Plant species diversity and traditional management in Eastern Carpathian grasslands

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1   Executive Summary

Agri-environment payments have been available in Romania since 2007. Payment is available for farmers living in zones classified as High Nature Value Areas in two packages: HNV grasslands and non-mechanized farming. While the system is functioning administratively, affecting hundreds of thousands of farmers and large areas of land, the biodiversity benefits are not fully understood. No distinction is made in the scheme, for example, in the amount of payment for pastures and meadows, while the management criteria are clearly different.

Also in 2007, implementation of the Natura 2000 system started in Romania. Natura 2000 sites have so far been designated in two waves, and as a result, more than 20% of the country is now designated as part of the network. Several managed grassland habitats are listed as priority habitats including mountain hay meadows. Compensatory payments related to the Natura 2000 network are available in Romania, as the elaboration of management plans is under progress. The management of Natura 2000 grassland habitats is currently done for free by rural communities over large areas of the country.

In the mountainous areas of Transylvania, abandonment of grasslands is a serious threat to biodiversity. Secondary succession towards forests may result in massive loss of meadow species. Yet, no quantitative biodiversity assessment has been conducted so far to document the plant species diversity of these traditionally managed grasslands. Abandonment is a delicate issue for biodiversity management, because it is very difficult for either government or NGOs to deal with the problem.

Another threat to biodiversity is an increase in the number of sheep and the conversion of former hay meadows to sheep pastures. While we acknowledge that mismanagement (e.g., use of hay meadows as pastures) may result in species loss or structural changes in the vegetation, there is no comparative biodiversity data in the region on which we can build conservation arguments. The present study addresses this issue by assessing the effect of two land use types - grazing and mowing - on plant diversity.

The results show important species loss following abandonment. The severity depends on the vegetation type, being higher in vegetation dominated by tor-grass (*Brachypodium pinnatum*). Clear differences in plant diversity patterns were revealed between hay meadows and pastures, hay meadows being richer and more evenly structured. Moreover, high altitude hay meadows are more diverse than the low altitude meadows. It is noteworthy that other abiotic conditions (moisture, slope, heat load) are additional modulators of floristic diversity in this area. In the light of our results, maintenance of traditional land-use practices is likely the most effective tool of biodiversity conservation in the area.

On the basis of plant diversity patterns (an ecological deliverable from land management), we therefore recommend that a distinction be made between hay meadows and pastures in agricultural policies, i.e. that higher payments are available for hay meadows. Otherwise there is a risk of conversion from hay meadows to pastures. On the other hand, the problem of abandonment should be addressed directly by agri-environment policies, with a shift in payment logic away from compensation for loss towards the additional cost of continuing management.

2   Introduction / Background

The abandonment of traditional management on European mountain meadows over the last decades has triggered rapid species loss and an increased interest in understanding biodiversity maintenance processes (MacDonald et al. 2000, Lemaire et al. 2005, Taff et al. 2010). It is now generally accepted that management-maintained grasslands are in dynamic equilibrium, and that changing the management regime introduces important shifts in nutrient conditions, disturbance and stress regimes (Grime 2001), which modulate competition-colonisation processes (Tilman 1990) and ultimately alters functional composition (Kahmen and Poschlod 2008, Drobnik et al. 2011) and species diversity (Jacquemyn et al. 2011).

Grazing is considered as an important form of disturbance (Grime 2001), being one of the best evaluated types of land use in terms of effect on species richness (Olff and Ritchie 1998) and plant traits (Diáz et al. 2007). The effect of herbivory on plant diversity boils down to the modulation of colonisation and extinction processes, caused primarily by disturbance. Colonisation is enhanced by the higher availability of regeneration microsites after trampling and higher propagule dispersal rates through zoochory, and is counteracted by the removal of reproductive structures of plants or mechanical damage to seedlings.

Extinction is prevented by the relaxation of competitive exclusion processes through the defoliation of dominant species and increased environment heterogeneity (soil disturbances, selective grazing) (Olff and Ritchie 1998, Adler et al. 2001), but is enhanced under high grazing pressure (lower survival chances of species that lack mechanisms of herbivory avoidance or tolerance). Moreover, nutrient deposition may enhance localised but frequent competitive exclusion processes (Adler et al. 2001).

Added to that, grazing animals cause a series of additional disturbances which are often overlooked in models of grazing impact on diversity. For instance, trampling by animals can destroy soil porosity and increase soil density through compaction and homogenisation (Abdel-Magid et al. 1987, Pietola et al. 2005) which may affect root development in plants. They may also cause a reduction in infiltration capacity (puddling effect), which can cause a concentration of nutrients (e.g. phosphorus from animal excreta) in the topsoil, and might interfere with seed germination. Documented trampling impacts are mechanical injury of stalks and roots and diminished shoot regeneration (Abdel-Magid et al. 1987, Pietola et al. 2005, Dunne et al. 2011), seedbed disturbance, e.g. increased seed burial (von Winkel and Roundy 1991), increased detachment of dead biomass and erosion (Abdel-Magid et al. 1987), etc. By necessity, the ability of vegetation to regenerate quickly (i.e., climate conditions and/or productivity), and the history and intensity of disturbance will impact on species diversity in pasture (Milchunas et al. 1988, Olff and Ritchie 1998). In sub-humid grasslands with a short history of herbivory, a rapid increase in diversity would be expected at low to intermediate grazing intensities and a rapid decline thereafter, as grazing mortality exceeds colonisation or boosts co-existence mechanisms (Milchunas et al. 1988).

In contrast to grazing, mowing generally causes less pressure on grassland ecosystems, mainly through even defoliation and the removal of nutrients. Colonisation is favoured on the newly-opened regeneration microsites, which enhance recruitment, and is counteracted by the total removal of reproductive structures. Competitive exclusion processes weaken as nutrients are gradually depleted as biomass is removed, enabling the persistence of weak competitors (Grime 2001, Tilman 1988, Tilman 1997). Biomass removal also creates more symmetric light competition conditions and enhances survival of shade-intolerant species (Zobel 1992). Fast growing and early season species that complete their life cycle

before mowing will also be favoured, which in turn will speed up nutrient recycling. As might be expected, mown areas are generally more diverse than pastures under similar land-use intensities (Hansson and Fogelfors 2000, Maurer et al. 2006), but exceptions are also recorded.

The abandonment of grazing or mowing is the main threat to the biodiversity of temperate humid grasslands, where forests are the climax vegetation. The cessation of disturbance impedes colonisation because a closed canopy prevents access of propagules to regeneration microsites, which also become unsuitable as litter accumulates. Moreover, light stress raises the mortality rates of heliophilous species (Kahmen and Poschlod 2004). Competitive exclusion processes are enhanced by the decreased nitrogen availability resulting from slower mineralisation and recycling, which favours the dominance of nutrient-conservative grasses (Robson et al. 2007). Reforestation and the decline of grassland species are gradual, with an early stage of only quantitative changes, when species richness is constant or even increases (Hansson et Fogelfors 2000), followed by a short period when fast-growing species able to take up external resources rapidly dominate before these are finally replaced by slow-growing species that manage resources more efficiently (Odum 1969, Tilman 1990, Garnier et al. 2004).

In addition, the impact of management depends upon the number of propagules available for colonisation from the regional species pool (Zobel et al. 1998), to which abiotic conditions and land-use history (Gustavsson et al. 2007, Reitalu et al. 2010) both contribute (Klimek et al. 2007). Nevertheless, the detail of how species diversity is modulated by the combined effects of management and environment is largely unexplored (Lavorel et al. 2011).

From another perspective, the functional character of dominant species is critical to species diversity (Emery and Gross 2007, Gilbert et al. 2009) and community stability (Sasaki and Laurenroth 2011). In communities governed by competition-colonisation (Tilman 1994) the best competitors will dominate, whereas good colonisers will play subordinate roles. A higher biomass of dominant species may also prevent colonisation processes and lower diversity (Gilbert et al. 2009). In other communities, dominant species increase diversity by facilitating effects (Callaway 2007). Consequently, understanding the diversity of management-maintained ecosystems means first and foremost identifying the functional role of dominant species and the effect of altered dominance hierarchies on species interactions (Chaneton and Facelli 1991) and ecosystem processes (Mokany et al. 2008, Laughlin 2011). Comparative studies of management impact on diversity of communities where dominants play contrasting functional roles are also scarce (Souza et al. 2011).

Very few studies have assessed the effect of land use and abandonment on species diversity in the Romanian Carpathians (Nagy et al, 2003, Coldea and Cristea 1998, Baur et al. 2006, Baur et al. 2007, Başnou et al. 2009) and to the best of our knowledge, only one relates to the Eastern Romanian Carpathians (Bărbos 2007).

Even fewer considered environmental factors as correlates of the management regime on diversity of Carpathian grasslands (Bărbos 2007, Başnou et al. 2009) and only Bărbos (2007) touched on the effect of abandonment on the diversity of different types of hay meadows in mountainous areas. Also, there are very few published data that allow the quantification of grassland abandonment in Romania, although empirical observations show that abandonment affects huge amount of land all over the country. Parallel with the present study, our team mapped mown and unmown meadows in the study area, showing

an alarming degree of abandonment in the case of mountain hay meadows (Demeter and Kelemen 2012).

In this study we highlight the variability of floristic composition in different localities, vegetation types and management regimes, and the presence of protected plant species in each study site. We assess the impact of abandonment on diversity of plant communities, as opposed to the effect of different management regimes (grazing and mowing). We also test whether the differences are traceable to abiotic environmental factors rather than to management. In addition, we assess whether the impact of management and abandonment on diversity is dependent upon the functional identity of dominant species. Finally, we summarise important messages that arise for the conservation and management of the grasslands under consideration.

