

Soil moisture and a legacy of prehistoric human activities have contributed to the extraordinary plant species diversity of grasslands in the White Carpathians

Půdní vlhkost a prehistorická činnost člověka v krajině přispěly k neobvyklé druhové diverzitě travníků v Bílých Karpatech

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Fajmonová Z., Hájková P. & Hájek M. (2020) Soil moisture and a legacy of prehistoric human activities have contributed to the extraordinary plant species diversity of grasslands in the White Carpathians. – *Preslia* 92: 35–56

The factors that determine the unique species richness and composition of some temperate grasslands are poorly known. Uniqueness of the extraordinarily species-rich grasslands in the White Carpathian mountain range (Czech Republic, Slovakia), with many disjunct occurrences of species, have been previously attributed to intermittently wet deep soils, which facilitate the co-occurrence of steppe and wet-grassland species, and Holocene continuity of open land, resulted in large species pools. Based on a detailed investigation of 23 well-preserved regularly mown grasslands differing in their vegetation composition and species richness, we tested the relative importance of the hypothetical determinants of compositional variability within semi-dry grasslands. For the first time we included measurements of seasonal moisture at different soil depths and landscape differences in the intensity of the effect of prehistoric humans. Soil moisture was measured using the frequency-domain reflectometry based Profile Probe PR2 sensor at depths of 10, 20, 30, 40 and 60 cm in the soil, repeatedly every four weeks from April to November. Soil samples were taken from the same depths in order to determine soil basicity and nutrient content. As a proxy of the intensity of the effect of prehistoric human activities, mean distances between sampled localities and the five nearest Neolithic or Aeneolithic settlements were used. The species richness was best explained by mean soil moisture, which increased towards the most species-rich grasslands, whereas the distance from prehistoric settlements had no effect. Basicity, moisture and the distance from prehistoric settlements had significant conditional effects on species composition. There was a high frequency of species of intermittently wet soils, thermophilous oak forests and forest fringes in the most valuable grasslands, which are located mostly in the south-western part of the area where the soils were moister and closer to prehistoric settlements, but the seasonal variation in moisture was not high. We conclude that coexistence of species from different habitats is dependent on regular management and high soil moisture throughout the growing season. Due to intermediate moisture conditions that are tolerated by multiple ecological groups of species, many species may locally coexist and form a species-rich grassland community of unique species composition. In addition, prehistoric human activities contributed to landscape openness and helped maintain a forest-steppe species pool during the Holocene forest optimum. Our results indicate that although this historical determinant shapes predominantly the species composition pattern, the extraordinarily species-rich spots are determined rather by abiotic factors, such as intermediate moisture and biotic interactions resulting from the type and duration of management practices.

Keywords: *Cirsio-Brachypodium pinnati*, forest-steppe, Neolithic settlement, semi-dry grasslands, soil moisture, species richness, White Carpathians

Introduction

Local plant species diversity results from ecological processes acting at different spatial scales. The ‘species pool’ hypothesis stresses large-scale processes: evolutionary and historical factors determining a set of species that are capable of coexisting in a particular community (Taylor et al. 1990, Eriksson 1993). On the other hand, theories based on inter-specific competition emphasize the importance of differential resource limitation and high spatial (or temporal) microhabitat heterogeneity for successful species coexistence (Grime 1979, Braakhekke & Hooftman 1999, Brauer et al. 2012). In fact, the two concepts are complementary in determining species richness and composition. Large-scale processes lead to the formation of a pool of species which are then subjected to local filtering driven by abiotic factors and biotic interactions (Zobel 1997, Pärtel & Zobel 1999).

Temperate grasslands are global hot spots of diversity with extraordinary fine-scale (up to 100 m²) species richness (Wilson et al. 2012, Chytrý et al. 2015). Distribution of the most species-rich grasslands in Europe is non-random and we can formulate conditions that seem to be a prerequisite for high local species diversity (see also Dengler et al. 2014, Roleček et al. 2014). Their location on rather base-rich soils corresponds to conditions that prevailed during the Pleistocene development of the Palaearctic flora and is hypothesized to be the reason for the existence of a large pool of species in these grasslands (Dengler et al. 2014). The species pool explanation is supported by the similarity in the species composition of extremely species-rich semi-dry grasslands in different areas (Roleček et al. 2014). In addition, there are indications that light-demanding species could have persisted in some areas throughout the Holocene. Since the early Holocene, open land in potentially wooded areas was maintained by climate, fires or herbivore pressure, followed by the first agro-pastoral activities of Neolithic people (Hájková et al. 2011, Pokorný et al. 2015, Feurdean et al. 2018). The continuity in land use over a long period of time has significantly contributed, not only to the persistence and migration of the species, but also to the formation of species-rich vegetation (Kull & Zobel 1991, Bruun et al. 2001, Poschod & WallisDeVries 2002, Pärtel et al. 2007). At the landscape scale, large areas of grassland, presence of solitary trees and shrubs, and high heterogeneity in land cover are considered to be the factors that support high species diversity in grasslands (Helm et al. 2006, Öster et al. 2007, Michalcová et al. 2014). The local conditions then include intermediate position along environmental gradients, resulting in the overlapping of the niches of many species (Löbel et al. 2006, Merunková et al. 2012, Dvořáková et al. 2014, Reitalu et al. 2014), and regular management that lowers competition and maintains the diversity (Hansson & Fogelfors 2000, Klimeš et al. 2000, Aavik et al. 2008, Jongepierová 2008, Bonari et al. 2017).

The White Carpathians (Bílé Karpaty Mts) are one of the few regions where the configuration of these factors facilitated the development of extraordinarily species-rich grasslands. The area has attracted attention of scientists over many years (e.g. Sillinger 1929, Podpěra 1930, 1951, Martinovský 1967, Tlusták 1975, Klimeš 1997) so we have detailed information on the species composition of these grasslands (Jongepierová

2008). The research carried out over the last 10 years focused on the determinants that may underlie the extremely high species richness of the most valuable semi-dry grasslands (Jongepierová 2008, Hájková et al. 2011, 2018, Merunková et al. 2012, Dvořáková et al. 2014, Michalcová et al. 2014, Bonari et al. 2017). The importance of such a research is not only regional, because compositionally similar extremely species-rich grasslands also occur at other sites in eastern central Europe where landscape configuration and history is analogous to that in the White Carpathians (Dengler et al. 2012, Roleček et al. 2014). Therefore, the White Carpathians could be a model area for doing research on the factors that determine the extraordinarily high biodiversity in these grasslands.

The species-richest grasslands, including the “world record holders” (Chytrý et al. 2015), are concentrated in the south-western part of the White Carpathians. They are further noteworthy for their remarkable species composition, which is currently insufficiently clarified. The historical processes determining the formation of a species pool are considered to be responsible for the occurrence of rare forest-steppe species with disjunct distributions in eastern central Europe (e.g. *Veratrum nigrum*, *Crepis sibirica*, *Pseudolysimachion spurium*, *Serratula lycopifolia*). Currently they frequently occur in meadow steppes in the forest-steppe zone in Eurasia (Erdős et al. 2018) and may hence represent relicts of Early Holocene forest-steppe communities (Hájková et al. 2011, Roleček et al. 2014, Dítě et al. 2018). Their occurrence hence provides an indication of continual existence of forest-steppe throughout the Holocene (Sillinger 1929, Hájková et al. 2011). Another piece of evidence comes from palaeoecological reconstruction of a continuous persistence of open land throughout the most prominent phases of woodland expansion, either in the area studied (Hájková et al. 2018, Novák et al. 2019) or nearby (Kuneš et al. 2015, Jamrichová et al. 2017), and from archaeological records since the Neolithic in the south-western foothills of the White Carpathians (Hájková et al. 2011). In contrast, palaeoecological evidence indicates that climax forests have been present in the central and north-eastern part of the White Carpathians since the Middle Holocene (Hájek et al. 2016, Hájková et al. 2018). However, there is currently no direct test of the effect of the configuration of the prehistoric landscape on present grassland diversity.

