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Environmental Impact and Yield of Permanent Grasslands: An Example of Romania

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Additional information is available at the end of the chapter

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1. Introduction

Organic farming is both a philosophy and a system of agricultural production. Its roots are to be found in certain values that closely reflect the ecological and social realities. Organic agriculture is a production method that takes into account the traditional knowledge of farmers and integrates the scientific progress in all agricultural disciplines, answering the social concerns of the environment and providing high quality products to consumers. The principles underpinning organic farming are universal, but the techniques used are adapted to the climatic conditions, resources and local traditions.

In other words, organic agriculture deals with the systematic study of material and functional structures of agricultural systems and the design of agro-ecosystem management capable to ensure the human needs for food, clothing and housing, for a long period of time, without diminishing the ecological, economic and social potential.

Organic farming methods to obtain food by means of culture that protect the environment and exclude the use of pesticides and synthetic fertilizers. No doubt that organic farming can also be defined as the activity of assembling the theoretical knowledge about nature and agriculture in sustainable technological systems based on material, energy and information resources of the agricultural systems. Also, organic farming is based on wisdom and as such, it involves detailed knowledge of land, living things and other economic and social realities, as well as intuition, moderation in choosing and implementing measures in practice.

Being a type of sustainable agriculture, the aim of organic farming can be expressed as a function of mini - max type: maximizing yields and minimizing the negative side effects of agricultural activities. Organic agriculture is a creation of farmers who love nature, as an al-



ternative to intensive farming of industrial type, based on efficient production methods and means, in particular, economically.

In accordance with the Council Regulation (EC) 834/2007 and Commission Regulation (EC) 889/2008, EU countries use, with the same meaning, the terms of *organic agriculture* (England), *biological agriculture* (Greece, France, Italy, Netherlands and Portugal) and *ecological agriculture* (Denmark, Germany and Spain). Since 2000, Romania has been using the term organic farming, according to the regulations stipulated in the Emergency Ordinance 34/2000.

Organic farming emerged in Europe as a result of health problems and negative experiences caused by the use of synthetic chemicals generated by the intensive industrial technologies, based on the forcing of production by over-fertilization of agricultural land and the use of stimulators in animal nutrition. Organic farming is a dynamic sector that has experienced an upward trend, both in the plant and animal production sector. Respect for every living organism is a general principle of organic farming, from the smallest micro-organism from the ground up to the largest tree that grows above. Because of this, each step of the ecological chain is designed to maintain, and where possible, to increase the diversity of plants and animals. Improvement of biodiversity is often the result of good practices of organic agriculture, as well as respect for the EU Regulation on organic agricultural production [39; 40].

1.1. In the world

Worldwide, nearly 31 million hectares are used for organic production, representing 0.7% of the total agricultural land. This farming system is practiced in over 633 890 farms [38].

The regions with the largest areas of organically managed agricultural land are Oceania (12.1 million hectares of 33 % of the global organic farmland), Europe (10 million hectares of 27 % of the global organic farmland) and Latin America (8.4 million hectares or 23 %). The countries with the most organic agricultural land are Australia (12 million hectares), Argentina (4.2 million hectares) and the United States (1.9 million hectares). The highest shares of organic agricultural land are in the Falkland Islands (35.9 %), Liechtenstein (27.3 %) and Austria (19.7 %).

1.2. In Europe

According to the study of World of Organic Agriculture, seven of the first ten countries of the world, ranked by the percentage of the agricultural land cultivated in organic system, are in the European Union [38].

The area under organic agriculture has increased significantly in the last years. In the period 2000-2008, the total organic area has increased from 4.3 to an estimated 7.6 mio ha (+7.4% per year). The Member States with the largest areas in 2008 are Spain (1.13 mio ha), Italy (1.00 mio ha), Germany (0.91 mio ha), the United Kingdom (0.72 mio ha) and France (0.58 mio ha). As of the end of 2010, 10 million hectares in Europe were managed organically by almost 280'000 farms.

