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Influence of the UAN Fertilizer Application on Quantitative and Qualitative Changes in Semi-Natural Grassland in Western Carpathians

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Abstract: Semi-natural grasslands are particularly important in mountainous areas of Romania, being the only source of forage for many farmers. The aim of this study was to investigate the changes in forage quantity and quality as a result of Urea Ammonium nitrate (UAN) liquid fertilization. The experiment was carried out in the eastern part of Apuseni Mountains, Romania on a *Festuca rubra* L.-*Agrostis capillaris* L. grassland located at 1240 m altitude. Studies were made over three years of experimental trial (2014–2016) and covered four experimental plots in three replicates, as follows: V1–control plot, unfertilized; V2–plot fertilized with 50 kg UAN ha^{-1} year^{-1}; V3–plot fertilized with 75 kg UAN ha^{-1} year^{-1}, and V4–plot fertilized with 100 kg UAN ha^{-1} year^{-1}. The experimental plots were harvested once per year and the botanical composition, dry matter yield and forage quality were assessed. Our results showed important changes in forage quantity, quality and diversity as a result of UAN fertilization. Starting from the second experimental year the dominance/co-dominance ratio changed favoring the species from *Poaceae* family. Dry matter increased as a result of UAN fertilization but forage quality was negatively affected by the higher percentage of participation of species from other botanical families which have higher crude fiber content and lower crude protein. Based on our results we recommend moderate fertilization with UAN up to 50 kg UAN ha^{-1} year^{-1} for semi-natural grasslands located in soil-climatic conditions similar to those in our experiment.

Keywords: mineral fertilization; floristic composition; *Festuca rubra* L.; *Agrostis capillaris* L.; forage quality

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1. Introduction

Grassland ecosystems cover from a third up to a half of the agricultural land in the countries in South-Eastern and Central Europe. They extend from the lowlands to the uplands and are a part of the agricultural landscape [1,2]. Grasslands are considered not only as a source of grazing and hay for herbivores, but they also play a multifunctional ecological and social-economic role, offering ideal conditions for a vast diversity of habitats and species [3]. The grassland area in Europe has been affected by significant changes in recent decades, including abandonment and conversion to arable land [4]. Most of the European countries have lost over 90% of their semi-natural grasslands because the productivity has been abandoned or intensified during the last century [5,6].

Romania has one of the largest grassland areas in Europe, still managed with traditional methods [7]. The pastoral area of Romania, covering of over 4.8 million ha, has been influenced, over the last decades, by natural and anthropogenic or human factors, which affect the normal functioning of the grassland ecosystem [8]. Among the most important natural factors affecting the primary productivity of grassland ecosystems the climatic (temperature and precipitation) and soil conditions play the most crucial roles. Previous studies have documented that changes in temperature and precipitation have a strong influence on temporal patterns in the biomass of grassland [9]. Nevertheless, grasslands are closely linked to the way they are being used and they can be very easily shifted from one extreme to the other, known as abandon-intensive exploitation [10]. Despite the fact that across much of Europe, the semi-natural grasslands, during the last 50 years, have become increasingly rare, the low intensification levels and the small-scale agriculture (the low-intensity agricultural habitats are one of the most valuable biodiversity sources), have led to the preservation of certain important areas in Romania. The regularly grazed or cut vegetation may contain some of the highest plant densities in Europe, or even in the world and thus the highest importance should be given to adopting the proper management for these ecosystems [11,12].

Most of experimental studies pointed out that the net primary production and plant diversity varies with the different locations and grassland ecosystems due to differences of resource availability [13]. The productive potential and quality of permanent grasslands could be increased by different fertilization regimes and types of mineral/organic fertilizers [14]. Several research works pointed out that biomass yield is positively influenced by fertilizer application. Among the most important nutrients, nitrogen (N) holds an outstanding role in agricultural production [15]. Liquid chemical fertilizers are also used for efficient grassland fertilization and not only for large crops [16], and among the most recommended ones is the Urea Ammonium nitrate (UAN). UAN provides prolonged nutrition of plants with nitrogen and because only one-half of the N is in the urea form, the loss potential is lower than with straight urea. However, volatile losses are greatly reduced if less than 0.25 inches of precipitation occurred within a day or two after the application of the urea [17]. Nitrogen losses in air and to groundwater by leaching are higher in intensively-managed grasslands than in arable crops [18]. Therefore, in order to improve the sustainability of grassland husbandry it is necessary to take into account the environmental risk through nitrogen losses associated with nitrogen management. Assessing the efficiency of nitrogen application is a valuable first step in establishing an optimum N fertilizer management plan [15,19].

