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Fertilizers' Impact on Grassland in Northeastern Romania

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Abstract: In order to obtain more data concerning the influence of fertilizers (organic and mineral) on different forage plants in the northeastern Romanian grassland, the mass fractions of 14 essential, enzymatic, or toxic elements were determined by instrumental neutron activation analysis together with the amount of crude proteins, ash, fibers, as well as fat ether extract. The final results showed a significant variance in the content of analyzed elements on organic as well as on mineral fertilized experimental plots. At the same time, increased content of crude protein and fat ether extract was evident in fertilized grasses for all applied fertilizers, while other global indicators such as neutral and acid fibers of sulfuric lignin content decreased, suggesting significantly higher nutritional values for fertilized forage plants.

Keywords: organic fertilizers; mineral fertilizers; grassland; dietary cation–anion difference; instrumental neutron activation analysis

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1. Introduction

Romania is a medium-sized country located in southeastern Europe. Bordering on the Black Sea, the country is halfway between the equator and the North Pole and equidistant from the westernmost part of Europe–the Atlantic Coast—and the most easterly–the Ural Mountains. At the same time, Romania has a well-balanced relief, in that about one-third of the country's surface is represented by plains, one-third by hills, and one-third by mountains with a maximum height of 2544 m [1,2].

Both geographical position and relief diversity determine a variety of climatic zones, beginning with humid subtropical in the south to subarctic and tundra at altitudes higher than 1800 m, and with a medium temperature varying between +11 °C in the south and +8 °C in the north. Under these conditions, the average precipitation is 589 mm for a relative humidity around of 70% [3].

These factors significantly influence the distribution of agricultural surface such that, from a total of 14.8 Mha of agriculture classes, the exploitable grassland (pasture and hay-field) covers about 4.9 Mha [4]. On the other hand, due to improper management, a significant proportion of pastures, which cover about 60% of the mountain area (18% of the entire country), are in many places overgrazed.

Overgrazing represents one of the most acute problems of any ecosystem, which can be extended to entire countries. As a rule, overgrazing appears when livestock graze on vegetation at a rate exceeding the system's capacity to regrow. Once the vegetation carpet disappears, the soil loses the most fertile topsoil, becoming prone to irreversible and total degradation. According to the Food and Agriculture Organization (FAO) of the United Nations [5,6], livestock represents one of the most significant factors that affect the environment, while the overgrazed area represents more than 20% of total pasture surface.

In Romania, grazing is well-regulated by the Land Fund Law 18/1991 [7], which was complemented in 2013 by Emergency Ordinance 34/2013 [8]. They establish the regime of exploitation of natural grasslands, including the fact that they can be organized for at least five years depending on the species of plants that inhabit them. As a corollary, grasslands cannot be turned into agricultural land or urban areas without government approval.

To prevent overgrazing, in addition to a series of severe regulations at the national level as mentioned above, the reintroduction of native fodder plants can mitigate overgrazing consequences. At the same time, better management and the administration of both natural and mineral fertilizer could be long-term solutions to prevent the consequences of overgrazing.

It has been shown that the application of natural fertilizers such as manure or N and P mineral fertilizers has increased the above-soil biomass and microbial community, stimulated plant diversity, and, at the same time, contributed to overgrazed topsoil regeneration [9–11]. The effect of this procedure has been shown to be beneficial for a great diversity of grassland [12,13], not only by restoring the vegetation but also by increasing the amount of forage crude protein (CP) and dried matter digestibility [14], which, in their own way, have a helpful influence on the grazing animals.

In this regard, it is worth mentioning that the use of fertilizer could significantly increase the forage biomass per hectare [15], and, at the same time, it could affect not only the nutritional value but also the content of a great diversity of elements, including Na, K, and Cl, whose concentration defines the dietary cation–anion difference (DCAD) [16], as well as the more enzymatic transition metal elements, i.e., Mn, Fe, Co, Zn, Mo, and Se [17], and even harmful ones such as Al [18,19] and As [20,21]. To this list, we could add Mg and Ca, whose role in plant metabolism and in the cell wall and membrane structures is essential [22,23], as well as Sr, due to its role in the process of osteosynthesis by stimulating bone formation together with the inhibition of bone resorption [24,25]. On the other hand, according to [26], Sr could cause harmful effects due to its mobility within plants. All these facts make the study of this category of elements that plays a major role not only in plants' physiology but also in the quality of derivative products currently used for human consumption, such as milk and or meat, very important [27,28].

