2.068	5	0.840
Canopy height	0.019	1	0.890	27.724	5	<0.001	1.175	5	0.947
Clonal index	1.047	1	0.306	44.084	5	<0.001	4.694	5	0.529
Start of flowering period	4.297	1	0.038	16.803	5	0.005	5.139	5	0.378
Duration of flowering period	9.524	1	0.002	17.284	5	0.004	10.094	5	0.073
Seed mass	0.405	1	0.524	36.129	5	<0.001	8.525	5	0.130

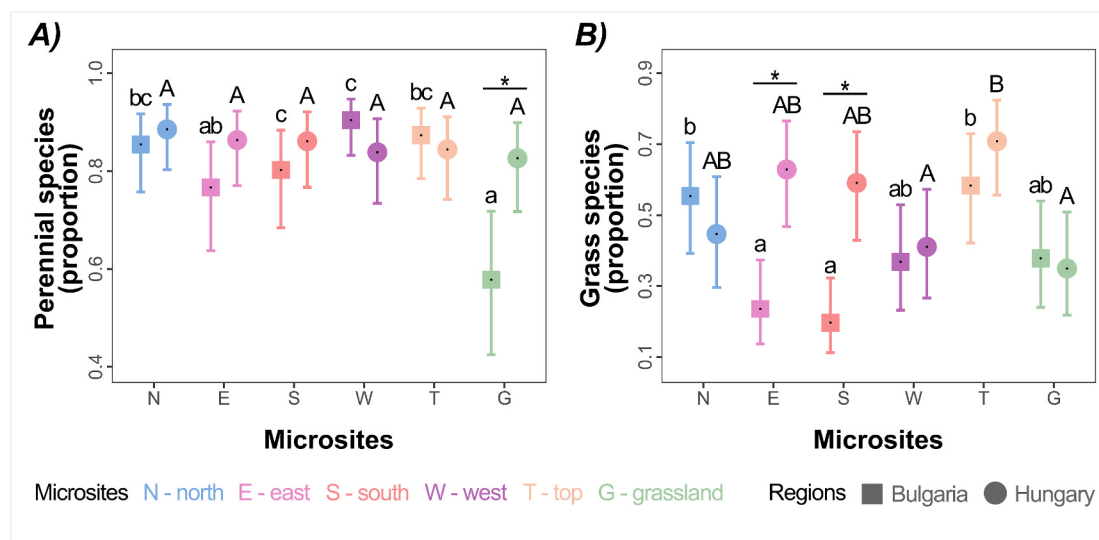


Fig. 1. Significant microsite:region interactions in community-weighted proportions of perennials and grasses. Plots represent the means, and error bars represent the 95 % confidence intervals based on the performed models. Lowercase letters denote significant differences between groups for the Bulgarian mounds, and uppercase letters code the within-microsite differences in Hungary. Asterisks denote significant differences within the same microsite.

4. Discussion

Our results show that despite the small size of mounds, mound microsites exhibiting different topography, microclimate, and edaphic conditions were characterized by plant communities with contrasting trait combinations. Both models based on CWMs and species abundances suggested that trait compositions differed considerably between constantly mild and harsh microsites. Therefore, abiotic factors existing in different microsites drive community assemblages by affecting functional traits. As a general pattern, we found that in mild microsites, species were characterized by traits enabling long-term on-spot persistence and competition, while species in harsh microsites had adaptive traits supporting stress tolerance. Mound microsites that experience large daily microclimatic fluctuations, forming a transition between the constantly mild and constantly harsh microsites, were often characterized by species with intermediate trait values. However, we found that in certain cases, environmental fluctuations selected certain specific trait values. When comparing the results of models based on CWMs and species abundances, we found that in many cases, the realized and optimal trait values were similar, suggesting that community-level trait patterns are resulted by a realized direct environmental filtering. However, for morphological groups, SLA, clonality, seed mass, start and duration of flowering only CWM models showed microsite-dependent

patterns. A possible explanation is that these traits were not directly affected by local environmental conditions but were either influenced by other filters (such as isolation) or were correlated with other traits that were affected by local environment.

