THE INFLUENCE OFMINERAL AND ORGANIC LONG-TERM FERTILIZATION UPON THE FLORISTIC COMPOSITION OF *Festuca rubra* L.-*Agrostis capillaris* L. GRASSLAND IN APUSENI MOUNTAINS, ROMANIA

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Abstract

The semi-natural grasslands in Apuseni Mountains are particularly important for the sustainable development of this region. The future development strategies must take into consideration the conservation of grassland plant diversity. In Apuseni Mountains there are numerous caves and a traditional landscape, these forming the area's wealth. Their joining with agro-tourism and performing a sustainable agriculture would significantly level up the wellbeing of the local population, who are in need of new income sources considering that the wood resources are more and more reduced. In Gârda de Sus there are considerable areas of semi-natural grasslands which lately have been traditionally used. Nowadays, some of them have been abandoned and others irrationally intensified. Therefore, the objective of this paper was to evaluate the effects of organic fertilization upon the sward in order to recommend certain versions which have minor repercussions upon the plant diversity. The research was carried out in Apuseni Mountains, in an experimental field with one experiences with organic fertilizers (T_1 control, T_2 10 t ha^{-1} manure, T_3 20 t ha^{-1} manure, T^4 30 t ha^{-1} manure). Giving organic fertilizers caused installation of *Festuca rubra* L. and *Trisetum flavescens* L. at the treatment using 10 t ha^{-1} manure and *Trisetum flavescens* L. at using 20 and 30 t ha^{-1} manure. The treatment with 10 t ha^{-1} manure induced a growth of the plant diversity and by raising the manure quantity did not cause important changes with respect to the species' number. For future management actions that are meant to maintain the plant diversity in Apuseni Mountains, the organic fertilization with 10 t ha^{-1} manure quantities annually administrated or once in two years is recommended.

Key words: mountainous grassland, organic fertilization, plant diversity, frequency of the species, abundance of the species

Mountain grasslands include some of the most important high nature value areas in Europe, and high-biodiversity mountain farmland can increase tourism incomes and is a potential seed source for local biodiversity restoration (Hopkins, 2011). The grassland system can provide ecosystem services such as soil conservation, water quality protection, biodiversity conservation, medicinal plants, pleasing landscapes, soil carbon storage and greenhouse gas mitigation (Sanderson and Wätzold, 2010). Grassland biodiversity is an important consideration in many agri-environmental policies (Orth et al. 2010). The grasslands in Europe suffer a dramatic loss of biodiversity features because of fertilization, land abandonment or the transformation of grassland into cropland (European Environment Agency, 2007). The first systematic assessment of Europe's most vulnerable habitat types and species has already shown that grasslands in particular have an unfavourable conservation status (Bruchmann and Hobohn, 2010). In less-favoured areas, this can take form of preserving plant diversity, e.g. through low-input farming system (Duru et al, 2010). Semi- natural grasslands need a management system which supports biodiversity conservation (Rotar et al., 2010). Being one of the most diverse plant communities, mountain meadows are the habitat for many rare species (Zarzycki and Misztal, 2010). Manure fertilization contributes to grasslands phyto-diversity, while spring overgrazing or frequent mowing are disadvantageous (Nettier et al., 2010). Questions remain as to what level of organic fertilization optimally maintains the plant diversity of meadows. Organic fertilization and rational use of fertilizers can produce substantial increases of the production and biodiversity and fodder quality improvement (Vîntu et al., 2008). In the central part of Apuseni Mountains, fertilization by manure is the most important component of the traditional management that means to increase the biomass yield, species diversity and identity of the cultural landscape. Nowadays, in Romania, on the

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grasslands of Apuseni Mountains, a management that involves manual labour, with horses-drawn carts and great consumption of time still is performed. This type of management has created over time great plant diversity (Auch, 2006). The meadows present within the perimeter of the Gârda de Sus commune, Apuseni Mountains, Romania, are highly plant diversified, due to traditional management performed over long periods of time. Most of the traditional management techniques use organic fertilization via manure combined with the mixed uses (Gârda, 2010). The recent changes (access roads improvement, better livelihood etc.) will influence the grasslands maintenance manner. Our paper's objective was to evaluate the effects of organic fertilization upon the sward in order to recommend certain versions which have minor repercussions upon the plant diversity.