Since Romania's admission to the European Union, European standards on food security have been adopted and implemented, and their application, according to Romanian legislation [29], is mandatory for all units that make up the food chain, agricultural production, the food industry, and public nutrition [30].

For a better characterization of the influence of fertilizer on the most common type of grasses, we have used instrumental neutron activation analysis (INAA) in both its thermal and epithermal variants [31] to determine the mass fractions of 14 major and trace elements in three types of perennial grasses in correlation with some of the most important biochemical descriptors of their nutritional values. It is worth mentioning that the forage grasses considered here are commonly used for cattle food.

To determine the mass fraction of a large category of elements, including the metabolic and structural, enzymatic, as well as toxic ones [17], we used INAA due to both its ability to determine the mass fractions of more than 40 elements with an accuracy on the order of 0.1–1 mg/kg and to the fact that INAA permits direct measurements of samples without any preliminary processing such as acid digestion [32]. Also, access to one of the best nuclear reactors still in use, i.e., IBR2 of the Joint Institute of Nuclear Research in Dubna, encouraged us to use INAA instead of other high-accuracy analytical methods such as ICP-MS or ICO-OAS.

2. Hypothesis and Research Objectives

Given the diversity of both forage grasses and their habitats, the main goals of this study are as follows:

(i) To investigate to what extent the use of natural and mineral fertilizers influences the content of some essential and harmful elements in three types of forage grasses common in Romanian medium- to high-altitude grasslands;

(ii) To quantify the influence of fertilizers as reflected by the parameters that characterize the nutritional value of the forages considered, i.e., the dietary requirements.

The results of our study performed under these circumstances will be further presented and discussed.

3. Materials and Methods

3.1. Sampling and Sample Preparation

For our study, we have chosen three types of perennial grasses, all of them currently populating the temperate climate grassland, i.e., *Agrostis capillaris* L., *Festuca rubra* L., and *Nardus stricta* L. The experiment was organized in four different places in Suceava County, northeastern Romania, at altitudes between 611 and 840 m, which are typical for Romania grasslands (Figure 1).

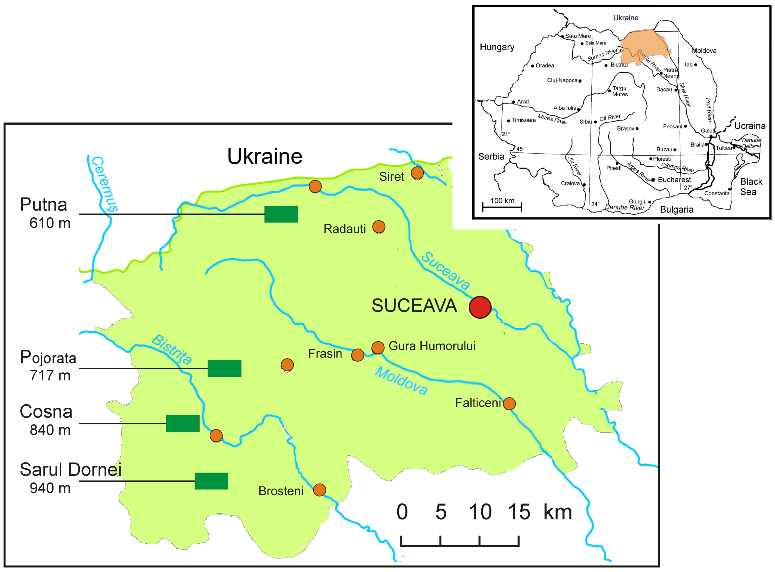

Figure 1. The location of experimental areas within Suceava County of the Romanian territory (inset).

All experiments consisted of applying a mixture of natural and mineral fertilizers for five consecutive years following different schemes as illustrated in Table 1. For this, 15 to 27 plots of land of 2×3 m, randomly disposed to form compact blocks of 90, 126, and 162 m^2, respectively, were kept unfertilized as reference or were fertilized as previously mentioned (Figure S1). For each experimental location, a detailed description of the different procedures used in fertilizer administration is reproduced in Tables A1–A3 (see Appendix A).

