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Influence of Fertilizers on the Biodiversity of Semi-natural Grassland in the Eastern Carpathians

Costel SAMUIL, Vasile VINTU*, Culita SIRBU, Mihai STAVARACHE

*University of Agricultural Sciences and Veterinary Medicine, Department Forage Crops, Mihail Sadoveanu Alley, No 3, Iaşi, 700490, Romania; vvintu@uaiasi.ro (*corresponding author)*

Abstract

This investigation examines the influence of fertilization with organic and mineral fertilizers on the biodiversity of grasslands containing *Festuca rubra*, *Agrostis capillaris* and *Nardus stricta*. Permanent meadows were studied in terms of production of food, and of biodiversity. The current strategy of using organic fertilizers has raised concerns about resource conservation and environmental protection. The increase in the number of species is due to the fertilizers that have been applied leading to changes in the soil fertility status. This change in soil fertility has allowed other mesotrophic and eutrophic species to become established in fertilized meadows. In Romania, meadows belonging to this category occupy an area of approximately 1,600,000 hectares and have relatively low production rates. The experiment was located at Pojorata, Suceava County (Romania), in two different natural grasslands that had different floristic compositions. Manure improved the growth of a number of species, especially in the "plants from other botanical families" category, because of the pool of seeds that it contains. Using a management system based on fertilization with small amounts of organic and mineral fertilizers can help preserve the biodiversity of these meadows. The results of this study, in an area considered representative for large parts of the mountainous areas of Romania, indicated that fertilization treatments were able to maintain a high species diversity.

Keywords: Carpathians, fertilizers, grassland

Introduction

Semi-natural grasslands, which are traditionally used as forage for ruminants, are an important type of land use in Europe, covering more than a third of the European agricultural area (Pacurar *et al.,* 2012). Fertilization with manure are considered an appropriate management technique to conserve biodiversity value (Assaf *et al.,* 2011; Chapin *et al.,* 2000; Kesting *et al.,* 2009; Louault *et al.,* 2005; Peeters *et al.,* 2004). Several management factors may affect the biodiversity of these grasslands, including fertilization, overseeding, grazing and cutting management (Duru *et al.,* 2010; Lehman *et al.,* 2000; Mauz and Rémy 2004; Paine *et al.,* 1999; Pasho *et al.,* 2011; Samuil *et al.,* 2012a; Sirbu *et al.,* 2012). Grasslands are an important forage resource in Romania, but poor management during the last few years have led to their present state of degradation.

The greatest reduction in permanent grassland productivity has been caused by unfavorable weather conditions and poor management (Samuil *et al.,* 2012b). An increase in the productive potential of these meadows can be achieved by fertilization with different rates and types of organic fertilizers (Hopkins *et al.,* 1990). Research conducted to date has demonstrated the positive effects of organic fertilizers on lawns (Hacala and Pflimlin, 1994; Ziegler, 1997). If regularly and sensibly applied, organic fertilizers can fully substitute for chemical fertilizers (Jeangros *et al.,* 1994, 2002).

Comparative studies that investigated the effects of different management practices on grasslands have demonstrated shifts in species diversity and in the composition of plant functional groups (Baudry, 2004; Dale *et al.*, 2012; Hopkins *et al.*, 1999). Each permanent grassland sward is a unique mixture of species and growing stages and this complexity makes it difficult to characterize and understand their feed value (Duru *et al.,* 1998). Floristic composition influences the nutritive value of permanent grasslands because of the differences in the chemical composition and digestibility of individual species and also because of variations in the growth rate of different species (Dale *et al.,* 2012; Duru *et al.,* 1998).

In this study, an experimental approach was used to evaluate the effects of management treatments on the biodiversity for two semi-natural grasslands in the Oriental Carpathian Mountains. The two following key questions were addressed: (i) are there any changes in plant species composition and in the functional groups of plants under the different fertilization treatments and (ii) what are the temporal trends over a period of six consecutive years?

Materials and methods

Study area

This paper presents the results of two experiments located at Pojorata, Suceava County (Romania), on two different natural grasslands with different floristic compositions. The first experiment took place on a meadow composed of *Agrostis capillaris* and *Festuca rubra* (experiment 1), and was located at an altitude of 707 meters on land with a slope of 20%. The second experiment took place on a meadow containing *Nardus stricta* (experiment 2), located at an altitude of 727 meters on land with a slope of 28%.

