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Patterns of fine-scale plant species richness in dry grasslands across the eastern Balkan Peninsula



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ABSTRACT

Fine-scale plant species richness varies across habitats, climatic and biogeographic regions, but the large-scale context of this variation is insufficiently explored. The patterns at the borders between biomes harbouring rich but different floras are of special interest. Dry grasslands of the eastern Balkan Peninsula, situated in the Eurasian forest-steppe zone and developed under Mediterranean influence, are a specific case of such biome transition. However, there are no studies assessing the patterns of fine-scale species richness and their underlying factors across the eastern Balkans. To explore these patterns, we sampled dry and semi-dry grasslands (phytosociological class *Festuco-Brometea*) across Bulgaria and SE Romania. In total, 172 vegetation plots of 10 × 10 m² were sampled, in which all vascular plant species were recorded, soil depth was measured, and soil samples were collected and analysed in a laboratory for pH and plant-available nutrients. Geographic coordinates were used to extract selected climatic variables. Regression trees and linear regressions were used to quantify the relationships between species richness and environmental variables. Climatic factors were identified as the main drivers of species richness: (1) Species richness was strongly positively correlated with the mean temperature of the coldest month: sub-Mediterranean areas of S and E Bulgaria, characterized by warmer winters, were more species-rich. (2) Outside the sub-Mediterranean areas, species richness strongly increased with annual precipitation, which was primarily controlled by altitude. (3) Bedrock type and soil pH also significantly affected dry grassland richness outside the sub-Mediterranean areas. These results suggest that fine-scale species richness of dry grasslands over large areas is driven by processes at the regional level, especially by the difference in the species pools of large regions, in our case the Continental and Mediterranean biogeographic regions. Local environmental factors are of secondary importance over broad extents, but their effect on fine-scale species richness increases within climatically and biogeographically homogeneous regions.

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1. Introduction

Eurasian temperate dry grasslands and steppes locally support the coexistence of a high number of vascular plant species (Kull and Zobel, 1991; Merunková et al., 2012; Wilson et al., 2012; Turtureanu et al., 2014). For several plot sizes smaller than 50 m², dry

grasslands of Central and Eastern Europe hold the world records for richness of vascular plant species (Wilson et al., 2012). However, not all dry grasslands are so extremely species-rich: their richness varies considerably, and knowing the relationships of this variation to environmental factors can help to predict and eventually avoid biodiversity loss if combined with wise nature conservation management.

Several observational and experimental studies have been carried out in temperate grasslands to explore the relationships between vascular plant species richness (henceforth 'species richness') and environmental factors at the fine scale, i.e. the scale of vegetation plots (Grace, 1999). Still, there is a lack of a general

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agreement as to which set of factors and which underlying mechanisms are the most important controls of species richness in dry grasslands. Local environmental factors, such as site conditions (e.g. soil properties, water availability, productivity; Löbel et al., 2006; Chytrý et al., 2007; Merunková and Chytrý, 2012; Axmanová et al., 2013), biotic interactions (e.g. competition for light; Kull and Zobel, 1991; Löbel et al., 2006), and management (Bonanomi et al., 2013; Turtureanu et al., 2014), were found to be important controls of fine-scale species richness in different temperate and (hemi-)boreal grasslands. However, the species richness–environment relationship is also influenced by historical and evolutionary processes at the regional level (large-scale migration, extinction, and speciation), which determine the size and composition of the species pool (the set of species potentially able to occur within a given environment; Zobel, 1992). Therefore, patterns of fine-scale species richness can differ among biogeographic and climatic regions. The more common a habitat was throughout history, the more time there was for speciation and large-scale migration, leading to the evolution of a larger species pool ('species pool effect'; Taylor et al., 1990; Ewald, 2003). In many cases, even in the very species-rich dry grasslands where biotic interactions are supposed to set a limit to the number of coexisting species (Bengtsson et al., 1994), fine-scale species richness patterns seem to be influenced by species pools (Pärtel et al., 1996).

Many dry grasslands in the eastern Balkan Peninsula are considered to be natural remnants of the forest-steppe that has persisted in the Lower Danube Basin and the Upper Thracian Plain since the Pleistocene, serving as an important migration corridor for grassland species between the Pontic, Anatolian, and Pannonian steppes (Magyari et al., 2008; Tonkov et al., 2011). Because of their distinct evolutionary and migrational history, diversity patterns reported from dry grasslands in other regions of Europe cannot be directly transferred to the eastern Balkan Peninsula. However, fine-scale species richness patterns of these dry grasslands have never been explored. Several previous studies gathered valuable data on the dry grassland vegetation of this area, but all of them dealt with smaller regions and focused on vegetation classification (e.g. Tzonev et al., 2006; Pedashenko et al., 2013; Sopotlieva and Apostolova, 2014; see Doniță et al., 2005 for references on the Romanian Dobrudzha) and the relationships between species composition (not richness) and the environment (Pedashenko et al., 2013).

Dry grasslands in the eastern Balkan Peninsula have developed under a unique combination of the Mediterranean and continental climatic influences. Therefore, they are a suitable model for assessing the relative effects of these two climate types on fine-scale species richness. Few previous studies have reported climate as a control of fine-scale species richness patterns in temperate grasslands (e.g. Adler and Levine, 2007; Chytrý et al., 2007; Reitalu et al., 2014; Turtureanu et al., 2014), possibly because most other research projects have not encompassed large climatic gradients or biogeographic transitions. In general, factors operating over broad scales such as macroclimate are considered to be poor predictors of fine-scale species richness (Pausas and Austin, 2001). Nevertheless, we hypothesize that, if filtering by local environmental factors is not too strong and the climatic gradient is large, climate may explain more variation in fine-scale species richness than local factors (as in Adler and Levine, 2007; Chytrý et al., 2007). Further, we hypothesize that climate should have a contrasting effect on the richness of different life forms, because Mediterranean and temperate grasslands considerably differ in the participation of different life forms (Cain, 1950).

Besides contrasting climates, the Balkan Peninsula is characterized by a very rich flora, especially in its Mediterranean and sub-

Mediterranean areas (Barthlott et al., 2005), with many endemics occurring mainly on calcareous substrates, and calcicole species that immigrated from diversity hotspots (such as Anatolia; Médail and Quézel, 1997) where calcareous soils prevail (Mücher et al., 2009). Thus, based on the species pool hypothesis (Taylor et al., 1990), we expect soil pH to be a strong correlate of fine-scale species richness in dry grasslands in the eastern Balkan Peninsula. Positive relationships with soil pH, explained by the prevalence of calcicole species in the existing species pools (Ewald, 2003), were found under continental, oceanic, and Mediterranean conditions across Eurasia (Pärtel, 2002; Schuster and Diekmann, 2003; Chytrý et al., 2003, 2007, 2010). However, the relationship between species richness and soil pH could be modified by the interaction with other factors such as climate (Löbel et al., 2006; Chytrý et al., 2007). The decrease in species richness on soils with very high pH might be due to the lower number of species adapted to the drought stress (low precipitation) associated with very basic substrates (Chytrý et al., 2007). Thus, we consider dry grasslands in the eastern Balkan Peninsula, occurring on different bedrock types and distributed over a large precipitation gradient, to be a good model for evaluating the species richness–soil pH relationship in different biogeographic contexts.