In contrast, the processes at the microhabitat scale may determine noteworthy combinations of species in grasslands. Coexistence of species of dry grasslands, mesic meadows and meadows on intermittently wet soils (e.g. *Molinia arundinacea*, *Serratula tinctoria*, *Potentilla alba*, *Sanguisorba officinalis*; Chytrý & Novák 2007) indicate intermittent changes in soil moisture (i.e. wet conditions in spring and after rainy periods, and summer droughts), which together with deep soils is suggested as one of the most important determinants of unique species richness and composition in White Carpathian grasslands (Jongepierová 2008, Merunková et al. 2012, Chytrý et al. 2017). Although recent studies demonstrate the importance of topsoil moisture for species richness (Dvořáková & Horsák 2012, Hettenbergerová et al. 2013), long-term measurements needed to verify this theory are still missing. In addition, the vertical variability in conditions in terms of moisture, nutrients or basicity has not been studied in deep and well developed soils. Although root density decreases with increasing soil depth, the roots of many species of central-European grasslands are reported at depths greater than 50 cm (Kutschera & Lichtenegger 1992). Properties of the topsoil in White Carpathian grasslands are considered to be favourable for high plant species richness (Kubíková & Kučera 1999,

Merunková et al. 2012), but it is still not clear whether vertical variability in soil conditions contributes to high species richness and a unique species composition.

In this study we combine both hypotheses in an attempt to explain the high diversity of semi-dry grasslands by testing the role of both large-scale historical processes and those acting at a microhabitat scale. We use archaeological data and measurements of soil moisture dynamics and soil chemistry in vertical profiles to explain the inter-regional differences in semi-dry grassland vegetation in the White Carpathians. We test the hypotheses that (i) distance from old-settlement areas codetermine the regional pattern in species richness and composition of semi-dry grasslands; (ii) soil moisture affects vegetation diversity and species richness, with the most species-rich grasslands being either moister or more dynamic in terms of moisture than other grasslands.

Methods

Study area

This study was conducted in the central and south-western part of the White Carpathians, both in the Czech and the Slovak part of the mountain range (48°49'–49°00'N, 17°24'–17°55'E; Fig. 1). The highest peak of the mountains reaches 970 m a.s.l. Geologically the area belongs to the Flysh Belt of the Outer Western Carpathians. Carpathian Flysh consists of alternating sedimentary rocks, mostly of sandstone and rock clay layers of variable thickness (from several centimetres to metres) and chemistry (Buday 1967, Pešl & Žůrková 1967). The flysh bedrock in the study area is formed mostly of lime-rich claystones, with the proportion of sandstone varying between sites and more abundant only in the summit areas (Cháb et al. 2007).

Soils are usually deep, clayey and difficult to classify, which results in inconsistent soil typology in the region. Most often, pellic and luvic chernozems with conspicuously dark topsoil are recorded at low altitudes, while pellic, luvic, gleyic and sometimes skeletal cambisols, pararendzinas and luvisols at middle altitudes and in the northern part of the area studied (Sedláček 2012). The soils in the species-rich grasslands included in this study are deep (1 m or more) and show signs of translocation of clay, brunification and redoximorfism of varying intensity (Novák et al. 2019).

Precipitation varies considerably from year to year. Annual precipitation ranged from 803 to 1058 mm in 2010 and from 497 to 684 mm in 2011. Mean annual temperature ranged from 6.9 to 8.4 °C in 2010 and from 7.8 to 9.4 °C in 2011 (measured at four climatic stations located in the area, see below; data from the Czech Hydrometeorological Institute). For localities in the south-western part of the study area, long-term mean annual precipitation is 759 mm and long-term mean annual temperature is 7.7 °C. For localities in the north-eastern part of the study area, it is 815 mm and 7.5 °C, respectively (Tolász 2007).

Grasslands in the south-western part of the White Carpathians, where there are the most species and more forest-steppe species, may be considered, according to the classification of Feurdean et al. (2018), as primary, ancient grasslands, maintained by climate and natural and later on anthropogenic disturbances. At low altitudes, grasslands have been continuously maintained since the pre-Neolithic times by natural or anthropogenic fires and grazing, whereas at middle and high altitudes grassland was intermixed with

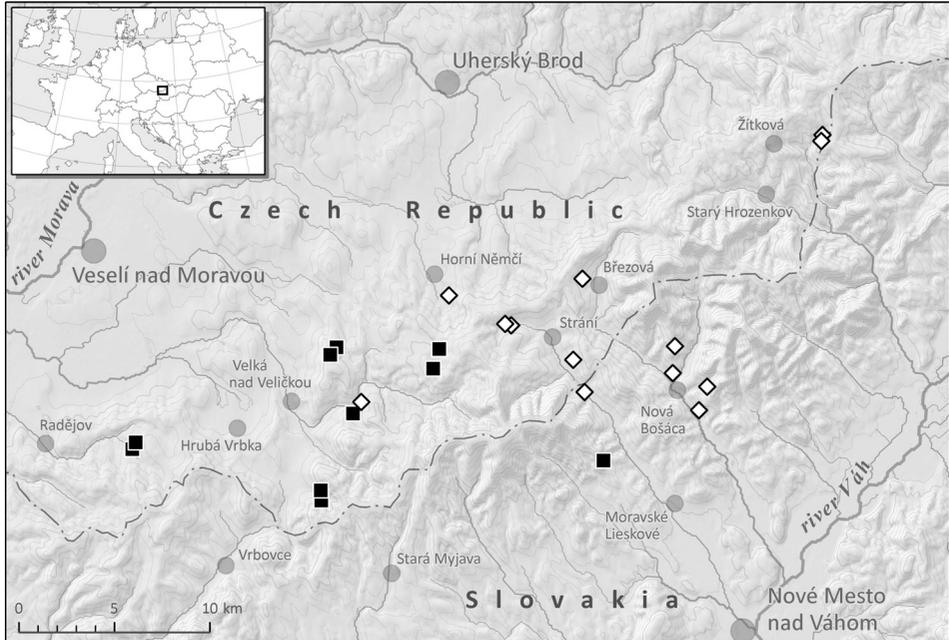


Fig. 1. – Map of the area studied showing the positions of the sites studied. Symbols refer to the assignment of the vegetation plots to the two groups based on species composition: ■ grasslands of south-western type, ◇ grasslands of north-eastern type.

mainly oak woodlands (Novák et al. 2019). The prehistoric management of these parklands and oak woodlands might include forest grazing, pollarding, shallow ploughing and burning. From medieval times onwards grasslands were transformed into hay meadows (Hájková et al. 2018). Grasslands have gradually become either productive and manured, easily accessible grasslands close to villages or species-rich, less accessible, distant grasslands, often located in places where there were settlements in prehistoric times. The distant grasslands were scythed once in late summer and developed into the extremely species-rich grasslands with a unique biodiversity that we have today (Hájek 2015).

