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Original paper

Technologies used for maintaining oligotrophic grasslands and their biodiversity in a mountain landscape

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Abstract

Conservation of oligotrophic grasslands and of biodiversity of mountainous landscapes continues to be a challenge in Europe. Numerous studies have identified technologies for ecological management and maintenance of oligotrophic grasslands especially in remote areas, confronted with large population exodus. The experiments were done in the Apuseni Mountains in Romania with the aim of identifying alternatives to traditional practices that are economically viable and easy to implement, leading to maintain oligotrophic grasslands at an adequate level of diversity. The effects of mulching and of mulching combined with organic fertilization on mountainous semi-natural grasslands were tested in 7 variants (treatment samples). The results, after 6 years, showed minimal changes on composition of vegetation cover, only some modifications on species level. Except for abandonment, all treatments applied and in particular mulching with 10 t/ha manure triennially can be used for conservation management of oligotrophic grasslands in the Apuseni Mountains.

Keywords Oligotrophic grassland, biodiversity, grassland management, mulching, organic fertilization.

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Introduction

Semi-natural grasslands with their high biodiversity are of great importance for Europe. Therefore, semi-natural grasslands especially in Eastern Europe are considered hotspots of biodiversity, some of them holding world records for the number of species per unit of area (WILSON [53]). Most semi-natural grasslands in E. Europe are oligotrophic or mesotrophic. Vascular plant species are adapted to oligotrophic habitats: from 7,394 species, 49.9% prefer soils that are oligotrophic or very oligotrophic (CHALMANDRIER & al [6]). In Southeast Europe, especially in Romania, there still exist large areas of oligotrophic grasslands (OG) which are subject to extensive management (VÎNTU & al [50]). Romania has a high level of species and ecosystem diversity, especially in the Carpathian Mts. where 75% of the endemic and sub-endemic species can be found (BRINKMANN & al [4], IORAS [18]). In Romania, most OG were included in HNV areas, totaling about 2 million hectares (out of 4.8 mil.) and stretching along the Carpathian Mts. (MADR 2018 [54]). The high diversity of OG provides many ecosystem services for farmers and society, including yields, release of nutrients from organic decomposition, protection against soil erosion, pollination (GIBON [17]; SANDERSON & al [42]). Biodiversity is also a pool of potential chemical compounds for pharmaceutics, plant breeding and other services for agriculture (CLERGUE [8]). TILMAN & DOWNING [46] showed that conservation of biodiversity is essential to maintaining the stability of ecosystem productivity. A significant decline in semi-natural grassland area has been observed in many places in Europe (TOKARCZYK [47]). After 1989, many agricultural and grassland areas were abandoned in Eastern Europe. Ca. 30% of the grassland area has been abandoned, especially in mountainous and Mediterranean areas (PEYRAUD & PEETERS [35]). Valuable, highly diverse habitats and species of the traditionally used cultural landscape become increasingly endangered (EMANUELSSON [13]). This is related to the livelihood of the people, with emigration from remote or mountainous rural regions, and increased urbanization (MCKINNEY [27]; FIGUEIREDO & PEREIRA [14]). OG are the result of extensive traditional land-use. The reduction of traditional farming practices, especially intensification or abandonment, has become a large-scale problem (DZWONKO [12]; WALLISDEVRIES [52]), related with deterioration of habitats, fragmentation and loss of specific diversity (MARINI & al [24]). Similar changes have occurred for grasslands all over Europe starting with the 19th century and accelerating over time, leading to significant losses of traditionally managed grassland (STOATE & al [44]). Economically less productive sites in remote areas were abandoned, while the productive sites were managed more intensively (ISSELSTEIN & al [20]; PLIENINGER & al [37]). Related to these changes was a dramatic decrease in the ecological value (COUSINS & ERIKSSON [9]; DAHLSTRÖM & al [10]) and species richness (VON GLASENAPP [51]; LENKA PAVLŮ [33]) of many grasslands. Management is altering soil nutrient levels, which also affects biodiversity, even in oligotrophic grassland communities (BARBARO & al [2]). Particularly the influence of fertilization with Phosphorus in oligotrophic grasslands needs to be considered more, since it may be a decisive and irreversible trophic parameter, as evidenced by the Rengen grassland experiments (MILAN & al [29]). Conservation of semi-natural grasslands is a sensitive topic that involves long term multidisciplinary studies (BRIEMLE [3]). It seems contradictive that improving grassland productivity and quality causes a drop in biodiversity and the reverse. However, based on detailed long term-studies, a compromise can be found. Many studies have focused on the effect of management type on the phytodiversity of grasslands (KAMPMANN & al [22]; REITALU & al [38]; PĂCURAR [31]; SAMUIL & al [40], PLEȘA & al [36], KARADAVUT & al [23]). A low-intensity type of management is required to conserve a semi-natural floristic composition (TONN & al [48]). In the Apuseni Mts. in Romania, OG present a great flora. These grasslands with high plant diversity (MICHLER & al [28]) are strongly dependent on management (STOIE & al [45]). Between 1956 and 2001, the population of Ghețari Plateau – Poiana Călineasa dropped from 2414 to 1478 persons (IORDAN & FRĂSINEANU [19]), a trend which continues (GARDA [16]). The decreased production has strongly modified grassland management and affected the diversity of the OG. The aim of this study is to identify alternatives to traditional practices that are economically viable and easy to implement, and to help to maintain OG at an adequate level of diversity. To achieve this objective, we investigated the effect of different management regimes including mulching on the phytodiversity of an oligotrophic grassland to discover the best management solutions for biodiversity conservation. GAISLER & al [15] and MAŠKOVÁ & al [25] suggested mulching as feasible practice for maintaining biodiversity and productivity in systems with low productivity (CARBONI & al [5]; DOLEŽAL & al [11]). TONN & al [48] found that mulching could be a less expensive practice for maintaining semi-natural and high-diversity grasslands. The specific aim of this study is to analyze the effect of mulching and mulching combined with organic fertilization at different doses on the phytodiversity of oligotrophic grassland.