MATERIALS AND METHODS

The experimental field is located in the Ghetari, Gârda de Sus village, Alba County, Apuseni Mountains, at 1130 m elevation and it was founded in 2001, using the random blocks method. The experiment fields included 4 treatments in 4 replications (T_1 control, $T^2$10 t ha^{-1} manure, T_3 20 t ha^{-1} manure, $T^4$30 t ha^{-1} manure). Each plot measures 10 m^2. The technological inputs administration took place annually in early spring. The organic fertilizer came from cattle and horses having the following elements 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. Thus, a medium supply in N and extremely low in P and K are noticed. The floristic studies were performed according to Braun-Blanquét method. For floristic data we have used PC-ORD program which performs multivariate analysis of ecological data entered in spreadsheets[19]. Our emphasis is on nonparametric tools, graphical representation, randomization tests and bootstrapped confidence intervals for analysis of community data. We also used the MRPP (Multi Response Permutation Procedures), which is a nonparametric procedure for testing the hypothesis of no differences between two or several groups of entities. The method implies the statistic T-test which describes the separation among the groups, meaning the more negative is T, the stronger is the separation. The agreement statistic A describes within - group homogeneity, compared to the random expectation. Also, Non-metric Multidimensional Scaling (NMS) was used, which is an ordination method well suited to data described as not normal or arbitrary, discontinuous, or otherwise questionable scales. NMS is generally the best ordination method for community data. NMDS does not alter the ordination too much and is replacing the correlation coefficient by the frequently used Bray-Curtis index which changes the result considerably 20. A Monte Carlo test of significance was included. The order-abundance and order- frequency correlations give us a synthetic view and are extremely useful in comparative studies 21.

RESULTS AND DISCUSSION

Administrating organic fertilizers produced a vegetation ordination in 4 floristic groups specific to each treatment (*figure 1*). Control floristic composition was much differentiated from the swards structure of all applied treatments (*p<0.01; table 1*).

The floristic composition of the 10 t ha^{-1} manure treatment differed very much from the one of the treatments with 20 and 30 t ha^{-1} manure (p<0.01, respectively, p<0.0.5). The plant community of the 20 t ha^{-1} manure treatment did not differentiate from that of the treatment with 30 t ha^{-1} manure (T = 0.9109, A = -0.0596), this statement not being totally asserted because it did not have statistical insurance (p>0.05).

Control type was represented by *Festuca rubra* L.- *Agrostis capillaris* L. composed by 51.31% Poaceae, 10.75% Fabaceae, 1.06% Cyperaceae-Juncaceae and 38.13% plants of other botanical families (*table 2*). Administrating 10 t ha^{-1} manure caused the installation of *Festuca rubra* L.- *Trisetum flavescens* L. type with 39.06% Poaceae, 15.38% Fabaceae, 0.38% Cyperaceae-Juncaceae and 49.63% plants of other botanical families. This type of plant community is marked by the species *Festuca rubra* L. which experienced a share decrease (p<0.01), by the species *Trisetum flavescens* L. which increased its share comparative to control (p<0.01) and by the species *Agrostis capillaris* L. which was slightly reduced (p<0.005).

The economic groups share was different from the control, but without statistical insurance. Treatments with 20 and 30 t ha^{-1} manure produced *Trisetum flavescens* L. subtype, where Poaceaes comprised 34.56-41.58%, Fabaceaes 12.13-15.13%; Cyperaceae- Juncaceaes 0.0-0.13% and plants of other botanical families 60-61.63 (*Table 2*). This type is marked, first of all, by the species *Trisetum flavescens* L. which significantly increased comparative to control (p<0.001 and p<0.01). The type was reported to be present on fertilized lands, at the slopes base on slightly acid soils In Apuseni Mountains, the association *Trisetum flavescens* was encountered on organically fertilized lands.

Poaceaes were reduced with 20 t ha^{-1} manure towards the control, but without statistical insurance and at the treatment with 30 t ha^{-1} manure the difference was significant (p<0.05). Fabaceaes experienced a slight increase comparative to control, but without statistical insurance ($p>0.05$). Cyperaceaes-Juncaceaes decreased and showed statistical insurance at the treatment with 30 t ha^{-1} manure ($p<0.05$). The plants of other botanical familieshad a strong increase up to 61.63% ($p<0.01$).

In the plant community of the *Festuca rubra* L. - *Agrostis capillaris* L. grassland type (control) certain specific species correlated with this type, like *Arnica montana* L., *Briza media* L., *Hieracium aurantiacum* L., *Polygala vulgaris* L., *Plantago media* L., *Scabiosa columbaria* L., *Potentilla erecta* L. etc. (*figure 2*). Certain species correlated with the *Festuca rubra* L. -*Trisetum flavescens* L. grassland type (10 t ha^{-1} manure): *Alchemilla vulgaris* L., *Colchicum autumnale* L., *Festuca rubra* L., *Gymnadenia conopsea* L., *Leucanthemum vulgare* Lam., *Luzula multiflora* Ehrh., *Taraxacum officinale* Weber ex F.H. Wigg., *Trifolium pratense* L., *Trifolium repens* L. etc. (*figure 2*).

Within the plant community of the *Trisetum flavescens* L. grassland type (20 t and 30 t ha^{-1} manure) the species, like *Achillea millefolium* L., *Agrostis capillaris* L., *Centaurea pseudophrygia* C. A. Mey., *Festuca pratensis* L., *Leontodon autumnalis* L., *Ranunculus acris* L., *Rumex acetosa* L., *Stellaria graminea* L., *Vicia cracca* L. etc., prefering large quantities of organic fertilizers were present.