Considering the potential effect of nitrogen fertilization on grassland ecosystems, several research works recommended that we should be carefully in adopting the optimum fertilization treatments since this change in management not only increases plant density and biomass, but also reduces the structural and floristic diversity of the sward [20,21]. The floristic composition is established based on scientific criteria, depending on the climate conditions, the exploitation manner and the agro-technique used [22]. The experiments conducted so far, nationally and internationally, show that species richness is reduced along with the intensification of the grassland systems, which leads to the installation of the species with a higher forage value and a better productivity [23].

Taking into account these concerns we strongly believe that a deeper understanding of how fertilization practices may induce changes in grassland productivity and quality is essential for the biodiversity management and semi-natural grassland conservation [24]. In this frame, the aim of our study is to track the changes occurred in quantitative and qualitative indices of semi-natural grassland as a result of UAN fertilization. We aim to determine the optimum fertilization regime for semi-natural grassland located in condition similar to those experimented in this trial. In order to achieve the proposed objectives we followed the effect of different rates of UAN fertilizer on dry matter production (DM), species composition and forage quality among three experimental years.

2. Materials and Methods

2.1. Study Site

The investigation was carried out during three experimental years (2014–2016) at a mountain semi-natural grassland located in Baisoara commune, Transylvania, Apuseni

Mountains, at an altitude of 1240 m above sea level (latitude of 46.56439 and longitude of 23.33726).

The daily weather data were collected by a fully automated weather station located close to the experimental field. In this research, we present the average monthly air temperature, the annual average temperature, and the long-time monthly average temperature (LTA; Table 1). The highest annual average temperature was recorded in the year 2014 (7.1 °C), followed by the year 2016 (7.0 °C) when the annual average temperature was pretty closed to that recorded in 2016. The lowest annual average temperature recorded during the 3-year experimental trial was reached in 2015 (6.6 °C). Comparing the average monthly air temperature for the 3-year experimental trial with the LTA (2004–2016), we observed that the monthly and annual average temperature recorded in the years 2014 and 2016 are much higher than the LTA, while the values recorded in the year 2015 are closer to the LTA.

Table 1. Average monthly air temperature for the three experimental years and the long-time average temperature (LTA).

Months	Experimental Years			
	2014	**2015**	**2016**	**LTA * 2004–2016**
January	1.1	−1.9	−4.7	−3.5
February	2.7	−2.8	0.3	−3.1
March	3.1	−0.1	0.5	−0.4
April	3.2	3.2	8.1	5.1
May	9.1	10.2	8.5	10.0
June	12.4	13.4	14.6	13.3
July	15.6	17.4	16.8	15.7
August	15.0	17.5	17.6	15.5
September	11.4	12.9	13.1	11.7
October	8.2	6.0	5.7	6.8
November	4.5	4.5	4.1	3.1
December	−0.9	−0.7	−0.6	−1.0
Annual average temperature	7.1	6.6	7.0	6.1

* LTA–long-time monthly and annual average temperature, between the years 2004–2016.

The data recorded for rainfalls are presented in this research as the monthly sum of rainfalls, annual sum and the long-time average rainfalls (LTA; Table 2). When analysing the weather data recorded during the 3-year experimental trial we observe that the annual sum of rainfalls occurred in the years 2015 and 2016 are much greater than the LTA. The highest amount of rainfalls peaked in the year 2016 (1181.0 mm), while the lowest annual sum of rainfalls was recorded in the year 2014 (908.7 mm). The rainiest months were May and August for 2014 and 2015 while the highest amount of rainfall in the year 2016 peaked in June, July and August.

Soil samples were randomly taken from the experimental field area at a depth of 0–25 cm, each year to estimate soil properties (Table 3). The dominating soil type is Lithic Regosols. The average soil properties for the 3-year experimental trial are presented in Table 3. The pH changed during the experimental years but still remained acidic.

Table 2. Total rainfall and rainfall distribution (mm) for the three experimental years and the long-time total rainfall (LTA).