Materials and Methods

The experiment started in 2010 in Poienile Ursului (1349 m), Ocoale village, Gârda de Sus commune, Apuseni Mts., using a randomized block design with 7 treatments groups in 5 replications: 1: Control, 2: Mulching (Mu). 1x / 1 year (y.), 3: Mu. 1x / 1 y. + 5 t/ha manure 1x / 1 y., 4: Mu. 1x / 1 y. + 5 t/ha manure 1x / 2 y., 5: Mu. 1x / 1 y. + 10 t/ha manure 1x / 2 y., 6: Mu. 1x / 1 y. + 10 t/ha manure 1x / 3 y., 7: abandoned. Grassland type for the experiment was *Agrostis capillaris – Festuca rubra*. The manure, derived from local horse and dairy farms, was applied in early spring. Chemical analysis of manure: pH 8.06, dry matter 16.7% t-P 385 mg/kg, t-K 11.37 mg/kg, Kjeldahl-N 18,235 mg/kg, P_2O_5 17,42 mg/kg, K_2O_5 14.83 mg/kg. Mulching was done at the end of July (traditional mowing period) with a special mulching machine. The biomass was shredded and left on the surface. Plant composition was recorded when the *Poaceae* species were in flower, with Braun-Blanquet method modified by PACURAR & ROTAR, 2014. We used the PC-ORD for the processing of species data. For data processing and interpretation, we used the Multi Response Permutation Procedure (MRPP), multidimensional scaling NMDS, Summary (for the Shannon index and the number of species) and average abundance and dominance (PECK [34]; MCCUNE & MEFFORD [26]). For the analysis of variance and to evaluate the effect of treatments we used the software STATISTICA. The analysis of variance was performed by Breakdown and One-Way ANOVA test. To analyze in detail the effect of treatment on species with significant reaction we used a post-hoc comparative analysis, Fisher LSD. This paper summarizes the results of the sixth experimental year (2016), showing the effect of treatments on grassland's plant species composition. NMS was carried out several times in autopilot mode to minimize the stress. Distance was measured with the Bray–Curtis distance.