Climatic conditions in the area are characterized by an average temperature of 6.3°C and 708.2 mm total annual precipitation. Between April and September, the average temperature is 12.8°C and rainfall amounts to 513.5 mm. In terms of climate, the areas are located on the northeast extremity of the European Central Province, with a temperate-moderate-continental climate that is also influenced by the eastern continental climate and the northern boreal climate. The species of plants found in the two experimental areas were sub-heliophile, micro-mezotermophile, mezophile, low acid-neutrophile and oligo-mezotrophile plants.

Permanent grassland occupies 156 000 hectares in the area where this research was conducted, with different plant distributions depending on altitude, slope, temperature and precipitation (Fig. 1).

The soil type was Typic Dystrudepts, according to the American System or Dystric Cambisol according World Reference Base for Soil Resources (WRB-SR). The soil had a pH (measured in H_2O) of 4.7 and the average nutrient contents were: total N content, 1.3 g kg^{-1}; P content, 0.33 g kg^{-1} and K content, 14.6 g kg^{-1}. The values for the available P and K in the soil are considered low in phosphorus, but adequate for potassium.

Experimental treatments

The experiments were subdivided into plots and were replicated four times. Each plot was 4 m × 5 m in size. They were subjected to the fertilizer treatments shown in Tab. 1.

The chemical composition of 1000 kg of manure was 5.19 kg N, 2.83 kg P and 6.72 kg K. The manure was applied during the autumn season, but the mineral nitrogen was applied in spring before vegetative growth began.

The Used Apparent Coefficient (UAC) was calculated so that the effects of the different types and combinations of organic and mineral nitrogen fertilizers could be compared. The UAC gives an indication of the effective use of organic nitrogen on a short-term basis. The UAC coefficient varies depending on the time of application and the type of vegetation cover and ranges in value from 0 to 70%. The UAC of the nitrogen equivalent for mineral fertilizer is the amount of nitrogen used by the plant. The results obtained in this experiment were based on a UAC value of 0.4 for manure applied annually, 0.45 for manure applied every 2 years and 0.55 for manure applied every 3 years (Bodet *et al.,* 2001).

Analyses and statistical interpretation

The mean indicator values for light (L), temperature (T), soil moisture (W), soil reaction (R) and soil trophicity (Tr) were calculated based on the number of species from a given category. Species indicator values followed the scales of Ellenberg *et al*. (1992). In order to calculate the biodiversity indicators, surveys were conducted at