Months	Experimental Years			
	2014	2015	2016	LTA * 2004–2016
January	29.9	59.9	60.3	49.4
February	14.7	39.3	83.9	38.0
March	59.9	50.5	65.1	64.6
April	87.9	51.8	102.6	75.1
May	172.3	129.8	83.5	112.9
June	92.3	212	144.2	141.8
July	95.6	40.8	168.5	115.1
August	116.5	127.5	144.2	100.9
September	49	194.8	133.7	74.8
October	74.7	64.1	61.1	59.4
November	34.1	91	97.2	52.0
December	81.8	7.7	36.7	56.2
Annual sum	908.7	1069.2	1181.0	940.1

* LTA–long-time monthly and annual average rainfalls, between the years 2004–2016.

Table 3. Soil characteristics in the experimental site *.

Agrochemical Index	Value
pH in H_2O	4.55
Humus (%)	2.16
Nitrogen index (IN; %)	0.12
Mobile P (ppm)	11.14
Mobile K (ppm)	72.50

* The chemical analysis of the soil weas delivered by specialists from the Department of Pedology, University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca, Romania.

2.2. Experimental Set Up

The experiment was placed after the randomized block method, in three replications. The research covered three blocks (parcels) and 4 levels of UAN fertilization: V1–control plot (unfertilized), V2–fertilized with 50 kg UAN ha^{-1} $year^{-1}$, V3–fertilized with 75 kg UAN ha^{-1} $year^{-1}$, V4–fertilized with 100 kg UAN ha^{-1} $year^{-1}$.

The fertilizer was applied annually, in early spring (early March), to avoid volatilization, in the form of liquid mineral fertilizer. UAN is a mixture of ammonium nitrate and urea, with the chemical formula: NH_4NO_3 NH_2-CO-NH_2. UAN is a liquid that ranges from colourless to yellow and has a total nitrogen content of 32% (the nitrogen in nitrate form is 15.5%). The fertilizer equipment was adapted using a 1.5 m ramp and special FD-type nozzles for liquid fertilization.

At each harvest floristic composition, dry matter yield (DM), crude protein content (CP), crude fat (%), crude fibre (%), ash content (%), NDF (%), and ADF (%) were assessed. Data presented in this paper are the result of 1 cut performed in July for each experimental year (2014–2016).

2.3. Species Composition

The floristic studies were described based on abundance-dominance after the Braun-Blanquet method [25,26], before mowing, when the *Poaceae* family plants were in the flowering stage.

The experiment was placed on a *Festuca rubra* L.–*Agrostis capillaris* L. natural grassland type. Floristic studies were performed each year, before harvest. The crop was harvested once every year, in 2014, 2015 and in 2016 (in July). The dry mass of the biomass was determined by oven-drying at 60 °C until constant mass was obtained.

2.4. Forage Quality

Forage analyses were performed by the Laboratory *Applied biological sciences*, located in the University for Agricultural Sciences and Veterinary Medicine, from Cluj-Napoca, Romania.

The protein content of forage was assessed following the Kjeldahl method, which has as a basis the digestion of the sample with sulfuric acid in the presence of catalysts. The crude fat was determined after Soxhlet method which has as a basis the extraction of total fat content by petroleum ether [27]. Ash content was determined using the burning the sample for 4 h at 600 °C. The determination of crude fiber was made following the Weende method and is based on the solubilization of non-cellulosic compounds by sulfuric acid and potassium hydroxide solutions. NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) were conducted following Van Soest [28].

2.5. Statistical Analyses

The statistical interpretation of the results for floristic data was conducted using the PC-ORD program Version 6, with Nonmetric Multidimensional Scaling (NMS) [29]. The data recorded for DM production and forage quality were analyzed with Statistica vs 10 (developed by StatSoft in the year 2010), *t*-test for single means and partial correlations. Effects were accepted as statistically significant if $p \leq 0.05$.

3. Results

3.1. Species Composition

The 2D graphical representation for the year 2014 allows us to explain 94.1% of the floristic changes. Axis 1 shows the greatest importance (82.3%) compared to axis 2 which is less represented (11.8 %). The 2D graphical representation for the year 2015 allows us to explain 94.7 % of the floristic changes. Axis 1 shows the greatest importance (87.7%) compared to axis 2 which is less represented (7.1%). In the year 2016 the 2D graphical representation allows us to explain 86.5% of the floristic changes. Axis 1 shows the greatest importance (64.5%) compared to axis 2 which is less represented (22%).