The countries in central and eastern Europe, like Poland, with areas of over 367,000 hectares cultivated organically and the Czech Republic, which had a market growth of 11% in 2009, are becoming increasingly important on the market of organic products [38].

Among arable crops, cereals represent the most important category with 1.2 mio ha in 2007, i.e. 18.3% of all EU organic land. The largest producers are Italy and Germany. Permanent grassland represents 2.51 mio ha (45.1% of the whole organic area and arable crops), in 2006. The higher level of permanent pastures in the organic sector stems from the more extensive production systems employed. In the EU-15, permanent pastures have represented more than 40% of all organic land. The area under permanent pastures is the highest in absolute terms in Germany, Spain and the United Kingdom where it is around 0.4 mio ha. In six Member States the organic sector amounts to more than 10% of the total area of permanent pastures: 25.8% in the Czech Republic, 16.0% in Greece, 16.2% in Latvia, 15.5% in Slovakia, 12.0% in Austria and 11.5% in Portugal.

Consumer food demand grows at a fast pace in the largest EU markets, yet the organic sector does not represent more than 2% of total food expenses in the EU- 15 in 2007 [38].

1.3. In Romania

In 2008, of the total area where organic farming was used, permanent pastures and forage crops represented 60,000 ha, the cereals 56,000 ha, oleaginous plants and protein plants 30,000 ha, while the collection and certification of plants and flowers from the spontaneous flora 59,000 ha. The data from 2011 show that the area cultivated organically was of about 300,000 ha. Of this area, the arable land occupies 158,825 ha, permanent grasslands and meadows 89,489 ha, permanent crops 54,840 ha, and the collection from spontaneous flora 47,101 ha [37].

Permanent grasslands, which are traditionally used as forage for ruminants, are an important land use in Europe and cover more than a third of the European agricultural area. In Europe extensive grazing by livestock and fertilization with their manure is considered an appropriate management strategy to conserve biodiversity value. The importance of permanent grasslands in Romania is shown by the area they occupy and by their comparatively high biodiversity. Currently, permanent pasture in Romania covers 4.9 million ha [37]. This area accounts for 33% of the total agricultural area of the country. In terms of area occupied by natural grasslands in Europe, Romania occupies fifth position after France, Britain, Spain and Germany. The permanent grasslands from Romania, situated on soils with low natural fertility, are weakly productive and have an improper flower composition. The main means for improving these grasslands consist in adjusting soil fertility, changing the dominance in the vegetal canopy and their good management. The organic fertilization and the rational use lead to substantial increases of the production, biodiversity and the fodder quality improvement. Increasing the productive potential of these grasslands can be achieved through fertilization with different rates and types of organic fertilizers. Previous studies have demonstrated the positive effects of organic fertilizers on grassland. Comparative studies, which investigated the effects of different management practices on grasslands, have demonstrated that changes do occur in species diversity and the composition of plant functional groups depending on management practices.

Each permanent grassland sward can be considered as a unique mixture of species at different growth stages and this complexity makes it difficult to characterize and understand their feed value. Floristic composition influences the nutritional value of permanent grasslands

due to differences in the chemical composition, digestibility of individual species and variation in the growth rate of different species.

The problem of the biodiversity reached in the top of the actual preoccupations because the modern agriculture was lately focused on developing some methods and procedures to allow the management of a relatively restrained number of species, the immediate economic interest being primary, without making a deep analysis of the long and medium -term consequences. Often, the preoccupations concerning the productivity left no place for the quality of the products or for the environment's health.

The experience of the developed countries underlines the fact that taking decisions in the problem of biodiversity must be made only after conducting thorough, professional, inter-disciplinary studies, which allow the projection of a sustainable management of the natural resources, among which the permanent pasture lands occupy an important place. Comparing the data from the specialty literature, regarding the Romania's pasture lands' vegetation from almost 40 years ago, we will observe that many of those aspects have modified. There are numerous technical solutions for making a compromise between the function of production of the meadows and maintaining their biodiversity.

