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SPECIES WITH INDICATIVE VALUES FOR THE MANAGEMENT OF THE MOUNTAIN GRASSLANDS

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ABSTRACT

The evaluation of grasslands is a current challenge of the agricultural policies in Romania. In the developed European countries this evaluation mode has been working for several years. To implement this initiative, it is necessary to elaborate a list of species with indicator value for the intensity of the applied management. The purpose of our research is to draw up a list of indicator species for the degree of fertilization intensity. The research activity was carried out in the Apuseni Mountains, as part of long-term experiences started in 2001. The technological inputs were applied annually. Within this paper, the data for 3 years was processed. Species with indicator value were identified using the "*Indicator Species Analysis*" (PC ORD) package. Following the application of fertilizers (organic and mineral), significant changes have been found at the level of vegetation cover. Based on the analysis of the indicator species, two lists of species with indicator value were produced. The evaluation of the grassland, according to the results, can be made based on the lists of indicator species elaborated within this study. These lists can provide support for beneficiaries of environmental and climate measures to self-assess agricultural practices on the farm and support for officials from institutions involved in verifying compliance with commitments.

Keywords: oligotrophic grassland, indicator species, grassland management, organic and mineral inputs.

INTRODUCTION

In South-Est Europe, there still exist important areas with oligotrophic grasslands that are the result of extensive management (Rotar et al., 2010; Vîntu et al., 2011). In Romania, most of the oligotrophic grasslands were classified as HNV (High Nature Value) farmland, representing an area of 2 million hectares (out of 4.8 M ha) and spanning across the Carpathians. The extensive and traditional agricultural systems, which have created a habitat mosaic with rich biodiversity and specific ecologic characteristics, are essential for maintaining naturalness. The socio-economic conditions existing today have led to a massive population exodus from the area of the Apuseni Mountains, which has led to the abandonment of grasslands and their reforestation (Gârda, 2010). This situation leads to loss of habitat and biodiversity. Since 2014, the locals have accessed measures and packages offered by the Ministry of Agriculture and Rural Development (MADR) through the Agency for Payments and Intervention for Agriculture (APIA) programs. These measures have partially halted the phenomenon of abandonment. The dangers and problems for biodiversity in the Apuseni Mountains area can be solved by applying a sustainable management system responsible for the existing biodiversity. Grassland management must be used by locals and, with the help of long-term studies, a balance in simultaneously maintaining biodiversity and economic productivity can be found. In recent years, there have been more and more studies on the effect of the intensity of the management on the productivity and biodiversity of grassland systems, but especially on grasslands with high natural value (Reif et al., 2008; Rotar et al., 2010; Čop and Eler, 2017; Hoffmann et al., 2017). In developed European countries, this evaluation model has been in operation for several years. In order to implement this initiative, it is necessary to elaborate a list of

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species with an indicator value for the intensity of the applied management in the framework of long-term experiments to ensure the validity of experimental data (Păcurar and Rotar, 2017; Ioana Vaida, 2018). Concerns about the assessment of HNV using indicator species have been around in Europe for a long time (Matzdorf et al., 2010; Schmitz and Isselstein, 2013). In Western Europe, the use of grasslands under intensive management has had a negative effect on biodiversity, a situation that is more backed by indicator species evaluation (Pál-Fám et al., 2013). In Germany, the effect of agri-environment measures has been validated based on indicator species (Wittig and Zacharias, 2006; Kaiser et al., 2010). The purpose of this paper is to identify some species with indicator value in order to evaluate the management and conservation status of HNV areas.

MATERIAL AND METHODS

The studies comprised of two long-term experiences: organic and mineral fertilizers experience.

The floristic studies were made within long-term experiments (since 2001) located in the Gheţari, Gârda de Sus village, Alba County, Apuseni Mountains, at 1130 m elevation, using the random blocks method. The field was composed of two experiments, one with mineral fertilizers and the other with organic fertilizers. The paper presents data from 3 experimental years: 2015, 2016, 2017, but also highlights the cumulative effect of organic and mineral inputs after 17 years.