To explore fine-scale species richness patterns in dry grasslands in the eastern Balkan Peninsula, we sampled vegetation plots along a broad spatial extent and long climatic and altitudinal gradients, and assessed the relative importance of these and other measured variables on species richness. Our main questions were:

1. Is there a geographical pattern in fine-scale species richness in the eastern Balkan Peninsula coinciding with the transition between the subcontinental and the sub-Mediterranean biogeographic regions?
2. Which factors are the most important drivers of the variation in fine-scale species richness of dry grasslands across the eastern Balkan Peninsula: soil properties, climate, or management?
3. Do species of specific life forms or biogeographic histories (e.g. sub-Mediterranean vs. subcontinental) contribute differently to species richness patterns?

2. Materials and methods

2.1. Study area

The study area is located in the eastern Balkan Peninsula (41.87°–45.15° N, 22.59°–29.08° E), and includes two major lowlands separated by the Stara planina (Balkan) mountain range: the Dobrudzha (Dobrogea) plateau and the Lower Danube basin in the north, and the Upper Thracian plain in the south. Dry grasslands were sampled from the Black Sea coast up to the mid-altitudes, including the karstic low mountains in western Bulgaria, the Stara planina, and the Strandzha-Sakar massif (14–1211 m a.s.l., Fig. 1). Limestones and other calcareous rocks of different ages and origins prevail in the study area. An exception is the Upper Thracian plain, with predominating Quaternary alluvial-diluvial sediments and Neogene continental deposits, and the Strandzha-Sakar massif, with predominating volcanic, metamorphic, and granitoid rocks. But even so, dry grasslands in the latter regions are mainly associated with outcrops of carbonate bedrocks, such as crystalline limestone. In the northern Dobrudzha and the Lower Danube basin, Quaternary loess deposits form a 20–60 km broad band parallel to the river (Kopralev, 2002).

The climate is temperate-continental (henceforth 'subcontinental') to the north of the Stara planina and in western Bulgaria, continental-Mediterranean (henceforth 'sub-Mediterranean') in

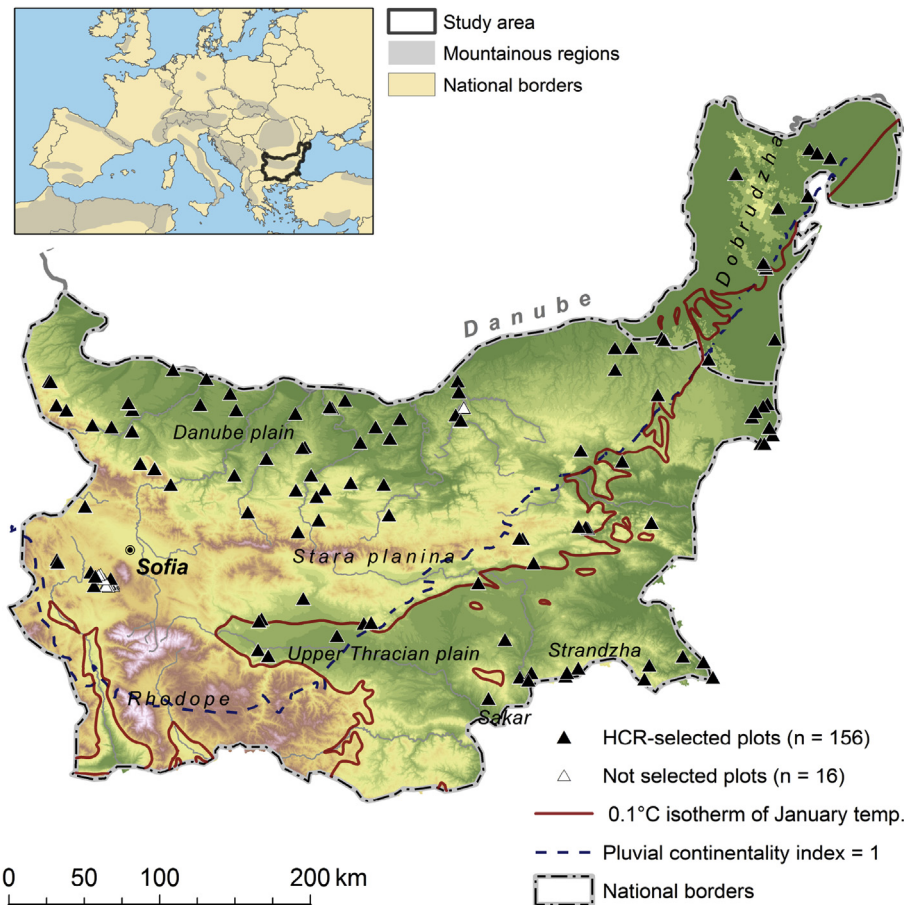


Fig. 1. Study area (Bulgaria and Romania south of the Danube river) with locations of vegetation plots ($n = 172$; not all plot symbols are visible due to the coarse scale). The solid line corresponds to the 0.1°C isotherm of January mean temperature. January temperatures increase to the east and south of the isotherm. The dashed line corresponds to the pluvial continentality index (I_{cont}) equal to 1; I_{cont} increases to the north and west, and with altitude.

the Strandzha-Sakar massif and along the Black Sea coast, and transitional in the Thracian plain, the eastern Stara planina, and the easternmost Dobrudzha (Koprarev, 2002). Continentality decreases and mediterraneity (i.e. the length and severity of summer drought and benign winter temperatures) increases from north to south and with proximity to the Black Sea, and both decrease with altitude (see Fig. 1). Precipitation decreases with continentality and increases with altitude (Koprarev, 2002). The precipitation maximum is in summer in the subcontinental areas, in autumn in the sub-Mediterranean areas, and in spring and autumn in the transitional areas. The precipitation minimum is in winter in the subcontinental areas, in summer in the sub-Mediterranean areas, and in autumn and summer in the transitional areas. A persistent snow cover is regularly formed only in the subcontinental areas, whereas in the sub-Mediterranean areas snow cover lasts normally for less than 20 days, and usually does not form every year (Koprarev, 2002). Temperature decreases with altitude, with July being the warmest month, and January the coldest. Summer temperatures vary little throughout the study area, whereas winters get colder and longer with increasing continentality.