4.1. Differences between mild and harsh mound microsites

Abundance models revealed that while perenniality was an effective strategy for increasing species abundances in the mildest north-facing slope, short-lived species were favored in harsh south-facing slopes. In accordance with other studies, our abundance models showed that under mild and productive conditions with low resource limitation perennial species could effectively exclude short-lived ones, and persist longer in the occupied space, and dominate the vegetation (Janečková et al., 2017). The advantage of short-lived plants over perennials on stressed south-facing slopes was directly driven by environmental filtering. These species likely could occupy establishment gaps formed under drought and heat stress, as they can adjust their life cycle to be able to utilize short periods with feasible conditions for growth and reproduction. In the intermediate periods, these species generally can survive for a long time in the seed bank (Lhotsky et al., 2016). By this strategy, to some extent, short-lived species can also avoid competition with more vigorous perennial competitive species (Lhotsky et al., 2016).

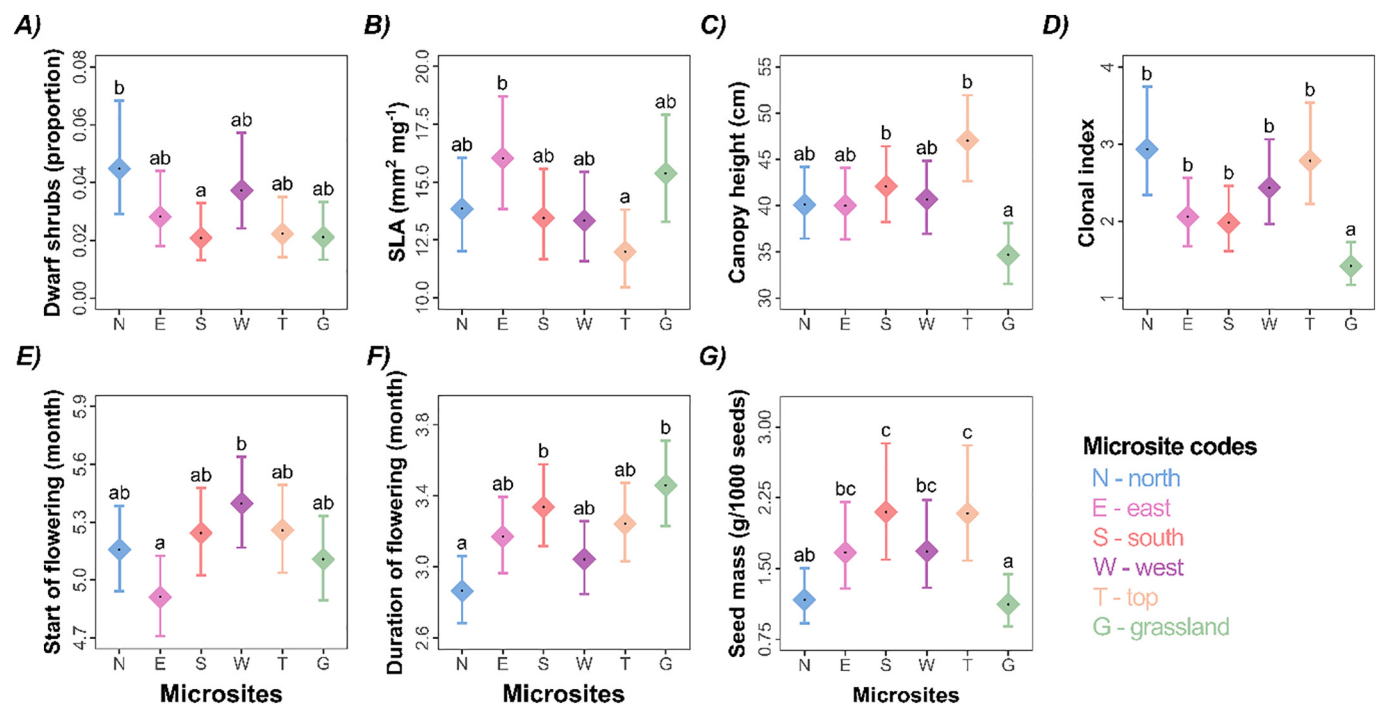


Fig. 2. Proportions of categorical traits and community-weighted means of functional groups and traits that showed significant differences among the studied microsites regardless of the region. Plots represent the means, and error bars represent the 95 % confidence intervals based on the performed models. Letters denote significant differences between groups. SLA was used as the abbreviation of specific leaf area.