3 Methods

3.1 Site description

The study was conducted in the Csík Mountains (Romanian: Munţii Ciucului) and the adjacent Csík Basin (Depresiunea Ciucului) in the Eastern Carpathians, Romania (Fig. 1), at altitudes between 671-1369 m. The climate is boreal-mountainous, with the average total annual precipitation ranging between 580 mm in the Csík Basin and 1000-1200 mm at high altitudes (Kristó 1994). The geology of the area is a mixture of volcanic rocks and alluvial sediments in its western part, and Mesozoic flysch in its central and eastern parts (Fig. 2).
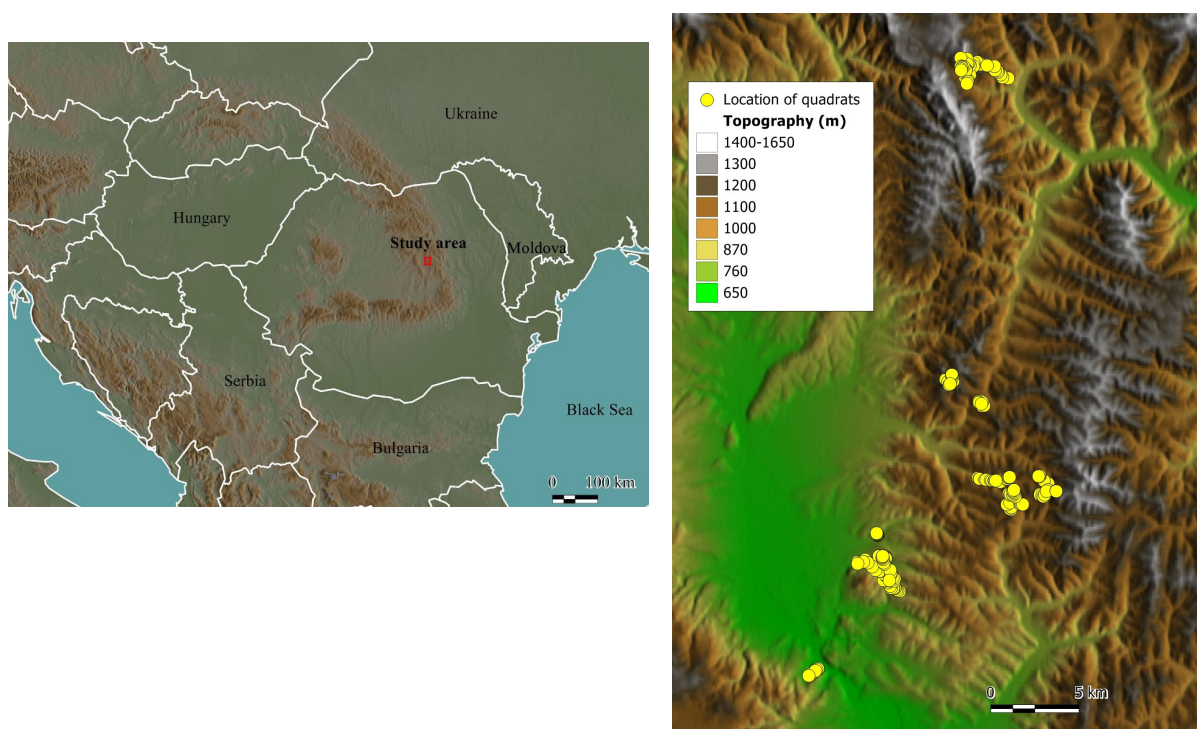

Figure 1: Left: Study area (red rectangle) in a digital elevation model of South-Eastern Europe. Right: The location of the sampled quadrats in the Csík Mountains (Munţii Ciucului).

The vegetation is a mosaic of spruce and mixed spruce forests with meadows and pastures, and arable fields. Three types of grasslands are more widespread in the area: wet lowland hay meadows dominated by meadow foxtail (*Alopecurus pratensis*); mesophilous mountain hay meadows co-dominated at lower elevations by red fescue (*Festuca rubra*) with common bent (*Agrostis capillaris*) and at higher elevations by Alpine Chewing's fescue (*Festuca nigrescens*) with *Agrostis* and meso-xerophilous hay meadows dominated by tor-grass (*Brachypodium pinnatum*) at both higher and lower elevations. The four dominant species display contrasting resource allocation: *Brachypodium pinnatum* and *Alopecurus pratensis* develop runners and extravaginal tillers, allocating more to rhizomes, whereas *Festuca rubra, F. nigrescens* and *Agrostis capillaris* develop small tussocks and

intravaginal tillers, allocating more to seed than to roots. The first two species are therefore the competitor and late-successional species whereas *Festuca* spp. and the co-dominant *A. capillaris* are the "coloniser" types in the light of competition-colonisation trade-offs (Tilman 1990).
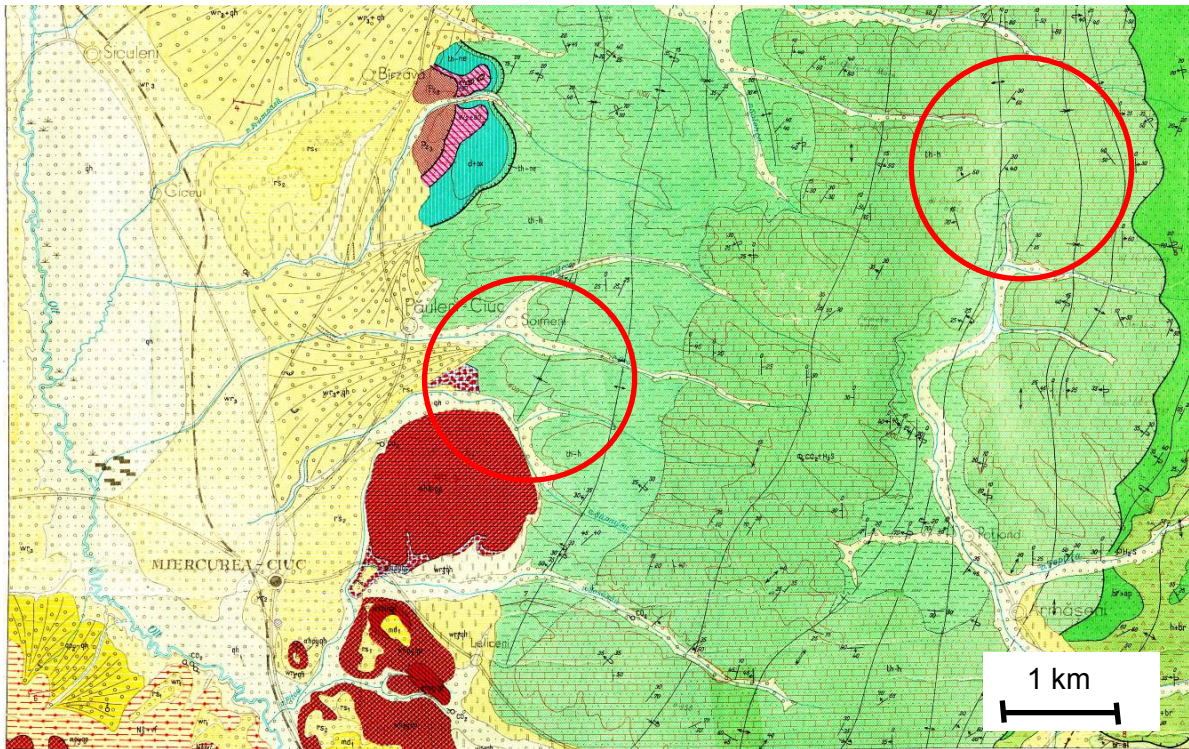

Figure 2: Geological map of a part of the study area. Dark red: Pliocene and Pleistocene volcanic; yellow and white: Pliocene and Pleistocene alluvial deposits; green: Mesozoic flysch; blue-pink-brown: dolomite. Red circles show the 2 main sampling areas.

The grasslands were formed following almost complete deforestation of deciduous forests at lower altitudes (below 900 m) and partial deforestation of spruce or mixed spruce forests at higher altitudes (Csűrös et al. 1980). The history of land use mirrors that of many other areas of Romania (Cioacă and Dinu 2010), but in remote mountainous areas deforestation occurred rather late, following the appearance of human settlements in the 13th century (Molnár and Babai 2009). The foothills of the Csík Basin were terraced at some unknown time, probably starting in the Middle Ages as in other areas of Romania (Cioacă and Dinu 2010), but especially from the 18th century onwards. The terraces were ploughed until the second half of the 20th century. The use of the grasslands saw little change during the second half of the 20th century, except for on the terraces of low-altitude sites in the Csík Basin, where hay making was abandoned. However, since the breakdown of the socialist system two decades ago large areas of grassland have been abandoned (Demeter and Kelmen 2012) and large parts of the landscape are currently experiencing secondary forest succession, as described by Taff et al. (2010).

Figure 3: Landscape at site 1, with great burnet (*Sanguisorba officinalis*) in the foreground
and terraced hillside in the distance.

Figure 4: Landscape at Site 2. Note the patchwork of woody and herbaceous vegetation.

Figure 5: Landscape at Site 3. In the centre, a parcel mown with a small two-wheeled mowing machine.
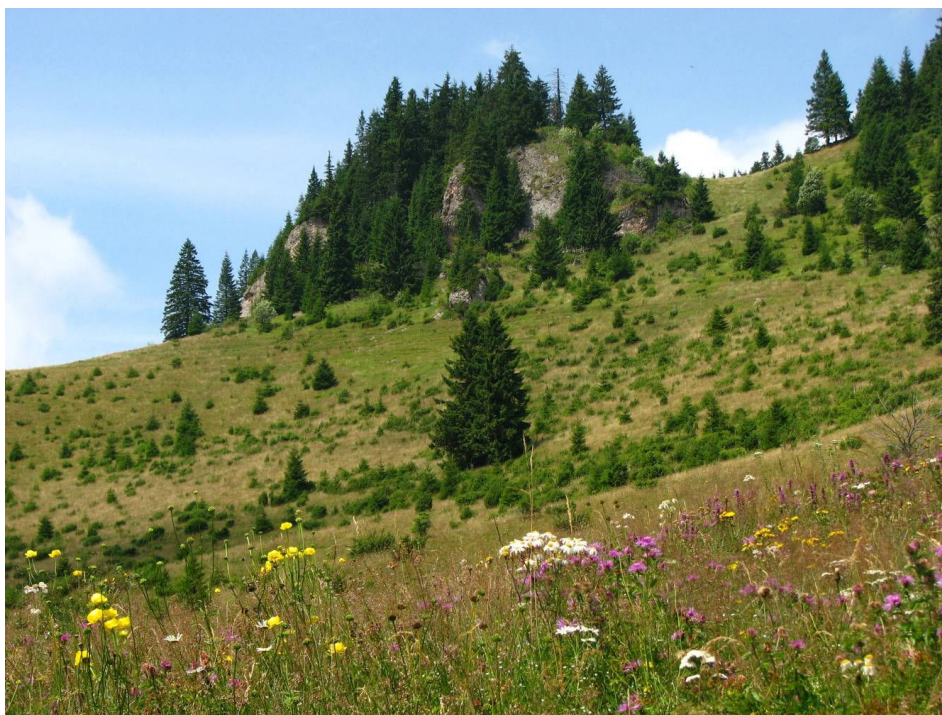

Figure 6: Landscape at site 4. Hay meadows in the foreground, cow pastures behind, under a dolomite outcrop.