2. Management of organic fertilizers

2.1. Importance of organic fertilizers

In the twentieth century numerous studies were made on the role of organic matter in defining soil fertility. Experimental fields were established in Rothamstead England (1843), Morrow, the U.S. (1876) Askov, Denmark (1894), Halle / Saale, Germany, Groningen, Netherlands, Dehéreim, France, Fundulea, Podu Iloaiei Suceava, Romania. The long-term experiments made in these fields contributed importantly to the knowledge of the effect of organic and mineral substances on improving soil fertility [20].

These long-term researches conducted worldwide established the utility of organic fertilizers for maintaining or increasing the organic component of the soil. The introduction of organic residues in soil means turning to good account the energy included in these livestock excreta. About 49% of the chemical energy contained in the organic compounds of the food consumed by animals is excreted as manure, where significant percentages of macro and micro- elements are to be found [20].

Consumption of organic products is a growing process, so agriculture must keep up and produce ever more. Obtaining products by producingno harmful effects to nature is almost impossible. One thing is sure, that farmers try to minimize these negative effects as much as possible.

Soil, which is the focus of organic farming, is considered a complex living environment, closely interacting with plants and animals. By its specific techniques, organic farming aims to increase the microbiological activity of the soil, to maintain and increase its fertility.

The organic substance used as fertilizer is an important component in order to maintain or restore the soil fertility. Collection, storage and fermentation of vegetal wastes so as to decrease their volume and improve their physicochemical properties are a requirement of organic farming.

For many considerations, the organic fertilizers are preferred in organic farming as poorly soluble nutrients are mobilized with the help of soil microorganisms.

Fertilization is an important means of increasing the amount of organic products and the methods of fertilization used vary from one farm to another. For fertilization, the natural fertilizers represented by animal or vegetal remains are used in organic farms.

The fertility and biological activity of the soil must be maintained and improved by the cultivation of legumes, green manure crops or deep-rooting plants in an appropriate rotation. Also, the fertility must be maintained by incorporating organic substances in the soil as compost or from the production units, which respect specific production rules.

Besides the use of legumes in rotations, the role of animals in the organic system facilitates nutrient recycling. The potential for recycling the nutrients through fertilizer application is high. Thus, both the nutrients from the grazing period and the nutrients from the stall period are concentrated in solid manure and urine which are available for redistribution. By grazing, the animals retain only 5-10% of the nitrogen existing in the grass consumed. Together with the manure, they remove about 70% of nitrogen in the urine and 30% in the solid manure.

Not all initial nitrogen in manure is used by herbs in the production of dry matter in the crop. Much of the nitrogen may be retained in roots, immobilized in organic matter in the soil or lost by leaching or denitrification. Also, the loss of nutrients during storage may occur due to leaching and volatilization, which depend largely on how these fertilizers are managed. The nitrogen losses as ammonia or nitrogen gas in the fertilizer can be of 10% of the total weight when it is tamped in the pile and reach 40% when the pile is loose and turned. The gaseous losses of urine can be of 10-20% and even higher when it is shaken. Because of this, the application in spring is more efficient because the leaching losses are lower than in the case of application in autumn or winter.

The organic fertilizers positively contribute to the modification of physical conditions in the soil by increasing the field capacity for water, aeration, porosity and brittleness, and the black colour of organic matter will lead to easier and faster heating of these soils [20].

It should be mentioned that, when using organic fertilizers it is very easy to overcome the nutrient dose that needs to be applied. Therefore, the amount applied for a complete rotation of the cultures should be limited to the equivalent of nutrient from the manure produced by maximum 2.5 to 3 units of cattle / ha.

2.2. Organic fertilizers used in Romania

2.2.1. Manure

The manure is composed of animal manure and bedding material, in variable amounts and in different stages of decomposition.

Because different types of bedding are used, in various amounts, and the animals are fed on different diets for long periods, the chemical composition of manure can vary widely.