Table 3
Analysis of deviance tables for the final reduced CATS fitted on selected plant traits ($n = 9$). Notations: T – Trait; M – Microsite; R – Region. Significant effects are marked with boldface. Partial effects were tested by excluding the higher-order interactions from the model.

Response variable	Trait: region			Trait ² : region			Trait: microsite		
	Chi-square	df	p	Chi-square	df	p	Chi-square	df	p
Life span	2.922	1	0.087	–	–	–	41.323	5	<0.001
Morphological group	0.478	1	0.489	–	–	–	2.332	5	0.802
Specific leaf area (SLA)	1.903	1	0.168	0.193	1	0.660	6.234	5	0.284
Leaf dry matter content (LDMC)	5.649	1	0.017	3.436	1	0.063	15.52	5	0.008
Canopy height	4.752	1	0.029	0.012	1	0.911	4.244	5	0.515
Clonal index	2.958	1	0.085	–	–	–	8.631	5	0.125
Start of flowering period	5.677	1	0.017	0.451	1	0.502	7.719	5	0.172
Duration of flowering period	0.408	1	0.523	1.008	1	0.315	10.055	5	0.074
Seed mass	0.049	1	0.826	0.001	1	0.982	3.255	5	0.661

Response variable	Trait ² : microsite			Trait: microsite: region			Trait ² : microsite: region		
	Chi-square	df	p	Chi-square	df	p	Chi-square	df	p
Life span	–	–	–	5.857	5	0.320	–	–	–
Morphological group	–	–	–	4.087	5	0.537	–	–	–
Specific leaf area (SLA)	9.358	5	0.096	4.495	5	0.481	2.256	5	0.813
Leaf dry matter content (LDMC)	10.326	5	0.066	11.664	5	0.040	4.496	5	0.480
Canopy height	3.941	5	0.558	11.262	5	0.046	5.436	5	0.365
Start of flowering period	3.799	5	0.579	1.719	5	0.887	0.802	5	0.977
Clonal index	–	–	–	2.807	5	0.730	–	–	–
Duration of flowering period	8.214	5	0.145	4.927	5	0.425	5.228	5	0.389
Seed mass	4.268	5	0.512	7.852	5	0.165	3.850	5	0.571

High proportion of grasses was typical both on mild north-facing slopes (only in Bulgaria) and harsh mound tops (both regions). As suggested by the abundance models, high cover of grasses on north-facing slopes was a consequence of an effective environmental filtering. On north-facing slopes, tussock-forming grasses (*Festuca* spp.) characterized by a high content of structural carbohydrates and typical of dry to mesic forest-steppe grasslands became established. Although these species are adapted to cope with stressful environments, they have a wider niche

preference regarding soil moisture. Additionally, their compact clonal growth form makes them highly competitive even in mild and resource-rich environments. Due to the high plasticity and effective space occupancy of these species, they have a high potential for reaching a high abundance in the north-facing slope (see also similar results of Sudnik-Wójcikowska et al., 2011 in Ukrainian kurgans). On mound tops, we found that plant communities hold a considerable cover of grass species with high LDMC values. However, the