The floristic studies revealed that the type of grassland specific to the control plot in the year 2014 is *Festuca rubra* L. with *Agrostis capillaris* L. As shown in Figure 1 the type of grassland is not influenced by the treatments applied. The main species of the studied semi-natural grassland, *Festuca rubra* L. and *Agrostis capillaris* L., suffered moderate increases in the percentage of participation in the vegetation cover as a result of fertilization, and the effects were noticed particularly on plots fertilized with 75 and 100 kg UAN ha^{-1} year^{-1}.

The phytocoenosis specific to the control plot is represented by *Festuca rubra* L.-*Agrostis capillaris* L. grassland type which shows moderate changes in dominance/co-dominance as a result of the treatments applied in the year 2015 (Figure 2). *Festuca rubra* L. remains the dominant species in plots fertilized with 50 kg UAN ha^{-1} year^{-1}. Increasing the fertilizer rate above 50 kg UAN ha^{-1} year^{-1} seems to be favor *Agrostis capillaris* L. since all plots fertilized with 75–100 kg UAN ha^{-1} year^{-1} have as dominant species the *Agrostis capillaris* L.

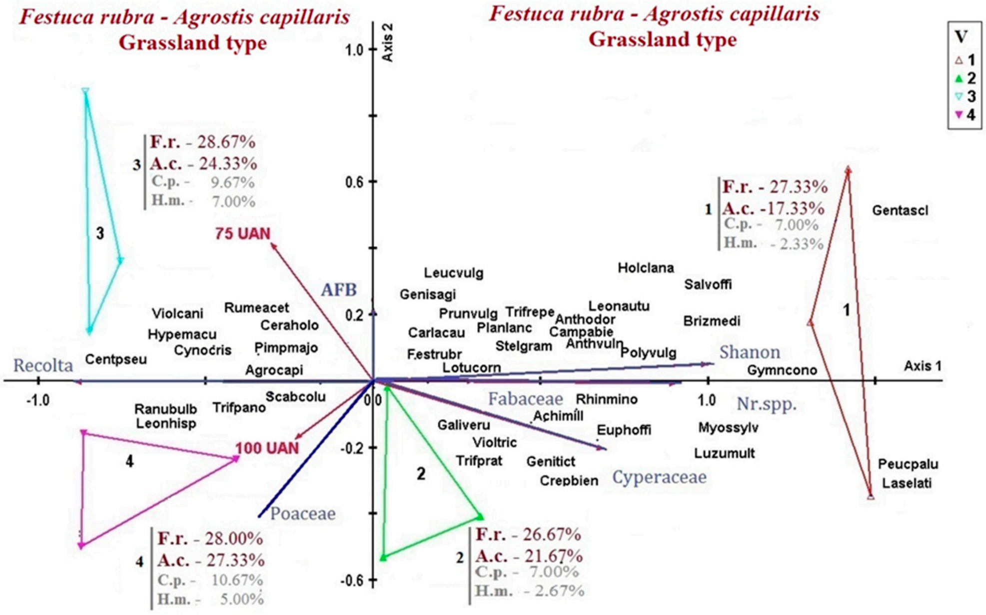

Figure 1. Changes in floristic composition as a result of the treatments applied in the year 2014. V1—control plot, (unfertilized); V2—fertilized with 50 kg UAN ha^{-1} year^{-1}; V3—fertilized with 75 kg UAN ha^{-1} year^{-1}; V4—fertilized with 100 kg UAN ha^{-1} year^{-1}; F.r.-*Festuca rubra* L.; A.c.-*Agrostis capillaris* L.; C.p.-*Centaurea pseudophrygia* C.A.Mey.; H.m.-*Hypericum maculatum* Crantz.