After four years of controlled fertilization, for two consecutive years, the grass was harvested, cleaned of foreign vegetation, dried, ground, and analyzed for major and trace elements as well as for the nutritional quality specific parameters.

Table 1. General data concerning the location of the experimental parcel, grass species involved in the experiment, as well as the type and amount of administered fertilizers.

Locality Grass Species	Natural Fertilizer (t/ha)	Chemical Fertilizer (t/ha)
Cosna (840 m altitude) *Nardus stricta* L.	20 to 50 t of manure applied annually/biannually (1–4 years)	$N_{100}P_{100}$; $N_{140}P_{140}$; $N_{200}P_{200}$; $N_{100}P_{100}$ + $N_{40}P_{40}$; $N_{100}P_{100}$ + $N_{100}P_{100}$; $N_{80}P_{80}$ + $N_{640}P_{60}$
Pojorata (717 m altitude) *Agrostis capillaris* L. *Festuca rubra* L. *Nardus stricta* L.	10 to 50 t of manure applied annually/biannually (1–4 years)	30–50 kg mineral nitrogen + 10 to 30 t of manure applied annually, biannually, or every three years
Putna (611 m altitude) *Agrostis capillaris* L. *Festuca rubra* L.	20 to 50 t of partially or totally fermented manure	—
Sarul Dornei (940 m altitude) *Festuca rubra* L. *Nardus stricta* L.	20 t to 30 t of manure applied annually and biannually following the schedule: 50 + 0 + 40 + 0 t over 4 years	—

3.2. Analytical Techniques

INAA was used to determine the mass fractions of the following elements: Na, Mg, Al, Cl, K, Ca, Mn, Fe, Co, Zn, Se, As, Br, Sr, and Mo. Among them, Mn, Fe, Co, Cu, Zn, Mo, and Se enter, according to Markert et al. [17], into the composition of some enzymes that are indispensable for plants' vital processes. Also important are the alkali elements Na and K, whose average mass fractions can reach up to 1.5% wt., together with the structural elements Cl and Ca, considered to be some of the main constituents of plant cells [17]. As mentioned before, Al [18,19] and As [20,21] are considered toxic for animals and especially for human consumption, while Sr could be harmful to plants [26]. For this reason, their presence needs special attention.

Related to this, it is worth mentioning that the mass fractions of Na, K, and Cl define the dietary cation–anion difference (DCAD) [16] as follows:

$$\mathrm{DCAD} = (\mathrm{Na}^{+} + \mathrm{K}^{+}) - \mathrm{Cl}^{-}, \tag{1}$$

where Na^{+}, K^{+}, and Cl^{-} represent the concentrations of mentioned elements expressed in milli-equivalents per 100 g of dietary dry matter.

The DCAD represents a valuable proxy in estimating the influence of the grasses' major elements (Na, K, and Cl) on cattle lactation and meat productivity. Accordingly, in dry cows, a negative DCAD can help prevent metabolic problems, while in the case of lactating cows, a positive DCAD helps increase milk production and nutritional potential.

All INAA measurements were performed by using the fast-pulsed reactor IBR2. To determine the mass fraction of the above-mentioned elements, both short- and long-time irradiation were used. More details on the experimental setup and quality control are provided in [33–36].

Special attention was paid to quality control, accomplished by simultaneous use of Standard Reference Materials (SRMs) assembled to compose the so-called Group of Standard Samples (GSSs) proprietary software [33–35]. The aim of this procedure consisted of selecting the most suitable SRM lines to maximize both accuracy and precision of INAA determinations for all considered elements. This allowed us to attain an accuracy between 3 and 15% for each INAA measurement, expressed using combined statistical uncertainty (CSU) [36,37].

Simultaneously with the INAA measurements, five chemical parameters essential for characterizing the nutritional qualities of grasses, i.e., the ash content (AC), CP, fat ether extract (FEE), acid detergent fiber (ADF), and neutral detergent fiber (NDF), were also determined.

The ash content was gravimetrically determined by muffle furnace ignition at 550 °C [38]; CP and FEE were determined by the Kjeldahl [39] and Soxhlet extraction [40] methods, respectively; and the Van Sosest method was used to determine both ADF and NDF, as well as the sulfuric lignin content (SLC) [41]. For these methods, the CSU was no greater than 7%. All these parameters were experimentally determined in the Chemical Analysis Laboratory of the Iasi University of Life Sciences.