In the aerobic composting of manure, the long time of composting increases the biological stability of the nitrogen compounds and the nitrogen availability decreases accordingly. Although the application of high doses of manure results in increasing the production of nitrogen, however the crops use less nitrogen of the manure applied in high doses.

The highest losses during waste storage are those occurring in gaseous form. The ammonia is lost each time the manure pile is moved, while inside the well compacted piles de-nitrifications can be caused due to the anaerobic conditions created. The losses by leaching from piles of uncovered manure can be considerable. The nitrogen losses by washing are reduced, being of only 4-6%, in case of the covered heaps, when compared to the losses of 10-14% in the case of unprotected piles [20].

The experiences showed that 60 to 90% of ammonia nitrogen from cattle manure can volatilize between the 5th and 25th day after the application on the soil surface. The losses by administration can be reduced by incorporating the manure in the soil as soon as possible. It should be noted that the standards of organic farming prohibit the use of manure derived from breeding systems, ethically unacceptable, such as batteries of cages and intensive poultry units.

There are two essential ways of approaching the manure management used in organic farming practices. The first approach involves the application of fresh manure in dose of about 10 t ha⁻¹. The alternative is the storage of manure in a wide range of possible conditions and its use in the moment it attained the over-maturation stage, but usually not later than six months.

Some farmers laid great emphasis on composting manure as a way of approaching the use of fresh manure, due to the microbiological activity associated with the decomposition occurring in the soil. The increased microbiological activity means that a larger amount of nutrients can be synthesized from the organic matter present in the soil.

During storage, several important chemical processes take place in the pile of manure. At first, the urea is converted into ammonia compounds, while carbohydrates from the bedding after the fermentation are converted into energy, different gases (e.g. CO₂, methane and hydrogen). At the same time, the proteins from the bedding are decomposed in simple nitrogen compounds and the nitrogen is assimilated and fixed by different bacteria.

A traditional approach to storing manure in central Europe is the "cold manure" technique, where the manure is carefully stored and compacted, thus creating complete conditions of anaerobiosis. However large losses are recorded during administration, because the material must be left at the soil surface for the toxic products synthesized during fermentation not to prevent root growth and microbiological processes from the soil.

The careful control of the conditions in which the decomposition takes place allows the decomposition process to be optimized. The microbiological activity increases rapidly at temperatures around 60°C, and after a few weeks the pile is turned over to allow a second heating.

The high temperatures developed during composting help destroy the weed seeds and pathogens. The insects present in compost will eat the eggs of cabbage root fly, but the

problem can be solved only if the distribution of compost is made in the adequate stage of fly development [20].

This is one of the reasons why the standards of organic agriculture recommend manure be composted before use.

In Britain, large quantities of organic fertilizers are produced on stubble, where their accumulation is allowed for a certain period of time. In case the composting was made too strongly it results a paste that can be used only when it corresponds to the proposed specific goals.

2.2.2. Vinassa

Vinassa is a by-product obtained after the evaporation of waste waters from factories that produce bakery yeast [11]. The waste waters from production, after the separation of yeast from the culture medium, represented by molasses derived from sugar factories, are subjected to concentration by evaporation, turning into a valuable product called vinasse, CMS (Condensed molasses solubles) FEL (Fermentation end Liquor), Dickschlempe). The vinassa product looks like a dark brown liquid, with relatively low viscosity, caramel odor slightly unpleasant because of the presence of phenols and sweet bitter taste.

Vinassa has a very low level of fermentable sugars (1.5 to 2.0%), and the product is very stable in time and does not have storage problems. The valuable composition of vinasse makes it widely used in western Europe as an organic fertilizer, encapsulating material for fertilizers and feed additive for ruminants, pigs and poultry [6; 21; 32].