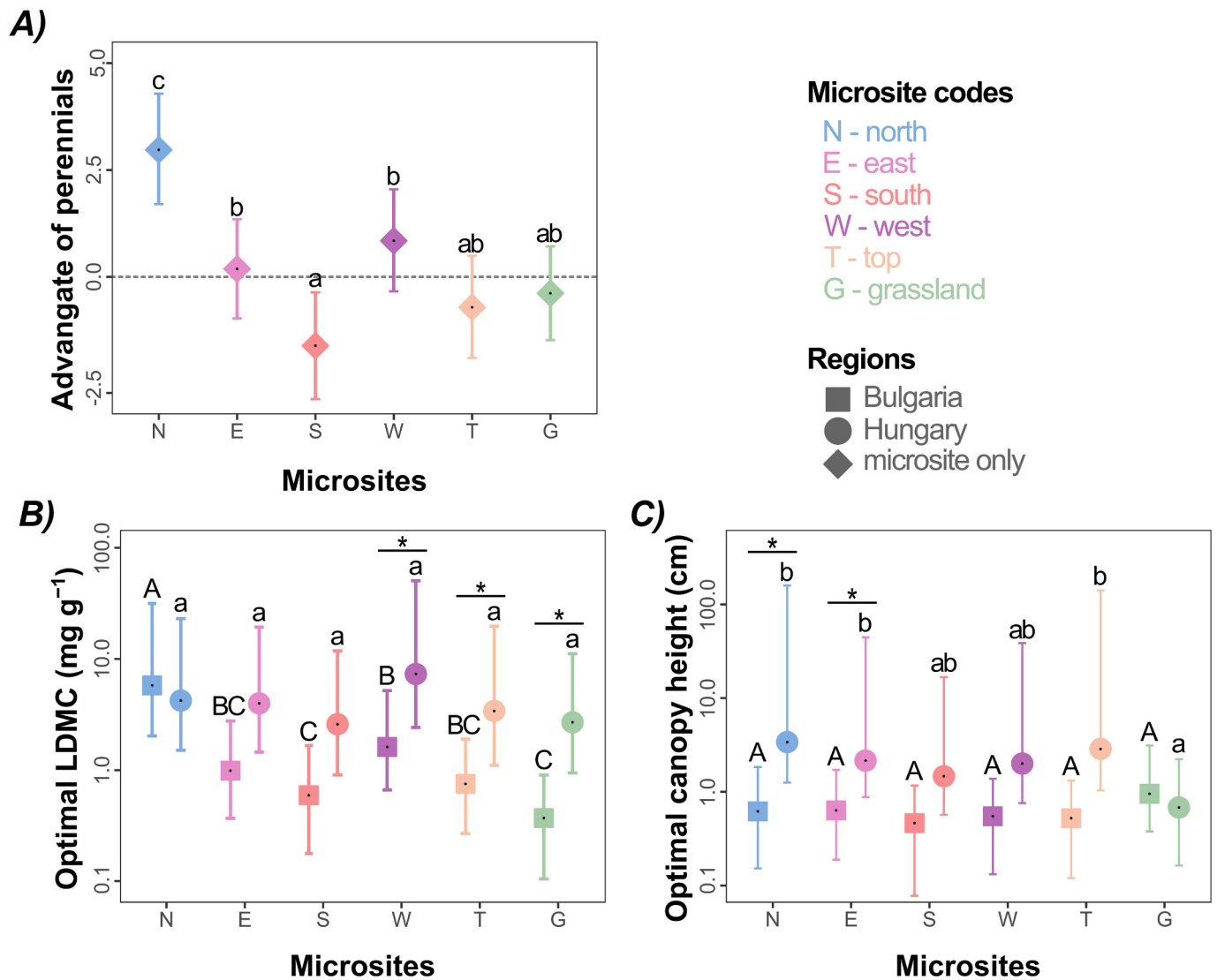


Fig. 3. Significant microsite:region interactions in the abundance models. Plots represent the means, and the whiskers represent the 95 % confidence intervals based on the performed models. Lowercase letters denote significant differences between groups for the Bulgarian mounds, and uppercase letters code the within-microsite differences in Hungary. Asterisks denote significant differences within the same microsite. In panel A, the zero lines indicate that there are no advantages/disadvantages of perennials (i.e., the expected abundance of a perennial and short-lived species is equal). Confidence intervals that do not cross this line indicate a significant advantage (i.e., a higher expected abundance of a species randomly chosen from the species pool) for perennials (positive values) or short-lived species (negative values). LDMC was used as the abbreviation of leaf dry matter content.

abundance models showed that intermediate LDMC values are optimal in this microsite. One possible explanation for this apparent paradox is that, in addition to drought-tolerant grasses, drought-tolerant forb species with intermediate LDMC values (such as *Astragalus hamosus* and *Dianthus carthusianorum*) were also typical on mound tops. On mound tops, one of the most characteristic grass species was the *Agropyron cristatum*, which is a typical stress-tolerant species of dry steppes and semi-deserts (Villasor et al., 2024). It can cope well with harsh conditions due to the low level of evapotranspiration and high LDMC that can support resistance against temperature extremities and low soil moisture values (Villasor et al., 2024; Backes et al., 2021). Despite the different macroclimates and bedrock materials, due to the presence of a resource-poor environment and high stress level, communities on mound tops had similar functional characteristics as ‘monocot mats’ on South American inselbergs (granite rock formations acting as habitat islands in Brazil; de Paula et al., 2019) where harsh microsites also held many stress-adapted monocot *Orchidaceae* and *Bromeliaceae* species.