Protocol of experience - the organic experiment: 4 treatments in 4 replications (T1 – control, T2 – 10 t manure/ha, T3 – 20 t manure/ha, T4 – 30 t manure/ha); the mineral experiment: 4 treatments in 4 replications (T1 – control, T2 – 50 kg N, 25 kg P_2O_5, 25 kg K_2O/ha, T3 – 100 kg N, 50 kg P_2O_5, 50 kg K_2O/ha, T4 – 150 kg N, 75 kg P_2O_5, 75 kg K_2O/ha). Each plot measured 10 m^2. The administration of inputs took place annually in early spring, the mineral fertilizer was the complex type NPK 20:10:10, and the organic fertilizer came from cattle and horses, having the following nutrient content: 0.40 N, 0.39 P and 0.45 K. The experimental field was placed on terra rossa soil having the following features: soil pH 5.34, N total 0.212%, P mobile 3 ppm, K 25 ppm, which means it had a medium supply of N and a low supply of P and K. The floristic studies were performed according to the Braun-Blanquét method modified by de Păcurar and Rotar (2014).

Floristic data processing was performed with PC-ORD, version 7, which uses the multivariate analysis of the botanical data (McCune and Mefford, 2011). The ordination of floristic composition was made based on applied fertilizer and was done with the Principal Coordinates Analysis (PCoA) method, described in detail by Legendre & Legendre, 1998. PCoA is best suited to the analysis of matrices of environmental/biotic data, particularly where variables are of different data types, including ranked and multistate data (Kent, 2012). PCoA operates by finding a final solution in which the measured distances among sample units (on the ordination axes) correspond as well as possible to the measured distances observed in the original data; this is a form of metric multidimensional scaling (Peck, 2010). All distances are calculated based on Sorenson (Bray – Curtis) formulas. In order to extract indicator species, the *indicator value indices* (*INDVAL*) will be used, which is fit for assessing the predictive values of species as indicators for the conditions prevailing in site groups, like field determination of community types or ecological monitoring (De Cáceres et al., 2012). Indicator species will be calculated with the method based on the medium abundance-dominance (AD_m) and constancy (K) of a species in all groups (Dufrene and Legendre, 1997). The product of these phytopopulation indices will be reported as percentages and the species' indicative value will be the result. This value can be between 0 (no indicator value) and 100 (a perfect indicator value).

RESULTS AND DISCUSSION

In the experience with organic fertilization, the type of grassland was identified as *Festuca rubra - Agrostis capillaris* (control) which had 17 species of indicator value in the floristic composition (Table 1). Most species in this group are oligotrophs and oligomesotrophs. Eight of the species with indicator value had 0.5% participation; the species *Festuca rubra* (21.1%) had the highest coverage (AD_m), followed by *Anthoxanthum odoratum* (4.2%), *Colchicum autumnale* (4.0%), etc. Two species had just over 3% participation [*Plantago lanceolata* – 3.1%, *Potentilla erecta* (3.1%), and 4 species had a participation between 0.8-3% (*Leucanthemum vulgare* – 2.9%; *Plantago media* – 2.9%; *Viola declinata* – 1.2%, *Leontodon autumnalis* – 0.8%)]. Eight species have a minimum weight of only 0.5% (*Hieracium aurantiacum, Luzula multiflora, Gymnadenia conopsea,* etc.). The highest indicator value (*INDVAL)* species were *Hieracium aurantiacum*, *Hieracium pilosella, Polygala comosa, Thymus pulegioides, Viola declinata* (100.0%). Even if the species *Gymnadenia conopsea* had no maximum indicator value, it is indicative for the phytocoenosis of the control, because the treatment with 10 t manure/ha led to its disappearance from the grass cover. This treatment (10 t manure/ha) also led to the extinction of species *Thymus pulegioides* and *Viola declinata*, these being present in the phytocoenosis of the control with a maximum indicator constancy and value (Table 1). The lowest indicator value (under 50%) species were: *Anthoxanthum odoratum* (40.7%), *Cynosurus cristatus* (33.3%), *Colchicum autumnale* (38.2%), etc. The low indicator value of these species was determined by their presence in the other phytocoenoses.