In the plant community of the *Festuca rubra* L. - *Agrostis capillaris* L. grassland type (control) the most considerable shares, besides the dominant species, were *Alchemilla vulgaris* L., *Lotus corniculatus* L., *Potentilla erecta* L. *Trifolium pratense* L. etc., and the lowest shares were shown by the species: *Arnica montana* L., *Briza media* L., *Hieracium aurantiacum* L., *Hypericum maculatum* Crantz, *Prunella vulgaris* L., *Polygala vulgaris* L. etc (*figure 3*). The most frequent species of this semi-natural grassland type were: *Alchemila vulgaris* L., *Leontodon autumnalis* L., *Lotus corniculatus* L., *Potentilla erecta* L., *Trifolium pratense* L., and the poorest presentation were for the species: *Arnica montana* L., *Briza media* L., *Hieracium aurantiacum* L., *Hypericum maculatum* Crantz, *Taraxacum officinale* Weber ex F.H. Wigg. etc (*figure 4*).

Within the plant community of the *Festuca rubra* L.- *Trisetum flavescens* L. grassland type (10 t ha^{-1} manure) the most dominant species were the following: *Agrostis capillaris* L., *Alchemilla vulgaris* L., *Centaurea pseudophrygia* C. A. Mey., *Pimpinella major* L., *Trifolium pratense* L., *Trifolium repens* L., *Vicia cracca* L. etc., and the lowest participation was specific to the following species: *Festuca pratensis* L., *Carlina acaulis* L., *Hieracium aurantiacum* L., *Polygala vulgaris* L. etc (*figure 5*). The most frequent species out of these types plant community were: *Alchemila vulgaris* L., *Centaurea pseudophrygia* C. A. Mey., *Vicia cracca* L., and less frequent were: *Agrostis capillaris* L., *Festuca rubra* L., *Trifolium pratensis* L. etc. (*figure 6*).

The plant community of the *Trisetum flavescens* L. type (20 and 30 t ha^{-1} manure) had in its structure, besides the most dominant species, the following: *Alchemilla vulgaris* L., *Centaurea pseudophrygia* C. A. Mey., *Pimpinella major* L., *Trifolium pratense* L., *Veronica chamaedrys* L., *Vicia cracca* L., and the ones with the poorest share were: *Carlina acaulis* L., *Festuca pratensis* L., *Prunella vulgaris* L., *Ranunculus bulbosus* L., *Rhinanthus minor* L. etc. (*Fig 7*). The most frequent species within this type were: *Agrostis capillaris* L., *Veronica chamaedrys* L., *Vicia cracca* L., and least frequent were: *Gymnadenia conopsea* L., *Leontodon autumnalis* L., *Lotus corniculatus* L., *Luzula multiflora* Ehrh., *Prunella vulgaris* L. etc. (*figure 8*).

Administrating organic fertilizers caused some changes in the plant diversity level. Control plant community was represented in average by 29.75% species that slightly rised at 10 t ha^{-1} manure application (*Festuca rubra* L.- *Trisetum flavescens* L. grassland type), after which they were reduce at the treatment with 20 and 30 t ha^{-1} manure (*Trisetum flavescens* L. grassland type) down to 26.75. This evolution of the species number did not show statistical insurance, thus the results cannot be confided. If the Shannon Index was to be analysed, it was noticed that at control this was 2.26 and increases up to 2.62 at the treatment with 10 t ha^{-1} manure (*Festuca rubra* L. - *Trisetum flavescens* L. grassland type, $p<0.001$). Increasing the manure quantity up to 20 t ha^{-1} produced a slight increase of the Shannon Index, but with no statistical insurance ($p>0.05$). The treatment with 30 t ha^{-1} manure generated a rise of the Shannon Index up to 2.43 ($p<0.05$; *table 3*), with significant statistical insurance. Similar results were obtained for an *Agrostis capillaris* L. grassland in the Moldavian region (Romania), where administrating organic fertilizers caused a slight increase of the species number 24.

Table 1

The pairwise comparison with MRPP (T – the t test, A – group homogeneity, p – the statistical significance)

Treatments	T	A	P
control vs 10 t ha^{-1} manure	-4.04161127	0.27468564	0.00610061
control vs 20 t ha^{-1} manure	-4.10028142	0.44153932	0.00668743
control vs 30 t ha^{-1} manure	-4.27548712	0.46160655	0.00597436
10 t ha^{-1} manure vs 20 t ha^{-1} manure	-2.02769523	0.12006600	0.04461151
10 t ha^{-1} manure vs 30 t ha^{-1} manure	-3.00087974	0.17128965	0.01249794
20 t ha^{-1} manure vs 30 t ha^{-1} manure	0.91097218	-0.05960591	0.81752838