Forest-steppe is the natural vegetation on chernozems, humus-carbonate and other soils in the Dobrudzha and along the Danube. Elsewhere in the lowlands of the subcontinental and transitional areas, i.e. up to 700 m a.s.l., the potential vegetation would be thermophilous summer-green oak forests with *Quercus robur* subsp. *robur* (in the river valleys), *Quercus robur* subsp. *pedunculiflora*, *Quercus cerris*, *Quercus frainetto*, *Quercus pubescens*, and *Carpinus orientalis* (Koprarev, 2002). Most of the natural vegetation

(steppe, forest-steppe, and thermophilous forests) in these lowlands was turned into arable land. Nowadays, natural and secondary grasslands (pastures) are restricted mainly to steep slopes or stony soils unsuitable for agriculture. In many places where the forests were cleared, secondary scrub with *Paliurus spina-christi*, *Syringa vulgaris*, *Prunus spinosa*, and *Crataegus monogyna* develops after the cessation of agricultural management, but in a few places, meadow-steppes remain at regularly mown sites. In the mountainous areas, above 600 m, mesophilous oak-hornbeam forests with *Quercus dalechampii* and *Carpinus betulus* predominate, being replaced at altitudes above 1300 m by beech forests with *Fagus sylvatica*, except in the Strandzha, where beech forests of *Fagus orientalis* occupy humid and cool valleys below the oak (mostly *Quercus polycarpa*) forest belt. Within the oak-hornbeam and beech forest belts, dry grasslands are considered natural only on steep south-facing slopes with shallow soils over carbonates in the continental climate of western Bulgaria, where *Festuca* spp. and *Bromus* spp. co-dominate, accompanied by many steppe specialists and regional endemic species. Grasslands dominated by *Chrysopogon gryllus* or *Bothriochloa ischaemum*, with many generalist species, are thought to be of a secondary origin, developed at sites of cleared forests.

2.2. Field survey and data compilation

We wanted to cover the maximum ecological variation of dry and semi-dry grasslands (phytosociological class *Festuco-Brometea*) across a large climatic gradient and different bedrock types.

Therefore, study sites were selected based on the literature and local expert suggestions with the aim of covering all major types of dry grasslands (petrophytic steppes, loess steppes, and semi-dry grasslands). The intensity of sampling in each climatic area (see Section 2.1.) reflected its size, environmental heterogeneity, and the range of different dry grassland types. Abandoned, recently strongly disturbed, or intensively grazed grasslands were avoided. However, as most of the loess steppes we visited were burnt in the previous year, we sampled some post-fire sites there.

At each dry grassland site, $10 \times 10 \text{ m}^2$ plots were sampled in the centre of physiognomically uniform vegetation stands with homogeneous abiotic conditions. Dry grasslands were selected based on the presence of species characteristic of the phytosociological class *Festuco-Brometea*. If a site was considerably topographically or edaphically variable, and this was reflected in the grassland structure and composition, we recorded more than one vegetation plot there. In order to sample dry grasslands at the peak of their growing season, field work was carried out each year from the end of May till the end of June, and in the first week of July at higher altitudes.

A total of 172 vegetation plots were sampled in 2010–2012. Within each 100-m^2 plot, all vascular plant species were recorded (root presence). In addition to total species richness (the number of all vascular plants per 100-m^2 plot), we also counted the number of species sharing the same life form: annuals, biennials, perennial

hemicryptophytes (henceforth ‘perennials’), geophytes, suffruticose chamaephytes (henceforth ‘suffruticose’), shrubs, and trees. Bunch grasses such as *Stipa capillata* were regarded as perennials, and rhizomatous grasses such as *Elymus repens* as geophytes. Regarding its main centre of origin and distribution in relation to the study area, each species was classified into one of eight geoelement categories based on Assyov and Petrova (2012): Balkan endemic and sub-endemic (main distribution area on the Balkan Peninsula, or the mountains of SE Europe), sub-Mediterranean (main distribution area to the south of the study area), sub-Pontic (main distribution in the steppes north of the Black Sea), subcontinental (distributed mainly in continental Eurasia), European (centre of distribution in temperate Europe), sub-boreal, cosmopolitan, and adventive.

For each plot, geographical coordinates and altitude were measured with a GPS device. Slope and aspect were measured by a clinometer and compass. The cover of bare rock and gravel was estimated visually in percentages. Soil depth was measured within each plot at 10 systematically placed points with a 30-cm metal rod. The mean soil depth was considered as a surrogate for moisture availability, and the coefficient of variation (CV) of soil depth was calculated as a measure of soil depth heterogeneity. In the analyses, soils deeper than 30 cm were considered as 30 cm deep. Soil samples were taken from each plot at five points (in the centre and

Table 1

Descriptive statistics of the variables used in the analysis based on a dataset of 156 HCR-selected plots. For categorical and ordinal variables, the number of cases (plots) for each class is given in brackets. Species richness is given as numbers of vascular plant species per 100 m^2 . Variables used for the regression tree are in bold.

	Variable name	Mean \pm SD	Minimum	Maximum	
Topography	Altitude [m]	306 \pm 263	14	1033	
	Inclination [°]	9.8 \pm 6.9	0	34	
	Radiation (topographic index)	0.92 \pm 0.06	0.65	1.01	
	Heat (topographic index)	0.92 \pm 0.07	0.68	1.02	
Soil	Bedrock type: carbonate (103), loess (26), other sedimentary rock (12), siliceous rock (11), volcanite (4)				
	Soil depth [cm] ^a	18.0 \pm 9.1	3	30	
	Soil depth CV [%] ^a	27.4 \pm 21.0	0	101	
	Cover of gravel and rock [%]	9.7 \pm 15.0	0	70	
	Soil pH (H ₂ O)	7.3 \pm 0.9	4.9	8.5	
	Soil pH CV [%] ^b	2.1 \pm 1.9	0.1	12.0	
	Soil Ca [mg/kg dry soil]	10207 \pm 8277	724	39041	
	Soil K [mg/kg dry soil]	279 \pm 123	85	705	
	Soil P [mg/kg dry soil]	5.6 \pm 8.5	0.0	71.0	
	Soil C _{org} [g/kg dry soil] ^a	65.1 \pm 25.7	14.4	175.7	
	Soil N_{tot} [g/kg dry soil]	3.4 \pm 1.6	1.1	9.6	
	Soil C/N ratio	19.8 \pm 5.0	11.1	41.9	
	Climate	Mean annual temperature [°C]	10.8 \pm 1.2	7.1	13.5
		Mean January temperature [°C]	−0.6 \pm 1.4	−3.7	3.8
Mean July temperature [°C]		21.2 \pm 1.5	16.6	23.0	
Annual temperature range [°C]		21.8 \pm 1.3	18.9	24.1	
Annual precipitation sum [mm]		578 \pm 69	403	711	
Spring precipitation sum [mm]		179 \pm 31	116	241	
Summer precipitation sum [mm]		132 \pm 16	98	172	
Autumn precipitation sum [mm]		147 \pm 25	92	218	
Winter precipitation sum [mm]		121 \pm 19	85	176	
Pluvial continentality index [$p_{III-VIII}/p_{IX-II}$] ^b		1.18 \pm 0.19	0.61	1.41	
Length of cold period [no. of months with $t < 0^\circ\text{C}$] ^b		0.9 \pm 0.8	0	3	
Length of summer drought [no. of months with $I_{DM} < 20$] ^b		2.7 \pm 1.6	0	7	
Management type: abandoned (13), burned (14), grazed (120), mown (9)					
Species richness	All species	52.2 \pm 17.6	14	107	
	Annuals	16.1 \pm 12.1	0	54	
	Biennials	2.9 \pm 1.8	0	8	
	Geophytes	3.0 \pm 2.0	0	9	
	Perennials	24.8 \pm 8.4	10	52	
	Balkan endemic and sub-endemic	2.7 \pm 2.2	0	12	
	European species	2.2 \pm 1.9	0	12	
	Subcontinental species	14.6 \pm 6.0	3	31	
	Sub-Mediterranean species	23.2 \pm 12.0	2	68	
	Sub-pontic species	7.3 \pm 4.0	0	19	

^a Soils deeper than 30 cm were arbitrarily given a value of 30 cm.