The type of grassland *Agrostis capillaris - Festuca rubra* (T2 – 10 t manure/ha) had in their grass cover 4 species with indicator value (Table 1). The species in this group are mesotrophs. They had a participation between 1.5-6.8% (*Trifolium pretense* – 5.3%; *Trifolium repens* – 6.8%; *Achillea millefolium* – 3.5%, etc.). In a study by Santa Maria in 2004, *Trifolium repens* had been identified contrary to our studies, namely as an indicator species in the meadows under high-intensity management, irrigated and rich in organic matter. In our experience, the highest indicator value (*INDVAL)* species were *Trifolium pratense* (57.4%), *Trifolium repens* (56.3%) and *Lotus corniculatus* (40.9). The lower indicator value of the above species is explained based on their presence and in the phytocoenoses determined by the other treatments.

In the floristic composition of the type *Agrostis capillaris - Trisetum flavescens* codominant *Centaurea pseudophrygia* (T3 – 20 t manure/ha) had 3 species with indicator value (Table 1). The species in this group were more mesotrophic. The species with the highest participation (AD_m) were *Centaurea pseudophrygia* (11.8%), *Pimpinella major (*6%) and *Rumex acetosa* (2.5%), which denoted their presence in the other phytocoenoses of the experience. Considering that these species had the highest presence in the phytocoenoses of this treatment, they can be considered as indicative for the application of 20 t/ha at that AD_m level. Species cannot be considered having an indicator value without considering their participation. The indicator values of the species are quite appropriate, all being below 50 (*Rumex acetosa* – 39.5; *Centaurea pseudophrygia* – 34.3; *Pimpinella major* – 31.1).

The type of grassland *Agrostis capillaris - Trisetum flavescens* (T4 – 30 t manure/ha) had 8 species with indicator value. The species in this group had a mesotrophic to eutrophic character. Two of the species showed participation of 15% in the vegetable cover: *Trisetum flavescens* (15.8%) and *Agrostis capillaris* (20.3%). In the study by Lavorel in 2004, in the mountain and subalpine areas, the species *Trisetum flavescens* appeared dominant in the fields used intensively and fertilized with manure, which confirms the results of the present research. Regarding our experience, two species had a share of over 5% (*Taraxacum*

officinale – 5.5%, *Veronica chamaedrys* – 8.1%). Four species had a participation in the vegetable cover ranging from 0.8%, *Poa trivialis,* to 4.2%, *Festuca pratensis*). The highest indicator values were for the species *Poa trivialis* (62.5%), *Festuca pratensis* (52.6%) and *Dactylis glomerata* (51.0%). *Poa trivialis* was present both in phytocoenosis treated with 20 t manure/ha as well as the one treated with 30 t manure/ha. Due to this situation, the indicator value was well below 100%. It is difficult to say that the species had an indicative value only for treatment with 30 t manure/ha. A similar situation was presented in the study undertaken in Sweden by Scherer-Lorenzen and collaborators (2002-2003), where it was found that, when applying small quantities of manure (10-20 t/ha), no major changes of the grass cover were observed in the studied phytocoenoses; the only aspects observed were the changes of the dominance of the species. The authors showed the appearance of the species *Dactylis glomerata* in the studied phytocoenoses only after fertilization with 50 t manure/ha. The low indicator value of these species is explained by the fact that they are present in all phytocoenoses. Indicative value for treatment with 30 t manure/ha was given by the greater participation in this variant. Comparing the participation of these species from the treatment with 30 t/ha manure with those from the treatment with 20 t manure/ha revealed that the differences were very small (1-3%) and difficult to appreciate during fieldwork.