All the sampling sites are part of a recently designated Site of Community Interest, ROSCI0323 (Csergő et al. 2011, Romanian Environment Ministry Order nr. 2387/2011).

The main features underlying the designation are different types of species-rich grasslands[1].

3.2 Sampling procedure

A 45 km transect was undertaken in a roughly north-south direction in an area where hay meadows are a dominant landscape feature, and there is a high variation of typical geomorphology, geology and management. The combinations of two factors, management regime with three variants (mown, grazed, abandoned but previously mown) and vegetation type with three variants (*Alopecurus*, *Festuca* and *Brachypodium*-dominated) were identified along the transect. Four localities were chosen as replicates. Site 1 (Somlyó and the adjacent Csomortán and Zsögöd, 1.8 km^2) is situated at lower altitude in Csík Basin. Site 2 (Kolos, 1.3 km^2), Site 3 (Pogányhavas, 0.2 km^2) and Site 3 (Jávárdi, 1.2 km^2) are situated at higher altitudes in Csík Mountains (see Fig. 3-6 for photos and Fig. 7-10 for maps).
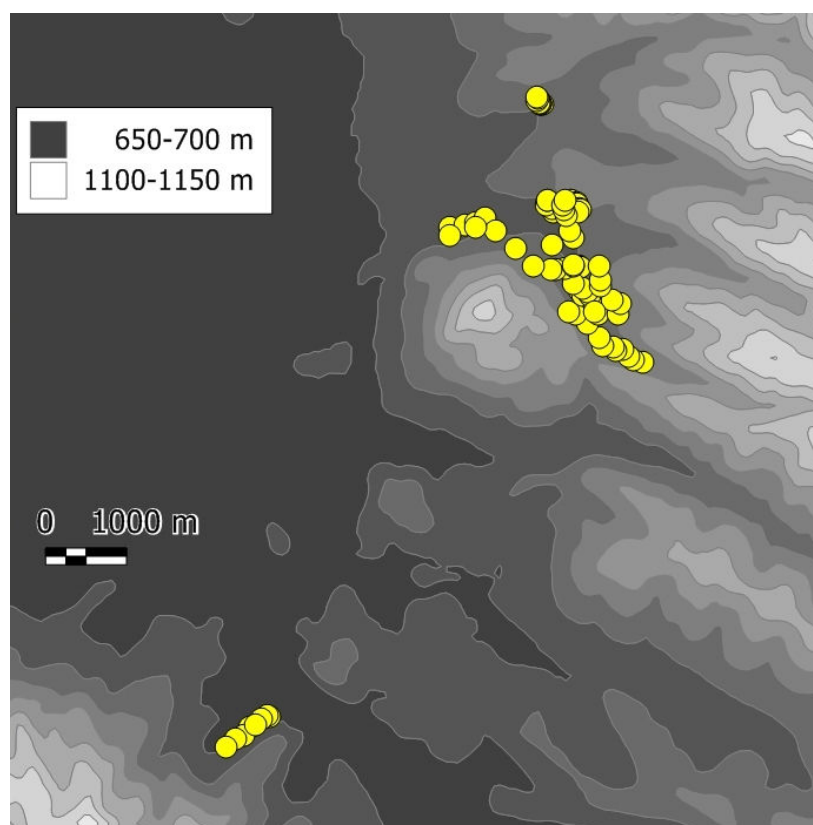

Figure 7:   Topography of Site 1, with the location of the quadrats.

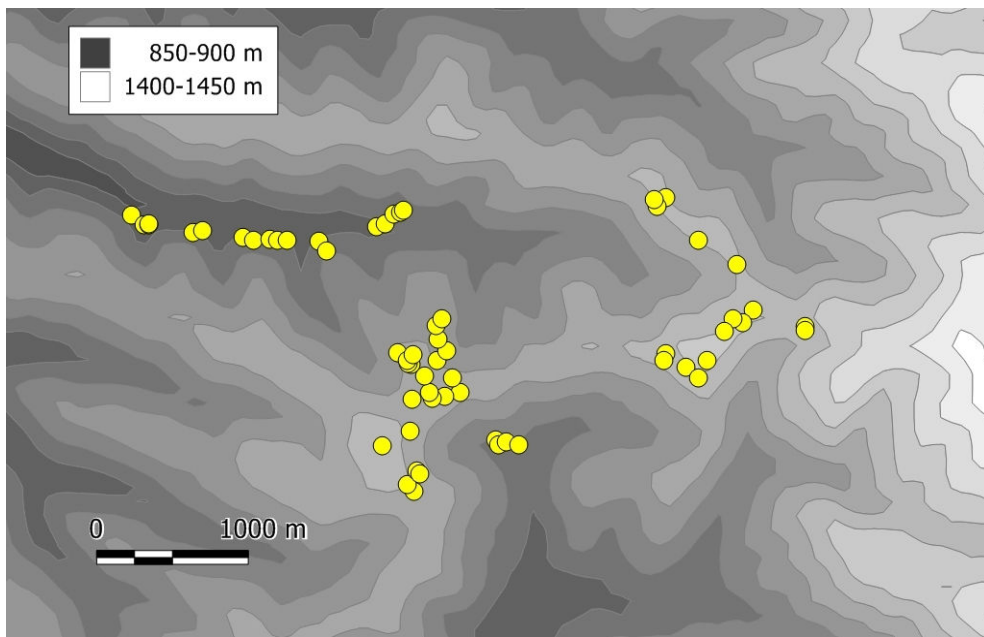[1] http://www.natura2000proposals.ro/images/pdf/muntii_ciucului.pdf

Figure 8: Topography of Site 2, with the location of the quadrats.
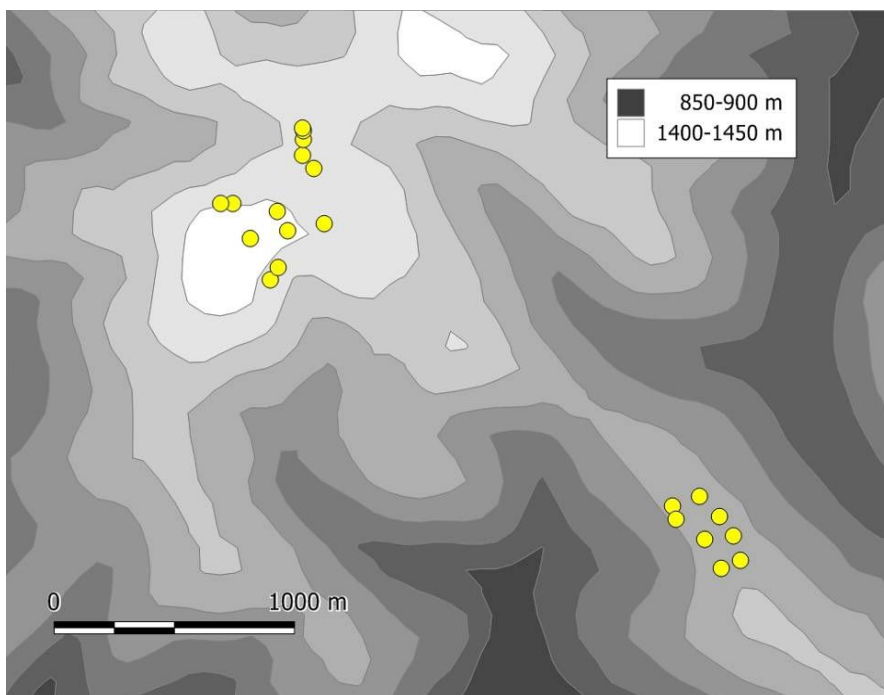

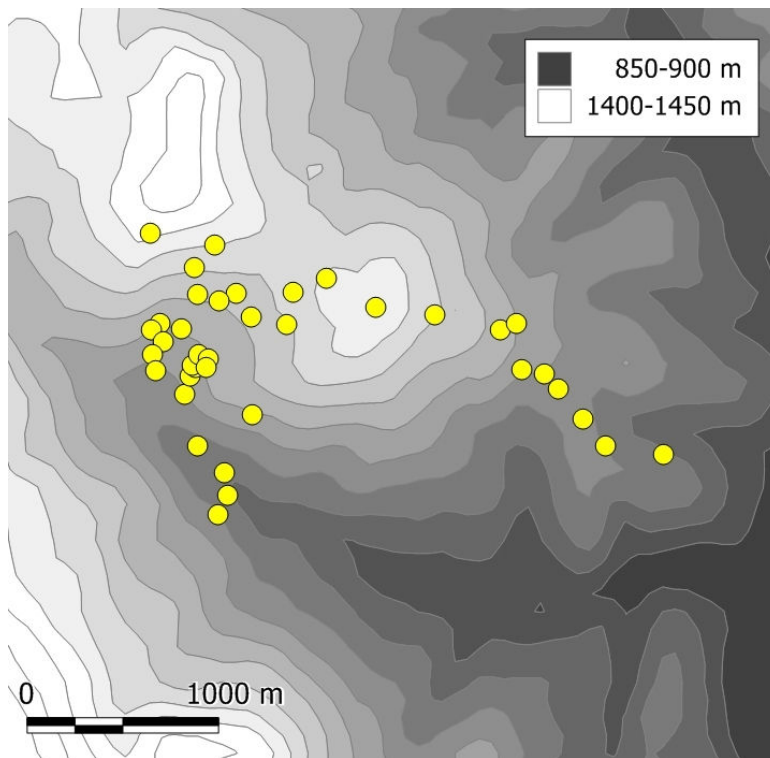Figure 9: Topography of Site 3, with the location of the quadrats.