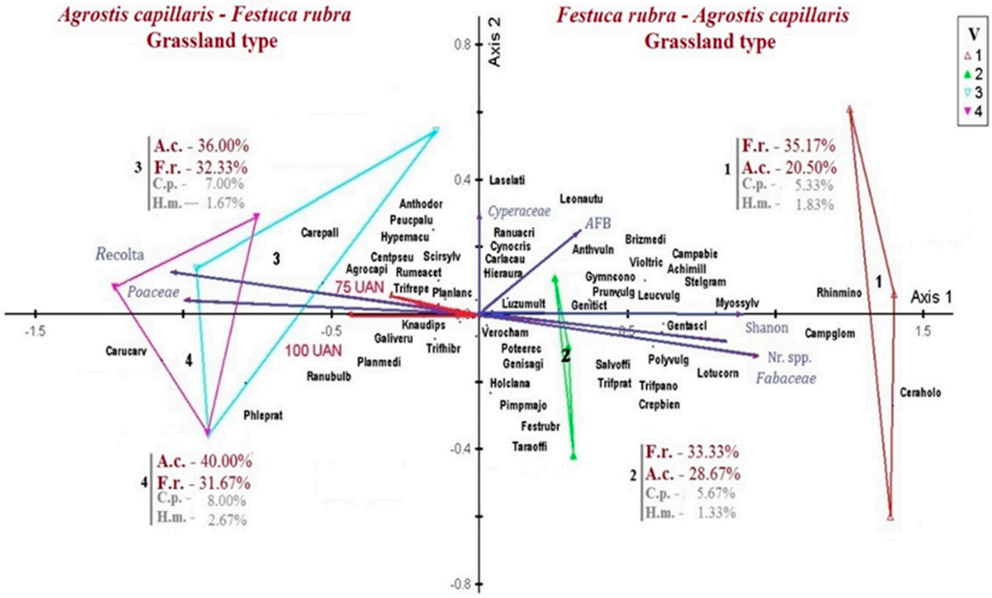

Figure 2. Changes in floristic composition as a result of the treatments applied in the year 2015.V1—control plot, (unfertilized); V2—fertilized with 50 kg UAN ha^{-1} year^{-1}; V3—fertilized with 75 kg UAN ha^{-1} year^{-1}; V4—fertilized with 100 kg UAN ha^{-1} year^{-1}; F.r.-*Festuca rubra* L.; A.c.-*Agrostis capillaris* L.; C.p.-*Centaurea pseudophrygia* C.A. Mey.; H.m.-*Hypericum maculatum* Crantz.

The results recorded in the third experimental year revealed that *Agrostis capillaris* L. is positively influenced by the treatments applied such that changes in floristic composition are registered (Figure 3). Thus *Agrostis capillaris* L. becomes the dominant species on all the experimental plots fertilized with UAN (increases its percentage of participation from 15.83% in control plot up to 22.25% in plot fertilized with 100 kg UAN ha^{-1} year^{-1}).

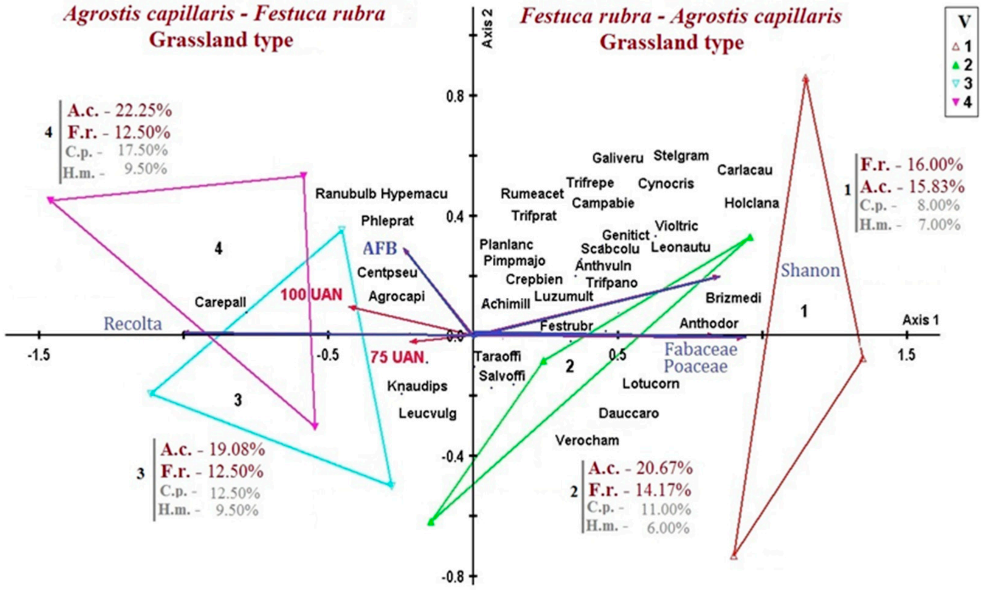

Figure 3. Changes in floristic composition as a result of the treatments applied in the year 2016. V1—control plot, (unfertilized); V2—fertilized with 50 kg UAN ha^{-1} year^{-1}; V3—fertilized with 75 kg UAN ha^{-1} year^{-1}; V4—fertilized with 100 kg UAN ha^{-1} year^{-1}; F.r.-*Festuca rubra* L.; A.c.-*Agrostis capillaris* L.; C.p.-*Centaurea pseudophrygia* C.A.Mey.; H.m.-*Hypericum maculatum* Crantz.