Table 1. Species with indicative value for applied treatments

Species	T	INDVAL	Mean	Dev. Std.	Signif.	N	AD_M (%)
Anthoxanthum odoratum L. s. str.	1	40.7	29.4	2.1	***	X	4.2
Cynosurus cristatus L.	1	33.3	25.5	3.3	*	4	0.5
Festuca rubra L.	1	57.3	31.8	3.3	***	X	21.1
Carex pallescens L.	1	50.0	21.3	4.8	***	4	0.5
Luzula multiflora (Ehrh.) Lej.	1	52.2	20.8	4.9	***	3	0.5
Colchium autumnale L.	1	38.2	29.1	2.0	***	X	4.0
Gymnadenia conopsea (L.) R. Br. s. l.	1	58.3	11.8	5.7	***	3	0.5
Hieracium aurantiacum L.	1	100.0	15.1	5.5	***	2	0.5
Hieracium pilosella L.	1	100.0	15.1	5.5	***	2	0.5
Leontodon autumnalis L.	1	62.5	23.0	5.9	***	5	0.8
Leucanthemum vulgare Lam. s. str.	1	38.3	30.0	2.4	***	3	2.9
Plantago lanceolata L.	1	49.3	31.8	3.3	***	X	3.1
Plantago media L.	1	67.0	34.7	5.3	***	3	2.9
Polygala comosa Schkuhr	1	100.0	15.1	5.5	***	2	0.5
Potentilla erecta (L.) Raeusch.	1	75.8	29.0	5.4	***	2	3.1
Thymus pulegioides L. s. l.	1	100.0	15.1	5.5	***	6	0.5
Viola declinata	1	100.0	16.3	6.4	***	6	1.2
Lotus corniculatus L	2	40.9	31.7	3.6	***	4	1.5
Trifolium pratense L.	2	57.4	52.9	2.3	***	6	5.3
Trifolium repens L.	2	56.3	52.5	2.1	*	6	6.8
Achillea millefolium L.	2	36.6	29.5	2.1	***	5	3.5
Centaurea pseudophrygia C. A. Mey.	3	34.3	27.5	1.3	***	4	11.8
Pimpinella major (L.) Huds.	3	31.1	27.8	1.4	***	7	6.0
Rumex acetosa L.	3	39.5	29.6	2.3	***	X	2.5
Agrostis capillaris L.	4	30.3	26.4	0.8	***	4	20.3
Dactylis glomerata L. s. str.	4	51.0	27.6	4.6	***	6	3.2
Festuca pratensis Huds. s. l.	4	52.6	27.1	4.2	***	6	4.2
Poa trivialis L.	4	62.5	23.1	6.0	***	7	0.8
Trisetum flavescens (L.) P. Beauv.	4	34.8	28.0	1.45	***	6	15.8
Vicia cracca L. s. str.	4	41.3	30.0	2.45	***	6	4.0
Taraxacum officinale Weber s. l.	4	34.6	29.1	1.96	*	6	5.5
Veronica chamaedrys L. s. str.	4	43.4	29.9	2.33	***	6	8.1

T – treatment; INDVAL – indicator value; N – the preferences of nitrogen species; ADm – abundance - dominance - medium; Dev. Std - standard deviation; Signif.: *** $p<0.001$; ** $p<0.01$; * $p<0.05$; ns – not significant.

In the phytocoenosis of the control (type *Festuca rubra – Agrostis capillaris)* 23 species were identified (Table 2), 5 species more than in the organic fertilizer experience. This difference can be explained by the stronger effect of mineral fertilizers compared to organic fertilizers when a deeper separation is observed in phytocoenosis. Most species in this group are oligotrophs and oligomesotrophs. There were 13 species that had a coverage of over 0.5%; the largest participation was for the species *Festuca rubra* (19.5%), *Anthoxanthum odoratum* (4.4%), *Plantago media* (3.8%), *Trifolium repens* (3.3%), etc. Three of the species had a ponderance slightly above one percent in the grass cover: *Lotus corniculatus* – 1.2%; *Thymus pulegioides* – 1.2%; *Scabiosa columbaria* – 1.4%. Two species had coverage close to the minimum value (0.5%), *Briza media* (0.7%) and *Polygala vulgaris* (0.7%). Ten of the species had only minimal participation (0.5%): *Carex pallescens, Luzula multiflora, Gymnadenia conopsea, Carlina acaulis,* etc.; (Table 2). The highest indicator value was for the species *Thymus pulegioides* (100.0%). Even if the species *Gymnadenia conopsea* had no maximum indicator value, it was indicative for the control variant because the treatment with 50 N, 25 P_2O_5, 25 K_2O kg/ha led to its disappearance from the grass cover. Poor indicator value (less than 50.0%) was found in the following species: *Anthoxanthum odoratum* (48.6%), *Cynosurus cristatus* (46.3%), *Leontodon autumnalis* (45.0%), etc. The low indicator value of these species was determined by their presence in the other phytocoenoses.