Grasslands in the central and north-eastern part of the mountain range started to form as late as after a long history of dense woodlands dominated by lime, ash, maple and elm, later beech (Hájek et al. 2016). There is evidence of local prehistoric management dating back to the Bronze Age (Hájková et al. 2011, Hájek et al. 2016). Origin of hay grasslands in the area is connected with human colonization from the Middle Ages onwards (Jongepierová 2008, Hájková et al. 2018). The largest deforestation and establishment of hay grasslands, mostly occurring around scattered homesteads, was associated with the so-called Kopanice colonization in early modern times (Jamrichová et al. 2017). The grasslands of present-day importance for their biodiversity are mostly scythed each year in summer and then sometimes grazed (Hájek 2015).

Though these grasslands are still widespread in both subregions, the present number and area of the species-rich semi-dry grasslands is only a fragment of what they were up to the 1950s because of undesirable agricultural measures (e.g. ploughing, fertilization) or wide scale abandonment (Jongepierová 2008).

The most valuable and species-richest of the semi-dry grasslands belong to the most mesic community of the class *Festuco-Brometea*, the *Brachypodio pinnati-Molinietum arundinaceae* association. According to the synthesis of Willner et al. (2019), this plant community is associated with mesic meadow steppes of the forest-steppe zone in central Europe, classified within the *Cirsio-Brachypodium pinnati* alliance. They are characterized by the presence of steppe and thermophilous species in combination with species of meadows on mesic and intermittently wet soils, open forests or forest fringes (Chytrý & Novák 2007, Škodová & Ujházy 2014). This community is distributed predominantly in the south-western part of the area studied (Jongepierová 2008). Impoverished forms can be found also in the north-eastern part, which are in transition towards other communities of *Cirsio-Brachypodium pinnati* or *Arrhenatherion elatioris*, which can also be relatively species-rich although the presence of the diagnostic species of *Brachypodio pinnati-Molinietum arundinaceae* is less constant (Škodová & Ujházy 2014). Henceforth we shall refer to these grasslands as of north-eastern type along with communities in transition towards dry meadow steppes, with rather short vegetation and a higher representation of thermophilous species that occur locally at warm, lime-rich sites (Škodová et al. 2011, Willner et al. 2019). Nomenclature follows Kubát et al. (2002) for vascular plants and Chytrý (2007) for syntaxa.

Field sampling

The study sites were selected during the 2010 growing season. In cooperation with local experts, localities with well-preserved regularly mown species-rich semi-dry grasslands were selected and a site with the best-developed vegetation and highest expected species richness at a given locality was marked with metal nails. Except for five large grassland complexes, only one site was established at each locality to reduce spatial autocorrelation. The final number of study sites was limited by number of suitable localities and need to take the soil moisture measurements within a short time span in order to reduce the effect of weather changes on the results. Hence, some well preserved but hardly accessible grasslands were omitted. Finally, 23 sites were selected. The altitudinal range of these sites is 315–577 m. All but two of them are situated in nature reserves.

Volumetric soil moisture was measured using frequency-domain reflectometry and Profile Probe PR2 sensor (with a HH2 portable moisture meter). It detects the ratio between the voltage of an oscillator operating at 100 MHz and that of the reflected signal using a sealed polycarbonate rod (25 mm diameter with electronic sensors arranged at fixed intervals along its length) installed in the soil. The ratio of the two voltages is dependent on the apparent dielectric permittivity of the soil (ϵ), which depends on the soil-water content (dos Anjos et al. 2011). The reliability of such indirect measurements is strongly affected by the calibration procedure used for calculating the water content based on the dielectric constant. The manufacturer provided the calibration curve for mineral soils, but for the most accurate measurements a site-specific soil calibration is required. This, however, is unrealistic in complex studies like this one. A direct comparison of the values obtained using the manufacturer calibration curve and real soil moisture data based on a gravimetric analysis demonstrates that the manufacturer calibration curve may differ significantly from the absolute moisture contents (Huang et al. 2004, Iwata et al. 2017, Di Matteo et al. 2018), but the relationship between the moisture content measured

gravimetrically and the dielectric method is very close; different studies report r-squared regression values between 0.70–0.92 (Qi & Helmers 2010, Bezerra et al. 2013) and > 0.97 (Gebregiorgis & Savage 2006, Di Matteo et al. 2018). Comparison of seasonal trends, i.e. as examined in this study, indicates the same trends in soil moisture content are obtained using the manufacturer calibration curve and soil-specific calibrations in terms of the relative differences between the samples and in overall variation (Qi & Helmers 2010). Based on these arguments, we use the default calibration curve for mineral soils, but only use it to interpret the relative differences between sites and extent of the temporal variation. The absolute values of soil moisture are nevertheless not reliably comparable with other soil moisture measurements reported in the literature.

When taking a reading, the probe is inserted into an access tube. These are specially constructed thin-wall tubes, which maximize the penetration of the electromagnetic field into the surrounding soil. For purposes of this study, the long version of the PR2 probe with sensors at 10, 20, 30, 40, 60 and 100 cm of its length was used. In March 2011, a hole was augered into a soil profile using a 25 mm gouge auger and 110 cm long access tube was installed at each study site. The measurements were recorded repeatedly every ca four weeks from April to November. Altogether, there were nine measurements of the soil moisture content. To maximize the sampling at each location, three readings were taken with the probe rotated through 120° each time and the average of the three values was used.

In June, plant species composition was sampled using square plots of 16 m² positioned at the study sites with the access tubes situated in the centre of the plots. Covers of all the vascular plants were recorded using the nine-grade Braun-Blanquet scale (Dengler et al. 2008). In agreement with actual management practices, the vegetation in the plots and their vicinity was cut at the beginning of July. In November, the access tubes were removed and soil samples were taken nearby from six depths corresponding to the positions of the electronic sensors.

Soil analyses

The soil samples were air dried and ground in a Fritsch planetary ball mill PULVERISETTE 6 for 30 sec at 450 rpm with five steel balls of 30 mm diameter. The following extracts of the soil samples were then prepared: water extract prepared by mixing soil and distilled water in the ratio 1:5 and Mehlich III extract prepared by mixing soil and Mehlich III solution in the ratio 1:10. The extracts were then filtered through Munktell quantitative filter papers (150 mm diameter, Grade 390). The pH (ISO 10523) and conductivity (EN 27888) values of the water extract were measured immediately after preparation.

Determination of total nitrogen (TN) following oxidation to nitrogen oxides (EN 12260) was done using the water extract in a Shimadzu TOC-VCPH analyzer with a TNM-1 module. Based on a similar oxidation process, three forms of carbon were determined simultaneously using the same analyzer. Total carbon (TC) and inorganic carbon (IC) were measured and total organic carbon (TOC) was determined using the difference method, i.e. the difference between TC and IC (EN 1484). C/N ratio was calculated as the ratio of TOC and TN.

The water extract was further used for determination of water-soluble calcium (Ca-H₂O) and potassium (K-H₂O) using the flame atomic absorption spectrometer novAA 350. The Mehlich III extract was used for determining phosphate phosphorus (P-PO₄; EN ISO 6878, Part 6) using a Helios Delta VIS Spectrometer and iron (Fe-Meh) using the flame atomic absorption spectrometer novAA 350.

Explanatory variables

Of the data collected from the soil profiles, values from the depth of 1 m were excluded from the analyses. In most cases, a soil sample from the depth of 1 m appeared like a mass of grey clay with no roots. Because the physical parameters of heavy clay soils may not correspond to the default calibration defined for mineral soils, it is possible to record confusing moisture measurements due to large sampling errors (Delta-T Devices Ltd 2008). Hence, all the analyses were based on data recorded for depths of 10, 20, 30, 40 and 60 cm in the soil. For those who are interested in measurements recorded at a depth of 1 m, the data are included in Electronic Appendix 1.