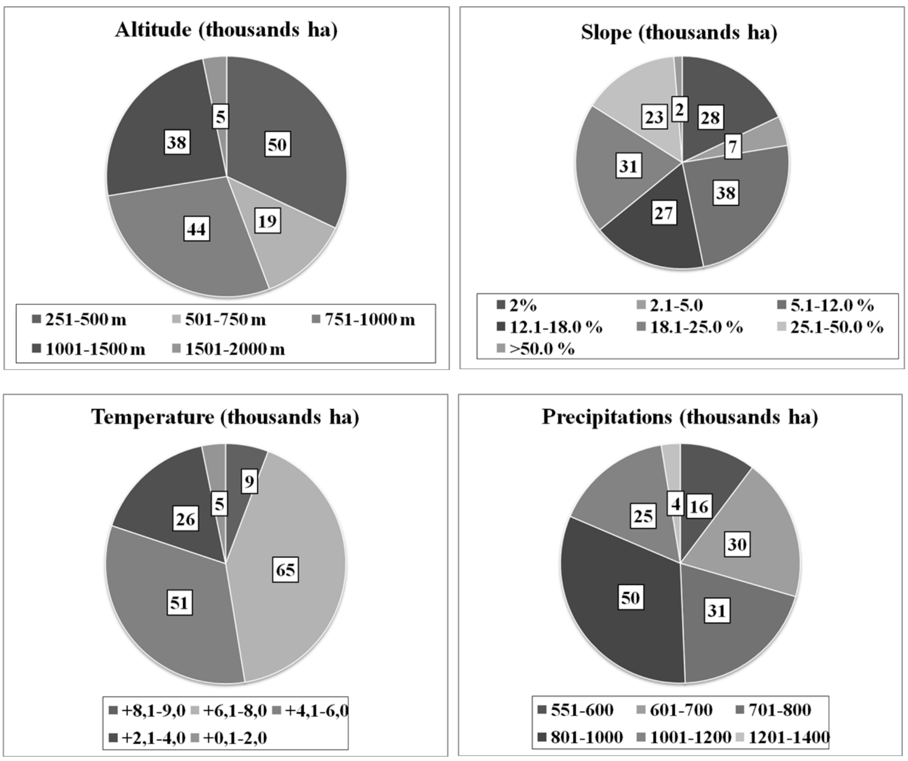

Fig. 1 Natural conditions of the permanent grassland research areas used in this study

Tab. 1 Fertilization methods applied and the quantities of nutrients supplied to the treatments

Cod variant	Fertilisation treatment	Manure t ha^{-1}	Total N* rates	Fertilisation treatment	N kg ha^{-1}	Manure t ha^{-1}	Total N rates
	Experiment 1			Experiment 2			
V1	A_0b_0	0	0	$A_0b_0c_0$	0	0	0
V2	A_1b_1	10	20	$A_1b_1c_1$	30	10	50
V3	A_2b_2	20	23	$A_1b_1c_2$	50	10	70
V4	A_3b_3	30	29	$A_2b_2c_1$	30	20	53
V5	$A_2b_1+A_1b_2+A_0b_0$	20+10+0	54	$A_2b_2c_2$	50	20	73
V6	$A_2b_1+A_0b_0+A_1b_3$	20+0+10	52	$A_3b_3c_1$	30	30	59
V7	$A_2b_1+A_1b_2+A_1b_3$	20+10+10	64	$A_3b_3c_2$	50	30	79

A– manure application rate: A_0 – 0 t ha^{-1}, A_1 – 10 t ha^{-1}, A_2 – 20 t ha^{-1}, A_3 – 30 t ha^{-1}; B – length of time the manure was applied: b_0 – no manure, b_1 – annually, b_2 – every 2 years, b_3 – every 3 years; C – mineral nitrogen application rate each year: c_1 – 30 kg ha^{-1}, c_2 – 50 kg ha^{-1}; UAC–apparent coefficient of use (0·4 for manure applied annually; 0·45 for the manure applied every 2 years and 0·55 for the manure applied every 3 years)

five points for each experimental parameter. Presence/absence data and plant cover were recorded. The cover of all vascular plant species was visually estimated in each plot based on the Braun-Blanquet methodology (Cristea *et al.,* 2004). In mid-June 2011, in order to eliminate edge effects, relevés were taken in the center of each 4 m ×5 m plot which covered an area of 2 m ×3 m.

The results obtained by the Braun-Blanquet methodology were transformed in cantitative value. The mean cumulative coverage of the plants was calculated from the abundance dominance values of speciesafter they had been converted to percentages (median percent values of the corresponding Braun-Blanquet classes). These results were used to calculate the Shannon Weaver index, the Simpson index and the Eveness index. Species richness was a count of the number of plant species present in a sample plot (Cristea *et al*., 2004) and when used in combination with diversity indices, revealed further trends in plant diversity that the more complex indices could not reveal.

The statistical data was analyzed by SPSS using ANOVA and the Lowest Significant Difference test (LSD).

Results and discussion

Botanical composition was very weak and was represented by species with low forage values (Tab. 2, Fig. 2). A total of 35 species were identified at the beginning of the experiments, of which 12 belonged to the Poaceae family, seven belonged to the Fabaceae family and 16 plant species belonged to other families. For each of these species, the ecological indicators were specified.

In the first experiment, 65.8% of the species had a high percentage tolerance to temperature and 63.2% had a high tolerance to soil acidity. In the second experiment, 61.9% of the species had a high percentage tolerance to temperature and 57.1% to soil acidity.

Constancy is an expression of a species relative to the area taken up by each species depending on the experimental variants assessed and provides information on the degree of fidelity. It can be seen that over 60% of the species had a high and a very high constancy, (classes IV and V; Fig. 3).