3.2. Dry matter Production

The results registered in 2014 highlighted a strong reaction of dry matter production (DM) as a result of fertilization (Table 4). Thus DM yields increased proportionally with increasing the amount of fertilizer applied, from 2.99 t DM ha^{-1} year^{-1} (control plot) up to 6.39 t DM ha^{-1} year^{-1} (plot fertilized with 100 kg UAN ha^{-1} year^{-1}).

Table 4. Changes in dry matter yield (DM) as a result of UAN fertilization (2014–2016).

Experimental Plot	Yield (t DM ha^{-1} year^{-1})	*p*-Value
Experimental Year 2014		
V1	2.99	Ct. plot
V2	3.88	*
V3	5.64	***
V4	6.39	***
Experimental year 2015		
V1	2.33	Ct. plot
V2	3.30	***
V3	4.26	***
V4	4.85	***
Experimental year 2016		
V1	3.22	Ct. plot
V2	3.52	*
V3	4.70	***
V4	5.33	***

Note: Ct. plot = control plot; V1—control plot (unfertilized); V2–fertilized with 50 kg UAN ha^{-1} year^{-1}; V3–fertilized with 75 kg UAN ha^{-1} year^{-1}; V4–fertilized with 100 kg UAN ha^{-1} year^{-1}; *: $p > 0.05$—non-significant (NS). ***: $p < 0.001$—highly significant (HS, confidence 99.9%).

DM yields recorded in the second experimental year (Table 4) varied between 2.33 t DM ha^{-1} year^{-1} (control plot) and 4.85 t DM ha^{-1} year^{-1} (plot fertilized with 100 kg UAN ha^{-1} year^{-1}).

DM yields recorded in the year 2016 (Table 4) ranged between 3.22 t DM ha^{-1} year^{-1} (control plot) and 5.33 t DM ha^{-1} year^{-1} (plot fertilized with 100 kg UAN ha^{-1} year^{-1}). Our results show, generaly, the phytocoenosis analyzed responded very well to UAN fertilization in terms of DM production.

3.3. Forage Quality

The results concerning forage quality revealed small changes in forage quality indices in the first year of research as a result of UAN fertilization (Table 5). The crude protein, crude fat and ash content were negatively influenced by mineral fertilization such that these indices suffered decreases on plots fertilized with 50 kg UAN ha^{-1} year^{-1} and 75 kg UAN ha^{-1} year^{-1}. Crude protein decreased from 8.01% in control plot up to 7.24% at plot fertilized with 75 kg UAN ha^{-1} year^{-1}. Following the same pattern, the crude fat suffered decreases from 4.11% in control plot up to 3.74% at plot fertilized with 75 kg UAN ha^{-1} year^{-1}. Fertilization with 100 kg UAN ha^{-1} year^{-1} seemed favor these indices since the values recorded on the plots fertilized with 100 kg UAN ha^{-1} year^{-1} are higher than those recorded on plots fertilized with 50 and 75 kg UAN ha^{-1} year^{-1} but still lower than those registered on control plots, unfertilized. Crude fibre, NDF and ADF showed positive response to UAN fertilization. Thus, crude fibre increased from 33.39% in control plot up to 34.11% in plot fertilized with 100 kg UAN ha^{-1} year^{-1}. NDF content ranged between 51.06% in control plot and 70.21% in plot fertilized with 100 kg UAN ha^{-1} year^{-1} and ADF recorded increases from 42.11% in control plot up to 45.61% in plot fertilized with 100 kg UAN ha^{-1} year^{-1}.

The results recorded in the second year of the experiment revealed a similar reaction of the studied forage quality indices to that recorded in the year 2014 (Table 5). Therefore the crude protein, crude fat and ash content decreased proportionally with increasing mineral fertilization with values ranging between 6.03% (75 kg UAN ha^{-1} year^{-1}) and 8.01% (control plot) for crude protein, 3.74% (75 kg UAN ha^{-1} year^{-1}) and 4.11% (control plot) for crude fat and 5.36% (75 kg UAN ha^{-1} year^{-1}) and 6.09% (control plot) in ash content. Crude fibre, NDF and ADF showed positive response to UAN fertilization. Thus, the crude fibre increased from 35.10% in control plot up to 38.52% in plot fertilized with 75 kg UAN ha^{-1} year^{-1}. The NDF content ranged between 50.86% in control plot and 68.62% in plot fertilized with 100 kg UAN ha^{-1} year^{-1} and ADF recorded increases from 43.24% in control plot up to 46.35% in plot fertilized with 100 kg UAN ha^{-1} year^{-1}.