Means and coefficients of variance (CV) for the soil profile (vertical trends) were calculated from the parameters recorded at a particular depth in the soil. For soil moisture measured repeatedly during the growing season, vertical means and CV were calculated separately for each measurement (moist-April, ... moist-November). Further, temporal trends in soil moisture were expressed by means and CV of values recorded at particular depths in the soil (moist-10, ... moist-60). Overall mean and CV for all measurements and soil depths were also calculated.

Following parameters were log-transformed: conductivity, concentration of IC, Ca-H₂O and K-H₂O recorded at all the specified depths in the soil profile and their mean values.

Because the total number of soil moisture and chemical parameters measured is several times greater than the number of sites studied, it was not possible to use them directly as explanatory variables in statistical analyses. Instead, based on their character and mutual correlations (not shown), the parameters were clustered in following groups: (i) mean soil moisture comprised of the vertical means for individual measurements (moist-April-mean, ... moist-November-mean), temporal means for individual soil depths (moist-10-mean, ... moist-60-mean) and mean of values from all soil depths; (ii) temporal variability in soil moisture comprised of the temporal CV for individual soil depths (moist-10-CV, ... moist-60-CV) and the overall CV calculated from moist-April-mean–moist-November-mean; (iii) vertical variability in moisture comprised of the vertical CV for individual measurements (moist-April-CV, ... moist-November-CV) and the overall CV calculated from moist-10-mean–moist-60-mean; (iv) basicity comprised of the pH, conductivity and concentrations of IC, Ca-H₂O and Fe-Meh at particular depths and their means in the soil profile; (v) vertical variability in basicity comprised of CV of pH, conductivity, IC, Ca-H₂O and Fe-Meh; (vi) nutrients comprised of the concentrations of TOC, TC, TN, C/N, K-H₂O and P-PO₄ at particular depths and their means in the soil profile; (vii) vertical variability in nutrients comprised of the CV of TOC, TC, TN, C/N, K-H₂O and P-PO₄.

Principal components analysis (PCA) was used for each group of parameters with the parameters used as response variables after centering and standardization. The ordination axes which accounted for at least 50% of total variability in data of individual PCAs

(i.e. the first ordination axes in all but the analysis of nutrients where the first two axes were of similar importance) were used as surrogate variables in the statistical analyses.

As a proxy for intensity of prehistoric human effect on the structure of vegetation at the localities and in their surroundings, distances between sampled localities and past Neolithic settlements were used. Information on human settlement during the Neolithic (including Aeneolithic, i.e. Chalcolithic, ~5500–2000 BC) in the area studied was compiled both from published and unpublished sources and digitalized after critical review (Hájková et al. 2011). The distance was calculated in two ways. In addition to direct distances, we calculated real distances that take into consideration differences in altitude. Each site studied was connected by a vector line with each archaeological site (settlement). The resulting 759 lines overlaid the digital altitude model with a resolution of 30 m, using the Add Surface Information tool in the 3DAnalyst extension of the ArcGis software. In both methods, the distances to the five nearest settlements were averaged for each site studied and log-transformed. The direct distances were strongly correlated with the real distances (Pearson $r = 0.99996$) and the method of calculation had no effect on the results of the numerical analyses. For the sake of parsimony, we present the analyses based on the simplest method, the calculation of direct distances.

For the visual comparison of direct soil moisture measurements and monthly precipitation, data from four climatic stations located at Radějov, Velká nad Veličkou, Strání and Žitková (for their position in the area studied, see Fig. 1; data from the Czech Hydro-meteorological Institute) were assigned to particular sites based on the shortest distance.

Statistical analyses

In order to explain the variability in species composition, three redundancy analyses (RDA) were performed with the surrogate variables representing trends in soil moisture, basicity and nutrients, respectively, used as explanatory variables. Marginal effect of the variables was then considered and only the variables with a statistically significant effect on species composition, together with distance from prehistoric settlements, were included in the model used to partition variation and quantification of its effect on species composition. For calculating the variation fractions, adjusted percentages of explained variation were considered. The main gradients in species composition and trends of significant variables along them were determined using PCA with species as response variables. Spearman correlations of the variables with the scores for the plots sampled on the first four PCA ordination axes were calculated. In ordination analyses where species were used as response variables the species abundances were log-transformed. The null hypothesis that the response data is independent of the explanatory variables was tested based on a Monte Carlo permutation test with 999 permutations. In all cases of multiple testing, significance levels were adjusted using a Holm correction. The ordination analyses were performed in CANOCO v. 5.11 (ter Braak & Šmilauer 2012).

In some cases during field sampling it was not possible to unequivocally classify grasslands into south-western or north-eastern type, respectively. Because we were interested in causes of different species compositions in the grasslands, we applied numerical classification of vegetation plots to the two groups based on species composition. We used the ISOPAM algorithm (Schmidtlein et al. 2010), which is based on the classification of ordination scores from isometric feature mapping, using presence-absence data. During

the classification process, ordination and classification are repeated in a search for groups rich in diagnostic species and high overall fidelities of individual species to individual clusters. We applied a default threshold for diagnostic species filtering and the Bray-Curtis distance. The groups were identified based on the diagnostic species of association *Brachypodio pinnati-Molinietum arundinaceae* (according to Chytrý & Novák 2007). The differences between the groups were tested for the variables with a significant effect on species composition using a two sample t-test.

The effect of the surrogate variables and the distance from prehistoric settlements on the species richness was estimated using multiple regression. The model predicting species richness was selected by stepwise selection using F-statistic ($P < 0.05$) at each step. The amount of explained variation was expressed using adjusted R^2 penalizing the number of parameters included in the model. The Spearman correlation coefficient, two sample t-test and the multiple regression were performed in Statistica v. 13 (www.statsoft.com).

Results

Variability in species composition

The numerical classification distinguished two groups of semi-dry grasslands. The first group corresponds to the grasslands of south-western type. According to the species composition, the vegetation can be assigned to the association *Brachypodio pinnati-Molinietum arundinaceae* (Fig. 2, Electronic Appendix 2). Distribution of these grasslands covers mostly the south-western part of the area (Fig. 1). The grasslands can be characterized by a high soil moisture (Fig. 3A, B). Further, localities are situated closer to prehistoric settlements (Fig. 3C). The second group includes the impoverished though still quite species-rich grasslands of north-eastern type (Fig. 1, Electronic Appendix 2, Fig. 3F).

There was no difference in soil basicity in the two groups of grasslands, for which the pH varied between 5.5 and 7.7 (Fig. 3D, E). Higher values of soil moisture in the grasslands of south-western type are apparent from spring and summer measurements (Fig. 4A) and for depths of 20 and 60 cm (Fig. 4B). Although, monthly sums of precipitation did not reveal consistent differences between the south-western and north-eastern localities (Electronic Appendix 1). Temporal trends in soil moisture were similar for both groups, actual measured values were high in spring and then corresponded, with delay, to rainfall fluctuations, with maximum in late summer and minimum in autumn. This temporal variability was apparent at all soil depths, with less pronounced summer fluctuations at 60 cm (Electronic Appendix 1). Moisture increased with soil depth but in different ways in the grasslands of south-western and north-eastern type (Fig. 4B, Electronic Appendix 1).

From the surrogate variables describing soil conditions, only two variables, mean moisture and basicity, accounted for a significant part of variability in species composition (Table 1). Therefore, they were included together with the distance from prehistoric settlements in the model for partitioning variation. Basicity had the strongest effect on species composition. Lower but still significant effects were recorded for mean moisture and distance from prehistoric settlements (Table 2). These three variables are associated with different parts of variability in the species composition, which is apparent also from their correlations with the scores of plots on PCA ordination axes (Fig. 2).