^b Abbreviations: CV – coefficient of variation; I_{DM} – monthly de Martonne index of aridity: drought episodes could be observed within a month in which $I_{DM} < 30$, while $I_{DM} < 20$ indicates a month with a severe drought; p , t – mean monthly precipitation and temperature.

the four corners) at a depth of 3–10 cm, and subsequently air-dried. Soil pH was measured separately for each soil sample after an 8–10 h extraction in distilled water (2:5 soil:water weight ratio). The mean value of soil pH per plot and its coefficient of variation (CV) were used for analyses, the latter being considered as a measure of soil pH heterogeneity. Nutrients were measured in a mixture of the five soil samples following standard protocols. Total nitrogen (N_{tot}) was determined using the Kjeldahl method. Plant-available phosphorus (P), potassium (K), and calcium (Ca) were extracted by the Mehlich III method. The P content was determined by spectrophotometer (Spekol 210, Carl Zeiss, Jena, Germany), and K and Ca contents by atomic absorption spectrophotometer (AAS 933 Plus, GBC Scientific Equipment, Melbourne, Australia). Total organic carbon (C_{org}) was determined by loss on ignition at 550°C for 16 h. The C/N ratio was calculated as $C_{\text{org}}/N_{\text{tot}}$.

Management of the dry grasslands was assessed in the field, and assigned to the following four categories: grazed (parts of plants missing, animal dung present, livestock present at the site or nearby), mown (no or little litter accumulated), burned (charcoal present, no litter), and abandoned (litter accumulated).

Radiation and heat-load indices (McCune and Keon, 2002, eq. 3 in their Table 2) were calculated based on latitude, slope, and aspect. The radiation index has the highest value of 1 on the steep south-facing slopes and the lowest value of 0 on the steep north-facing slopes; for the heat load index, the highest and lowest values are shifted to SW- and NE-facing slopes, respectively.

Bedrock types were extracted from the geological maps of Bulgaria and Romania (Anonymous, 1968; Cheshitev et al., 1989) and cross-checked in the field. The categories given in the maps were combined into five broad categories: carbonate (including crystalline limestone and flysch), loess, other sedimentary rock (sandstone, siltstone, and conglomerate), siliceous rock (e.g. granitoid, rhyolite, and quartzite schist), and volcanite (basalt and andesitobasalt).

The mean monthly precipitation sum and mean monthly air temperature were extracted from the WorldClim database at a spatial resolution of ca. 1 km (Hijmans et al., 2005; www.worldclim.com). In order to approximate the degree of continentality or mediterraneity of the climate, the pluvial continentality index, I_{cont} , was calculated as the precipitation of the six warmest months (March to August) divided by the precipitation of the six coldest months (September to February; Tuhkanen, 1980). In the study area, September is warmer than March, but we included it in the colder half-year, since higher precipitation in September is an indication of sub-Mediterranean conditions. Thus, $I_{\text{cont}} > 1$ indicates continental type, and $I_{\text{cont}} < 1$ Mediterranean type of precipitation seasonality. Additionally, we calculated (1) the length of the cold period, expressed as the number of months with mean monthly temperature below 0°C; and (2) the length of the drought period, as the number of months with $I_{\text{DM}} < 20$. I_{DM} is the de Martonne index of aridity ($\text{mm}/^{\circ}\text{C}$), calculated for each month as $12p/(t + 10)$, where p and t are the monthly mean precipitation (mm) and air temperature ($^{\circ}\text{C}$). Within the study area, drought episodes could be observed within a month in which I_{DM} is lower than 30; value below 20 indicates severe drought (Tuhkanen, 1980).

A summary of the descriptive statistics for the variables used in the analysis is provided in Table 1.

2.3. Data analysis

Besides environmental factors and biogeographic context, grassland species richness is also influenced by management. In particular, abandoned sites tend to be species-poorer than regularly managed sites. As we were able to obtain only rough data on the recent management of all sampled sites, we decided to also analyse

a subset of those plots that were the species-richest in each area, removing those that became species-poor due to possible suboptimal management. We overlaid the study area by a 10×10 km grid, and within each cell we selected the plot with the highest richness. To reduce the effects of oversampled regions, we made another selection using grid cells of 40×50 km cells. In each grid cell that contained at least five plots, we selected up to five plots representing various species compositions following the heterogeneity-constrained random (HCR) resampling procedure (Lengyel et al., 2011) using the JUICE program (Tichý, 2002). We made parallel analyses with the whole dataset ($n = 172$), the richest-plot selection ($n = 95$), and the HCR-resampled plots ($n = 156$). As the results were almost identical between the full dataset and its subsets, we describe and discuss here only the results from the HCR-resampled dataset.

We chose regression trees as an effective exploratory tool for describing the relationships between the response and explanatory variables (De'ath and Fabricius, 2000). To estimate the prediction error of the trees, we used the 10-fold cross-validation procedure. We ran 100 series of the 10-fold cross-validation, and from each series selected the best tree size according to the minimum error rule. From the distribution of the 100 'best' tree sizes, the most frequently occurring size was chosen (De'ath and Fabricius, 2000). For each split of the tree, we also selected the first five surrogate variables, i.e. those that allocate the most cases similarly to the allocation by the primary splitter. As a criterion for including a variable as a surrogate, we required its association value to the main splitter to be higher than 0.7. As the trees may be unstable if there is a strong correlation among the explanatory variables, collinearity between continuous predictor variables was checked by pairwise Pearson correlations. Two predictors were considered to be collinear when Pearson's $|r| > 0.70$. In such cases, we kept only the predictors that we considered more ecologically meaningful for explaining richness. Details on the correlations between explanatory variables are given in Appendix A.

Table 2

Ranked values of the relative importance of explanatory variables based on the optimal regression tree for species richness. The relative importance of a given variable is calculated as the average of the overall improvement of the model (measured as a deviance explained) by this variable stemming both from the variable's role as a primary splitter of a node, and its role as a surrogate to any of the primary splitters. Note that due to rounding the sum of relative importances across individual variables or groups slightly exceeds 100.