		Average values	Quality indexes	U.M.	Average values
		61-63	Zinc	mg/100g	0,5-0,6
Humidity	%	39-37	Organic carbon	%	18,26
Sugars	%	1,5-2	Lactic acid	%	1,28-1,29
Raw protein	%	18-21	Formic acid	%	0,001-0,011
Ash	%	21-23	Acetic acid	%	0,47-0,475
Potassium	%	5-7	Malic acid	%	0,28-0,281
Calcium	%	0,99-1,1	Glucose	%	0,04-0,044
Magnesium	%	0,11-0,12	Fructose	%	0,05-0,06
Sodium	%	6-6,2	Betaine	%	13,3-14,5
Phosphor	%	0,3-0,5	Glycerin	%	2,03-2,07
Nitrites	%	0,005-0,006	Total nitrogen	%	2,8-3,2
Nitrates	%	0,8-1,1	Free amino acids		
Ph		7-8	glutamic acid	g/kg	4,57-4,76
Iron	mg/100g	27-30	methionine	g/kg	0,08-1,29
Copper	mg/100g	0,60-0,65	lysine	g/kg	1,1-1,6

Table 1. Chemical composition of vinassa [32].

Vinassa has a complex chemical composition (Table 1), being rich in total nitrogen (3.0 to 3.2%), very rich in potassium (5-7%) and low in phosphorus (0.3 to 0.5 %). It also contains appreciable quantities of sodium (6.0 to 6.2%), calcium (0.99 to 1.1%), magnesium (0.11 to 0.12%), iron (27-30 mg / 100 g soil), copper (0.60 to 0.65 mg/100 g soil) and zinc (from 0.50 to 0.60 mg/100 g soil) etc.

Due to its chemical composition, vinassa leads to the formation of bacterial flora in the soil which accelerates the degradation of cellulose material and enables fast incorporation in the natural circuit of vegetal residues in the cellulose material. This property recommends vinassa for use in direct spraying on the stubbles left after harvesting the cereals. In addition, because of the high content in potassium and nitrogen, vinassa is considered a valuable organic fertilizer.

Following the research carried out, the product was approved in 2003 as the vinassa-Rompak or just "vinassa". Used in dilution with water in 1:5 ratio on permanent pastures, "vinassa" reacts as a semi-organic fertilizer, with beneficial effects on productivity and quality of the forage. An important role of "vinassa" is also present in the formation of bacterial flora responsible for the degradation of cellulose material in the soil and due to its content of potassium and nitrogen it can replace totally or partially the application of mineral fertilizers.

3. Organic fertilizers used on permanent grasslands: an example of Romania

3.1. Manure used on Festuca valesiaca and Agrostis capillaris+Festuca rubra grasslands

The experiment has investigated the influence of organic fertilizers, applied each year or every 2-3 years, at rates of 10-30 t ha⁻¹, in a *Festuca valesiaca* grassland, situated at the height of 107 m, at Ezareni-Iasi County, and at rates of 10-30 t ha⁻¹, in an *Agrostis capillaris+Festuca rubra* grassland, situated at the height of 707 m at Pojorata-Suceava County, on yield and flower composition. Even if permanent grasslands from north-eastern Romania are found at a rate of 70% on fields affected by erosion, which highly diminishes their productive potential, the most important reduction in their productivity is due to unfavourable climatic conditions and bad management [29; 30]. Increasing the grassland productive potential can be achieved by different fertilization rates and types of organic fertilizers [2; 28]. The investigations carried out until today have demonstrated the positive effects of manure on grasslands and, if applied reasonably, it can replace all the chemical fertilizers [15; 33].

These trials was set up at two different sites: Ezareni – Iasi site, from the forest steppe area, on a *Festuca valesiaca* L. grassland, and Pojorata – Suceava site, on *Agrostis capillaris* + *Festuca rubra* grassland, from the boreal floor; both sites present a weak botanical composition. The trial from Ezareni – Iasi was set up at the height of 107 m, on 18-20% slope, and the one from Pojorata – Suceava, at the height of 707 m, on 20% slope. The climatic conditions were characterized by mean temperatures of 9.5 0C and total rainfall amounts of 552.4 mm at Ezareni – Iasi, and by mean temperatures of 6.3 °C and total rainfall amounts of 675 mm at Pojorata - Suceava. An im-

portant fact was that the year 2007 was very dry at Ezareni – Iasi, and the climatic conditions were unfavourable to the good development of vegetation on grasslands.