Figure 10: Topography of Site 4, with the location of the quadrats.

Lowland hay meadows were found only in the Csík Basin (Site 1) and were either mown or abandoned. Grazed parcels were located only on *Festuca* meadows within the study area. A few other combinations occurred in only some of the localities and thus the number of replicates finally varied between two for lowland meadows (Site 1) and three to five for other meadows (Site 1-4). Each available combination of factors was sub-sampled with 4-13 1 m^2 pseudo-replicate plots placed randomly along the transect, totalling 196 plots. Individual species were identified and their abundance was visually estimated in each plot (expressed as percentage cover). Total vegetation cover was estimated visually and vegetation height was measured with a ruler. Topographic parameters such as altitude, slope and aspect were also recorded for each plot using a handheld GPS and visual estimations.

3.3 Data analysis

The variability in floristic assemblage determined by the local environment and management was first quantified using canonical correspondence analysis (CCA, Ter Braak 1986). Rare species occurring in less than 1% of the plots were eliminated due to method constraints (Ter Braak et Šmilauer 1998) and abundance data were square root transformed, to dampen the effect of dominant species. Explanatory environmental variables (elevation, heat load, slope) were not transformed for the purpose of this computation and were introduced to the model with forward selection. Because we only used three environmental variables, only the first three axes of the CCA were canonical,

and the forth axis remained unconstrained. Analyses were made using Canoco 4.5 software (Ter Braak and Šmilauer 2002).

Species diversity was expressed in terms of species density (number of species per 1 m^2 subplot) and species evenness (E_{var} formula from Smith and Wilson 1996). An Analysis of Covariance was run in SAS (Version 9.2) to obtain differences in density and evenness between different management regimes and meadow types after correction for altitude. Because of the incomplete set of combinations of the two factors along the transect, the dataset was arranged in two different scenarios, as follows:

- *model 1:* mown vs. formerly mown (abandoned) *Alopecurus*, *Festuca* and *Brachypodium* meadows (factors: management regime and vegetation type, five localities as replicates, 152 pseudo-replicates)

- *model 2:* mown vs. formerly mown (abandoned) vs. grazed *Festuca* grasslands (factor: three localities as blocks, management regime, 85 pseudo-replicates).

To obtain an estimate of total species richness in each locality and each type of managed meadow, expected species accumulation curves (sample-based rarefaction curves) were computed from the presence-absence floristic matrix, using the bootstrap method (Colwell et al. 2004) in Estimate S (Colwell 2009). Because each locality was represented by a different number of plots, the expected number of species was compared on the steepest part of all accumulation curves. This part of the slope is a measure of the inter-plot diversity in a given region (Olszewski 2004).

To model the relationship between the diversity estimates and other variables (vegetation height as a surrogate of light conditions, vegetation cover, elevation, slope, and aspect), multiple linear regressions were performed.

4 Results

4.1 Species composition

A total of 334 species were identified. In the Canonical Correspondence Analysis, the first three (constrained) axes explained 7.3% and together with the fourth (unconstrained) axis explained 13.2% cumulative variance of species data (Fig. 11).

The first axis represented a strong (canonical eigenvalue=0.424, Fig. 11) elevation gradient and separated all low altitude plots of Site 1 from the high altitude plots (Sites 2-4), regardless of dominant species identity and management regime (Fig. 12, 13). High elevation mesophilous grasslands were represented by a series of high altitude (*Phleum alpinum, Ranunculus oreophilus, Campanula polymorpha* ssp. *rotundifolia*), acidophilous (*Botrychium lunaria, Potentilla aurea, Vaccinium myrtillus*), endemic (*Hypericum richeri* ssp. *transsilvanicum, Viola jooi, Centaurea kotschyana*) and forest (*Melampyrum sylvaticum, Lilium martagon*) species.

The second axis (canonical eigenvalue=0.174, Fig. 11) represented a heat load gradient, which contained a variety of management regimes and meadow types from different localities (Fig. 13). We could not interpret the third axis, which explained a low amount of variance (canonical eigenvalue=0.064, Fig. 11). Interestingly, the fourth axis (eigenvalue=0.534, Fig. 11), which was left unconstrained by environmental variables represented a clear humidity gradient based on species composition (this residual variance in our dataset could not be captured by the canonical axes as no humidity variable was available). This axis contrasted, regardless of management type, moist *Alopecurus pratensis* lowland meadows with dry *Brachypodium pinnatum* meadows, leaving *Festuca* spp. and more mesophilous *Brachypodium pinnatum* meadows at an intermediate position (Fig. 12). Characteristic mesohygrophilous species of lowland meadows were *Lysimachia nummularia, Thalictrum lucidum, Alopecurus pratensis, Cirsium palustre, Polygonum bistorta, Sanguisorba officinalis*, etc. Xeromesophilous species of dry *Brachypodium* communities were *Campanula sibirica, Aster amellus, Cytisus nigricans, Galium glaucum, Linum flavum, Centaurea micranthos, Sanguisorba minor*, etc.

Of the total variance of species-environment relation, elevation explained 41%, followed by slope with 16% and heat load with an additional 9% (conditional effects, F test, p<0.002) (Fig. 11).
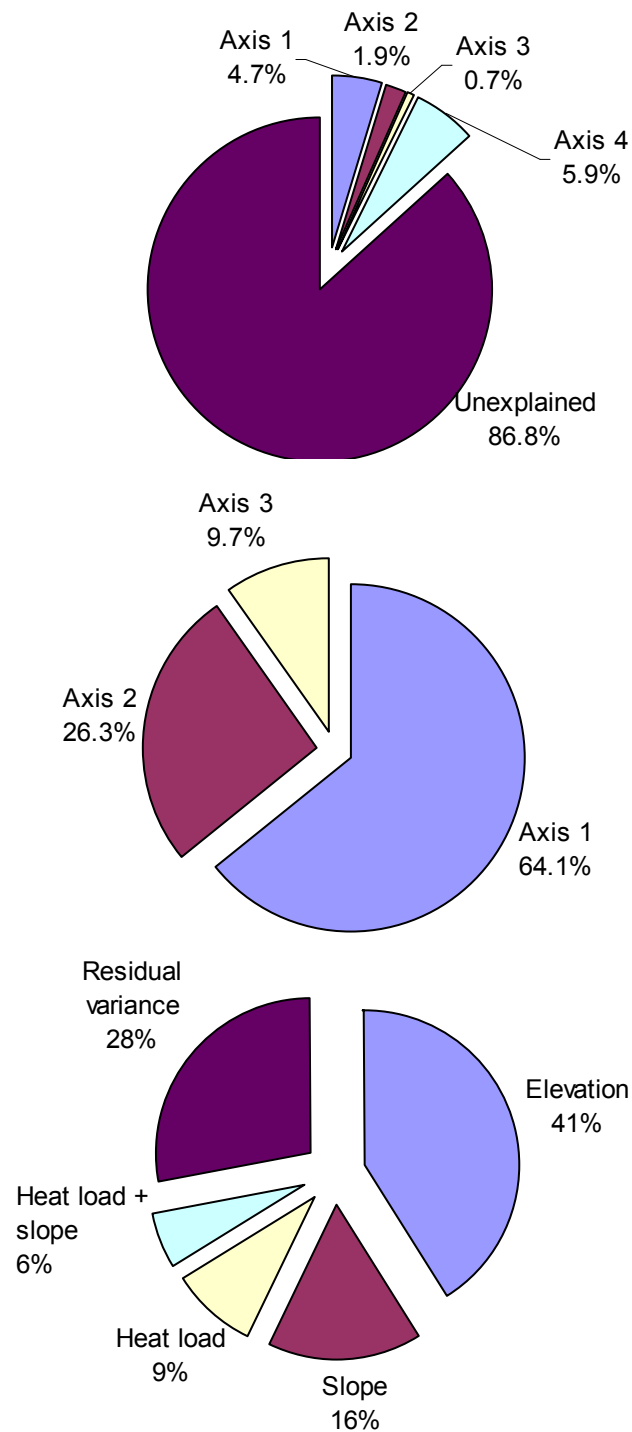
Figure 11: Percentage variance of species data explained by the four axes (top), percentage variance of species-environment relation explained by the first three constrained axes (middle) and the species-environment variance partitioned between the three environmental variables taken into consideration (bottom).
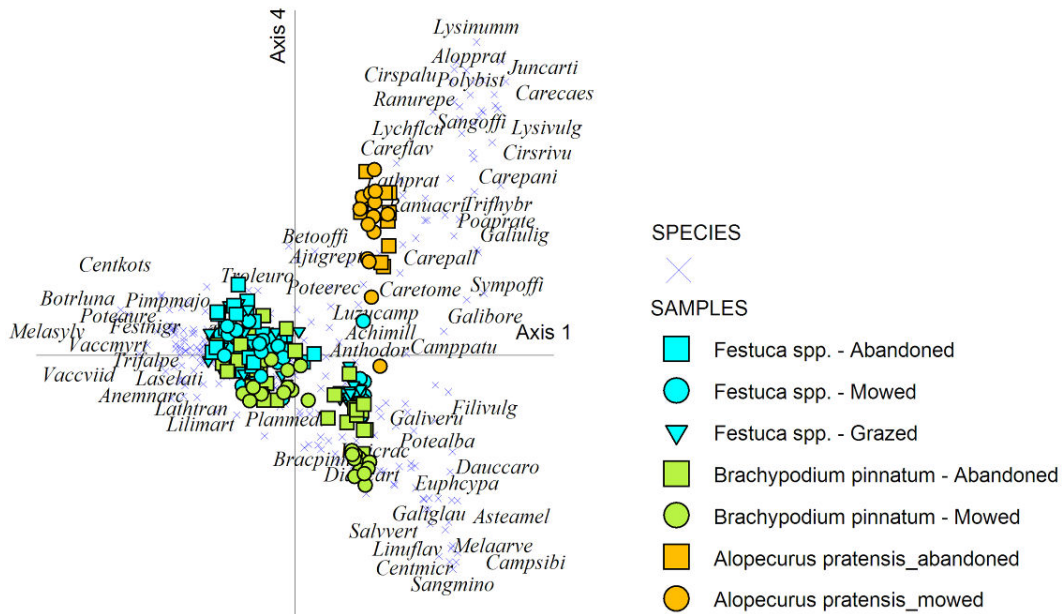
Figure 12: Axes 1 and 4 (unconstrained) of the Canonical Correspondence Analysis of 1 x 1 m^2 plots in different communities under different management regimes.
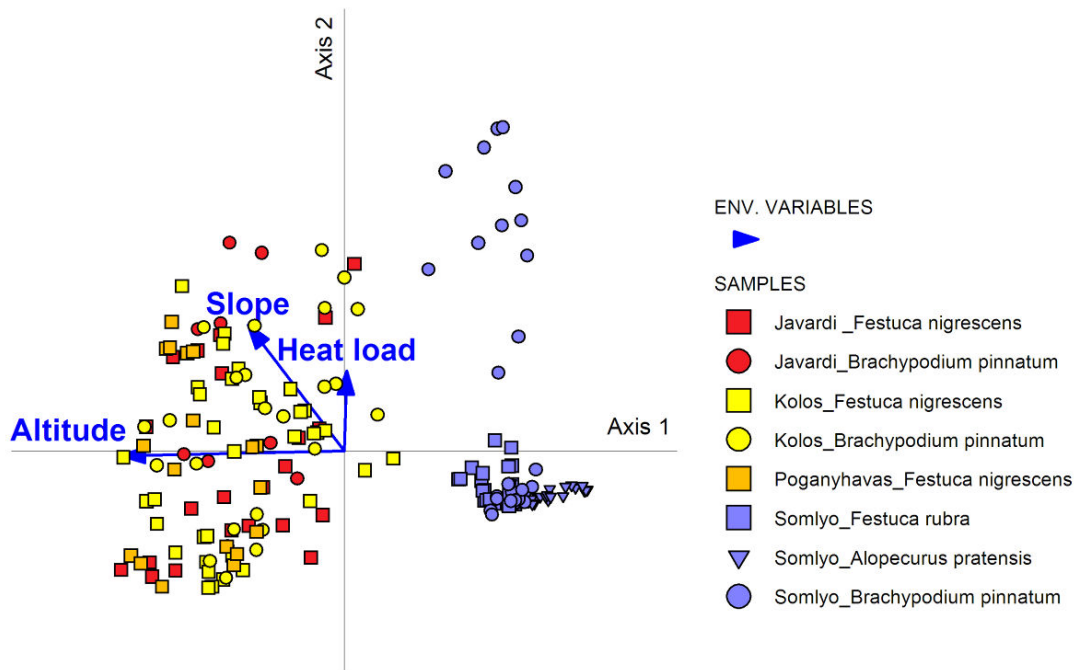
Figure 13: Axes 1 and 2 of the Canonical Correspondence Analysis of 1 x 1 m^2 plots in different communities under different management regimes.