Results and Discussion

The recommended solution for data presentation was tridimensional (stress 15,987; Table 1). The total variance explained by ordination, obtained as an intersection between ordination distances and distances in the original n-dimensional space was 76,9% (as a sum of axis importances reported to entire variation – 100%).

Table 1. Importance of axis, final stress and recommended ordination space (2013)

Axis	Axis importance	Cumulative variance (axis*100)	Final stress	Sig.	Recommended solution
1	0.323	32,3	15.987	**	3D
2	0.248	57,1			
3	0.198	76,9			

r- coefficient of determination for the correlations between ordination distances and distances in the original n-dimensional space

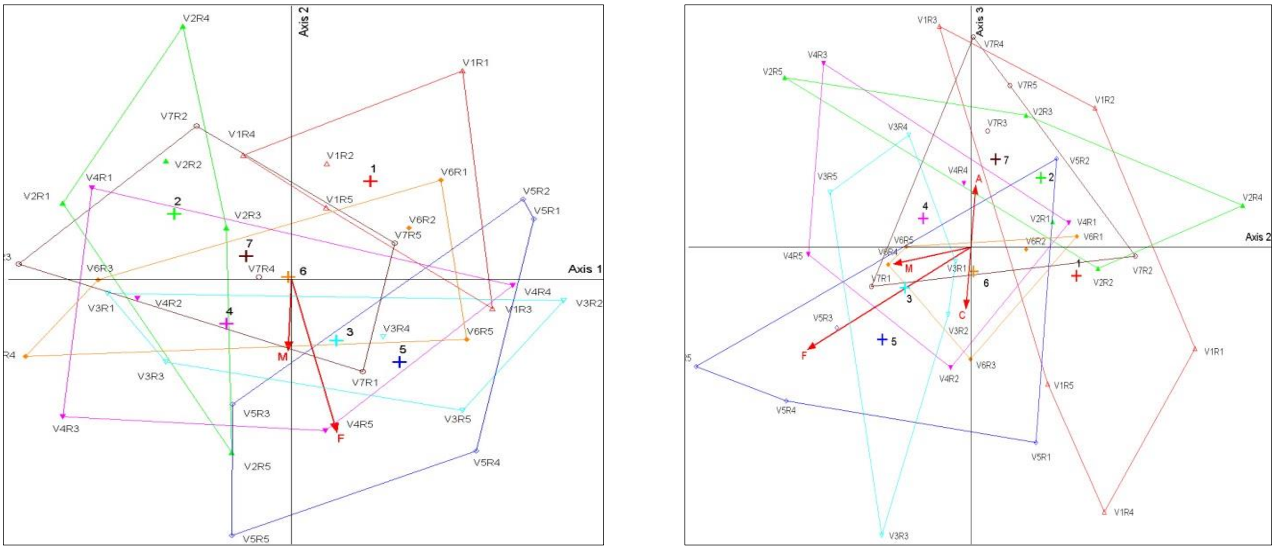
Mulching was weakly and negatively correlated with axis 2 (r = -0.337), while fertilization was very strongly and negatively correlated with axis 2 (r = -0.490) and weakly and negatively correlated with axis 3 (r = -0.364) (Table 2). Correlations for mowing and abandonment were statistically insignificant, which means they had only a minor role in describing the phenomenon.

Table 2. Correlation of experimental factors (vectors) with the ordination axis in (2013)

Experimental factors	Axis 1		Axis 2		Axis 3	
	r	Sig.	r	Sig.	r	Sig.
Mulching (M)	-0.066	ns	-0.337	*	-0.147	ns
Fertilization & M (F)*	0.245	ns	-0.490	**	-0.364	*
Mowing (C)	0.114	ns	-0.082	ns	-0.283	ns
Abandonment (A)	-0.114	ns	0.082	ns	0.283	ns

* F in Fig. 1.a and b refers to treatments that combine mulching and organic fertilization. ns – not significant.