3.3. Statistical Data Analysis

In order to examine to what extent the utilized fertilizers influenced the presence of the considered elements, Student's *t*-test was used. Both elemental statistics and Student's *t*-tests were performed using PAST software (version 4) [42].

4. Results

The final results concerning the mass fractions of the most important elements in both unfertilized (for reference) and fertilized soil grasses are reproduced in Table 2. Accordingly, to highlight the influence of fertilizers, we have provided not only the mass fractions of investigated elements but also the corresponding values of the reference plants [17], the recommended dietary mineral requirements (RDMR) [43], and the maximum tolerable level (MTL) [43]; all of the data can be found in Table 2. In this regard, it should be mentioned that, for a better statistical analysis, as well as to be closer to real grasslands, all presented results refer to the average values obtained by combining the corresponding parameters for all three types of grasses considered in this study.

In order to examine the influence of the fertilizing procedures investigated in this study, in Table 3, we have illustrated to what extent the presence of fertilizer influenced the mass fractions of the considered elements in the fertilized grasses. All values were significant at $p < 0.05$ (95% probability) according to Student's *t*-test. In this regard, it should be noted that the provided value is for the average results of all three grass species, i.e., *Agrostis capillaris* L., *Festuca rubra* L., and *Nardus stricta* L.

Table 2. Final results concerning the effect of fertilization on the mass fractions (in mg/kg) of the metabolic and structural elements (black ink), enzymatic elements (blue ink), as well as potentially toxic elements (red ink) in foraging grasses involved in the experiment. For a better comparison, the recommended dietary mineral reserve (RDMR) [41], maximum tolerable level (MTL) [43], and the reference plants (RPs) [17] are reproduced as well. Note: bdl—below detection limit.

Element	Cosna		Pojorata		Sarul Dornei		Putna		RDM	MTL	RP
	Unfertilized	**Fertilized**	**Unfertilized**	**Fertilized**	**Unfertilized**	**Fertilized**	**Unfertilized**	**Fertilized**			
Na	80 ± 50	110 ± 50	90 ± 50	100 ± 30	50 ± 20	50 ± 10	80 ± 20	90 ± 10	1000–2000	40,000	150
Cl	1960 ± 820	2730 ± 1320	1350 ± 820	1480 ± 610	4630 ± 2090	4500 ± 1730	3310 ± 1740	4290 ± 1300	1300–2900	40,000	2000
K	9500 ± 3700	12,200 ± 4900	16,500 ± 3700	15,800 ± 3700	16,900 ± 3500	19,000 ± 3700	20,300 ± 3700	21,700 ± 2500	4700–10,000	30,000	19,000
Mg	2800 ± 860	3860 ± 2530	5700 ± 860	5080 ± 1240	3020 ± 800	3280 ± 890	2100 ± 530	1940 ± 600	1100–2100	2000	2000
Ca	5160 ± 1410	7150 ± 2950	14,230 ± 1410	11,110 ± 4230	9530 ± 830	9650 ± 1050	5780 ± 1580	6290 ± 1310	2200–3800	7000	10,000
Mn	550 ± 210	590 ± 340	80 ± 10	70 ± 30	260 ± 40	240 ± 20	460 ± 90	440 ± 40	13–24	1000	200
Fe	300 ± 250	360 ± 180	250 ± 250	290 ± 120	220 ± 140	160 ± 30	210 ± 80	200 ± 70	12–40	500	150
Co	0.23 ± 0.1	0.27 ± 0.1	0.16 ± 0.1	0.16 ± 0.05	0.16 ± 0.05	0.15 ± 0.04	0.19 ± 0.04	0.18 ± 0.03	0.10–0.15	25	0.2
Ni	3.3 ± 0.4	3.1 ± 0.7	bdl	1.9 ± 0.4	3.8 ± 1.9	4.3 ± 2.5	4.9 ± 1.2	5 ± 1.5	21–55	500	1.5
Zn	70 ± 11	65 ± 15	40 ± 11	33 ± 9	69 ± 8	71 ± 7	55 ± 6	49 ± 9	0.1–0.3	2	50
Mo	1 ± 1.3	0.4 ± 0.4	0.5 ± 1.3	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2	5	0.5
Se	0.2 ± 0.03	0.22 ± 0.04	bdl	0.19 ± 0.04	0.28 ± 0.08	0.28 ± 0.09	0.29 ± 0.04	0.29 ± 0.03	13–24	1000	0.02
Al	510 ± 400	640 ± 440	490 ± 400	390 ± 290	350 ± 330	240 ± 50	180 ± 50	200 ± 60	—	1000	80
As	1.7 ± 0.7	1.8 ± 0.8	1 ± 0.7	1.1 ± 0.6	1.4 ± 0.7	1.4 ± 0.6	1.5 ± 0.5	1.4 ± 0.4	—	30	0.1
Sr	24 ± 12	30 ± 10	14 ± 12	13 ± 3	40 ± 9	42 ± 6	32 ± 8	33 ± 7	—	2000	50