We found that the constantly mild north-facing slopes were characterized by a high proportion of dwarf shrubs (such as *Artemisia pontica*

and *Chamaecytisus albus*) typical of forest steppes, and often present in forest edges of a favorable conservation status. These species are confined to mesic environmental conditions (Bátori et al., 2018); that implies that topography-related abiotic patterns on mounds can create microclimates similar to structural elements of forest steppes such as forest edges. Therefore, since mounds increase local topographic heterogeneity, they can provide refuge for rare dwarf shrubs adapted to special microsite conditions.

The correlation between SLA and the mound microsites was weak according to the CWM models. However, we found that the east-facing slopes were typically associated with high SLA. Although high SLA is generally associated with moist and resource-rich environments, where fast growth can support species in competition for light (Backes et al., 2021), in our study system, we assume that it was related to the high nutrient content of the soil. The total soil organic carbon content was highest in this microsite, which could counterbalance the poor availability of water and allow the establishment of several fast-growing, short-lived weed species that could emerge in wet periods (please see also Fig. S5 for trait correlations).

The timing of flowering can considerably be influenced by environmental factors such as temperature, moisture, and photoperiod (Olliff-Yang and Ackerly, 2020). Our CWM models showed that plant communities were characterized by the earliest flowering in the east-facing slopes. Early flowering might be a good drought stress avoidance strategy in harsh microsites such as east-facing slopes, since early in the spring, they warm up slower than later in the season, and morning dew might be efficiently utilized by plants, resulting in less stressed habitat conditions compared to later periods of the year when this microsite is affected by severe drought stress. However, it should be noted that this pattern might not be exclusively shaped by local environmental filters (missing trait:environment interaction in the abundance models), but probably partly by the high abundance of forbs that in general, have an earlier flowering period.

The CWM models showed that north-facing slopes, which are the coldest microsites within the area of mounds, were characterized by the shortest mean flowering period and in this regard contrasted with harsh south-facing slopes. This phenomenon was described by Körner (2003) at larger spatial scales in alpine habitats. Körner pointed out that the short duration of flowering can be considered an evolutionary adaptation in cold environments present in mountainous areas along an increasing altitudinal gradient. In our study system, similar patterns were observed on a much smaller scale. However, as we did not find significant environment-related effects in the abundance models, this pattern could also be influenced by other factors, such as the high abundance of grasses on north-facing slopes, which in general have a short flowering period (Fig. S4).

Patterns of seed sizes within plant communities on mounds reflected the marked differences between constantly mild (north-facing slope) and constantly harsh (south-facing slope and mound top) microsites. Although mild north-facing slopes were characterized by plants with light seeds, in harsh microsites, heavy seeds were typical. Increased productivity in mild microsites likely resulted in a reduced importance of seeds in regeneration compared to clonal spread, which caused a reduction in seed mass (Leishman and Westoby, 1994; Lhotsky et al., 2016). This pattern can also be explained by the fact that the dominant grass species (*Festuca* spp.) have light seeds. Under stressed conditions on south-facing slopes, the large nutrient supplies provided by heavy seeds typical of short-lived forbs could improve the establishment rates of cotyledon-stage seedlings and support established individuals to grow fast, effectively occupy gaps, and survive various hazards typical of stressed habitats (Westoby, 1998; Dainese et al., 2015).

4.2. Mounds versus plain grasslands

We detected markedly contrasting trait patterns when we compared mound microsites with plain grasslands. This pattern was likely driven by differences in local environmental factors, but also by differences in habitat size, level of isolation, and land use on mounds and in plain grasslands.