Minor changes were detected produced by treatments without a shift in grassland type (Fig. 1, Table 3). We found highly significant differences in plant composition between mulching once per year (V2) and annual mulching + 10 t/ha manure biennially (V5), and significant differences between annual mulching + 10 t/ha manure biennially (V5) and abandonment (V7, Table 4). The dominant species (*Agrostis capillaris* L.) did not respond to the treatments, while the co-dominant species (*Festuca rubra* L.) slightly reduced its cover with annual mulching + 10 t/ha manure biennially (V5). This treatment also produced a decrease in *Poaceae* cover. Abandonment (V7) promoted *Cyperacee* and *Juncacee*. The establishing of *Fabaceae* was significant under the treatments (1) annual mulching + 5 t/ha manure yearly (V3); (2) annual mulching + 5 t/ha manure biennially (V4); and (3) annual mulching + 5 t/ha manure triennially (V6). In studies by MAŠKOVÁ [25] mulching produced a larger proportion of *Fabaceae* than of *Poaceae*. In our study, mulching combined with organic fertilization produced only strong establishment of *Fabaceae*, without surpassing the total cover of *Poaceae*.



a) axis 1 & axis 2 b) axis 2 & axis 3

Figure 1.a and **b.** Ordination of treatments influenced by organic fertilizers. Mulching (M), Fertilization & M (F), Mowing (C), Abandonment (A)

Table 3. Comparison of the floristic composition of experimental variants (MRPP, 2013)

Treatments	T	A	p	Sig.	Treatments	T	A	p	Sig.
V1 vs. V2	-2.582	0.077	0.012	*	**V3 vs. V4**	-0.264	0.010	0.311	ns
V1 vs. V3	-1.800	0.061	0.045	*	**V3 vs. V5**	0.759	-0.027	0.761	ns
V1 vs. V4	-2.467	0.089	0.017	*	**V3 vs. V6**	0.569	-0.026	0.662	ns
V1 vs. V5	-2.225	0.085	0.024	*	**V3 vs. V7**	-0.478	0.018	0.272	ns
V1 vs. V6	-1.895	0.065	0.039	*	**V4 vs. V5**	-0.367	0.012	0.313	ns
V1 vs. V7	-1.843	0.055	0.039	*	**V4 vs. V6**	0.272	-0.012	0.518	ns
V2 vs. V3	-2.011	0.071	0.043	*	**V4 vs. V7**	0.222	-0.008	0.514	ns
V2 vs. V4	0.188	-0.007	0.493	ns	**V5 vs. V6**	-0.282	0.011	0.312	ns
V2 vs. V5	-3.345	0.120	0.004	**	**V5 vs. V7**	-2.072	0.069	0.028	*
V2 vs. V6	-0.853	0.030	0.188	ns	**V6 vs. V7**	0.213	-0.008	0.527	ns
V2 vs. V7	0.592	-0.019	0.693	ns					

T – t test, A – group homogeneity, p – statistical significance, Sig – significance level