Category	Individual variables	Relative importance (%)
Climate	January temperature	37.1
Topography	Altitude	12.0
Climate	Annual temperature range	11.4
Soil	Bedrock type	8.8
Climate	July temperature	7.4
Soil	Soil pH	4.7
Climate	Annual precipitation	4.6
Soil	C/N ratio	3.2
Soil	Soil Ca	2.5
Soil	Soil K	2.4
Soil	Soil pH CV	1.6
Topography	Radiation	1.6
Soil	Soil N	1.4
Soil	Cover of rock and gravel	0.9
Management	Management	0.5
	By group	
	Climate	60.5
	Soil	25.5
	Topography	13.6
	Management	0.5
	Total	100.0

Linear models were built separately for the relationship involving the richness of all vascular plants and soil pH and other selected environmental variables. Where a unimodal response was theoretically expected, we tested for the significance of the negative quadratic term. The response and explanatory variables were analysed on their original, non-transformed, scale.

To assess the strength of the relationship between the richness of separate life forms/geoelements and environmental variables, Spearman's rank correlation coefficient was used. The Monte-Carlo permutation test was used with 1000 permutations to test the significance of the relationship.

The statistical analyses were carried out in the R environment (R Core Team, 2012) using the package 'vegan' (Oksanen et al., 2013) for the linear regression models, and 'rpart' (Therneau and Atkinson, 2012) and 'partykit' (Hothorn and Zeileis, 2013) for building and visualizing the regression trees.

3. Results

Total species richness varied between 14 and 107 vascular plant species per 100 m² (both for the whole dataset and the subset of HCR-selected plots). An overview of species richness by life forms and geoelements is given in Appendix B.

Climatic variables explained most of the variation in total species of dry grasslands in the study area, followed by soil properties

and topography (Table 2). January temperature was the strongest predictor, with its split reducing the variation in species richness by more than a third (Fig. 2). Species richness increased strongly with January temperature, reaching the highest values at sites with positive January means (Fig. 2, Appendix C). Within the study area, January temperature is strongly negatively correlated with the length of the cold period and the pluvial continentality index (see Appendix A). Thus, positive January temperature indicates mild and precipitation-rich winter, typical of the sub-Mediterranean climate.

The most species-poor dry grasslands were at sites with negative January temperatures (i.e. outside the sub-Mediterranean areas) on soils over loess or siliceous bedrock, characterized by a low Ca and N content and, in a few cases, by burning. These grasslands are distributed in the subcontinental plains as indicated by the low altitude and low precipitation. Elsewhere in the sub-continental lowlands, where the precipitation was higher, or the bedrock was calcium-richer, dry grasslands were species-richer. In general, outside the sub-Mediterranean areas, richness tended to increase with both altitude and precipitation (surrogates in the bedrock and annual temperature range splits), with dry grasslands in mountainous and precipitation-rich areas being comparably rich as some rocky steppes in the sub-Mediterranean areas. An exception to this pattern were the dry grasslands on very basic soil (pH > 8), which were more species-poor than other dry grasslands at higher altitudes.

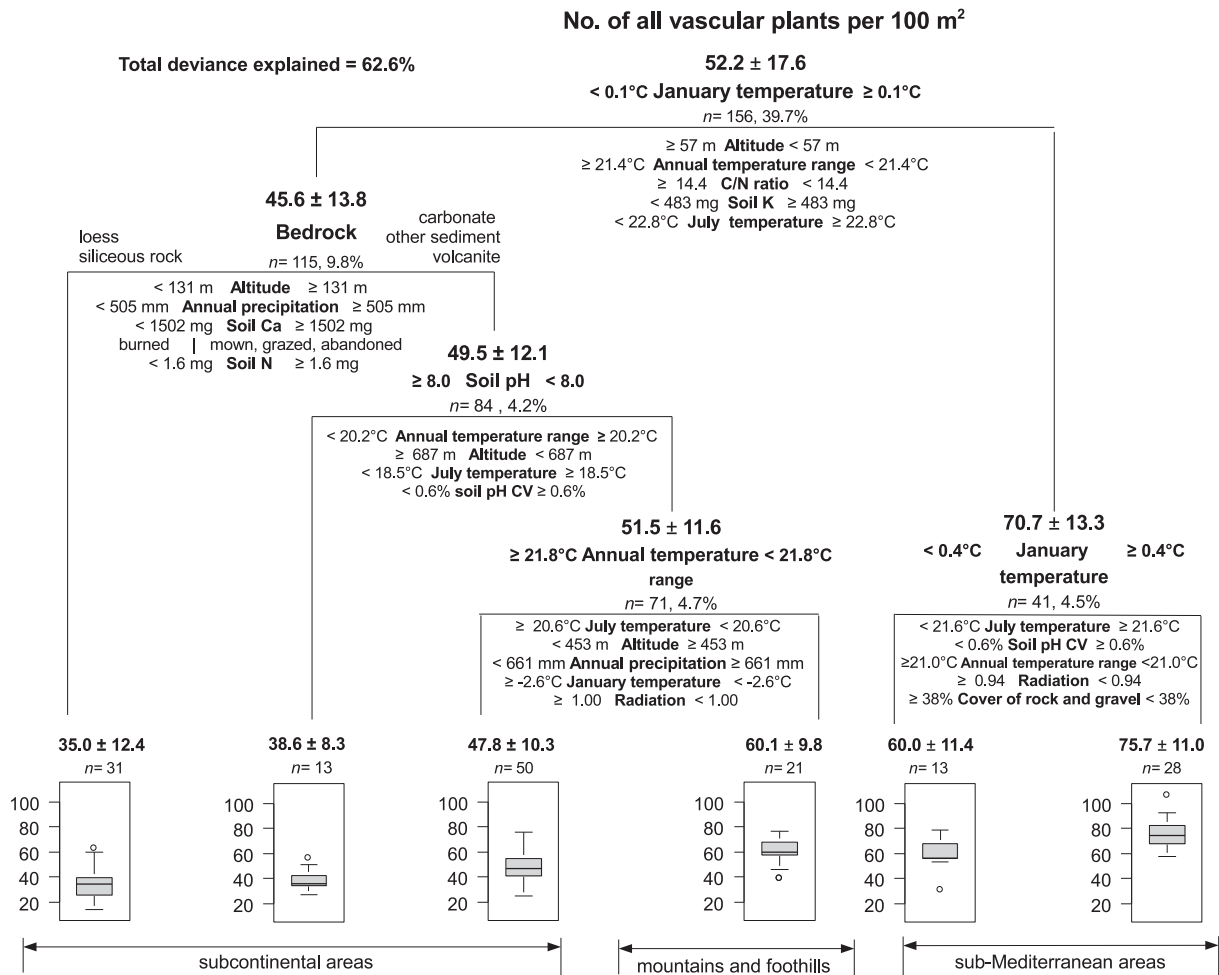


Fig. 2. The optimal regression tree for the total species richness cross-validated using the minimum rule. Numbers at each split indicate the mean number of vascular plant species per 100m² ± standard deviation. The primary splitter variable is in bold, surrogates (variables that allocate most cases to the same group as the primary splitter) are in smaller letters below the primary splitter. Number of plots assigned to each node, and the deviance explained are in smaller font below the main splitter name.

After taking the climate effect into account (Fig. 3), a decrease in species richness with soil pH was observed in the foothills and the mountains. In the sub-Mediterranean areas and subcontinental plains no pattern was detected. Soil depth had contrasting effects on species richness in different regions. In the foothills and the mountains, species richness increased with soil depth; in the sub-Mediterranean areas, the relationship was unimodal (Fig. 3).