The difference in the proportion of perennial species between mounds and plain grasslands was due to the millennia-long intensive land use typical of the studied Bulgarian landscape, which resulted in the degradation of many grassland habitats. Although perennial species typical of forest steppe communities could persist on the relatively undisturbed mounds, plain grasslands in Bulgaria were characterized by a high proportion of short-lived weedy forb species due to intensive use (e. g., overgrazing) (Deák et al., 2021b). Furthermore, in the lack of disturbance, perennial species are more capable of survival in isolated habitats, such as the studied burial mounds (Deák et al., 2021b).

As the CWM models showed that, clonal species were generally more typical on mounds compared to plain grasslands, it can be assumed that the ability for clonal growth and reproduction is an effective strategy for long-term persistence in isolated habitat patches (Heinken and Weber, 2013; Lindborg et al., 2014). Clonality can considerably increase the chance of species persistence in habitat islands by supporting on-spot

persistence and space occupancy in horizontal dimensions (Heinken and Weber, 2013). In highly isolated small populations, perennial clonal species show a considerably longer extinction time compared to non-clonal ones, because the latter rely less on sexual reproduction assuming functioning metapopulation connections at the landscape level (Lindborg et al., 2014; Deák et al., 2021b). Clonal species are also effective in occupying proper establishment gaps by clonal spread, where otherwise seedling growth would be limited, and they can also bridge unfavorable patches, which is a useful ability in abandoned sites characterized by thick litter layer (Heinken and Weber, 2013). On the contrary, in plain grassland communities, non-clonal herbaceous species with a short life cycle had an advantage for establishment. These gap-strategist species with low competitive ability can effectively establish in disturbed grasslands, and this can be especially important in the Bulgarian sites with sub-Mediterranean climatic influence where more gaps are created due to the interaction of human disturbance and long summer droughts (Palpurina et al., 2015; Marini et al., 2012; Kahmen and Poschlod, 2008).

The higher canopy at certain mound microsites compared to plain grasslands could be related to the isolated character of the mounds and the lack of management. As previous studies showed, habitat islands in agricultural landscapes often have tall vegetation capable of effective light capture that provides a competitive advantage for species (Marini et al., 2012; Lindborg et al., 2014). As Janečková et al. (2017) pointed out, tall stature can also support species survival in non-managed grasslands with a thick litter layer, which can physically suppress short-growing species. Another viewpoint was presented by Conti et al. (2017) from dune vegetation, who found that under arid conditions tall species can facilitate each other by improved shading and by an improved level of water uptake from deeper soil layers. The latter is supported by the fact that taller species generally have deeper roots and more effective hydraulic uplift (Janečková et al., 2017).

We found that plain grassland communities had a longer flowering period compared to north- and west-facing mound slopes. As Janečková et al. (2017) reported from semi-natural grasslands, managed sites were characterized by longer flowering periods than abandoned ones. In plain grasslands, where biomass is frequently removed, a prolonged flowering and seed maturation period can contribute to risk avoidance (Kahmen and Poschlod, 2008). Another reason for short flowering on mounds may be that in isolated habitats, pollinator populations are often small, which makes asexual (i.e. clonal) reproduction a more efficient tool for survival than sexual reproduction (Janečková et al., 2017; Olliff-Yang and Ackerly, 2020).

We found that plant communities on mounds (except the grass-dominated north-facing slopes) were characterized by heavier seeds compared to plain grasslands. As mounds are more affected by drought stress than plain grasslands, producing heavy seeds can be a successful stress avoidance strategy on them (Leishman et al., 2000). This theory is supported by the observed pattern that light-seeded plants were characteristic of the mildest north-facing slopes. Unlike plain grasslands where some level of disturbances reduced the amount of litter, increased litter accumulation on unmanaged mounds reduced the availability of establishment gaps and hindered the germination of light-seeded species. Compared to heavy ones, light seeds have a lower chance of penetrating the thick litter layer and reach the soil surface (Thomson et al., 2011). Also, seedlings of light seeds are more light-dependent than seedlings of heavy ones; thus, reduced amount of light under the litter layer might hamper their establishment. Plants with heavier seeds, and thus with larger amounts of nutrient supply, can outcompete light-seeded species under such conditions, therefore, they can be better recruiters (Ruprecht and Szabó, 2012; Loydi et al., 2013). The presence of heavy seeds on mounds can also be explained by the landscape context, as the lower dispersal ability and higher recruitment ability (both supporting local persistence) provided by heavy seeds can be a successful strategy in highly isolated habitats (Riba et al., 2009).