Analyzing the production data concerning the *Festuca valesiaca* grassland from Ezareni, we have noticed that in 2006, they were comprised between 1.56 t ha⁻¹ DM at the control and 2.71 t ha⁻¹ DM at the fertilization with 40 t ha⁻¹ cattle manure, applied every 3 years (Table 2). The highest yields were found in case of 40 t ha⁻¹ manure fertilization, applied every 3 years; the yields were of 2.57 t ha⁻¹ DM in case of sheep manure and 2.71 t ha⁻¹ DM in case of cattle manure. In 2007, the vegetation from permanent grasslands was highly affected by the long-term draught that dominated the experimental area from Ezareni, since September 2006 until August 2007, so that the productivity of these agro-ecosystems was greatly diminished, the effect of fertilization on production becoming negligible. The mean yields during 2006-2007 were comprised between 1.09 t ha⁻¹ DM at the control and 1.96 t ha⁻¹ DM in case of fertilization with 40 t ha⁻¹ cattle manure, every 3 years.

Fertilization variant	2006	2007	Average
V ₁ . Unfertilized control	1.56	0.61	1.09
V ₂ . 10 t ha ⁻¹ sheep manure applied every year	2.16	0.91	1.54*
_{v3} ₋ 20 t ha-1 sheep manure applied every 2 years	2.35	1.02	1.69**
V ₄ . 30 t ha ⁻¹ sheep manure applied every 3 years	2.12	1.01	1.57**
V _{5.} 40 t ha ⁻¹ sheep manure applied every 3 years	2.57	1.12	1.85***
V ₆₋ 10 t ha ⁻¹ cattle manure	2.28	1.13	1.71**
V _{7 -} 20 t ha ⁻¹ cattle manure applied every 2 years	2.50	1.09	1.80***
V ₈ . 30 t ha ⁻¹ cattle manure applied every 3 years	2.69	1.04	1.87***
V _{9.} 40 t ha ⁻¹ cattle manure applied every 3 years	2.71	1.21	1.96***
Average	2.33	1.02	1.68

Table 2. Influence of organic fertilization on DM yield (t ha-1), Ezareni, Iasi [29].

 $*=P \le 0.05$; $**=P \le 0.01$; $***=P \le 0.001$; NS= not significant

In the trial conducted on the *Agrostis capillaris+Festuca rubra* grassland from Pojorata in 2006, the yields were between 2.95 t ha⁻¹ DM at the control and 4.17 t ha⁻¹ DM at 30 Mg ha⁻¹ manure fertilization, applied every 3 years (Table 3). In 2007, the yields were higher than in 2006, being comprised between 4.34 t ha⁻¹ at the control and 5.51 t ha⁻¹ in case of fertilization with 30 t ha⁻¹ manure, applied every 3 years. The mean yields during 2006-2007 have been influenced by climate and the type and level of organic fertilization, being comprised between 3.65 t ha⁻¹ at the control and 4.84 t ha⁻¹ in case of fertilization with 30 t ha⁻¹ manure, applied every 3 years.

The analysis of canopy has shown that the mean values of the presence rate were of 68% in grasses, 13% in legumes and 19% in other species (Table 4) and 39% in grasses, 32% in legumes and 29% in other species (Table 5).