The results concerning forage quality recorded in the year 2016 revealed a different reaction of some of the quality indices studied to UAN fertilization compared to that registered in the first two experimental years (Table 5). Therefore crude protein followed the same reaction and its values decreased proportionally with increasing UAN fertilization reaching values between 6.11% (plot fertilized with 100 kg UAN ha^{-1} year^{-1}) and 8.54% (control plot). Crude fat reacted differently to UAN fertilizer compared to the first two years of experiment such that in the year 2016 crude fat recorded increases as a result of fertilization with 50 and 75 kg UAN ha^{-1} year^{-1}. Crude fibre, NDF, and ADF showed positive response to UAN fertilization. Crude fibre increased from 33.95% in control plot up to 36.34% in plot fertilized with 100 kg UAN ha^{-1} year^{-1}. NDF content ranged between 50.15% in control plot and 69.64% in plot fertilized with 100 kg UAN ha^{-1} year^{-1} and ADF recorded increases from 41.05% in control plot up to 47.95% in plot fertilized with 100 kg UAN ha^{-1} year^{-1}.

Table 5. Changes in forage quality as a result of UAN fertilization (2014–2016).

Forage Quality Indices	Experimental Plot	2014		2015		2016	
		[%]	*p*-Value	[%]	*p*-Value	[%]	*p*-Value
Crude protein	V1	8.01	Ct. plot	8.38	Ct. plot	8.54	Ct. plot
	V2	7.75	***	7.62	***	7.45	***
	V3	7.24	***	6.03	***	6.19	***
	V4	7.38	***	7.53	***	6.11	***
Crude fat	V1	4.11	Ct. plot	4.44	Ct. plot	3.28	Ct. plot
	V2	4.16	***	4.48	***	3.33	***
	V3	3.74	***	3.41	***	3.30	***
	V4	4.05	***	4.35	***	3.05	***
Ash	V1	6.09	Ct. plot	5.42	Ct. plot	5.46	Ct. plot
	V2	5.90	***	5.23	***	4.73	***
	V3	5.36	***	4.80	***	4.46	***
	V4	5.36	***	5.06	***	4.35	***
Crude fiber	V1	33.39	Ct. plot	35.10	Ct. plot	33.95	Ct. plot
	V2	32.70	***	35.13	***	35.47	***
	V3	34.06	***	38.52	***	34.31	***
	V4	34.11	***	35.55	***	36.34	***
NDF	V1	51.06	Ct. plot	50.86	Ct. plot	50.15	Ct. plot
	V2	56.75	**	55.92	**	54.22	**
	V3	57.44	**	65.38	**	54.19	**
	V4	70.21	***	68.62	***	69.64	***
ADF	V1	42.11	Ct. plot	43.24	Ct. plot	41.05	Ct. plot
	V2	43.45	**	44.32	**	42.18	**
	V3	45.72	**	44.89	**	45.08	**
	V4	45.61	**	46.35	**	47.95	**

Note: V1—control plot (Ct. plot; unfertilized); V2–fertilized with 50 kg UAN ha^{-1} year^{-1}; V3–fertilized with 75 kg UAN ha^{-1} year^{-1}; V4–fertilized with 100 kg UAN ha^{-1} year^{-1}; **: $p < 0.01$—significant (S, confidence 99%). ***: $p < 0.001$—highly significant (HS, confidence 99.9%).