4.3. Regional differences

The CWM models revealed that the region had a much smaller effect on trait patterns in general than microsites. Of the nine traits studied, the CWMs of five traits were affected by the region and eight by the microsites. This result highlights that meso-scale environmental heterogeneity can override the effects of macroclimatic factors in shaping plant trait distributions. The differences in community weighted proportions and means and optimal trait values between the two study regions were likely mediated by the slightly different macroclimates. For instance, the cooler and moister macroclimate in Hungary allowed the formation of grasslands typical of forest-steppes with a higher proportion of grasses, while in Bulgaria, the sub-Mediterranean climatic influence favored more forb species (Wesche et al., 2016). This finding is in agreement with the results of Sudnik-Wójcikowska et al. (2011), who focused on mound vegetation along a large macroclimatic gradient in the steppe biome, and found that in the northern regions, where macroclimate favored forest-steppe vegetation, grass species with rhizomatous growth were dominant. The high proportion of species with large LDMC values in Hungary was probably due to the dominance of perennial grasses. Another regional difference was detected in the flowering phenology of the species in our study. We found an earlier and elongated flowering period in Bulgaria, which is probably due to the stronger sub-Mediterranean macroclimatic influences.

5. Conclusions

Our study revealed that isolated landscape features with high topographic complexity can considerably increase variability in plant functional traits and ecological strategies both at the site and landscape levels, supporting a unique combination of environmental variables different from those in the neighboring landscape. Our results also highlight that environmentally heterogeneous landscape features such as mounds not only preserve a subset of trait values that are otherwise typical of the landscape, but often expand the trait spectrum due to their special environmental conditions. Despite the large geographical scale of our study regions, trait composition patterns were mostly consistent and independent of the macroclimate. This is explained by the large relative differences in environmental parameters between the (studied) mounds and the neighboring plain landscapes.

We found that topographic complexity can lead to the formation of environmentally different microsites within the studied sites, selecting for different adaptive trait values. Consequently, the co-occurrence of mild and harsh environmental conditions can support the small-scale co-existence of species with contrasting trait values. Although there were only a few meters' distance between the different microsites, large contrasts were present in their environmental parameters leading to such differences in trait values that otherwise would be expected along larger altitudinal and/or latitudinal gradients. We found that the community-weighted means of species were not always the same as predicted by the abundance models, which means that in certain cases, the optimal strategy cannot be realized. Such scenarios can occur in cases when the legacy effects shaping the species pool overrode the actual trait:environment interactions. Furthermore, in the case of certain traits, environmental factors do not affect trait optima directly (as shown by the applied abundance models), but influence them indirectly through correlating traits. Finally, in some cases, traits can be influenced by other factors such as landscape characteristics or management.

We assume that the large trait variation on topographically complex landscape features makes the plant communities more resilient against the potential effects of ongoing climate change or other stochastic disturbances. This underlines the conservation importance of these landmarks, as they can be considered adaptive resources for the maintenance of natural vegetation, and can also be used as propagule sources for restoration projects aiming to use seed mixtures of locally adapted

species. As mounds and analogous landscape features, such as inselbergs, rock glades, dunes, and dolines, are widespread throughout the world, our results may be applied directly to other regions as well.

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CRediT authorship contribution statement

Balázs Deák: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Zoltán Botta-Dukát:** Writing – original draft, Formal analysis. **Bence Kovács:** Writing – original draft, Visualization, Formal analysis. **Iva Apostolova:** Writing – review & editing, Project administration, Methodology, Investigation. **Zoltán Bátori:** Writing – review & editing, Conceptualization. **András Kelemen:** Writing – review & editing, Investigation. **Katalin Lukács:** Writing – review & editing, Investigation. **Réka Kiss:** Writing – review & editing, Investigation. **Salza Palpurina:** Writing – review & editing, Investigation. **Desislava Sopotlieva:** Writing – review & editing, Investigation. **Orsolya Valkó:** Writing – original draft, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

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