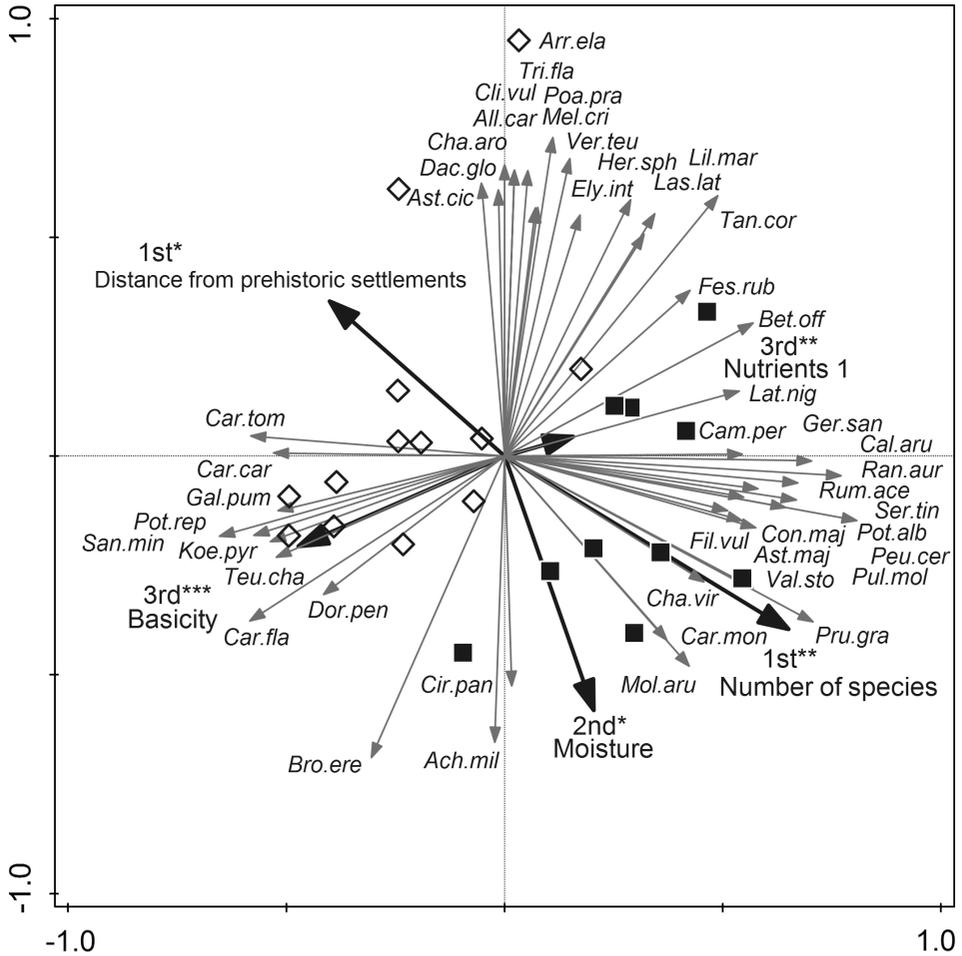


Fig. 2. – PCA scatter with species shown as response variables. Symbols refer to the assignment of the vegetation plots to the two groups based on species composition: ■ grasslands of south-western type, ◇ grasslands of north-eastern type. Black arrows refer to selected variables passively projected onto the scatter. Significant correlations between the individual variables and sample scores are presented: *** P < 0.001, ** P < 0.01, * P < 0.05. Ach.mil – *Achillea millefolium* agg., All.car – *Allium carinatum*, Arr.ela – *Arrhenatherum elatius*, Ast.cic – *Astragalus cicer*, Ast.maj – *Astrantia major*, Bet.off – *Betonica officinalis*, Bro.ere – *Bromus erectus*, Cal.aru – *Calamagrostis arundinacea*, Cam.per – *Campanula persicifolia*, Car.car – *Carex caryophylllea*, Car.fla – *Carex flacca*, Car.mon – *Carex montana*, Car.tom – *Carex tomentosa*, Cha.aro – *Chaerophyllum aromaticum*, Cha.vir – *Chamaecytisus virescens*, Cir.pan – *Cirsium pannonicum*, Cli.vul – *Clinopodium vulgare*, Con.maj – *Convallaria majalis*, Dac.glo – *Dactylis glomerata*, Dor.pen – *Dorycnium germanicum/herbaceum*, Ely.int – *Elytrigia intermedia*, Fes.rub – *Festuca rubra*, Fil.vul – *Filipendula vulgaris*, Gal.pum – *Galium pumilum*, Ger.san – *Geranium sanguineum*, Her.sph – *Heraclium sphondylium*, Koe.pyr – *Koeleria pyramidata*, Las.lat – *Laserpitium latifolium*, Lat.nig – *Lathyrus niger*, Lil.mar – *Lilium martagon*, Mel.cri – *Melampyrum cristatum*, Peu.cer – *Peucedanum cervaria*, Poa.pra – *Poa pratensis*, Pot.alb – *Potentilla alba*, Pot.rep – *Potentilla reptans*, Pru.gra – *Prunella grandiflora*, Pul.mol – *Pulmonaria mollis*, Ran.aur – *Ranunculus auricomus*, Rum.ace – *Rumex acetosa*, San.min – *Sanguisorba minor*, Ser.tin – *Serratula tinctoria*, Tan.cor – *Tanacetum corymbosum*, Teu.cha – *Teucrium chamaedrys*, Tri.fla – *Trisetum flavescens*, Val.sto – *Valeriana stolonifera* subsp. *angustifolia*, Ver.teu – *Veronica teucrium*.