Table 2

The floristic structure of the grassland types under the influence of organic inputs (dominant species and economic groups, Fr - Ac = *Festuca rubra* L.- *Agrostis capillaris* L., F.r - T.f = *Festuca rubra* L. - *Trisetum flavescens* L., T.f = *Trisetum flavescens* L., Mt. = control, *=*p<0.05*, **=*p<0.01*, ***=*p<0.001*, ^{0}=*p<0.05*, ^{00}=*p<0.01*, ^{000}=*p<0.001*, ^{ns} – not significant)

	Grassland type	Treatments/Signif.							
		Control	Signif.	10 t ha^{-1} manure	Signif.	20 t ha^{-1} manure	Signif.	30 t ha^{-1} manure	Signif.
		F.r-A.c		F.r-T.f		T.f		T.f	
Dominant and co-dominant species	*Agrostis capillaris* L.	15.94	Mt.	11.25	0	12.81	Ns	12.81	Ns
	Festuca rubra L.	32.50	Mt.	14.38	00	7.56	000	3.31	000
	Trisetum flavescens L.	1.63	Mt.	12.81	**	20.00	***	16.88	**
Economic groups	*Poaceae*	51.31	Mt.	39.06	ns	41.38	Ns	34.56	0
	Fabaceae	10.75	Mt.	15.38	ns	12.13	Ns	15.13	Ns
	Cyperacea and *Juncaceae*	1.06	Mt.	0.38	ns	0.13	Ns	0.00	0
	OBF	38.13	Mt.	49.63	ns	60.00	**	61.63	**

Table 3

The effect of organic fertilization upon the plant diversity (T-treatment, Fr-Ac = *Festuca rubra* L.- *Agrostis capillaris* L., F.r-T.f = *Festuca rubra* L.- *Trisetum flavescens* L., T.f = *Trisetum flavescens* L., *=*p<0.05*, **=*p<0.01*, ***=*p<0.001*, ^{0}=*p<0.05*, ^{00}=*p<0.01*, ^{000}=*p<0.001*, ^{ns} – not significant)

Biodiversity index	Treatments			
	Control	10 t ha^{-1} manure	20 t ha^{-1} manure	30 t ha^{-1} manure
Specie's number	29,75	33.00^{ns}	28,00^{ns}	26,75^{ns}
Shannon Index	2.26	2.62***	2.37^{ns}	2.43*

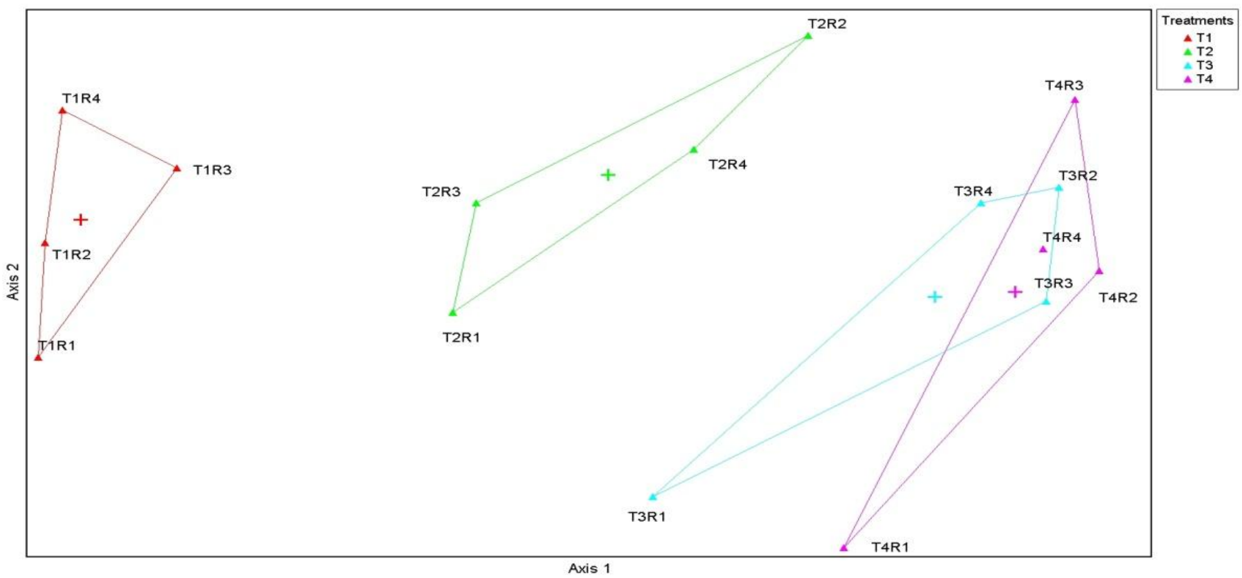

Figure 1 **Ordination of floristic composition depending on the organic treatments (T-treatments, T_1- control, T_2-10 t ha^{-1} manure, T_3- 20 t ha^{-1} manure, T_4-30 t ha^{-1} manure, R-replication)**