The grassland of type *Trisetum flavescens* - *Agrostis capillaris* (T2 – 50 N, 25 P_2O_5, 25 K_2O kg/ha) had, in its floristic composition, 4 species with indicator value. The species in this group are mesotrophs.

The highest participation (AD_m) was for the species *Trisetum flavescens* (23%). The other 3 species had a ponderance between 0.5% – 8% (*Hieracium aurantiacum* – 0.5%, *Hypericum maculatum* – 4%, *Alchemilla vulgaris* – 8%). The highest indicator values were shown by the species *Hypericum maculatum* (48.0%), *Trisetum flavescens* (47.0%), *Alchemilla vulgaris* (36.9%) and *Hieracium aurantiacum* (30.0%). The lower indicator values of these species were explained based on their presence in the other phytocoenoses with the other treatments.

In the grassland with the type *Agrostis capillaris* - *Trisetum flavescens* (T3 – 100 N, 50 P_2O_5, 50 K_2O kg/ha) there were two species with indicator value. The species in this group have a mesotrophic character. These species have a ponderance of 3% (*Rumex acetosa*) and 3.4% (*Veronica chamaedrys*), which denoted their presence in the other phytocoenoses of the experience. Given that these species had the highest weight in the phytocoenoses of this treatment, they can be considered as indicative value for application with 100 N, 50 P_2O_5, 50 K_2O kg/ha but relative to the ponderance of the species; they cannot be considered species with indicator value without taking into account their participation in the grass cover. The indicator value of the following species was similar (*Rumex acetosa* – 37.1%; *Veronica chamaedrys* – 40.0%). These species with indicator value were also identified in the variants with treatments of 20 t/ha and 30 t/ha of manure, when grassland type had been identified as *Agrostis capillaris* with *Trisetum flavescens*.

For the grassland of type *Agrostis capillaris* (T4 – 150 N, 75 P_2O_5, 75 K_2O kg/ha), there were two species with indicator value in the composition. The species in this group have a mesotrophic to eutrophic character. The largest participation in the grass cover was with the species *Agrostis capillaris* (64.6%) and *Taraxacum officinale* (3.1%). The highest indicator values were shown by the species *Agrostis capillaris* (45.5%), *Taraxacum officinale* (38.5%). As with the previous variants, the reduced indicator value of these species was explained by the fact that they are present in all phytocoenoses. Indicative value for treatment with 150 N, 75 P_2O_5, 75 K_2O kg/ha was given to species with the highest ponderance in this variant.