4.2   Species diversity

Generally, mountain meadows (*Festuca* and *Brachypodium*) supported a higher number of species than lowland (*Alopecurus*) hay meadows (Fig. 14, top). Species density ranged

between 12 species/m^2 in an abandoned *Brachypodium* meadow and 50 species/m^2 in a mown *Brachypodium* meadow. The mean number of species was the highest in mown *Festuca* grasslands (mean±SD = 38.1±6.5) and the lowest in abandoned *Alopecurus* grasslands (mean±SD = 24.8±6.1).

Management regime, vegetation type and locality exerted significant effect on species richness after correction for elevation (*model 1*: F test, p<0.001, $p_{vegetation\ type}$=0.043, $p_{management\ regime}$=0.047; *model 2*: F test, p<0.001, $p_{management\ regime}$=0.016, $p_{locality}$=0.022). In model 1, abandonment of hay meadows resulted in a significant overall decrease of species number, from 35.0±7.9 to 29.9±7.5 (mean±SD). Species loss was significant in *Festuca* meadows (average 4.1 species lost), double that much in *Brachypodium* meadows (average 8.1 species lost), and insignificant in lowland meadows (average 0.8 species lost).

Festuca grasslands had on average 36.1±6.5 (mean±SD) species; *Brachypodium* meadows had 31.2±8.4 (mean ±SD) species and lowland meadows had 25.3±5.5 (mean ±SD) species, i.e., *Festuca* grasslands had overall 10.8 species more than lowland meadows and 4.9 species more than *Brachypodium* meadows. In model 2, grazing decreased the species richness in *Festuca* grasslands with an average of 6.6 species compared to the mown and 1.7 species compared to the abandoned *Festuca* grasslands. Still, grazing maintained a higher number of species (30.4 ±5.9) than abandoned *Brachypodium* (27.5±7.5) and lowland *Alopecurus* (24.8±6.1) meadows (Fig. 14 above). In the low elevation Site 1, species richness of *Festuca* grasslands was significantly lower than that of its higher altitude counterparts (Bonferroni-corrected post-hoc t test, p<0.021).

Species evenness showed a similar pattern, being generally higher in mountain (*Festuca* and *Brachypodium*) than in lowland meadows (Fig. 14 below). Values ranged between 0.179 in a grazed *Festuca* meadow and 0.433 in an abandoned *Festuca* meadow.

Only vegetation type had significant effect on species evenness (model 1: F test, p<0.001, $p_{vegetation\ type}$<0.001, model 2: F test, p=0.133). Overall, there was significant difference between each type of grassland, with *Festuca* grasslands having higher values (mean±SD = 0.268±0.05) than *Brachypodium* (mean±SD = 0.244±0.04) and *Alopecurus* (mean±SD = 0.215±0.07) meadows (Bonferroni-corrected post-hoc t tests, p<0.030). There was no significant overall difference between mown (mean±SD = 0.251±0.05) and abandoned (mean±SD = 0.248±0.05) meadows in terms of species evenness, values being almost unchanged in *Festuca* meadows, dropping in *Brachypodium* meadows and raising in lowland hay meadows following abandonment (Fig. 14 below). Grazing produced lower evenness (mean±SD = 0.245±0.04) than mowing (mean±SD = 0.271±0.05) and abandonment (mean±SD = 0.267±0.05) in *Festuca* dominated pastures, yet the difference was not significant in this model.

The expected species accumulation curves confirmed the highest species richness of mown *Festuca* grasslands relative to all other types of meadows and management, as opposed to the lowest richness of *Alopecurus* grasslands (Fig. 15). At a scale of 10 m^2 situated on the steep part of the curve, mown *Festuca* grasslands had the highest average of 155.1±6.7 (mean±SD) species, followed by mown *Brachypodium* meadows (mean±SD = 144.0±9.6), grazed *Festuca* meadows (mean±SD = 138.3±9.3), abandoned *Festuca* meadows (mean±SD = 137.0±9.5) and abandoned *Brachypodium* meadows (mean±SD = 135.4±10.5) and finally mown *Alopecurus* meadows (mean±SD = 101.3±6.4) and abandoned *Alopecurus* meadows (mean±SD = 85.5±1.4) which had the lowest number of species. Importantly, the actual difference in species richness between abandoned