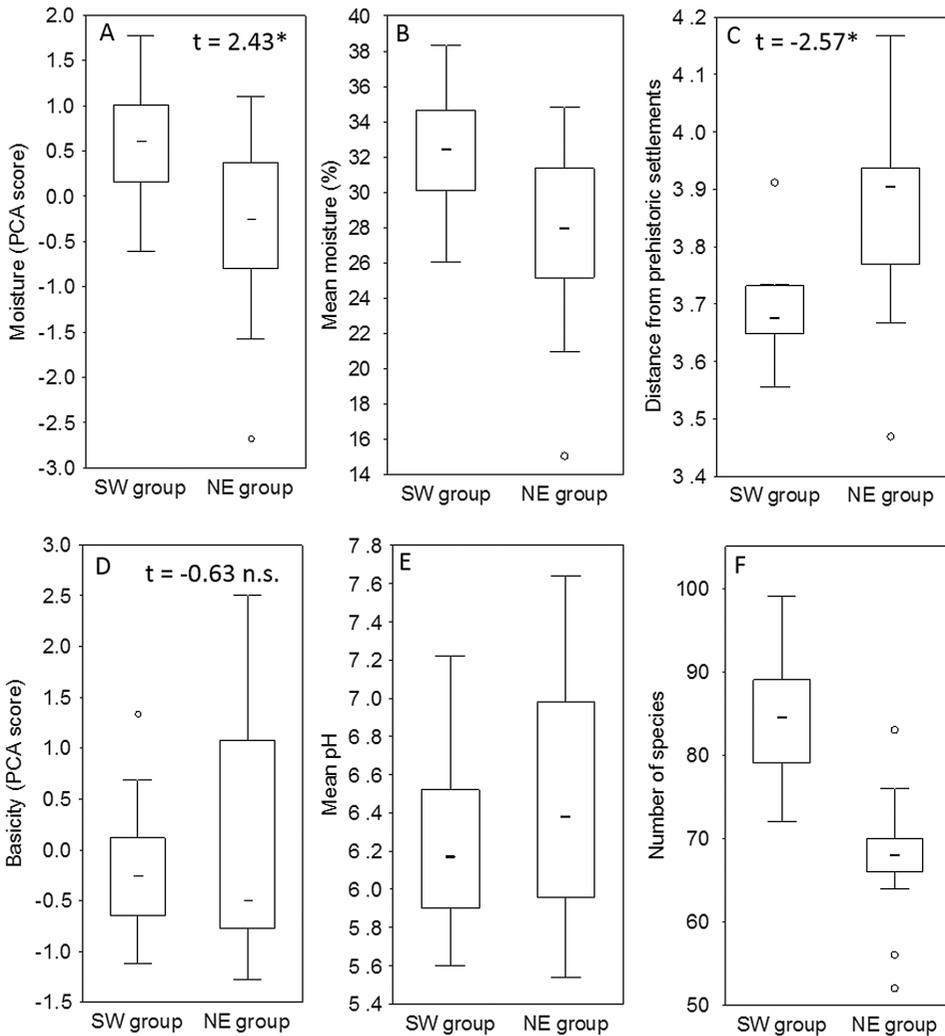


Fig. 3. – Differences in soil moisture, basicity (surrogate variables based on PCA; original values), distance from prehistoric settlements and species richness in the two groups of vegetation plots defined by species composition. Results of two-sample t-test are displayed if calculated: * $P < 0.05$, n.s. – not significant. Boxes include 25–75 % of the values, short lines represent medians, whiskers encompass the range of non-outlying values (up to 1.5 × the height of the box) and points are outliers.

Variability in species richness

The lowest number of species recorded in a vegetation plot was 52, but more than 65 species were recorded in most of the plots. Species richness was significantly correlated with mean soil moisture and basicity ($R^2_{\text{adj}} = 0.379$, $F = 7.7$, $P = 0.003$; Table 3), with species richness increasing with increase in soil moisture. Where soil pH was high (> 7.0), the number of species was low regardless of soil moisture (Fig. 5). The distance from prehistoric settlements did not account for a significant part of the variability in species richness.

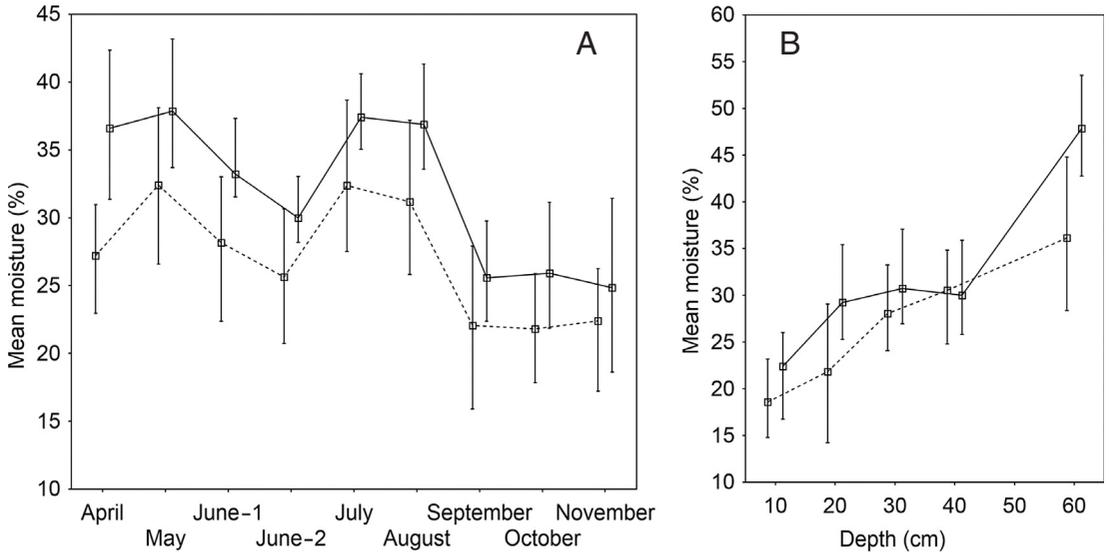


Fig. 4. – Trends in (A) vertical mean of soil moisture throughout the growing season and (B) temporal mean of soil moisture throughout the soil profile in grasslands of south-western type (solid line) and grasslands of north-eastern type (dashed line). The middle points represent means; whiskers encompass the range of non-outlying values (up to $1.5 \times$ range (mean \pm standard error)). For detailed information about measured soil moisture see Electronic Appendix 1.

Variables characterizing temporal trends in soil moisture or vertical trends in soil conditions were not significantly associated with the variability in species composition (Table 1) or variability in species richness.

Table 1. – Marginal effects of the surrogate variables on the species composition tested using three RDAs performed separately with the explanatory variables representing soil moisture, basicity and nutrients, respectively. The significance levels were tested using a Monte Carlo permutation test with 999 permutations and adjusted using a Holm correction. The statistically significant effects are in bold.

| | Explained variation (%) | pseudo-F | P(adj) |
|-----------------------------------|-------------------------|----------|--------------|
| RDA 1 | | | |
| Moisture | 7.6 | 1.7 | 0.046 |
| Temporal variability in moisture | 4.8 | 1.0 | n.s. |
| Vertical variability in moisture | 3.3 | 0.7 | n.s. |
| RDA 2 | | | |
| Basicity | 9.4 | 2.2 | 0.004 |
| Vertical variability in basicity | 5.4 | 1.2 | n.s. |
| RDA 3 | | | |
| Nutrients 1 | 6.6 | 1.5 | n.s. |
| Nutrients 2 | 4.0 | 0.9 | n.s. |
| Vertical variability in nutrients | 3.1 | 0.7 | n.s. |

Table 2. – Pure and shared effects of variables included in variation partitioning. Values refer to adjusted percentages of explained variation in the species composition. Negative values of a shared variation fraction indicate that the joint explanatory effect of the two variables is stronger than the sum of their marginal effects. The significance levels were tested using a Monte Carlo permutation test with 999 permutations.

| | Adjusted explained variation (%) | P |
|---|----------------------------------|-------|
| Moisture | 4.1 | 0.003 |
| Basicity | 7.4 | 0.001 |
| Distance from prehistoric settlements | 4.3 | 0.004 |
| Moisture \cap Basicity | -1.2 | |
| Basicity \cap Distance from prehistoric settlements | -1.3 | |
| Moisture \cap Distance from prehistoric settlements | 0.2 | |
| Moisture \cap Basicity \cap Distance from prehistoric settlements | 0.1 | |
| Total explained variation | 13.6 | 0.001 |

Table 3. – Multiple linear regression model of the relationship between species richness and variables representing soil moisture and basicity. The partial t-test of individual predictors had 20 degrees of freedom. b* represents standardized regression coefficients allowing comparison across the predictors.

| | b* | SE(b*) | b | SE(b) | t | P |
|----------|--------|--------|--------|-------|--------|-------|
| Moisture | 0.588 | 0.171 | 6.672 | 1.947 | 3.426 | 0.003 |
| Basicity | -0.438 | 0.171 | -4.974 | 1.947 | -2.554 | 0.019 |