The application of mineral and organic fertilizers to the *Agrostis capillaris* L. with *Festuca rubra* L. grassland and the *Nardus stricta* L. grassland produced significant changes within the sward, depending on the applied treatments. However, the floristic composition in both experiments was quite similar. Analysis of the biodiversity parameters highlighted the fact that the number of species increased across all the variants compared to the control when fertilizers were applied (Tab. 3).

In experiment 1, the number of species increased from 15, in the control plots, to 17-26 in the fertilized plots. The Shannon index increased from 2.504 in the A_0b_0 fertilized plots (control) to 2.611-2.855 in the fertilized plots. Shannon evenness values were 0884 in the A_0b_0 plots and 0.875-0.932 in the fertilized plots. The Simpson index was

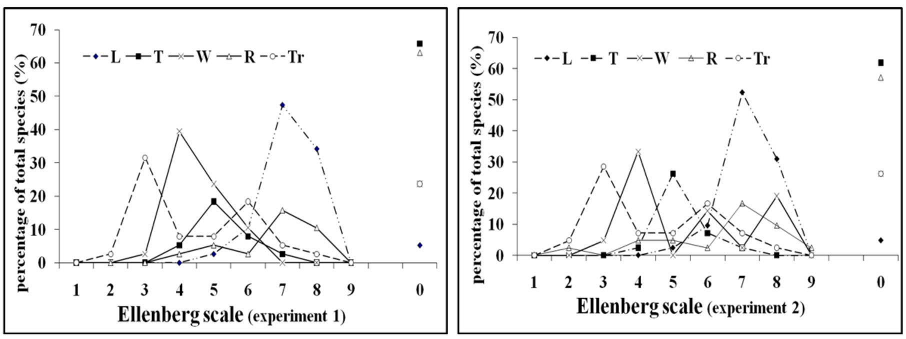

Fig. 2. Distribution of the Ellenberg indicators (1-9 Ellenberg scale) for both experiments

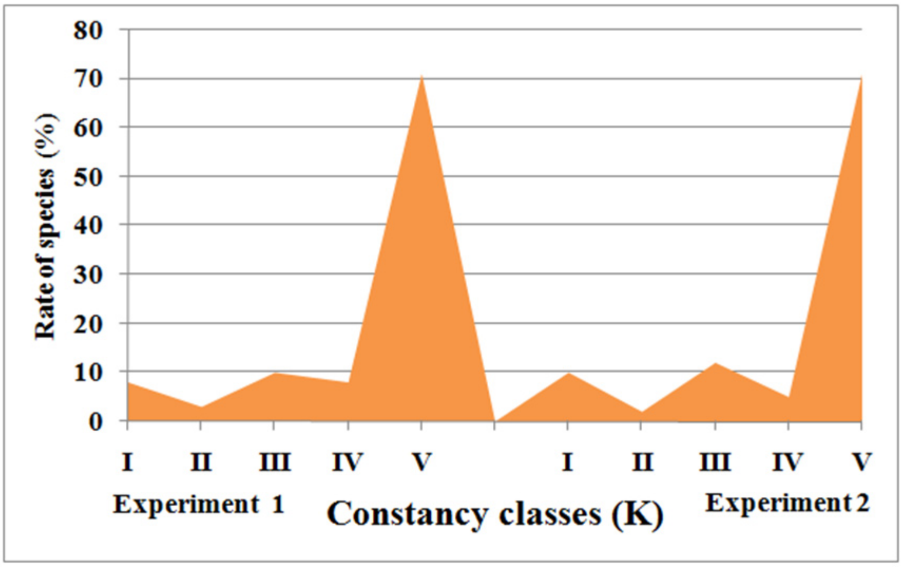

Fig. 3. J-shaped curve for Raunkiaer interfered from the constant value classes of the species found in the second natural grassland (I-V represent the classes of the constancy: I for the plant with low presence <20%; V for the plant present with high presence >80%)

0.100 in the A_0b_0 plots and 0.075-0.098 in the fertilized plots.

In experiment 2, the number of species increased from 14 in the control plots to between 15 and 19 in the fertilized plots. The Shannon index was 2.534 in the A_0b_0 plots and between 1.936 and 2.355 in the fertilized plots. The Shannon Evenness values were 0.914 in the A_0b_0 plots and between 0.715 and 0.849 in the fertilized plots. The Simpson index was 0.097 in the A_0b_0 plots and between 0.149 and 0.253 in the fertilized plots (Fig. 4).