Alopecurus meadows and their managed counterpart was much lower than might had been expected (Fig. 14).
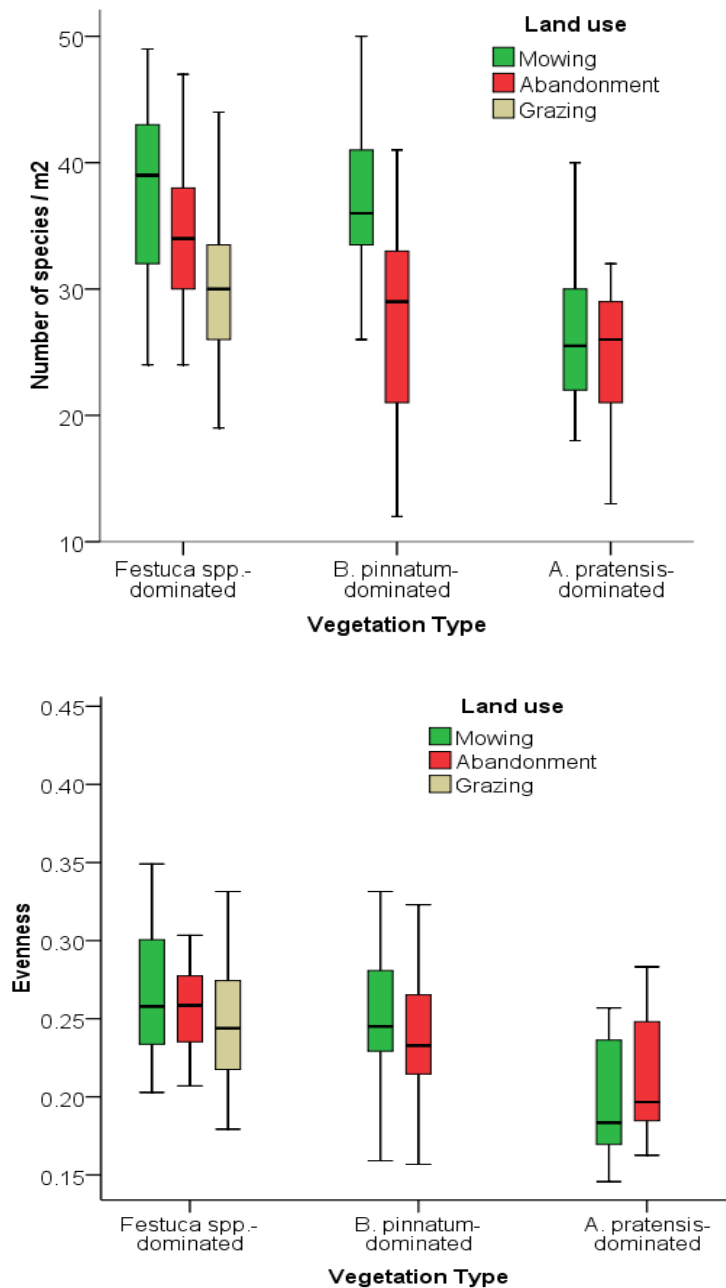
Figure 14: Box diagrams (median and quartiles) of species density (above) and Simpson's evenness (below) for different land-use categories and vegetation types.
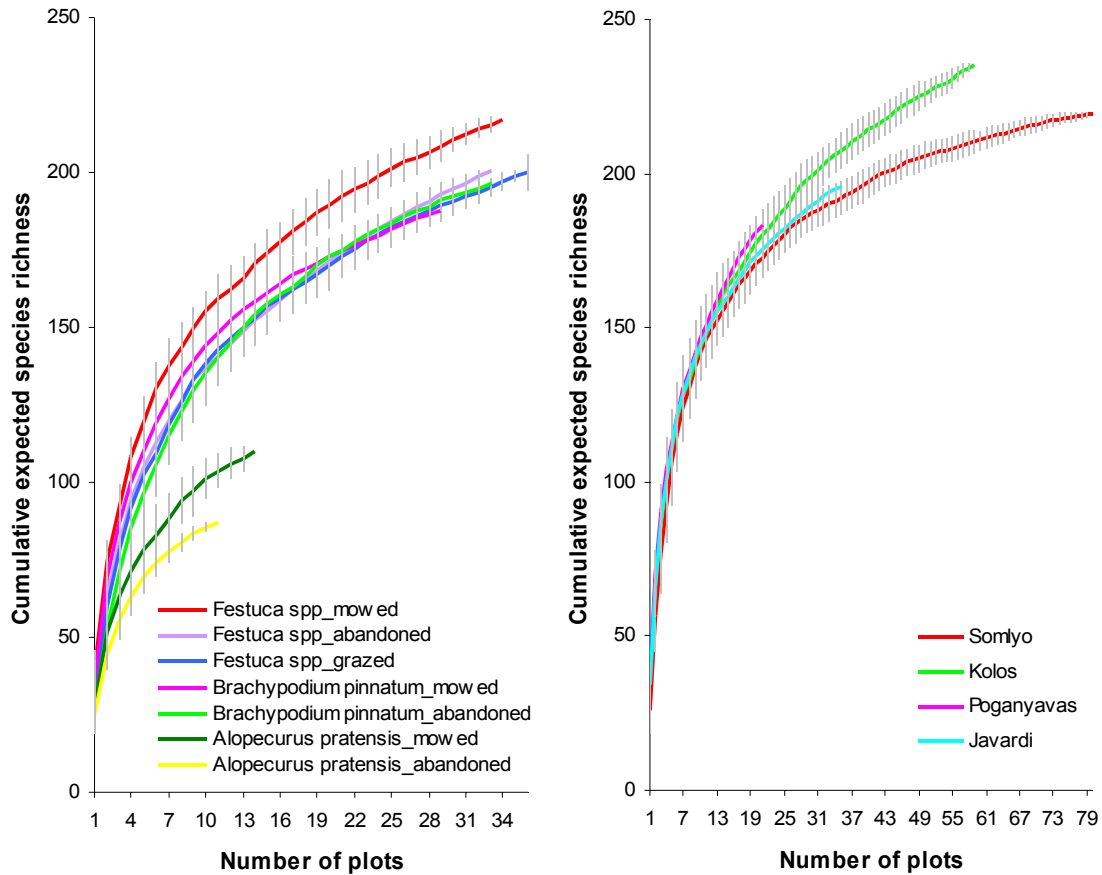
Figure 15: Accumulation curves of grassland richness based on expected (bootstrapped) species number in each combination of meadow type and treatment (a) and in each locality (b). Means and standard deviations are shown at each point.

On the other hand, the overall estimated species pool did not differ significantly between the sites (Fig. 15). At a scale of 10 m^2 situated on the steep part of the curve, Pogányhavas accumulated an average of 146.9±10.4, Jávárdi 144.9±9.2, Kolos 144.2±10.4 and Somlyó 142.2±9.3 species. Overall, Pogányhavas accumulated the highest number of species relative to the sampling effort, and at the scale of 21 m^2 had significantly higher species than Somlyó locality. The total number of observed and expected species for each locality is given in Table 1.

In terms of rare and endemic species, high altitude localities performed better (Table 2). Of a total of 38 species of high conservation value, the highest number was registered on Site 2 (26 species), followed closely by Site 3, whereas in the low altitude Site 1 only eight species of high conservation value were identified. Almost all 12 endemic species occurred at the high elevation sites.

Table 1: Total number of observed and expected species, and sampling effort expressed as number of 1 m^2 plots in each locality

Locality	Observed number of species	Expected number of species	Number of 1m^2 plots
Site 1	204	219	80
Site 2	211	235	59
Site 3	162	183	21
Site 4	177	196	35

Table 2. List of endemic and rare species identified within or close to the study plots in each locality. E = endemic species, R = rare species, according to the Romanian Red List of Vascular Plant Species (Oltean et al. 1994).

No.	Category	Taxon	Site 1	Site 2	Site 3	Site 4
1	E (Carpathian Mts.)	*Aconitum moldavicum*		X	X	X
2	R	*Anemone narcissiflora*		X	X	
3	R	*Aquilegia vulgaris*		X	X	
4	R	*Arnica montana*		X		
5	R	*Botrychium multifidum*				X
6	E (Carpathian-Balkan Mts.)	*Centaurea kotschyana*		X	X	X
7	R	*Cimicifuga europaea*		X		
8	R	*Dactylorhiza maculata*			X	
9	R	*Delphinium elatum*				X
10	R	*Dianthus superbus*	X	X		
11	E (Carpathian Mts.)	*Dianthus tenuifolius*				X
12	R	*Epipactis atrorubens*			X	
13	R	*Epipactis helleborine*		X	X	X
14	R	*Gentiana pneumonanthe*	X			
15	R	*Gentiana acaulis*		X	X	
16	R	*Gymnadenia conopsea*	X	X	X	X
17	E (Carpathian Mts.)	*Hepatica transsilvanica*		X		
18	E (SE Carpathian Mts.)	*Hypericum richeri* ssp.	X	X		
19	E (Alps-Carpathian Mts.)	*Hypochoeris uniflora*		X	X	X
20	R	*Iris sibirica*	X			
21	R	*Listera ovata*	X	X	X	X
22	R	*Lonicera nigra*		X		
23	R	*Malaxis monophyllos*			X	
24	End (Dacic)	*Melampyrum bihariense*		X	X	X
25	R	*Nigritella nigra* ssp. *rubra*		X	X	
26	R	*Ophioglossum vulgatum*				X
27	R	*Orchis mascula* ssp. *signifera*		X		
28	R	*Orchis ustulata*	X	X	X	X
29	E (Carpathian Mts.)	*Phyteuma tetramerum*		X	X	
30	R	*Plantago atrata* ssp. *carpatica*		X	X	X
31	E (E Carpathian Mts.)	*Primula elatior* ssp. *leucophylla*		X	X	X
32	R	*Pulsatilla patens*			X	
33	R	*Ribes rubrum*		X	X	
34	E (Carpathian-Balkan Mts.)	*Silene heuffeli*				X
35	E (Carpathian Mts.)	*Silene nutans* ssp. *dubia*		X		X
36	R	*Traunsteinera globosa*		X	X	X
37	R	*Trollius europaeus*	X	X	X	X
38	E (Carpathian Mts.)	*Viola jooi*	X	X	X	X
Total			8	26	25	19

4.3 Relationship of environmental and biotic conditions to diversity

From the variables taken into account, only altitude and vegetation height (as a surrogate of light conditions within plot) were selected by the model (F test, p<0.001 for both variables). In the univariate models, elevation had a weak positive effect (R^2=176, p<0.001), whereas vegetation height had a weak negative effect on species richness (R^2=0.128, p<0.001) (Fig. 16).
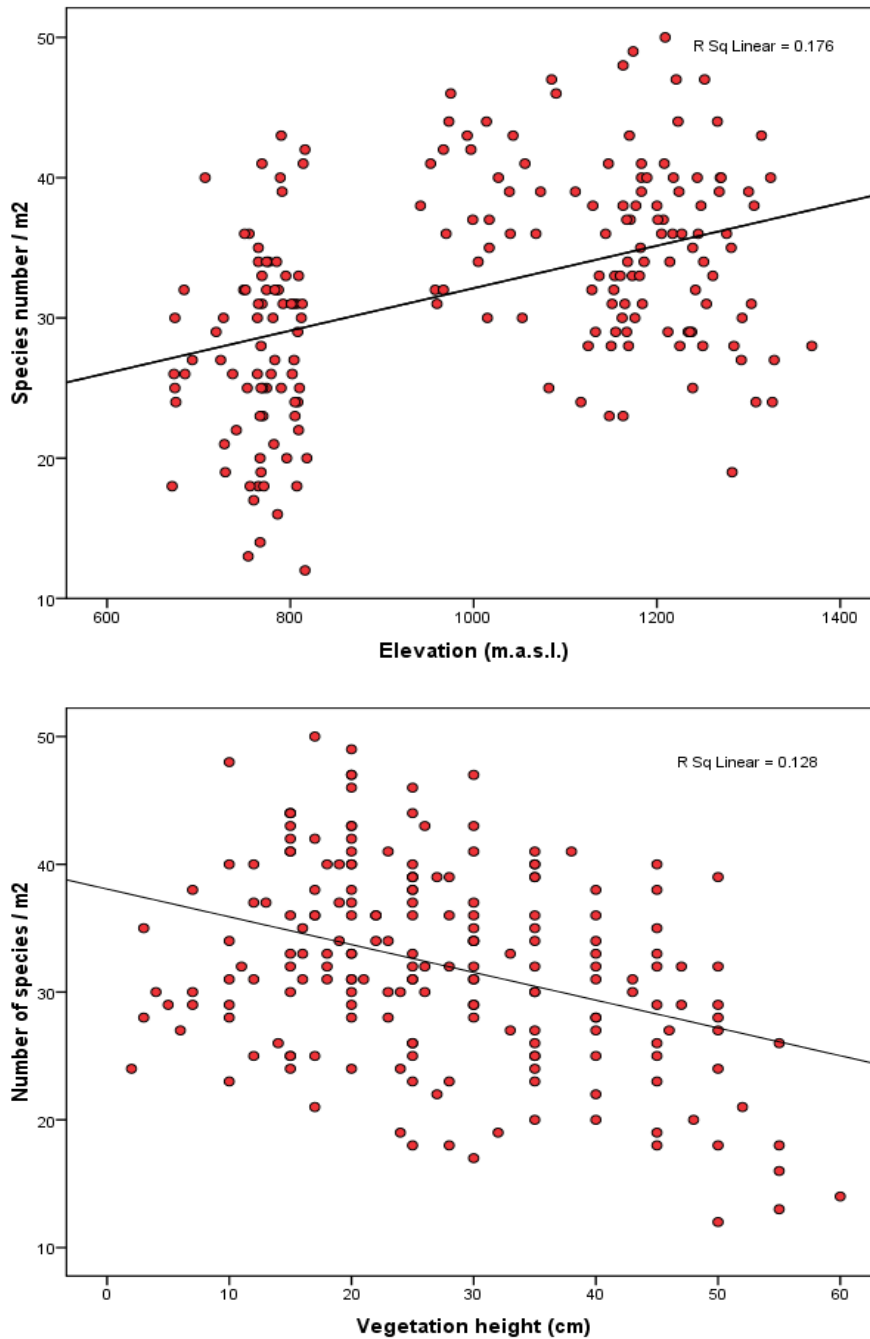