Within the study area, higher species richness was associated with a strong increase in the number of sub-Mediterranean species, most of them being annuals (Fig. 4). The total species richness was not much related to the number of perennials, while the numbers of geophytes, biennials, and suffruticose species per plot were low and did not vary much. Most of the plots contained no trees or shrubs (Fig. 4, Appendix B). The positive relationship between total species richness and January temperature arose mainly from the strong increase in the richness of sub-Mediterranean (and annual) species with January temperature (Table 3, Appendix C). The numbers of sub-Mediterranean and annual species were moderately to strongly negatively correlated with the length of the cold period, the pluvial continentality index, and the annual temperature range (i.e. their richness increased strongly with mediterraneity and decreased with continentality), and were weakly correlated with topographic or soil factors (Table 3). The other most abundant life form, perennials, showed no strong relationship with

either local factors or climate. Within a plot, subcontinental and sub-Pontic species were on average the second and third most represented geoelements after sub-Mediterranean species (Appendix B). Similarly to sub-Mediterranean species, the number of sub-Pontic species increased strongly with the mediterraneity of the climate (i.e. decreased with the length of the cold period and increased with January temperature, characteristic for the mild winter along the coast), but was also strongly positively correlated with soil factors such as pH and Ca content (Table 3). The number of subcontinental species was not correlated with climate, but was strongly correlated with soil depth (positively) and with the cover of rock and gravel (negatively).

4. Discussion

4.1. Species richness changes at the transition of two biogeographic regions: Macroclimatic control or migration constraints?

Our data suggest that climate is the most important driver of fine-scale species richness patterns across dry grasslands in the eastern Balkan Peninsula. Based on the strong decrease of sub-Mediterranean species with decreasing January temperature (Appendix C) and the absence of many of them from the subcontinental areas (Appendix E), we suggest that the zero January

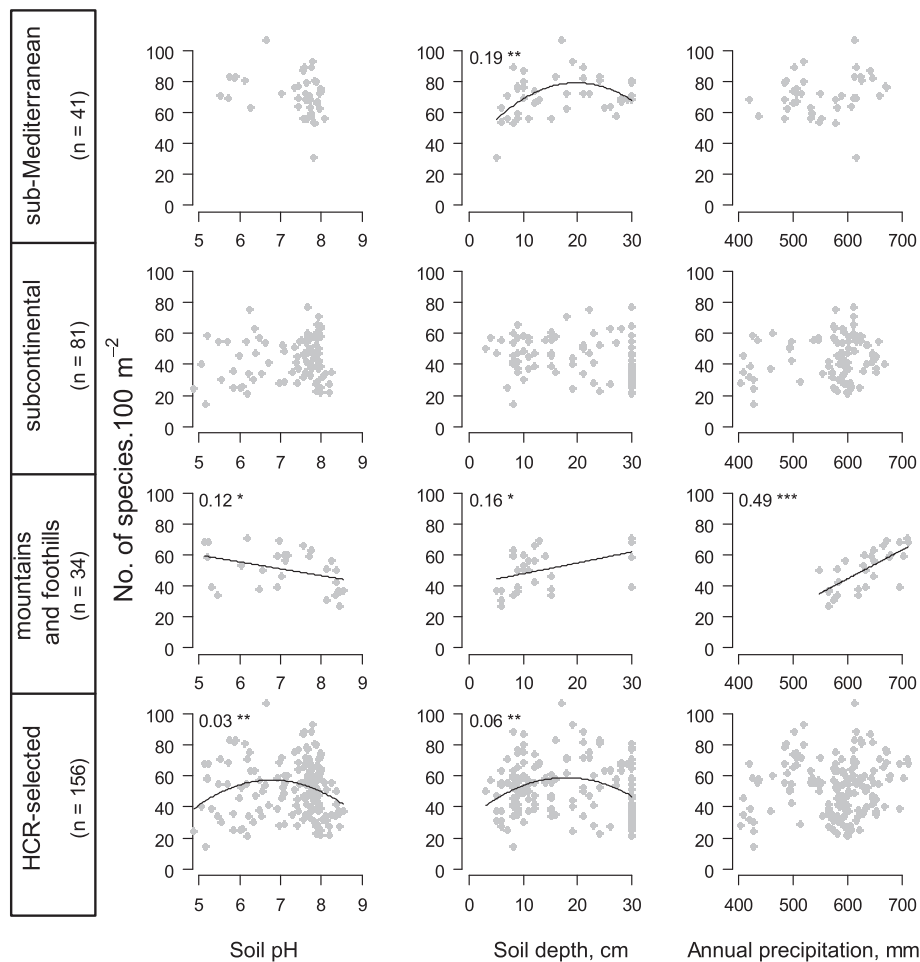


Fig. 3. The relationships between total species richness and annual precipitation, soil depth, and soil pH within climatic areas. Climatic areas were delineated based on the January temperature in the first split of the optimal regression tree, and the annual temperature range in the last split (Fig. 2) as follows: sub-Mediterranean areas (January temperature $\geq 0.1^\circ\text{C}$); subcontinental areas (January temperature $< 0.1^\circ\text{C}$, and the annual temperature range $\geq 21.8^\circ\text{C}$); mountains and foothills (January temperature $< 0.1^\circ\text{C}$, and annual temperature range $< 21.8^\circ\text{C}$). Only significant trends were fitted. Significance levels: ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$. The number before the significance level is adjusted R^2 .