Table 3. Influence of fertilizer type on the content of metabolic and structural elements (black ink), enzymatic elements (blue ink), as well as potential toxic elements (red ink) in foraging grasses involved in the experiment. All results were significant at $p < 0.05$ according to Student's *t*-test. Note: nd means below the detection limit.

Element	Organic Fertilizers			Mineral Fertilizers		
	Increased (%)	Unchanged (%)	Decreased (%)	Increased (%)	Unchanged (%)	Decreased (%)
Na	50	37.5	12.5	50	50	nd
Cl	87.5	12.5	nd	25	25	50
K	50	37.5	nd	nd	75	25
Mg	50	12.5	37.5	25	25	50
Ca	62.5	12.5	25	nd	25	75
Mn	nd	12.5	87.5	50	25	25
Fe	62.5	12.5	25	50	25	25
Co	25	75	nd	50	25	25
Zn	25	50	25	25	25	50
Se	nd	nd	nd	nd	nd	nd
Mo	75	nd	25	25	nd	50
Al	25	12.5	62.5	25	25	50
As	nd	62.5	37.5	50	25	25
Sr	75	nd	25	nd	50	50

5. Discussion

Regarding the first objective of our study, we have compared the content of the above-mentioned category of elements in the foraging grasses cultivated on fertilized grassland with the content of the same elements in grasses collected from unfertilized plots.

A first remark concerning the global data provided in Table 2 points toward a great spread of final mass fractions of almost all elements, although the CSU, as previously mentioned, never exceeded 7%.

In our opinion, this fact could be attributed to either the reduced experimental surfaces that determined the significant variability in the numerical data or to the diversity of pedologic conditions. On this subject, it should be taken into account that the experiment took place in four different places at heights varying between 610 m for Putna and 940 m for Sarul Dornei (Figure 1), with different local climate and soil types.

At the same time, the results reproduced in Tables 2 and 3 clearly show that, in all cases, Na presents a chronic deficit, as its mass fraction is significantly lower than the RDMR. Also, it should be remarked that, in Cosna, Pojorata, and Sarul Dornei, the Al mass fraction locally exceeded the MTL, which should be a concern for local authorities. In all other cases, the content of investigated elements was either lower or higher than the RDMR, never exceeding the MTL.

The same results confirmed a relatively dispersed reaction to fertilizer administration, which most probably, as mentioned before, can be attributed to local variability of soil and micro-climates. From this point of view, the most positive results were observed in the Cosna experimental plots with organic fertilizers, where the presence of almost all elements showed a statistically proven growth at $p < 0.05$.

We have also noted the opposite tendency in the Cosna experimental plots, but this time for mineral fertilizers, where the content of almost all considered elements decreased to that of non-fertilized areas.

The extreme variation we have noticed in Na and Ca content in fertilized grasses increased in six locations, mainly as a result of applying organic fertilizer, and in the case of Mn, whose mass fraction decreased in six locations that were mostly fertilized with manure.

Although V and Cr can play a significant role in plant metabolism, their content was, in many cases below the detection limit, so we did not consider them in this report.

More detailed information concerning the influence of different fertilizing procedures is provided in Table 3. Here we have illustrated to what extent the presence of the considered elements increased, decreased, or remained unchanged after fertilizer administration. In all cases, the probability was more than 95% ($p < 0.05$). Accordingly, the contents increased in 24% of plots, decreased in 23%, and remained unchanged in 45%, while in 8%, their content was below the detection limits, as in the case of Se. It should be remarked that, according to the data in Table 3, Fe remains the single element whose content, excepting the Sarul Dornei (organic fertilizer) and Pojorate (mineral fertilizer) experimental areas, increased in all other locations; see Table 3.