4. Discussion

Natural and semi-natural grasslands have played an important role in people's livelihoods for millennia as areas producing fodder for animals [30]. At the same time, semi-natural grasslands act as carbon sinks, erosion preventives, birds' directive areas, habitat for small animals, and nitrogen fixation source. Therefore studies aiming to investigate the optimum management required for higher forage yields and better forage quality while preserving species biodiversity are still required. Being a mixture of different grass species, legumes and herbs, most grasslands are in harmony and in balance with the environment, excepted intensively used ones [31]. The intensification of grassland management through the use of fertilizers, pesticides and the use of more efficient, mechanical mowing techniques, are among the most important threats for grassland ecosystems [20,21]. Our research pointed out that the application of UAN fertilizer caused changes in the ratio between the dominant and co-dominant species in the studied grassland (Figures 1–3). Changes occurred starting with the second year of fertilization [32,33]. Increasing the rates of UAN fertilizer over 50 kg UAN ha^{-1} year^{-1} changed the dominant species such that *Agrostis capillaris* L. became dominant (Figures 2 and 3). As a result of mineral fertilization with UAN, the species from *Fabaceae* family decreased their percentage of participation in the vegetation cover, favoring the installation of *Poaceae* species. In the same time the plants from other botanical families increased their percentage of participation in the vegetation cover with increasing fertilizer rates. Briemle [23] also pointed out that mineral fertilizer has a negative influence on *Fabaceae* species and plants from other botanical families favoring the occurrence and development of *Poaceae* species. Our results highlighted that generally species diversity is reduced as a result of fertilizer treatments thus the highest diversity was being recorded in the control plot, unfertilized during the entire three-year period of the experimental trial (Figures 1–3). Most of the species found in the studied semi-natural grassland showed preferences for lower rates of fertilizer and among the most sensitive to fertilization where *Festuca rubra* L., *Genista sagittalis*, *Peucedanum palustre, Laserpitium latifolium, Gymnadenia conopsea* L. R. Br., *Briza media* L., *Trifolium repens* L. etc. At the same time, some species like *Agrostis capillaris* L., *Centaurea pseudophrygia* C.A.Mey., *Hypericum maculatum* Crantz, *Leontodon hispidus* etc., increased their percentage of participation in the vegetation cover as a result of UAN fertilization. The occurrence of these species was influenced also by the climatic condition specific to the three years experimental trial which favored their participation in the vegetation cover. When analysing the climatic conditions specific to the experimental period we observed that the temperature recorded in each of the 3 studied year are higher than the long-term average temperature (Table 1). Thus *Agrostis capillaris* L. reaction could be more or less related to these temperature increases since other researchers already pointed out a better resistance of this species to higher temperatures. Previous studies have documented that changes in temperature and precipitation have a strong influence on temporal patterns in the biomass of grassland [9]. At the same time, *Agrostis capillaris* L. reacts very well to acid soil conditions which are found in our experiment.

Important changes as a response to UAN fertilization were also recorded also when dry matter yield is considered (Table 4). Our results pointed out that dry matter yield increased proportionally with increasing fertilizer rates such that the highest yields were recorded in the plots fertilized with 100 kg UAN ha^{-1} year^{-1} in all 3 years of the experimental trial. Our results are in accordance with other investigations conducted so far [34]. The highest dry matter yield (6.39 t DM ha^{-1} year^{-1}) was recorded in the year 2014 on the experimental plot fertilized with the maximum amount of UAN fertilizer (Table 4). Several studies emphasized that grassland yield and quality are necessary to assess in relation to changes in the botanical composition [35]. Our results also confirm that dry matter yields are influenced by the floristic composition such that the highest yields are recorded in plots where *Poaceae* species recorded higher percentage of participation (Table 4; Figures 1–3).

The investigation conducted by us highlighted important changes in forage quality as a result of UAN fertilization. Forage quality seemed to be negatively influenced by the application of UAN fertilizer in all 3 years of the experimental trial (Table 5). When analysing the results recorded we observed that UAN fertilization caused decreases in crude protein, crude fat and ash content while favoring the crude fibre, NDF and ADF content. NDF recorded values ranging between 50.15% and 70.21%. [36,37] also reported values similar for NDF content. ADF varied between 41.05% and 47.95%, results confirmed by other investigations [38–40]. These changes in forage quality could be explain also through species composition. Species from *Fabaceae* family reduced their percentage of participation favoring the appearance of plants from other botanical families [34,41] like *Centaurea pseudophrygia* C.A.Mey. and *Hypericum maculatum* Crantz (17.50%), *Centaurea pseudophrygia* C.A.Mey. and *Hypericum maculatum* Crantz (9.5%), which have a higher content in crude fibre and a lower crude protein and crude fat content.

5. Conclusions

The investigation conducted at the mountain semi-natural grassland from Baisoara commune, Apuseni Mountains, Romania pointed out important changes in forage quantity and quality as a result of UAN fertilization. A strong correlation between species composition and dry matter and forage quality was also noted. Based on our results we recommend moderate fertilization with UAN up to 50 kg UAN ha^{-1} year^{-1} for semi-natural grasslands located in soil-climatic conditions similar to those in our experiment.

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