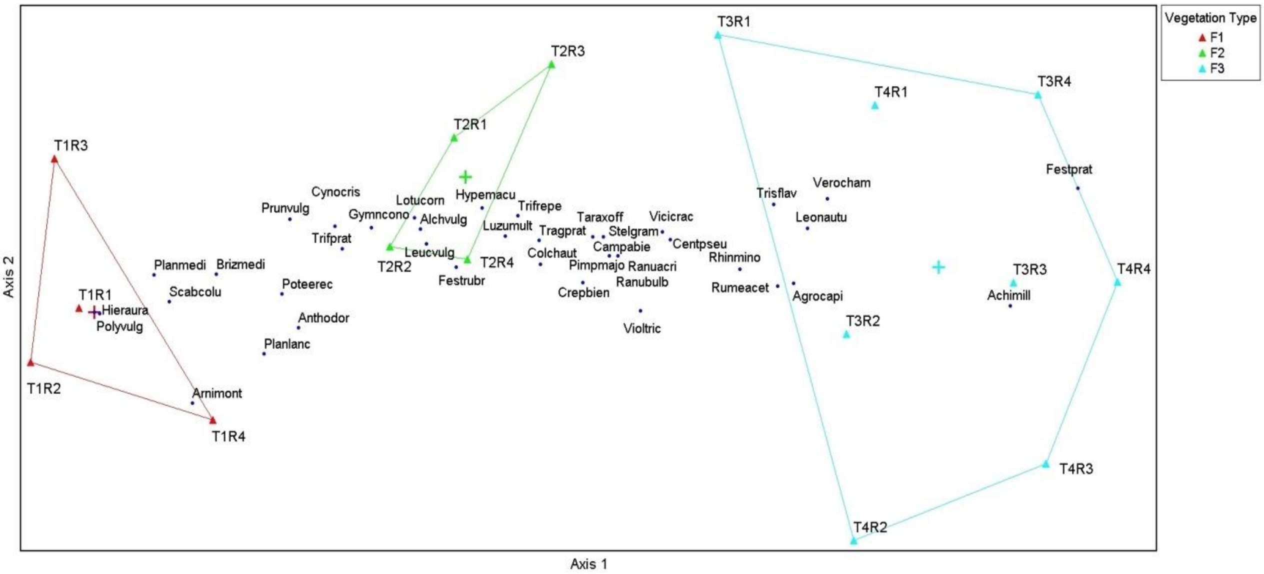

Figure 2 **Ordination of floristic composition on types and species depending on the organic treatments (T-treatments, T_1- control, T_2-10 t ha^{-1} manure, T_3- 20 t ha^{-1} manure, T_4-30 t ha^{-1} manure, R-replication, F-grasslands types, F1 = *Festuca rubra* L. – *Agrostis capilllaris* L., F2 = *Festuca rubra* L. – *Trisetum flavescens* L., F3 = *Trisetum flavescens* L., Achimill = *Achillea millefolium* L., Agrocapi= *Agrostis capillaris* L., Alchvulg= *Alchemilla vulgaris* L., Anthodor= *Anthoxanthum odoratum* L., Arnimont = *Arnica montana* L., Brizmedi = *Briza media* L., Campabie= *Campanula abietina* Griseb., Centpseu = *Centaurea pseudophrygia* C. A. Mey., Colcautu = *Colchicum autumnale* L., Crepbien = *Crepis biennis* L., Cynocris = *Cynosurus cristatus* L., Festprat = *Festuca pratensis* L. , Festrubr= *Festuca rubra* L. , Gymncono = *Gymnadenia conopsea* L., Hieraura = *Hieracium aurantiacum* L., Hypemacu= *Hypericum maculatum* Crantz, Leonautu =*Leontodon autumnalis* L., Leucvulg =*Leucanthemum vulgare* Lam., Lotucorn = *Lotus corniculatus* L., Luzumult = *Luzula multiflora* Ehrh., Pimpmajo = *Pimpinella major* L., Planlanc = *Plantago lanceolata* L., Planmedi = *Plantago media* L., Polyvulg = *Polygala vulgaris* L., Poteerec = *Potentilla erecta* L., Prunvulg = *Prunella vulgaris* L., Ranuacri = *Ranunculus acris* L., Ranubulb = *Ranunculus bulbosus* L., Rhinmino = *Rhinanthus minor* L., Rumeacet = *Rumex acetosa* L., Scabcolu = *Scabiosa columbaria* L., Stelgram = *Stellaria graminea* L., Taraoffi = *Taraxacum officinale* Weber ex F.H.Wigg., Tragprat = *Tragopogon pratensis* L., Trifprat = *Trifolium pratense* L., Trifrepe = *Trifolium repens*, L., Trisflav= *Trisetum flavescens* L., Verocham = *Veronica chamaedrys* L., Vicicrac= *Vicia cracca* L., Violtric = *Viola tricolor* L.)**