Figure 16: Simple linear regressions showing the effect of elevation and vegetation height on species density (p<0.001 in both cases).

5 Discussion

5.1 Species diversity

The Csík meadows are among the most species-rich managed semi-natural grasslands in Europe (see Annex I for comparable data from the literature). At the scale of our study ($1 \times 1 m^2$), mean numbers of species ranging from 14.5 (Czech Republic, Mašková et al. 2009) to 40.8 (Estonia, Aavik et al. 2008) were reported for managed meadows. Most of the species-rich grasslands reported from Europe had on average about 30 species: 29±4.4, Sweden (Linusson et al. 1998); 32±3, Norway (Losvik 1999); "more than 30 is normal", Netherlands (Bobbink and Willems 1987). By comparison, the species-poor, abandoned lowland meadows in our study area had 24.8±6.1 and the species-rich mown *Festuca* meadows had 38.1±6.5 mean number of species/m^2. So far, the higher average of 38.1±6.5 and the maximum recorded in our study area (50 species/m^2) compares with the most species-rich meadows in Europe (average 40.8, maximum 63 species/m^2, Aavik et al. 2008).

5.2 Effect of management

Mowing maintains the highest grassland diversity in our area. More specifically, mown *Festuca* meadows were by far the most diverse and are expected to provide more species to the regional species pool than any other combination of management and vegetation type. Two major explanations arise: firstly, low competitive ability of *Festuca rubra* or *F. nigrescens* and of the co-dominant grass *Agrostis capillaris* may allow the co-existence of several species according to the "competition-colonization" hypothesis (Tilman 1990); secondly, those meadows benefited from a continuous mowing history over several decades, even centuries, which is likely to have allowed the beneficial "mowing effects" to take effect (low nutrient content of soil; rapid nutrient recycling; even light conditions; open microsites for colonisation). Mowing practice on remote mountain meadows in the area has been rather moderate, taking place at the end of July-beginning of August, and in some years being left fallow - something might have contributed to their high diversity, as has been suggested in the case of other meadows mown at intermediate intensities (Hansson and Fogelfors, 2000). Long-term uninterrupted mowing has similarly produced exceptionally high diversity in Estonian wooded meadows (Aavik et al. 2008).

Figure 17: One of the most species-rich *Festuca* meadows at the high elevation Site 2.

Figure 18: Species-rich mown *Festuca* meadow at the low elevation Site 1.

Grazed *Festuca* meadows had on average 6.6 species fewer than mown sites – a significant difference. Grazing has often been reported to decrease species diversity in the Carpathian Mountains, especially at high livestock density (Puşcaş et al. 2005, Baur et al. 2007, Başnou et al. 2009). Yet, even in the most heavily grazed grasslands of the studied area, species richness was still relatively high compared to that of other European pastures. By comparison, at this study scale, grazing maintained 10 to 20 species in a *Veronico spicatae-Avenetum pratensis* community (Dupré and Diekmann 2001). Overall, grazing maintained an acceptable level of diversity of nearly 30.5 mean number of species/m^2, which is comparable to the intermediate to high diversity values of managed meadows in other European countries (Annex I). We also report here a tendency towards lowered species evenness with grazing, which is an expected result. It is noteworthy that in this study we surveyed meadows subjected to different grazing intensities (light medium-heavy grazing pressure, cow pastures, sheep pastures), which can hide a wider heterogeneity of answers to grazing and even lower diversity values in pastures with high grazing pressure, as reported in the literature (Milchunas et al. 1988).

Figure 19: The effect of grazing on abandoned hay meadows on Site 2: trampling and lowered vegetation height.

Figure 20: Sampling on a grazed plot (sheep pasture) at site 2.

Figure 21: Sheep pasture at Site 2. Note short grass and abundance of unpalatable *Cirsium* and *Carduus* species.

5.3   Effect of abandonment

As expected, abandonment dramatically lowered the species richness of mountain meadows, but not that of lowland meadows. There are a number of possible explanations. *Alopecurus* meadows are nutrient-rich and light-stressed habitats developed on river floodplains, where competitively superior, tall species maintain naturally low species richness. Component species might be already filtered for survival in this competitive environment; hence abandonment effects may take longer to show up. On the other hand, the sampled meadows were closer to human settlements, and abandonment has been more recent (only 2-3 years). However, importantly, the lower expected species pool in abandoned *Alopecurus* meadows suggests significant species loss will occur over the next few years.

Figure 22: Mown lowland hay meadow with *Alopecurus pratensis*, *Sanguisorba officinalis* and *Galium palustre* at Site 1.

By contrast, the decrease of species richness with abandonment in mountain hay meadows is significant. Even the most diverse and most competitively-balanced plant communities lost on average 4 species in a relatively short time period (up to 5 years of abandonment), whereas in competitively-unbalanced communities, species loss was twice as high. One of the immediate causes is increased light competition with the higher canopy, which probably favours competitive exclusion processes and the dominance of tall plants such as *Laserpitium latifolium, Salvia pratensis, Trollius europaeus* etc. or plants with better resource allocation such as *Salix, Populus* and *Corylus* shrubs. The higher

amount of litter observed in abandoned plots most likely prevents seedling recruitment, and is expected to be the most important limiting factor to the biodiversity of the area, as in other similar areas in the Carpathians (Bărbos 2007).

Abandonment of mowing tends to increase species evenness in lowland meadows, which is attributable to the initial "flourishing" of forbs (herbaceous non-graminoids), which would normally be suppressed by mowing. This transient increase of species abundance is often documented in early stages of abandonment (Garnier et al. 2004) and was thus expected. However, there are data in the literature for increased diversity with relaxed mowing intensity (Huhta et al. 2001), which was not the case in our study area. By contrast, in *Brachypodium* meadows species evenness has slightly decreased, which is most often the typical change with abandonment (Aavik et al. 2008), due to increasingly higher cover of dominant grasses. Interestingly, we did not find evidence for changed evenness in *Festuca* grasslands with abandonment, which speaks out again for the lower competitive abilities of dominant grasses and probably easier structural recovery of communities after short-term abandonment.

Mountain hay meadows have been recognised as highly vulnerable habitats to abandonment, which can take a long and uncertain way to recover (Galvánek and Lepš 2008). Other authors have estimated that full restoration of species-rich grasslands can take several decades (Zobel et al. 1996). In our area, the available species pool for recolonisation and restoration of abandoned meadows is still high (334 species in our dataset alone, including 38 rare and endemic species); it is certainly an important germoplasm and seed bank reserve for present and future restoration efforts.

Figure 23: Species-rich hay meadow with *Corylus* bushes at Site 2.

Figure 24: Hay meadow invaded by poplar (*Populus tremula*) at Site 2.

Figure 25: Meadow abandoned for a few years at Site 4: high abundance of the tall forb *Laserpitium latifolium*.

Figure 26: Recently abandoned hay meadow with lush vegetation on Site 4. Roof of a hay barn at top left.

5.4   Effect of abiotic conditions

Recent studies have shown that plant richness varies in response to topography-related abiotic factors like slope or solar radiation (Klimek et al. 2007). We found similar significant effects of elevation and light conditions on diversity, and elevation, slope, heat load and moisture on species composition.

Higher altitude conditions added endemic and rare species, forest and acidophilous species to our system. Likewise, enriching effect of higher altitudes due to biogeographical history was reported from other limestone grasslands (White and Miller 1988). With a high number of species valuable for conservation, the Csík grasslands are representative of the Eastern Carpathian Mountains, which is an important area of endemism and shelters a high proportion of protected species in Romania (Pawłowski 1970, Institutul de Cercetari şi Amenajari Silvice 1996). The presence of forest species may be an indicator of late deforestation at remote high altitude habitats and perpetuation of woodland species from seed bank or reproduction. Moreover, presence of acidophilous species signals transition to *Nardus stricta* communities (some developed from *Festuca* grasslands under heavy grazing) or to sub-alpine heathlands. Lower land-use intensity might add to the species richness of these remote localities, and this seems to be acknowledged as a general pattern (Gustavsson et al. 2007).

Steeper slopes and higher heat load represent the second most important environmental factor in structuring species composition in our area, regardless of management or community type. In the mountain-boreal climate of the Csík region, warmer and most likely

drier micro- and mesohabitats may act as topoclimatic refugia for continental species like *Linum hirsutum, Pulsatilla patens*, *Aster amellus* etc. In the same time, xeric conditions might filter out a number of species, yet this effect was not prominent in our dataset or was not generally valid for all plant communities that we analysed together.