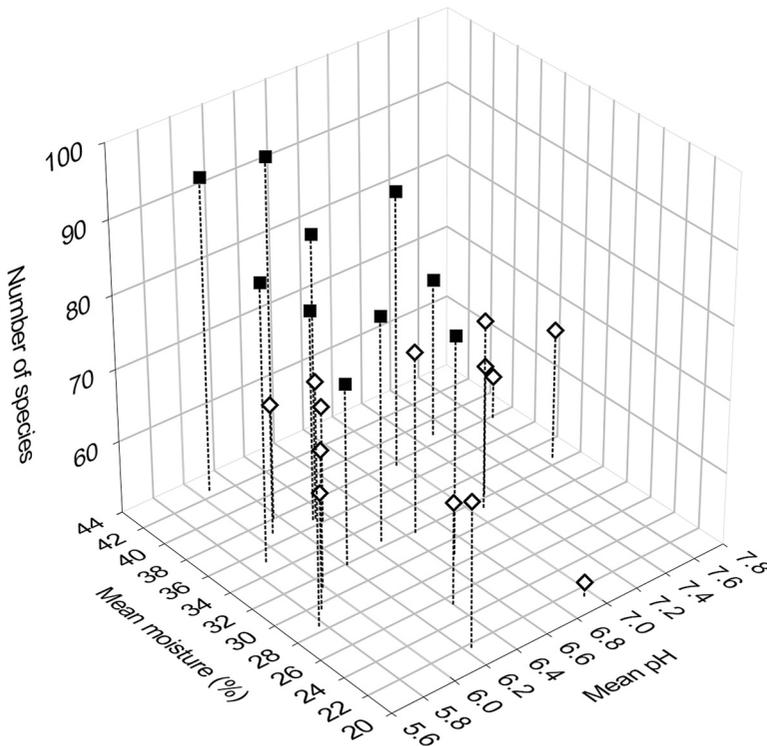


Fig. 5. – Scatter plot presenting relationship between species richness and either mean soil moisture or mean pH. ■ grasslands of south-western type, ◇ grasslands of north-eastern type.

Discussion

Local factors determining species richness and composition

Our results demonstrate that soil moisture is an important factor determining the diversity of semi-dry grasslands, with plant species richness increasing with increasing soil moisture. The explanation is that high soil moisture facilitates the coexistence of mesic and moist-meadow species with species of semi-dry grasslands (Hettenbergerová et al. 2013, Hájek et al. 2017). This result indicates that the higher species richness in grasslands of the south-western type is dependent among others on moisture conditions that are suboptimal for a wide set of coexisting species and hence limit competitive exclusions. Nevertheless, there was no difference in temporal variation in soil moisture in south-western and north-eastern types of grassland, which contradicts the hypothesis about the effect of an intermittent soil moisture regime on the high species richness and specific species composition in south-western grasslands (Jongepierová 2008, Merunková et al. 2012, Chytrý et al. 2017).

Vertical variability in soil moisture expressed by the coefficient of variance also did not appear to be significant, but the pattern recorded at particular depths differs in south-western and north-eastern type grasslands. The moisture of soils in the south-western part of the area is not consistently higher at all depths but there is a vertical structure that probably reflects physical soil properties. Indeed, under similar conditions in terms of the sum of the precipitation recorded in the two areas, properties influencing water retention seem to be the reason for the differences in the soil moisture. Water retention characteristics are determined by the soil's fine-fraction (silt and clay) content (Vereecken et al. 1989, Heiskanen & Mäkitalo 2002, Heiskanen et al. 2018). The amount of water retained increases with increasing fine-fraction content, because of the larger specific surface area of soil particles (van den Berg et al. 1997) or higher residual water content associated with higher water-filled pore volume (Dexter et al. 2012, Ding et al. 2016). Contrary to the results for moisture, air-filled porosity indicating availability of oxygen to plant roots is lower in fine-textured mineral soils than in coarser-textured soils (Wall & Heiskanen 2003). Oxygen deficiencies occur mostly in clayey soils caused mainly by the indirect effect of higher water content decreasing the permeability and diffusivity (Ben-Noah & Friedman 2018). Soil texture thus can underlie the essential conditions for plant growth and seems to be an important local factor that indirectly influences species richness and composition in grasslands. Further research on soil moisture and texture is needed to disentangle their conditional and shared effects on species composition and richness in grasslands. The next step should be an investigation of the general pattern in soil moisture in grasslands with different types of vegetation and in different regions to assess whether soil moisture can be integrated among the factors determining the extremely high species richness in grasslands.

The next local factor, soil basicity, also had a significant additive effect on the variability in species richness and composition. When soil moisture was partitioned out, species richness was lower where pH was high. Generally, our results correspond to the unimodal species richness-pH relationship reported for dry grasslands in climatically rather wet Eurasian regions (Dengler et al. 2014, Palpurina et al. 2017). The pH values recorded in top layers of soil in the five most species-rich grasslands (88–99 species in an area of 16 m²) ranged from 5.6 to 7.0, which is similar to the values recorded in extraordinarily

species-rich grasslands across Eurasia (Löbel et al. 2006, Dengler et al. 2012, Merunková et al. 2012, Dvořáková et al. 2014, Palpurina et al. 2017).

Soil nutrient content does not have an important role in accounting for the diversity of grasslands in this study, probably because the differences in nutrient availability were very small. Nevertheless, we assume that a prerequisite for successful species coexistence is suitable moisture conditions that are not accompanied by an enhanced nutrient, mainly phosphorus, availability, which can lead to suppression of dry-grassland specialists and shift from semi-dry grasslands to species-poor “mesic” vegetation, which is widely reported for grasslands recently enriched with nutrients (Hájek et al. 2017). In a recent meta-analysis of the species richness in dry grasslands whose productivity is co-limited by nitrogen and phosphorus, it was revealed that in northern Eurasia, it is higher in the dry grasslands that were the most productive (Palpurina et al. 2019). The phosphorus and nitrogen colimitation that occurs also in White Carpathian grasslands (Merunková et al. 2012) hence may facilitate a high species richness in wetter, i.e. more productive grassland ecosystems. In general, our results contribute to a growing body of evidence that intermediate positions along environmental gradients is a crucial condition for developing high fine-scale species richness (Dengler et al. 2012, Merunková et al. 2012, Dvořáková et al. 2014, Reitalu et al. 2014). This conclusion is, however, valid only when management is continuous and weakens the competitive pressure from highly competitive species.

Role of prehistoric human activities in determining the current diversity in grasslands

The intensity of human activities in the landscape during the Neolithic, expressed by the distance of sampled grasslands from old-settlement locations, appeared to be a significant factor influencing current species composition in grasslands. This supports the assumptions about continual existence of open habitats in the south-western part of the White Carpathians during the entire Holocene and their importance for the formation of a regional species pool (Hájková et al. 2011, 2018, Roleček et al. 2014). Although rare heliophilous species with disjunct distributions were largely not recorded in the vegetation plots studied, higher frequencies of species of forest-steppe habitats in eastern Europe (e.g. *Potentilla alba*, *Peucedanum cervaria*, *Pulmonaria mollis*, *Crepis praemorsa*, *Serratula tinctoria*) were recorded in grasslands of south-western type. We consider this result to be further evidence of the relict character of the grasslands of south-western type and the continuation of an ancient forest-steppe ecosystem maintained by prehistoric human activities since the Neolithic, or even Mesolithic times (Hájková et al. 2011, Jamrichová et al. 2017, Feurdean et al. 2018, Novák et al. 2019). Nevertheless, this result cannot be evidence of the continual existence of all grasslands sampled of the south-western type throughout the Holocene. Higher density of prehistoric human settlements and early human activities in the south-western part of the White Carpathians might have prevented the spread of closed-canopy forests during the Middle Holocene (Hájková et al. 2011). We see this as resulting in a spatiotemporal continuum of open land that facilitated regional survival of heliophilous species. Though some of the large grassland complexes may have originated in the Neolithic or a later prehistoric period, initially perhaps as grazed open-canopy woodland or parkland, the others originated as late as in the medieval or early modern times (Hájková et al. 2018). Their current species composition

partially derives from ancient regional species pool, but is largely shaped also by mowing over hundreds of years.