Numerous studies have revealed positive relationships between biodiversity and ecosystem function (Chytrý *et al.*, 2009; Ganatsas *et al.*, 2012, Reiss *et al.*, 2011). Grassland species richness was linked to the type of fertilizers

Tab. 2. Plant composition and ecological characteristics of the identified species (after Ellenberg *et al.*, 1992; Kovács, 1979)

Species	Ecological indicators*					Species	Ecological indicators*				
	L	T	W	R	Tr		L	T	W	R	Tr
Agrostis capillaris [a,b]	7	x	x	x	3	*Trifolium repens* [a,b]	8	x	x	x	7
Anthoxanthum odoratum [a,b]	x	x	x	5	x	*Achillea millefolium* [a,b]	8	x	4	x	5
Arrhenatherum elatius [a,b]	8	6	5	7	7	*Alchemilla vulgaris* [a,b]	6	4	6	x	6
Brachypodium pinnatum [a,b]	6	5	4	7	4	*Carex tomentosa* [b]	7	5	7	9	x
Briza media [a,b]	8	x	x	x	3	*Carum carvi* [a]	8	4	5	x	6
Cynosurus cristatus [a,b]	8	5	5	x	4	*Leucanthemum vulgare* [a,b]	7	x	4	x	3
Dactylis glomerata [a,b]	7	x	5	x	6	*Colchicum autumnale* [a,b]	5	5	6	7	x
Festuca pratensis [a,b]	8	x	6	x	6	*Filipendula vulgaris* [a,b]	8	7	4	x	3
Festuca rubra [a,b]	x	x	5	x	x	*Galium verum* [a,b]	7	5	4	7	3
Holcus lanatus [a,b]	7	6	6	x	5	*Hypericum perforatum* [a,b]	7	x	4	x	x
Nardus stricta [b]	8	x	x	2	x	*Knautia arvensis* [a,b]	7	5	4	x	3
Poa pratensis [a,b]	6	x	5	x	x	*Plantago lanceolata* [a,b]	7	x	x	x	x
Trisetum flavescens [a,b]	7	x	x	x	5	*Plantago major* [a,b]	8	x	5	x	6
Anthylis vulneraria [a,b]	8	x	4	8	3	*Plantago media* [a,b]	7	x	4	8	3
Genista tinctoria [b]	8	5	x	4	2	*Potentilla anserina* [b]	7	5	6	x	7
Lathyrus pratensis [b]	7	5	6	7	6	*Prunella vulgaris* [a,b]	7	x	x	4	x
Lotus corniculatus [a,b]	7	x	4	7	4	*Ranunculus polyanthemos* [a,b]	6	x	4	x	3
Medicago lupulina [a,b]	7	5	4	8	x	*Rhinanthus rumelicus* [a,b]	7	5	4	x	3
Trifolium alpestre [b]	7	6	3	6	3	*Rumex acetosa* [a,b]	8	x	x	x	6
Trifolium campestre [a]	8	6	4	6	3	*Taraxacum officinale* [a,b]	7	x	5	x	8
Trifolium montanum [a,b]	7	x	3	8	2	*Thymus pulegioides* [a,b]	8	x	4	5	3
Trifolium pratense [a,b]	7	x	x	x	x	*Tragopogon orientalis* [a,b]	7	x	5	7	6
Average	7.2	5.2	4.6	6.7	4.4	Average	7.2	5.3	5.5	6.4	4.5

[a]–Experiment 1; [b]–Experiment 2; * Ecological indicators according to Ellenberg *et al.*, 1992 and Kovacs, 1979: L = light value; T = temperature value; W = soil moisture value; R = soil (water) acidity (pH) value; Tr = trophicity value