These results highlight two important messages for conservation: both species composition and richness are important facets of biodiversity, and abiotic conditions of the environment should be used to optimise management or restoration strategies.

Figure 27: The beautifully scented *Centaurea kotschyana* is a Carpathian-Balkan endemic species found at high altitudes in the study area (Site 2, 3, 4).

5.5   Effect of dominant species

The character or identity of dominant species (i.e. vegetation type) has a strong effect on diversity of meadows following abandonment, and *Brachypodium* meadows lost significantly more species than *Festuca* meadows. Increased dominance of *Brachypodium pinnatum* in early successional stages of abandoned grasslands has long been recognized as a threat to biodiversity in chalk grasslands of Western Europe (Tansley and Adamson 1926). For example, Bobbink and Willems (1987) reported a dramatic decrease of species richness from 23.4±3.8 to 9.6±3.3 with increasing biomass and cover of *Brachypodium* in 30x30 cm plots in The Netherlands. Shading, litter accumulation and alteration of soil nitrogen content has been put forward to explain these negative effects (Hurst and John 1999).

Figure 28: South-facing steep slopes of terraced areas with the xeromesophilous continental *Aster amellus*.

Stronger clonal propagation of the *Brachypodium* through rhizomes makes it a superior competitor with a greater ability to accumulate nutrients and it is highly likely that as such it promotes nitrogen accumulation in the soil (Hurst et John 1999). Therefore, several authors suggested that simple mowing, grazing or mulching are not enough to prevent further expansion of this grass, and other complementary methods (e.g. removal of litter) are recommended to reduce the nitrogen level in the soil (Bobbink et Willems 1987, Hurst et John 1999). Interestingly, Bărbos (2007) did not consider *Brachypodium* as a threat to biodiversity in Maramureş Mountains of Eastern Carpathians, this community being reported as typical to forest edges and strongly restricted by topographical and nutrient conditions (dry, steep slopes and K^+ content). Although we perceived this general pattern in our region, it is possible that the drier mesoclimate of the Csík Mountains favoured a more widespread occurrence of this meso-xerophilous community. Climate change might further complicate the dominance relationship between these two types of communities in our area in the future.

Figure 29: Species-poor abandoned hay meadow with high cover of *Brachypodium pinnatum* at Site 4.

5.6   Effect of management systems and conservation efforts

Grassland conservation is linked to extensive agriculture all over Europe. Outstandingly within the EU, Romania still has entire communities whose main economic activity is traditional agriculture. These communities often possess high levels of knowledge concerning sustainable grassland management, acquired through centuries of experience. This is one important component of traditional ecological knowledge which has been studied in detail in our study area by Molnár and Babai (2009). This living ecological knowledge of rural populations is a powerful tool for the conservation of species-rich grasslands in the area and probably in the wider region. Mountain meadow management was probably the agricultural activity least affected by the political changes and modernisation trends of the 20th century and, at least in our study area, it preserves many elements of the medieval management and ownership systems. As our study suggests, traditional management is close to ideal management for delivering high plant diversity. Traditional agriculture is a largely self-sufficient sector, producing goods for the economy out of almost entirely local resources. Taken together, these facts suggest that local agricultural practices and traditional ecological knowledge should be taken into account when it comes to agricultural and conservation policies.

In the past half-decade, with the joining of the EU, much more emphasis has been put in Romania on, amongst other things, on conservation and environmental protection. Two significant innovations have been agri-environment subsidies and the Natura 2000 system. While the first is a working system, affecting many farmers and large areas of farmland in the Eastern Carpathians, the second is in its initial phase (Natura 2000 sites have been

designated, but the setting up of functioning management and compensation system associated with them is still a work in progress).

In the Romanian agri-environment payment system, no distinction is made between pastures and meadows in terms of payment levels, and the prescription for management by grazing is easier to fulfil. This is clearly visible in our study area, where many farmers report their mountain hay meadows as pastures. Our study shows that in terms of biodiversity, there are significant differences between pastures and meadows, and a long term shift towards pastures is not the best option for conservation.

The adoption of the Habitats Directive means that the maintenance of mountain hay meadows has become a legal obligation for Romania within SCIs. We consider it a priority to incorporate traditional practices and traditional ecological knowledge into the management plans that are currently being elaborated all over the country. Maintaining traditional or close to traditional agricultural systems is probably the cheapest and most sustainable way to achieve grassland conservation in this area.

6   Conclusions

The hay meadows of the Csík Mountains are among the most diverse in Europe from a plant perspective, and are therefore a natural value that should receive increased attention.

Our data show significant differences between the plant diversity of the same types of grasslands under different management regimes. Grasslands managed as hay meadows using traditional techniques (late mowing, no fertilisation, no surface seeding, little mechanisation) are superior from the plant diversity point of view to grasslands managed as pastures. This has implications for agri-environment policies. Current policies offer the same amount of payment for mowing and grazing in two packages (HNV grasslands and non-mechanised management). While the labour requirement of mowing is much higher, although concentrated in a shorter period of the growing season, it requires no additional cost to apply for the non-mechanised package in the case of the grazing as opposed to mowing.

Localities at higher elevation (Sites 2-4) are the most diverse and shelter an overwhelming percentage of endemic and protected species. This pattern may be explained by both natural processes and lower land-use intensity in these remote areas. Consequently, such localities should be the primary focus of conservation efforts. On the other hand, local topography modulates floristic composition of hay meadows, which suggests that at least major mesotopographical units (combinations of elevation and slope) should be selected for focused conservation efforts.

In the light of our results, abandonment of traditional land-use in this region causes loss of species richness more from *Brachypodium pinnatum* grasslands than from the species-rich *Festuca* spp. meadows before reforestation occurs. We suspect that *Brachypodium* is gaining terrain over *Festuca* grasslands following abandonment, probably in areas with drier meso- and microclimates. Although less widespread than *Festuca* grasslands and not representative of one of the study sites (Site 1), these communities pose at least preventative conservation issues. Because restoration of grasslands invaded by *Brachypodium* species is a difficult and long-term process, keeping alive traditional land-use is the best way to prevent expansion or local dominance gain of this grass. On the other hand, when mown, *Brachypodium pinnatum* grasslands maintain the second highest species diversity in the region and hence have a high conservation value.

The high species richness in our area is therefore maintained by a combination of traditional land-use, abiotic conditions and vegetation types. It is sobering that maintaining at least an important fraction of this diversity will cost less and is more effective than probably any restoration effort in the future.

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8   Annex 1

Species richness of managed meadows in several European countries and corresponding values in the Csík study area.

Number of species	Plot size (m2)	Locality	Source	Number of species in the study area
29±4.4	1	SE	Linusson, A., Berlin, G. & Olsson, E. 1998. Reduced community diversity in semi-natural meadows in southern Sweden, 1965-1990. *Plant Ecol.* 136: 77-94	38.1±3.16
32±3	1	NO	Losvik M (1999) Plant species diversity in an old, traditionally managed hay meadow compared to abandoned hay meadows in southwest Norway – Nordic J. Bot. 19: 473-487	38.1±3.16
Max. 63	1	EE	Kull, K. & Zobel, M. 1991. High species richness in an Estonian wooded meadow. Journal of Vegetation Science 2: 715-718	Max. 50
Ave. 40.8	1	EE	Aavik T, Jõgar Ü, Liira J, Tulva I, Zobel M (2008) Plant diversity in a calcareous wooded meadow – The significance of management continuity. J. of Veg. Sci. 19: 475-484	38.1±3.16
14.5	1	CZ	Mašková Z, Doležal J, Květ J, Zemek F (2009) Long-term functioning of a species-rich mountain meadow under different management regimes. Agric Ecosyst Environ 132:192–202	38.1±3.16
Max. 54	1	NL	Bobbink R, Willems JH (1987) Increasing dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grasslands: a threat to a species-rich ecosystem. Biol. Conserv. 40: 301-314	Max. 50
>30 normal	1	NL	Bobbink R, Willems JH (1987) Increasing dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grasslands: a threat to a species-rich ecosystem. Biol. Conserv. 40: 301-314	>30 normal
9±1.4 - 17±2.1	1	FI	Huhta A-P, Rautio P, Tuomi J, Laine K (2001) Restorative mowing on an abandoned semi-natural meadow: short-term and predicted long-term effects. J. Veg. Sci. 12: 677-686	24.8±6.1 - 38.1±6.5
range 7-31	1	DE	Schumacher J, Roscher C (2009) Differential effects of functional traits on aboveground biomass in semi-natural grasslands. Oikos 118: 1659-1668	12-50
29±1 - 39±0	16	Alps	Niedrist G, Tasser E, Lüth C, Dalla Via J, Tappeiner U (2009) Plant diversity declines with recent land use changes in European Alps. Plant Ecol. 202: 195-210	-
26.5 - 40.0	25	Alps	Kampmann, D., Herzog, F., Jeanneret, P., Konold, W., Peter, M., Walter, T., Wildi, O., Lu¨scher, A., 2008. Mountain grassland biodiversity: impact of site conditions versus management type. J. Nat. Conserv. 16, 12–25	-
30	32	IT	Marini L, Fontana P, Klimek S, Battisti A, Gaston KJ (2009) Impact of farm size and topography on plant and insect diversity of managed grasslands in the Alps. Biol. Conserv. 142, 394–403.	-
14-56	32	IT	Marini L, Klimek S, Battisti A (2010) Mitigating the impacts of the decline of traditional farming on mountain landscapes and biodiversity: a case study in the European Alps. Env. Science and Policy, in press	-
35-37	25	RO	Bărbos I (2006) Montane grasslands dominated by Agrostis capillaries and Festuca rubra in Maramureş county I. Phytosociological analysis. Contribuţii Botanice XLI(2): 41-52	-
52.3±4.2	2500	RO	Baur B, Cremene C, Groza Gh, Schileyko AA, Baur A, Erhardt A (2007) Intensified grazing affects endemic plant and gastropod diversity in alpine grasslands of the Southern Carpathian mountains (Romania). Biologia, Bratislava, 62/4: 438-445	-