Nevertheless, the prehistoric landscape processes do not explain the fine-scale species richness in the grasslands studied as was demonstrated in Northern Europe by Bruun et al. (2001) and Pärtel et al. (2007). Generally, the grasslands of south-western type were located closer to past Neolithic settlements and were species-richer than grasslands of north-eastern type, which implies that the grassland species pool is larger in the south-western than in north-eastern part. However, this does not apply to all localities. In line with the species-pool concept (Zobel 1997, Pärtel & Zobel 1999), the results indicate that the fine-scale species richness, in addition to large-scale processes, is strongly influenced by environmental filtering and biotic interactions. In the case of the extraordinary species richness recorded in grasslands of south-western type, it is dependent on an ancient regional species pool and long-term continuous management with suitable edaphic conditions (moderately alkaline soils of appropriate soil moisture and nutrient colimitation).

The seeming discrepancy between species richness and size of the grassland species pool is demonstrated at the regional level by Michalcová et al. (2014) who show that high fine-scale species richness in the White Carpathian grasslands is not supported by the size of the grassland species pool of the regional flora, which is comparable with species pools in nearby regions. Instead, low beta diversity rather than high species pool is a typical feature of the White Carpathian grasslands (Kubíková & Kučera 1999, Michalcová et al. 2014). In other words, many grassland species are rather common across the area so that there is a high probability that a species from the regional grassland flora is present in a given grassland. This is facilitated by homogeneous environmental conditions determined by low geological diversity of the area and large size of grassland patches (Michalcová et al. 2014). The comparison of regions conducted by Michalcová et al. (2014) hence illustrates that not only total size of the grassland species pool, but also its distribution over the region, dispersal abilities and proportion of grassland species that may significantly co-occur in mesic, quite productive hay grasslands, play a role in determining the extreme local species richness.

In addition to a large ancient species pool and specific edaphic conditions, local continuity in mowing or moderate grazing is an essential prerequisite for forming and maintaining high species richness. Across regions, the most fine-scale species-rich grasslands occur at sites managed by mowing once a year in most cases (Kull & Zobel 1991, Aavik et al. 2008, Roleček et al. 2014, Turtureanu et al. 2014; but see Roleček et al. 2019) and this is the case also in the White Carpathians (Jongepierová 2008, Bonari et al. 2017). Although the general history of hay grasslands goes back to the Iron Age (Poschlod et al. 2009, Hejcman et al. 2013), the area of hay grasslands in the White Carpathians expanded as late as in the 17th century (Hájková et al. 2018) and was preceded by burning or moderate grazing that probably had similar effects (Hájková et al. 2018, Novák et al. 2019). According to Hájek et al. (2017), the centennial stability in mowing might even have prevented the modern species pool of fertilized mesic grasslands from replacing the ancient species pool of mesic grassland, but instead enriched the ancient White Carpathian grassland communities. In conclusion, a combination of ancient species pool maintained by prehistoric human activities, landscape configuration, edaphic factors (intermediate moisture, nutrient colimitation) and long-term regular disturbances have been crucial factors in developing world-unique fine-scale species richness.

Acknowledgements

We thank Petr Dresler for calculation of distances between study sites and prehistoric settlements, Katarína Devánová, Karel Fajmon, František Fajmon, David Zelený, the late Ching-Feng Li and Eva Šmerdová for their assistance in the field, Michal Pavonič and Eva Šmerdová for laboratory analyses, David Zelený, Jan Roleček and anonymous reviewers for useful comments, and Ondřej Hájek for compilation of climate data and preparation of the map. The authors affiliated to Institute of Botany, the Czech Academy of Sciences (Z.F. and P.H.) were supported by the long-term developmental project RVO 67985939. During the paper finalization, since 2020, P.H. was further supported by the Czech Science Foundation (project no. 20-09895S). At Masaryk University, M.H. was supported by Centre for European Vegetation Syntheses (CEVS; project of Czech Science Foundation no. 19-28491X).

Souhrn

Unikátní druhová bohatost na malých plochách a neobvyklé druhové složení některých evropských širokolístých suchých trávníků jsou známy, ale jejich příčiny nejsou dosud zcela objasněny. Výzkum probíhal na 23 lokalitách zachovalých a pravidelným kosením udržovaných širokolístých suchých trávníků v jihozápadní a střední části pohoří Bílé Karpaty. Přestože se tato oblast zdá být z hlediska přírodních podmínek homogenní, travinobylinná vegetace v její jihozápadní části se od severovýchodní části liší vyšší druhovou bohatostí a přítomností některých skupin druhů. Zaměřili jsme se na faktory považované za možné příčiny diverzity širokolístých suchých trávníků v oblasti, které však nebyly dosud dostatečně studovány – vlhkost půdy, včetně její časové a prostorové variability, a intenzitu lidské činnosti v krajině od neolitu. Také jsme sledovali další půdní vlastnosti – bazicitu a obsah živin. Na lokalitách byla pomocí přístroje Profile Probe PR2 měřena vlhkost v pěti hloubkách půdního profilu, a to v devíti měřeních během celé vegetační sezony. Z měřených hloubek byly odebrány vzorky půdy pro chemický rozbor. Intenzita lidské činnosti v krajině od neolitu byla odhadnuta na základě hustoty neolitického a eneolitického osídlení v okolí lokalit. Průměrná půdní vlhkost, bazicita a vzdálenost od (e)neolitických sídel vysvětlily významný podíl variability v druhovém složení. Druhová bohatost se zvyšovala spolu s průměrnou půdní vlhkostí a snižovala s rostoucí bazicitou, se vzdáleností od (e)neolitických osídlení však nesouvisela. Specifické druhové složení a vysoká druhová bohatost trávníků vyskytujících se převážně v jihozápadní části studované oblasti souvisí s vyšší celkovou půdní vlhkostí, vliv její časové nebo prostorové variability se neprokázal. Zdá se, že vlhkostní podmínky, které jsou schůdné, ale ne optimální, pro mnoho druhů různých stanovišť, přispívají k utváření společenstev s neobvyklým druhovým složením a vysokou druhovou bohatostí na malé ploše. Zajímavé je, že vyšší půdní vlhkost není způsobena vyššími úhrny srážek, které jsou v celém území srovnatelné, ale zřejmě odlišnými fyzikálními vlastnostmi půd, např. zrnitostí, která ovlivňuje zadržování vody v půdě. Výsledky také poskytují další, byť nepřímý, doklad o tom, že lidská činnost v krajině před několika tisíci lety ovlivňovala formování zásobníku druhů, které se dnes uplatňují v travinobylinných společenstvech. Výsledky jsou v souladu s hypotézami o kontinuální existenci bezlesí na jihozápadním okraji Bílých Karpat a reliktním charakteru výskytu některých druhů v dnešní vegetaci suchých trávníků. Vysoká druhová bohatost je podmíněna součinností mnoha faktorů, kromě rozsáhlého zásobníku druhů v krajině jsou to příhodné půdní podmínky z hlediska vlhkosti, bazicity a přístupnosti živin, ale také způsob, doba trvání a kontinuita obhospodařování.

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Submitted 26 February 2019

Revision received 29 January 2020

Accepted 30 January 2020