Many studies have demonstrated that fertilization with manure promotes the establishment of *Fabaceae* (GAISLER & al [15]; NOWAK 2002; SAMUIL & al [39], SIMA [43]). Legumes play an important part in grass cover due to adding Nitrogen to soils and altering physical and chemical properties because of the symbiosis with N fixing *Rhizobium* bacteria. Like mulching, fertilization with manure at higher doses than ours promotes the establishment of *Fabaceae* in various types of grasslands (SAMUIL [41]; VÎNTU [49]). Unlike legumes, forbs reduced their cover to 47%, compared with control (61%) after annual mulching + 5 t/ha manure annually (V3). DOLEŽAL [11] could show that mulching promotes legumes and certain low forbs which are suppressed in mowed or abandoned treatments. We didn't find significant changes for species that had <5% cover, except for those with <1% cover which showed a very significant positive effect, growing to 19.25% compared with control (11.00%) with the annual mulching + 10 t/ha manure triennially treatment (V6). The applied treatments did not produce significant changes in the number of species (biodiversity), even if there were changes at the level of species groups. However, we found a very significant increase of the Shannon diversity index with the annual mulching + 10 t/ha manure triennially treatment (V6) and the abandoned treatment (V7), compared with control, thus promoting diversity (Table 4). Our results are in concordance with DOLEŽAL's findings [11], where the first changes in diversity indicators (Simpson) occurred after 3 years, and specific diversity after 6 years. BAKKER & al [1] had similar findings, showing an increase in vascular plant species in recently abandoned grasslands. For the *Agrostis capillaris* – *Festuca rubra* grassland, fertilizer doses bigger than 10 t/ha manure many studies have noticed significant decreases in the number of species, often leading to a change of grassland type (CHIDOVEȚ [7]; PĂCURAR [32]; SAMUIL & al [40]).

Table 4. Flora structure of grassland type in 2013 (ANOVA and LSD tests)

Phytocoenosis indicators		**Treatments**						
2013	**Species groups**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**
Grassland type: A.c. – F.r.	***Agrostis capillaris* L.**	23.50	18.25	23.50	17.00	21.50	22.25	22.25
	***Festuca rubra* L.**	16.25	15.00	13.75	12.50	10.00°	16.25	13.75
Economic groups	***Poaceae***	42.05	36.00	40.00	31.80	33.80°	41.70	36.95
	Cyperaceae & Juncaceae	0.30	0.40	0.20	0.30	0.40	0.30	**0.70***
	Fabaceae	2.20	4.10	**12.00***	11.25*	**12.70*****	**11.00***	7.70
	FORBS	60.55	59.70	**46.55°°**	51.70	51.05	52.60	57.15
Species under 5%	**<5%**	7.00	11.00	14.00	11.00	13.00	17.00	18.00
	<1%	11.00	12.65	14.30	15.95	13.20	**19.25****	15.40
	<0.1%	9.10	9.30	8.20	7.60	7.00	7.10	9.10
Phytodiversity	**Nr. of species**	27.20	28.80	27.40	26.40	24.80	27.60	30.20
	Shannon index	2.36	2.49	2.58	2.51	2.50	2.67**	2.68**

A.c.= *Agrostis capillaris* L., F.r. = *Festuca rubra* L., positive changes: * = p<0.05, ** = p<0.01, *** = p<0.001, negative changes: ° = p<0.05, °° = p<0.01, °°° =p<0.001, ns – not significant.

As we can see from Figure 2 a and b and Table 6, mulching has a positive effect on *Trifolium repens* L., *Colchicum autumnale* L., *Leucanthemum vulgare* Lam., *Plantago lanceolata* L., and a negative effect on *Arnica montana* L., *Knautia dipsacifolia* Kreutzer. GAISLER & al [15] found similar results where mulching promoted *Trifolium repens* L. from 1% up to 21%. The frequency of *Colchicum autumnale* L. is higher in grasslands with extensive management, which is undesirable due to its toxicity, and this has become a problem for extensively managed grasslands in Germany and Austria (JUNG & al [21]). *Plantago lanceolate* L. appears to be indifferent to nutrient supply, and is a heliophyte, tolerant to mowing (PĂCURAR & ROTAR [30]), which explains why it favors the conditions created by mulching. Heliophytes like *Arnica montana* L., *Knautia dipsacifolia* Kreutzer were suppressed by mulching, but are moderately tolerant to mowing. Organic fertilization with mulching has a positive effect on *Trifolium pratense* L., *Trifolium repens* L., *Alchemilla vulgaris* L., *Ranunculus acris* L., *Centaurea mollis* Waldst. & Kit., *Plantago lanceolata* L. and a negative effect on *Arnica montana* L., *Cerastium holosteoides* Fr., *Silene nutans* L. Through mulching promoted species are medium tolerant to the supply of soil N (except for *C. mollis* Waldst. et Kit.), which confirms their preference for this treatment, while the disfavored ones do not tolerate eutrophic environment (*A. montana* L., oligotrophic) or the shading produced by the accumulation of decomposing vegetation (*A. montana* L., extreme-heliophyte; *C. holosteoides* Fr., heliophyte; *S. nutans* L., heliophyte).