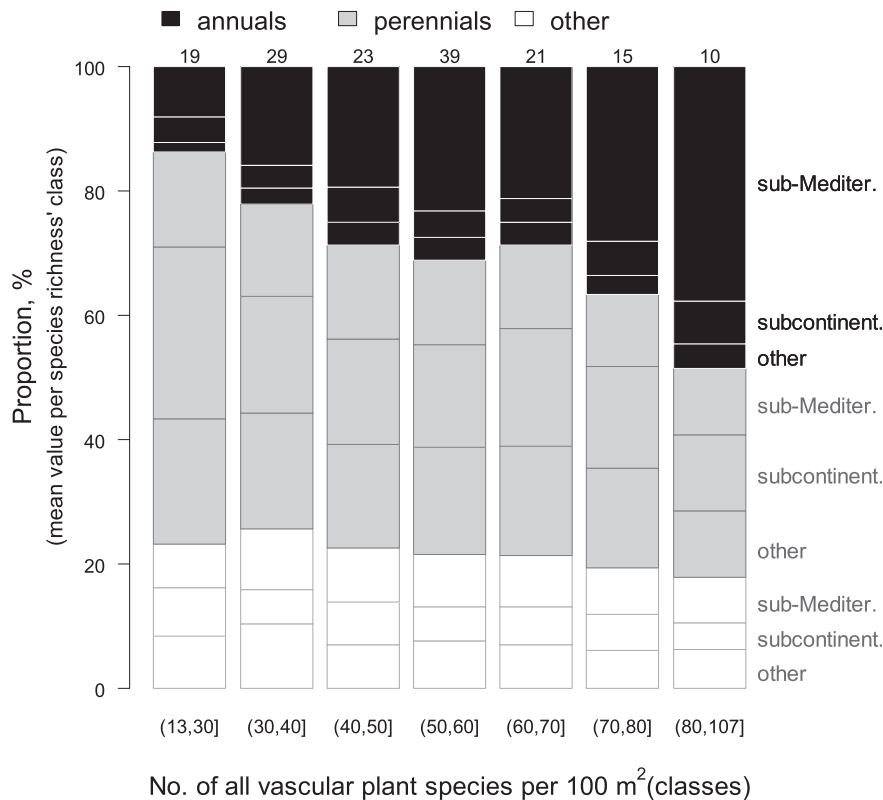


Fig. 4. Proportion of life forms and geoelements in plots with different species richness ($n = 156$). The range of species richness within each class is in brackets below the bars (squared bracket – value in the interval, rounded bracket – value not in the interval). The number of plots within a given class is above the corresponding bar. Shading intensities and sections of the bars correspond to the combinations of life forms and geoelements.

isotherm creates the main border between the richer (sub-Mediterranean) and poorer (subcontinental) dry grasslands by limiting mainly the distribution of sub-Mediterranean species. The limiting effect of January temperature on species distribution can be a direct one – due to the physiological effect of frost (Normand and Treier, 2009). However, it is difficult to separate the effect of physiological constraints from dispersal limitation in determining species ranges, especially in mountainous areas such as the Balkan Peninsula. It is possible that the mountain ranges in the study area influenced species migrations indirectly through their effect on climate patterns, rather than directly as migration barriers. The Stara planina and Rhodopes Mountains did not stop the northward migration for many sub-Mediterranean species, which were able to migrate northwards along the Black Sea coast, probably aided by milder winters near the sea. Additionally, the stability of climatic conditions (Davis et al., 2003) and the continuous persistence of forest-steppe in the area (Magyari et al., 2008; Tonkov et al., 2011) may have facilitated continuous migrations between the subcontinental and sub-Mediterranean areas. Still, given the large beta-diversity of the dry grasslands across the study area (not shown), large-scale dispersal limitation at the sub-Mediterranean/subcontinental boundary is not improbable. Dispersal limitation might explain the locally lower species richness in the dry grasslands of the western Bulgarian limestone mountains, as their connection with the sub-Mediterranean areas is not as open as in the mountains near the Black Sea.

Although some studies suggest that high temperature and drought can limit species ranges in some areas (Cahill et al., 2014), the greater summer aridity, associated with the sub-Mediterranean climate, appeared as a limiting factor only for the number of Balkan endemics and sub-endemics, probably because most of these species originated in the precipitation-richer and colder mountainous

areas. Otherwise, the length of the summer drought was not associated with any decline in species richness of particular life forms or geoelements in the study area. An explanation could be that the summer drought period is an intrinsic attribute of the dry grassland habitat, with most species of continental origin reaching the peak of their biomass production before the summer drought period. It is interesting to note that many typical dry grassland species of continental origin, e.g. *Filipendula vulgaris*, *Salvia nemorosa* and *Salvia nutans*, extend their range into sub-Mediterranean areas but not further south to the typical Mediterranean areas.

Although the species pool effects seem to have a dominant influence on species richness patterns within our study system, a difference in the species pool may not be the full explanation why dry grasslands in the sub-Mediterranean areas are species-richer locally, in small plots. Local biotic processes such as asymmetric competition can reduce diversity (Kull and Zobel, 1991; Löbel et al., 2006). Theoretically, competitively superior perennial species might outcompete most of the sub-Mediterranean species that are annuals, but this does not happen in the eastern Balkan sub-Mediterranean dry grasslands. An explanation can be that earlier growth and seed production allow annuals and geophytes to avoid competition with perennials (Shmida and Wilson, 1985), which produce most of their biomass later in the growing season. In addition, grasslands at sites with long summer droughts in areas with stronger Mediterranean climatic features provide more regeneration gaps for germination in autumn-winter (Ortega et al., 1997) favourable for the establishment of annuals (Huber, 1994). This is also in agreement with the fact that many species, especially annuals, respond positively to dry conditions in the previous year (Adler and Levine, 2007). Thus, beside frost, the observed decrease in species richness (especially of annuals) with continentality and altitude in the study area can be explained by a reduced availability

Table 3
Spearman rank correlations between fine-scale species richness (number of species per 100 m²) of the entire dry grassland community or respective life forms/geoelements, and environmental variables (HCR-selected plots, $n = 156$). Significance levels:***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns – not significant. Significance was tested using Monte Carlo test with 1000 permutations. Strong relationships ($r \geq |0.5|$) are in bold. ann – annual, bie – biennial, geo – geophyte, per – perennial, suffr – suffruticose, balk – Balkan (sub-)endemic; pont – sub-Pontic, cont – continental, med – sub-Mediterranean, eur – European, bor - subboreal.