Tab. 3. Diversity parameters measured in 2010 and 2011

Fertilisation treatment	Species richness (no)	Shannon index	Shannon evenness	Simpson index (D)
Experiement 1				
A_0b_0	15	2.504	0.884	0.100
A_1b_1	19*	2.744*	0.932*	0.075*
A_2b_2	26***	2.855*	0.876 ns	0.076*
A_3b_3	16 ns	2.516 ns	0.907*	0.098 ns
A_2b_1+A_1b_2+ A_0b_0	23***	2.745*	0.875 ns	0.090 ns
A_2b_1+ A_0b_0+ A_1b_3	19*	2.675 ns	0.909 ns	0.084 ns
A_2b_1+A_1b_2+ A_1b_3	17*	2.611 ns	0.921*	0.086 ns
Experiment 2				
$A_0b_0c_0$	14	2.534	0.914	0.097
$A_1b_1c_1$	15 ns	2.143 ns	0.791 ns	0.192*
$A_1b_1c_2$	15 ns	1.936 *	0.715 *	0.253*
$A_2b_2c_1$	16*	2.355 ns	0.849 ns	0.149 ns
$A_2b_2c_2$	16*	2.072*	0.747*	0.217*
$A_3b_3c_1$	19**	2.298 ns	0.780 ns	0.189*
$A_3b_3c_2$	16*	2.122 ns	0.784 ns	0.189*

ns - non significant, $^{*}p<0.05$, $^{**}p<0.01$

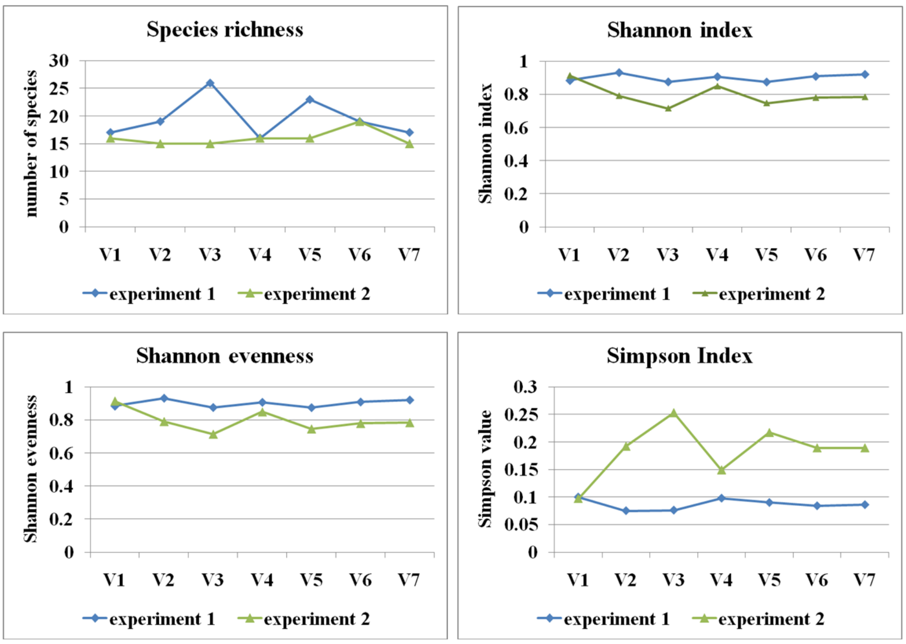

Fig. 4. Relationships between treatments and some diversity indexes (species richness, Shannon diversity index, Shannon evenness and Simpson index)

applied and to the intensity that animals grazed the grassland resource on a year-round basis (Cao *et al.,* 2011; Hejcman *et al.,* 2010).

The increase in the number of species is due to the application of fertilizers, which changed the soil fertility status. This change in soil fertility allowed other mesotrophic and/or eutrophic species to establish themselves in the fertilized plots. Manure fertilizer caused the greatest increase in the number of species, especially in the "plants from the "other botanical families" group because of the pool of seeds that it contains. This study demonstrated that low doses of manure, applied at different intervals, together with low doses of chemical fertilizers, significantly contributed to the increase in the number of species present in the vegetation cover.

Conclusions

The results showed that there were changes in the number of plant species and in the functional groups of plants under the different fertilization treatments. The increase in the number of species could have been caused by improved soil nutrient content after fertilizer application and/or by the species brought in with the applied manure. Using a fertilization management program based on small amounts of organic and mineral fertilizers could contribute to the conservation of biodiversity in these grasslands. The results of this study, in an area considered to be regionally representative of large parts of the mountain areas of Romania, indicated that fertilization treatments could maintain high levels of species diversity.

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