a) Axis 1 & axis 2 b) Axis 2 & axis 3

Figure 2.a and **b.** Ordination of species influenced by organic fertilizers (2013). Mulching (M), Fertilization & M (F), Mowing (C), Abandonment (A)

Table 6. Correlation of species (significance) with the ordination axis in 2013

Species	Axis 1		Axis 2		Axis 3	
	r	Signif.	*r*	Signif.	*r*	Signif.
Agrostis capillaris L.	0.785	***	0.249	ns	0.139	ns
Anthoxanthum odoratum L.	0.026	ns	0.013	ns	-0.532	**
Festuca rubra L.	-0.394	*	0.624	***	-0.106	ns
Trifolium pratense L.	0.140	ns	-0.377	*	0.144	ns
Trifolium repens L.	-0.103	ns	-0.690	***	-0.367	*
Alchemilla vulgaris L.	0.164	ns	-0.430	**	0.235	ns
Arnica montana L.	0.259	ns	0.374	*	-0.239	ns
Centaurea mollis Waldst. et Kit.	-0.475	**	-0.308	ns	-0.457	**
Cerastium holosteoides Fr.	-0.051	ns	0.090	ns	-0.439	**
Colchicum autumnale L.	-0.197	ns	-0.399	*	0.208	ns
Gymnadenia conopsea (L.) R.Br.	-0.355	*	0.169	ns	0.213	ns
Hypericum maculatum Crantz	0.474	**	0.291	ns	-0.397	*
Knautia dipsacifolia Kreutzer	-0.068	ns	0.391	*	0.139	ns
Leucanthemum vulgare Lam.	-0.368	*	-0.391	*	-0.071	ns
Pimpinella major (L.) Huds.	0.493	**	-0.261	ns	0.139	ns
Plantago lanceolata L.	0.339	*	-0.409	*	-0.085	ns
Polygala comosa Schkuhr	-0.244	ns	0.031	ns	0.335	*
Polygala vulgaris L.	-0.338	*	-0.242	ns	0.415	*
Ranunculus acris L.	0.080	ns	-0.577	***	0.045	ns
Rhinanthus minor L.	0.391	*	-0.275	ns	-0.029	ns
Rumex acetosa L.	0.353	*	-0.036	ns	0.112	ns
Silene nutans L.	-0.311	ns	0.386	*	0.151	ns
Thymus pulegioides L.	-0.680	***	0.303	ns	0.420	*
Veratrum album L.	0.485	**	0.324	ns	-0.013	ns
Veronica chamaedrys L.	0.638	***	-0.180	ns	0.276	ns

r - Pearson correlation coefficient, Significance

Conclusion

Treatments, lasting at least 6 years, with mulching and mulching combined with organic fertilization in different quantities did not cause profound changes in the general cover of the dominating grasses, i.e., leaving the vegetation defined as grassland type unchanged. However, there were changes with respect to the herbs. Legumes were promoted by almost all treatments. Forbs were negatively affected by annual mulching + 5 t/ha manure yearly. Mulching and mulching combined with organic fertilization influenced certain species, either positively or negatively. The treatments conserve phytodiversity in all variants. The species number has increased in the case of annual mulching + 10 t/ha manure triennially (V6). For the maintenance of oligotrophic grasslands and biodiversity in the Apuseni Mountains any of our treatments can be applied, especially annual mulching + 10 t/ha manure triennially. We recommend alternating mowing (and biomass removal) and mulching as the best management practice to avoid succession and to maintain biodiversity in a feasible, inexpensive way, for example conservation of *Arnica montana*.

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