		All species	Life forms							Geoelements						
			ann	bie	geo	per	suffr	shrub	tree	balk	pont	cont	med	eur	bor	
Topography	Altitude	ns	ns	-0.28**	ns	ns	0.14*	ns	0.15*	0.45***	-0.28***	-0.19**	ns	ns	0.19**	
	Inclination	ns	ns	ns	-0.20**	ns	0.26***	ns	ns	ns	ns	-0.28***	ns	ns	ns	
	Radiation	-0.15*	ns	-0.14*	-0.21**	-0.23***	ns	ns	ns	ns	ns	-0.18**	-0.14*	ns	-0.15*	ns
Soil	Heat	ns	ns	-0.15*	-0.18*	-0.15*	ns	ns	ns	ns	ns	ns	ns	ns	-0.17*	ns
	Soil depth	ns	-0.22**	ns	0.14*	0.15*	-0.37***	ns	0.13*	-0.39***	-0.30**	0.40***	-0.31***	0.39***	0.27**	
	Cover of gravel and rock	ns	0.19**	ns	-0.14*	-0.14*	0.33***	ns	ns	0.32***	0.27***	-0.42***	0.32***	-0.37***	-0.25**	
	Soil pH (H ₂ O)	ns	ns	ns	-0.20*	ns	0.51***	ns	-0.21**	0.18*	0.40***	-0.20**	ns	-0.32***	-0.37***	
	Soil pH CV ^a	ns	ns	ns	ns	ns	-0.36***	ns	ns	-0.21**	-0.49***	0.17*	ns	0.23**	0.16*	
	Soil Ca	0.19*	0.14*	0.24***	ns	ns	0.44***	ns	ns	0.17*	0.53***	ns	0.21**	-0.26**	-0.31***	
	Soil K	ns	ns	ns	ns	ns	0.23***	ns	ns	ns	0.18*	ns	0.17*	-0.15*	-0.24***	
	Soil P	-0.15*	ns	ns	ns	-0.14*	-0.24***	ns	ns	-0.24**	ns	ns	-0.21**	ns	ns	
	Soil C _{org}	0.22**	ns	ns	0.19*	0.15*	0.22**	ns	ns	0.33***	0.19*	ns	0.24**	ns	ns	
	Soil N _{tot}	0.20**	ns	ns	0.22**	0.15*	0.25**	ns	ns	0.37***	0.24**	ns	0.23**	ns	ns	
	Soil C/N ratio	ns	ns	ns	-0.21**	ns	ns	ns	ns	-0.19**	-0.15*	0.15*	ns	ns	ns	
	Climate	Mean annual temperature	0.22**	0.35***	0.28***	0.18*	ns	-0.16*	ns	ns	-0.19**	0.25**	0.14*	0.26***	ns	ns
		Mean January temperature	0.47***	0.51***	0.34***	0.29***	0.16*	ns	0.13*	ns	ns	0.46***	0.21**	0.50***	ns	ns
Mean July temperature		ns	0.15*	0.16*	ns	-0.24***	-0.21**	-0.26**	ns	-0.34***	ns	ns	ns	-0.13*	-0.13*	
Annual temperature range		-0.52***	-0.27***	ns	-0.39***	-0.39***	-0.25**	-0.31***	-0.14*	-0.49***	-0.27***	ns	-0.48***	ns	-0.14*	
Annual precipitation sum		ns	ns	-0.18*	ns	ns	-0.17*	ns	0.27***	0.23**	-0.44***	ns	ns	0.21**	0.18**	
Spring precipitation sum		-0.25**	-0.25***	-0.23***	-0.16*	ns	ns	ns	0.15*	ns	-0.55***	ns	-0.26***	0.18*	0.16*	
Summer precipitation sum		-0.27***	-0.27***	-0.18*	-0.20**	ns	ns	ns	ns	ns	-0.46***	ns	-0.30***	0.15*	ns	
Autumn precipitation sum		0.49***	0.37***	ns	0.40***	0.25***	ns	0.21**	0.24**	0.52***	ns	ns	0.50***	0.16*	ns	
Winter precipitation sum		0.20*	0.18*	-0.16*	0.20**	ns	-0.19*	ns	0.26***	0.33***	-0.39***	ns	0.24***	0.18*	0.19*	
Pluvial continentality index		-0.47***	-0.42***	ns	-0.37***	-0.19**	ns	-0.18**	ns	-0.37***	-0.34***	ns	-0.55***	ns	ns	
Length of cold period		-0.51***	-0.58***	-0.36***	-0.30***	-0.17*	ns	ns	ns	ns	-0.47***	-0.24**	-0.55***	ns	ns	
Length of summer drought	0.16*	0.22**	0.21**	ns	ns	ns	ns	ns	-0.25***	0.41***	ns	0.18*	ns	ns		

^a CV – coefficient of variation.

of regeneration gaps at higher water availability (that is in summer), increasing with both continentality and altitude.

4.2. The importance of bedrock and soil pH in subcontinental areas: Is it only a species pool effect?

In subcontinental areas, dry grasslands over loess and siliceous bedrock contained the fewest species. While we could explain the low richness of acidic grasslands with a limited number of species adapted to the double stress of soil acidity and drought, it is more difficult to explain why grasslands developed on fertile chernozem soils over loess were so species-poor. A combination of drought with the burning of some of the loess grasslands might be one of the reasons, as this management can have negative short-term effect on the species richness of temperate grasslands (Ryser et al., 1995).

Contrary to our expectations, the relationship between species richness and soil pH in dry grasslands across the eastern Balkan Peninsula was weak, but the unimodal shape with an optimum at pH about 7 was consistent with previous studies (e.g. Schuster and Diekmann, 2003; Löbel et al., 2006). Nevertheless, after taking climate in account (Fig. 3), a strong decrease in richness on soils with pH > 8 in the mountainous areas was observed. This pattern can be due to fewer species adapted to nutrient uptake limitation associated with very basic substrates (Tyler, 1994). However, considering that the few plots on high-pH soils are all from the karst mountains of western Bulgaria, this pattern can be locally idiosyncratic.

In the sub-Mediterranean areas, soil pH varied over a broad range, but in spite of that no relationship with species richness was detected. Both low-pH and high-pH soils were species-rich, indicating that probably most sub-Mediterranean species in the study area are generalists regarding soil pH. This is in contrast to the positive relationship of species richness to pH reported for the typical Mediterranean vegetation (Chytrý et al., 2010).

4.3. Species richness increases with precipitation: Moisture availability or spatial mass effect?

We found that outside of the sub-Mediterranean area of the eastern Balkan Peninsula species richness increased with precipitation, which in turn increased with altitude. This is consistent with patterns found in other temperate grasslands: in southern Siberia, meadow steppes at higher altitudes (with higher precipitation) were richer than dry lowland steppes (100-m² plots; Chytrý et al., 2007), or in North America, species richness increased with precipitation in temperate grasslands (1-m² plots; Adler and Levine, 2007). The existence of fewer species in the regional species pools adapted to drought-stress conditions was proposed as an explanation for this pattern (Chytrý et al., 2007).

Another explanation for the decrease in fine-scale species richness in dry grasslands from the mid-altitudes to the more continental lowlands could be the increased unpredictability and severity of summer droughts. Unpredictability and severity of climate increase the chance for local extinctions of species at the limit of their range or in isolated populations (Huston, 1979). This could be particularly valid for dry grasslands in the subcontinental lowlands in the eastern Balkan Peninsula, as most of them occur in isolated smaller patches, which implies limited species immigration and increased risk of extinction (MacArthur and Wilson, 1963).

An additional explanation for the higher species richness at higher altitudes could be related to the spatial mass effect (Shmida and Wilson, 1985). Unlike dry grasslands in the continental lowlands, scattered as isolated patches among agricultural fields, those in the mountainous areas are surrounded by a mosaic of mesic

grasslands and forests, and thus more mesophilous species can coexist with dry grasslands species.

5. Conclusions

This is the first study on fine-scale species richness patterns in dry grasslands of the eastern Balkan Peninsula. Our results suggest that climate is the most important factor influencing fine-scale species richness in these grasslands, possibly due to species-pool effects. Fine-scale species richness patterns within the study area exhibit strong geographical patterns in relation to climate: dry grasslands in the sub-Mediterranean areas are species-richer, mainly due to the higher number of sub-Mediterranean species, especially annuals. When focussing on different climatic regions separately, species richness increases with precipitation outside the sub-Mediterranean areas. In the subcontinental lowlands, lower precipitation in combination with specific substrate properties (e.g. greater acidity) or unfavourable management (burning) results in the low species richness of dry grasslands. Elsewhere in the subcontinental areas, species richness increases with precipitation but decreases if the substrate is too basic (pH > 8). Thus, local factors gain importance mainly within climatically and biogeographically homogeneous regions.

Statement of authorship

SP and MC designed the study. All authors participated in field sampling. SP prepared the dataset, performed all analyses in collaboration with MC, and wrote the manuscript. All authors discussed the results and were involved in revisions of the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.actao.2015.02.001>.

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