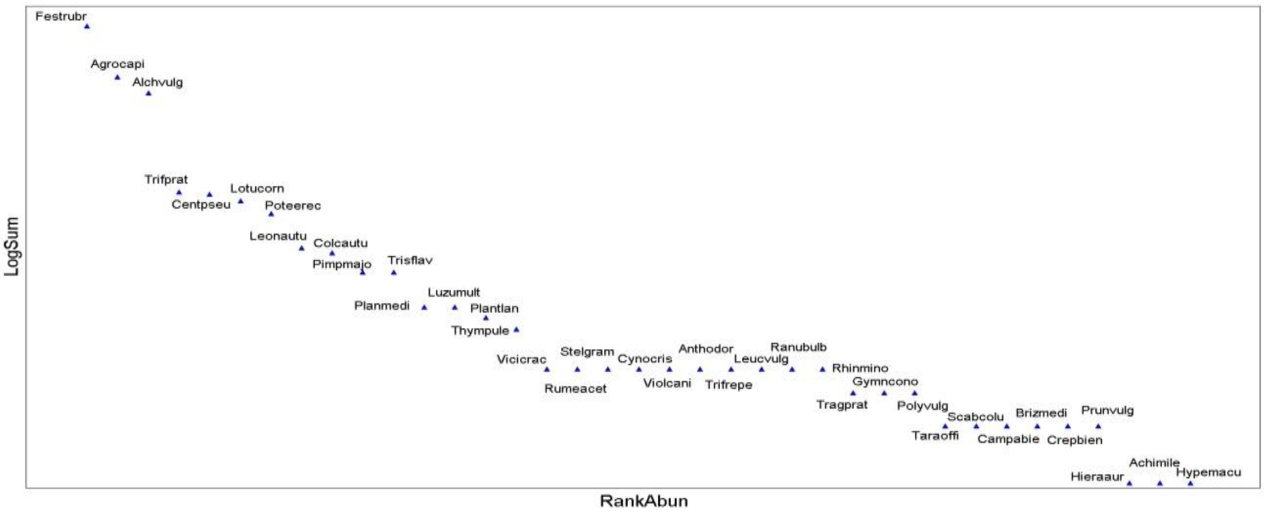

Figure 3 **Species abundance in *Festuca rubra* L.-*Agrostis capillaris* L. grassland (control, Rankabund = Rank abundance of the species, Logsum = Log base 10 of the species Sum, for species names see legend of Figure 2)**

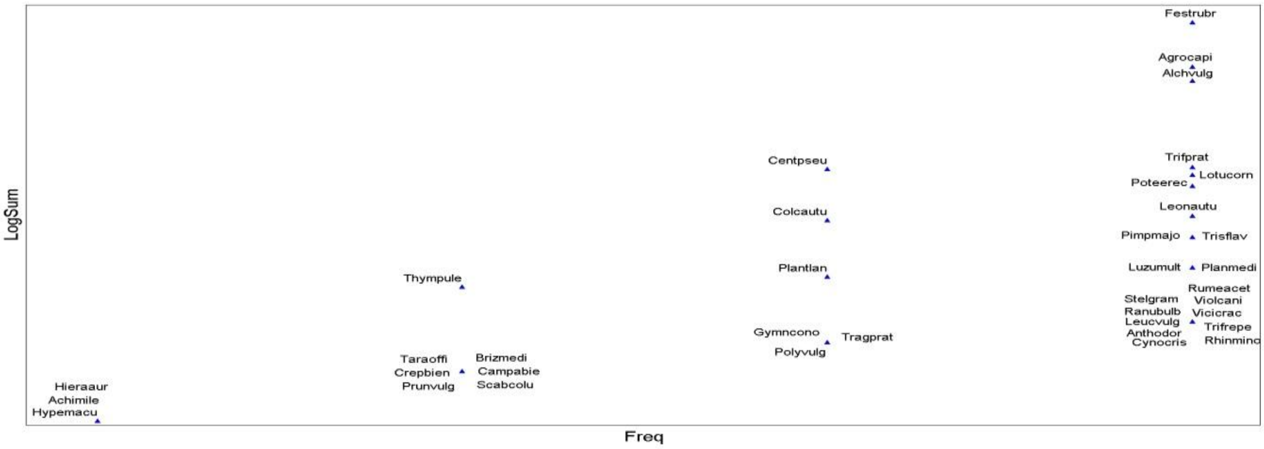

Figure 4 **Species frequency in *Festuca rubra* L. - *Agrostis capillaris* L. grassland (control, Freq = Frequency of the species, Logsum = Log base 10 of the species Sum, for species names see legend of Figure 2)**