The best results were observed for the Cosna plots (organic fertilizers, 2010) where the mass fraction of eight elements increased. On the opposite side was Pojorata (organic fertilizer, 2011), where the mass fractions of all elements either remained unchanged or even decreased.

In terms of the second objective, we have calculated the more representative descriptors, such as dietary cation–anion difference (DCAD), crude protein (CP), ash content (AC), fat ether extract (FEE), neutral detergent fiber (NDF), and acid detergent fiber (ADF), as well as the sulfuric lignin content (SLC), by using the results obtained by both INAA and chemical analysis.

The obtained results reproduced in Table 4 show that DCAD, whose values are determined by the content of Na, K, and Cl, as well as by the fertilizer type, either organic or mineral, seems to have greater values in the case of mineral fertilizers. In our opinion, this fact may be due to the reduced content of Na, which the use of organic and mineral fertilizers could not increase significantly. Despite this fact, DCAD presented positive values everywhere.

Table 4. The influence of organic fertilizers on the parameters characterizing the nutritional qualities of grasses for both organic and mineral fertilizers. All results were significant at $p < 0.05$ according to Student's *t*-test.

Fertilization Variant	DCAD	CP	AC	FEE	NDF	ADF	SLC
				Organic Fertilizers			
V1 Unfertilized Control	3100	6.4	5.5	1.6	74.7	47.1	11
V2 20 t/ha every year	3130	9.3	9.8	2	60.2	38.4	10.4
V3 30 t/ha every year	3720	11.5	9	1.6	55.1	37.5	10.4
V4 40 t/ha every year	3170	9.7	10.5	1.9	50.1	38.5	10.4
V5 50 t/ha every year	3940	10.8	10.2	2.2	53.8	40.7	9.9
V6 20 t/ha every 2 years	2100	11.7	8.8	1.7	57.5	37.7	9.9
V7 30 t/ha every 2 years	2150	12	9	1.3	57.8	39.4	9.5
V8 40 t/ha every 2 years	2590	12.8	9.4	1.3	55.9	38.6	9.6
V9 50 t/ha every 2 years	2620	13.1	10	1.5	54.8	43.1	9.2
				Mineral Fertilizers			
V1 Unfertilized Control	3200	6.5	7.6	2.7	72	46	10
V2 N100P100	3420	6.6	6.3	3	59	34	9.6
V3 N140P140	4430	8.1	6.2	3.1	59	33	9.8
V4 N200P200	3180	10	7.1	3.2	52	33	9.5
V5 N100P100 + N40P40	3370	11	6	3	55	34	9.7
V6 N100P100 + N100P100	3070	14	7.3	3.3	54	32	10.5
V7 N80P80 + N60P60	2670	11	6.2	2.6	59	40	9.6

At the same time, both organic and mineral fertilizers seem to have a positive role in increasing the quality of foraging grasses. According to results illustrated in Table 4, CP and FEE, the latter of which consists chiefly of fats and fatty acids, increased in almost all cases. A similar behavior could be seen for AC, which represents a measure of the total amount of minerals present within plants. The higher the AC, the more developed the aerial part of the plant. This characteristic could be well-correlated with an increased content of protein and fats for all three grass species, i.e., *Agrostis capillaris* L., *Festuca rubra* L., and *Nardus stricta* L.

On this subject, it is worth mentioning that the ash content increased only in the case of organic fertilizers, showing an opposite tendency for mineral ones. In our opinion, this fact, if future experiments can confirm it, could play a significant role in increasing the use of organic fertilizers, which are more environmentally friendly.

It is worth mentioning that the values of the other nutritional parameters, such as NDF, ADF, and SLC, significantly decreased in the presence of both mineral and organic fertilizers, which, in our opinion, confirms the positive role of fertilizer in the regeneration process of the overgrazed grasslands.

6. Conclusions

To examine the possible influence of different fertilizing procedures, instrumental neutron activation analysis and other analytical techniques were used to determine the mass fractions of 14 elements as well as the numerical values of seven nutritional descriptors in three species of *Gramineae* currently found on Romanian grasslands. The grass samples were collected from unfertilized and fertilized experimental plots, all of them located in Suceava County in northeastern Romania.

The analysis of the elemental content of the 14 most important structural, metabolic, enzymatic, or potentially toxic elements showed, first of all, a significant Na deficit for all plants harvested from both fertilized and unfertilized experimental plots.