Table 2. Species with indicative value for applied treatments

Species	T	INDVAL	Mean	Std. Dev.	Signif.	N	ADM (%)
Anthoxanthum odoratum L. s. str.	1	48.6	29.9	3.7	***	X	4.4
Briza media L.	1	83.3	15.3	6.3	***	3	0.7
Cynosurus cristatus L.	1	**46.3**	**18.4**	5.3	***	4	0.5
Festuca rubra L.	1	**66.2**	**33.0**	3.8	***	X	19.5
Carex pallescens L.	1	52.2	20.9	5.0	***	4	0.5
Luzula multiflora (Ehrh.) Lej	1	50.0	21.3	4.9	***	3	0.5
Lotus corniculatus L	1	54.9	24.6	5.9	***	4	1.2
Trifolium pratense L.	1	47.7	32.1	4.1	***	6	2.6
Trifolium repens L.	1	47.1	31.6	3.7	***	6	3.3
Carlina acaulis L.	1	58.3	11.8	5.3	***	2	0.5
Cerastium glomeratum Thuill	1	51.0	12.5	5.3	***	4	0.5
Gentiana lutea L.	1	58.3	11.6	5.4	***	2	0.5
Gymnadenia conopsea (L.) R. Br. s. l.	1	83.3	13.6	5.4	***	3	0.5
Leontodon autumnalis L.	1	45.0	16.8	5.4	***	5	0.5
Leucanthemum vulgare Lam. s. str.	1	75.4	21.4	5.7	***	3	2.3
Plantago lanceolata L.	1	87.5	21.7	6.2	***	X	2.6
Plantago media L.	1	90.2	22.5	6.3	***	3	3.8
Polygala vulgaris L.	1	80.9	17.0	6.3	***	2	0.7
Potentilla erecta (L.) Raeusch.	1	68.1	28.8	6.1	***	2	2.7
Scabiosa columbaria L.	1	91.7	18.8	6.7	***	2	1.4
Thymus pulegioides L. s. l.	1	100.0	16.3	6.2	***	6	1.2
Tragopogon pratensis L	1	46.3	18.4	5.3	***	6	0.5
Viola declinata	1	45.8	20.4	5.1	***	6	0.5
Trisetum flavescens (L.) P. Beauv.	2	47.3	29.6	2.2	***	6	23.0
Alchemilla vulgaris L.	2	36.9	28.4	1.6	***	6	8.0
Hieracium aurantiacum L.	2	30.0	13.8	5.5	*	2	0.5
Hypericum maculatum Crantz	2	48.0	31.5	3.2	***	2	4.0
Rumex acetosa L.	3	37.1	29.3	2.8	***	X	3.0
Veronica chamaedrys L.	3	40.0	30.8	3.01	***	6	3.4
Agrostis capillaris L.	4	45.5	29.9	2.32	***	4	64.6
Taraxacum officinale Weber ex Wiggers	4	38.5	29.6	2.72	***	6	3.1

T – treatment; INDVAL – indicator value; N – the preferences of nitrogen species; ADm – abundance - dominance - medium; Dev. Std - standard deviation; Signif.: *** p<0.001; ** p<0.01; * p<0.05; ns – not significant.

CONCLUSIONS

Both mineral and organic fertilization cause changes at the grass cover level, leading to changes in dominance and co-dominance between species. Responses varied, with the *Festuca rubra – Agrostis capillaris* type changing to different intensity-dependent (fertilization) specific types.

The phytocoenosis of the organic fertilizers control experience (type *Festuca rubra – Agrostis capillaris*) presented 17 species with indicator values, while the mineral fertilizers control experience had 23 indicator species.

The type *Festuca rubra – Agrostis capillaris* grassland (T1 – 10 t manure/ha) presented, in its floristic composition, 4 species with indicator value, the type *Agrostis capillaris - Trisetum flavescens* grassland (T3 – 20 t manure/ha) had 3 indicator species, and the type *Agrostis capillaris - Trisetum flavescens* grassland (T4 – 30 t manure/ha) had 8 species with indicator value.

In the mineral fertilizers experience, the type *Trisetum flavescens - Agrostis capillaris* grassland (T2) presented, in its floristic composition, 4 species with indicator value; type *Agrostis capillaris - Trisetum flavescens* grassland (T3) had 2 indicator species, and type *Agrostis capillaris* (T4) grassland had 2 species with indicator value.

Within the two experiences, the same phytocoenosis presented a different number of indicator species depending on the type of fertilization, a situation produced by the more

energetic effect of the mineral fertilizers which cause profound changes with a high degree of differentiation in the grass cover.

The response to fertilization of some species does not confirm their preference for Nitrogen according to Ellenberg (1992) and adapted by various Romanian specialists, which is why there is a need for reconsideration of species' ecological Nitrogen preferences based on our long-term experiments.

RECOMMENDATIONS

The indicator value species identified and presented here can be taken under consideration by the Ministry of Agriculture and the Agency for Payments and Intervention (APIA) in order to elaborate a methodology for evaluation and subsidization, with the aim of protecting and conserving landscape biodiversity in the Apuseni Mountains.

CONTRIBUTIONS

Ioan Rotar coordinated the research activity, Florin Păcurar coordinated the research activity and elaborated the method of study and interpretation of the vegetation of grassland, Ioana Vaida participated in floristic studies, worked with statistical analysis and identified indicator species for intensity of management.

Florin Păcurar and Ioana Vaida interpretation of results with PC-ORD software. All authors reviewed the manuscript.

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