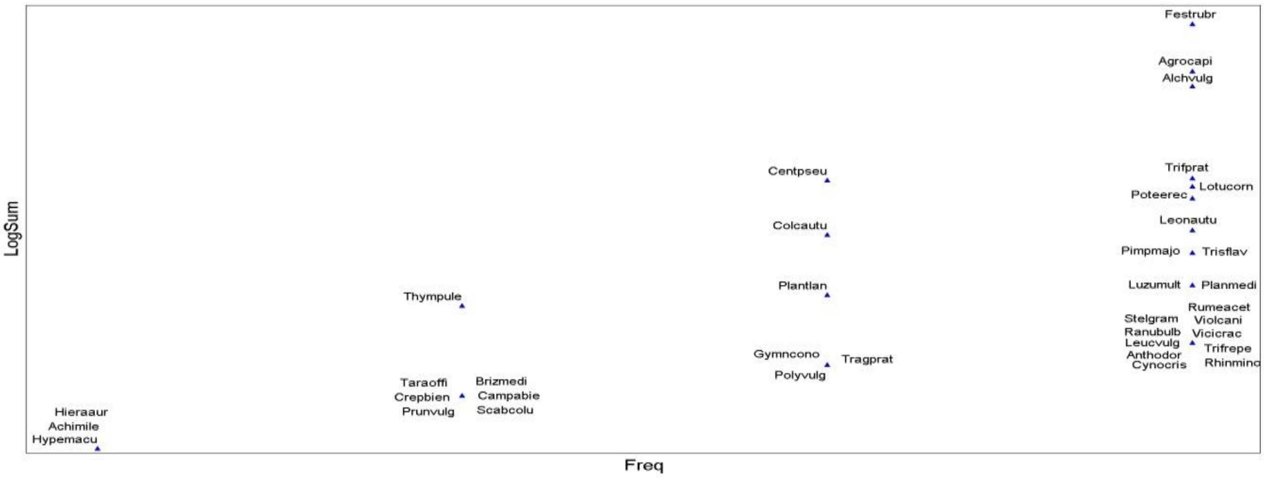

Figure 5 **Species abundance in *Festuca rubra L. - Trisetum flavescens* L. grassland (treatment with 10 t ha^{-1} manure, Rankabund = Rank abundance of the species, Logsum = Log base 10 of the species Sum, for species names see legend of Figure 2)**

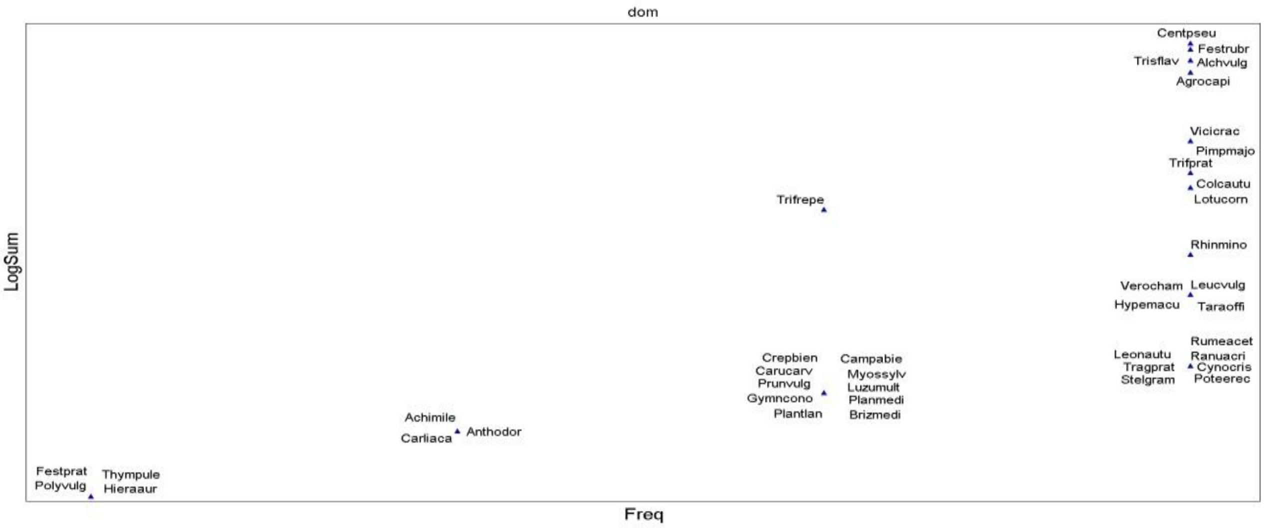

Figure 6 (**Species frequency in *Festuca rubra L .- Trisetum flavescens* L. grassland (treatment with 10 t ha^{-1} manure, Freq = Frequency of the species, Logsum = Log base 10 of the species Sum, for species names see legend of Figure 2)**