Also, we noticed a significant variance in the content of analyzed elements for both types of experimental plots; e.g., the elemental content increased in 24% of plots, decreased in 23%, and remained unchanged in 45%, while in 8%, it was below the detection limits, as in the case of Se.

A better influence was noticed for crude protein and ether extract, whose content increased in almost all fertilized grasses. Both indicators point towards an increased nutritional value.

The values of other global indicators, such as neutral and acid fibers of sulfuric lignin content, decreased in almost all cases. This finding again suggests the beneficial influence of both organic and mineral fertilization.

The great variability observed for all descriptors points towards the use, in future studies, of significantly larger experimental plots to reduce as much as possible the statistical uncertainties associated with small amounts of the analyzed material.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations11050139/s1.

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Abbreviations

The following abbreviations are used in this manuscript:

AC	Ash Content
ADF	Acid Detergent Fiber
CP	Crude Protein
DCAD	Dietary Cation–Anion Difference
FEE	Fat Ether Extract
INAA	Instrumental Neutron Activation Analysis
MTL	Maximum Tolerable Level
NDF	Neutral Detergent Fiber
RDMR	Recommended Dietary Mineral Reserve
SLC	Sulfuric Lignin Content
SRM	Standard Reference Material

Appendix A

Table A1. Detailed description of the fertilizing procedure for *Nardus stricta* L. in the Cosna grassland.

Experience 1: Organic Fertilizer		**Experience 2: Mineral Fertilizer**	
Plot		Plot	
V1	Unfertilized control	V1	Unfertilized control
V2	20 t/h manure applied every year	V2	$N_{100}P_{100}$
V3	30 t/h manure applied every year	V3	$N_{140}P_{140}$
V4	40 t/h manure applied every year	V4	$N_{200}P_{200}$
V5	50 t/h manure applied every year	V5	$N_{100}P_{100}$ + $N_{100}P_{100}$
V6	20 t/h manure applied every 2 years	V6	$N_{100}P_{100}$ + $N_{100}P_{100}$
V7	30 t/h manure applied every 2 years	V7	$N_{80}P_{80}$ + $N_{60}P_{60}$
V8	40 t/h manure applied every 2 years		
V9	50 t/h manure applied every 2 years		

Table A2. Detailed description of the fertilizing procedure for the Pojorata permanent grassland of *Agrostis capillaris* L. and *Festuca rubra* L. (Experience 1), as well as *Nardus stricta* L. (Experience 2).

Experience 1: Organic Fertilizer		**Experience 2: Mineral Fertilizer**	
Plot		Plot	
V1	Unfertilized control	V1	Unfertilized control
V2	10 t/ha manure applied every year	V2	30 kg/ha mineral nitrogen + 10 t/ha manure applied every year
V3	20 t/ha manure applied every 2 years	V3	50 kg/ha mineral nitrogen + 10 t/ha manure applied every year
V4	30 t/ha manure applied every 3 years	V4	30 kg/ha mineral nitrogen + 20 t/ha manure applied every 2 years
V5	20 t/ha manure annually + 10 t/ha manure every 2 years + no manure	V5	50 kg/ha mineral nitrogen + 20 t/ha manure applied every 2 years
V6	20 t/ha manure annually + no manure + 10 t/ha manure every 3 years	V6	30 kg/ha mineral nitrogen + 30 t/ha manure applied every 3 years
V7	20 t/ha manure annually + 10 t/ha manure every 2 years + 10 t/ha manure every 2 years	V7	50 kg/ha mineral nitrogen + 30 t/ha manure applied every 3 years

Table A3. Detailed description of the fertilizing procedure for *Nardus stricta* L. in the Cosna grassland.

Sarul Dornei: Organic Fertilizer		**Putna: Mineral Fertilizer**	
Plot		Plot	
V1	Unfertilized control	V1	Unfertilized control
V2	20 t/ha manure applied every year	V2	$N_{100}P_{100}$
V3	30 t/ha manure applied every year	V3	$N_{140}P_{140}$
V4	30 t/ha manure applied every 2 years	V4	$N_{200}P_{200}$
V5	50 + 0 + 40 + 0 t/ha manure applied	V5	$N_{100}P_{100}$ + $N_{40}P_{40}$

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