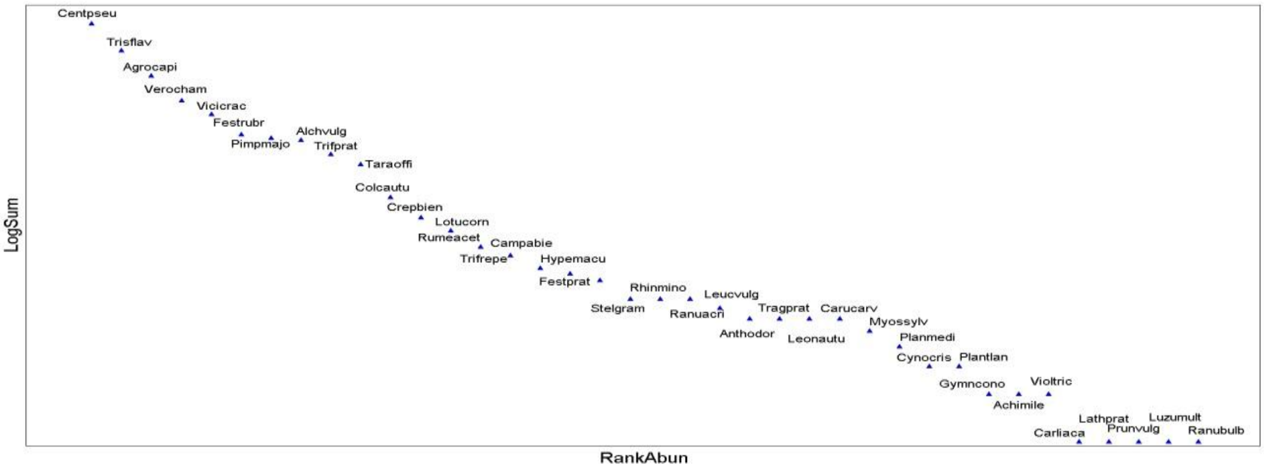

Figure 7 **(Species abundance in *Trisetum flavescens* L. grassland (treatments with 20 t and 30 t ha^{-1} manure, Rankabund = Rank abundance of the species,Logsum = Log base 10 of the species Sum, for species names see legend of Figure 2)**

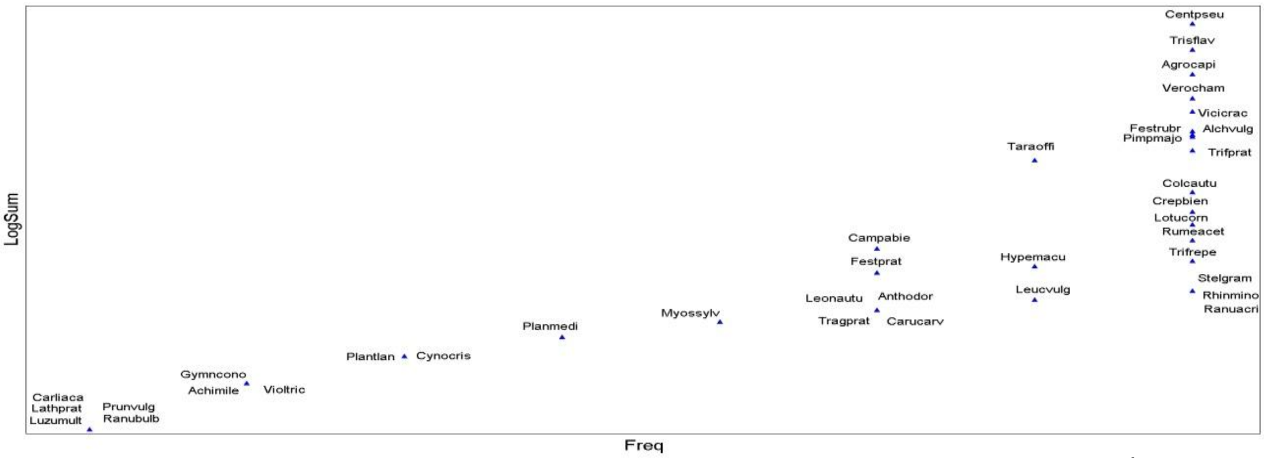

Figure 8 **(Species frequency in *Trisetum flavescens L.* grassland (treatments with 20 t and 30 t ha^{-1} manure, Freq = Frequency of the species, Logsum = Log base 10 of the species Sum, for species names see legend of Figure 2)**

CONCLUSIONS

The organic fertilizers administration on the *Festuca rubra* L. - *Agrostis capillaris* L. grassland type produced important changes within the sward depending on the applied treatments. Administration of 20 and 30 t ha^{-1} manure produced the installation of *Trisetum flavescens* L. grassland type. Administrating organic fertilizers induced plant diversity growth, especially at the treatment with 10 t ha^{-1} manure. For future management actions that are meant to maintain the plant diversity in Apuseni Mountains, the organic fertilization with 10 t ha^{-1} manure quantities annually administrated or once at two years is recommended.

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