Fertilization variant	2006	2007	Average
Unfertilized control	2.95	4.34	3.65
10 t ha ⁻¹ cattle manure applied every year	3.50	5.05	4.28**
20 t ha-1 cattle manure applied every 2 years	3.90	4.90	4.40**
30 t ha ⁻¹ cattle manure applied every 3 years	4.17	5.51	4.84***
20 t ha ⁻¹ cattle manure applied in the first year+10 t ha ⁻¹ cattle manure applied in the second year+0 t ha ⁻¹ manure applied in the third year	ne 3.86	4.87	4.37**
20 t ha ⁻¹ cattle manure applied in the first year+0 t ha ⁻¹ manure applied in the secon year+10 t ha ⁻¹ cattle manure applied in the third year	nd 3.78	5.25	4.52**
20 t ha ⁻¹ cattle manure applied in the first year+10 t ha ⁻¹ cattle manure applied in the second year+10 t ha ⁻¹ cattle manure applied in the third year	ne 4.03	4.81	4.42**
10 t ha ⁻¹ cattle manure applied in the first year+20 t ha ⁻¹ cattle manure applied in the second year+10 t ha ⁻¹ cattle manure applied in the third year	ne 3.63	5.12	4.38**
Average	3.72	4.98	4.36**

Table 3. Influence of organic fertilization on DM yield (t ha⁻¹), Pojorata, Suceava [30].

At Ezareni - Iasi, a total number of 40 species was registered, of which 6 species from grass family, 10 species from Fabaceae and 24 species from others, while at Pojorata - Suceava, the total number of species was of 45, of which 12 grasses, 9 legumes and 24 species from others. The species with the highest presence rate from Ezareni – Iasi were Festuca valesiaca (39%), Trifolium pratense (7%), Plantago media (3%), Achillea setacea (4%), and from Pojorata – Suceava, Agrostis capillaris (14%), Festuca rubra (7%), Trisetum flavescens (6%), Trifolium repens (16%), Trifolium pratense (8%) and Taraxacum officinale (5%).

Fertilization variant	Grass	Legumes	Other species
Unfertilized control	69	10	21
10 t ha ⁻¹ sheep manure applied every year	76	13	11
20 t ha-1 sheep manure applied every 2 years	59	16	25
30 t ha ⁻¹ sheep manure applied every 3 years	70	11	19
40 t ha ⁻¹ sheep manure applied every 3 years	67	15	18
10 t ha ⁻¹ cattle manure	62	11	27
20 t ha ⁻¹ cattle manure applied every 2 years	68	16	16
30 t ha ⁻¹ cattle manure applied every 3 years	71	12	17
40 t ha ⁻¹ cattle manure applied every 3 years	69	11	20
Average	68	13	19

Table 4. Influence of the organic fertilization on the canopy structure (%), Ezareni, lasi [30].

Fertilization variant	Grass	Legumes	Other species
Unfertilized control	44	25	31
10 t ha ⁻¹ manure applied every year	38	33	29
20 t ha-1 manure applied every 2 years	43	30	27
30 t ha ⁻¹ manure applied every 3 years	37	33	30
20 t ha ⁻¹ manure applied in the first year+10 t ha ⁻¹ manure applied in the second year+0 t ha ⁻¹ manure applied in the third year	36	36	28
20 t ha ⁻¹ manure applied in the first year+0 t ha ⁻¹ manure applied in the second year+10 t ha ⁻¹ manure applied in the third year	42	30	28
20 t ha ⁻¹ manure applied in the first year+10 t ha ⁻¹ manure applied in the second year+10 t ha ⁻¹ manure applied in the third year	36	33	31
10 t ha ⁻¹ manure applied in the first year+20 t ha ⁻¹ manure applied in the second year+10 t ha ⁻¹ manure applied in the third year	33	37	30
Average	39	32	29

Table 5. Influence of the organic fertilization on the canopy structure (%), Pojorata, Suceava [30].

The yields obtained were influenced in both experiencing sites by climatic conditions, type and level of organic fertilization. Our results demonstrated the positive effects of organic fertilizers on canopy structure, biodiversity and productivity in the studied permanent grasslands. In both trials, we noticed that the highest number of species (24 species) was represented by others, proving that the management of organic fertilizers did not affect the biodiversity of these grassland types.

3.2. Manure used on Nardus stricta L. Grasslands in Romania's Carpathians

In Romania, the grassland area, dominated by *Nardus stricta* L., covers 200,000 hectares. Meadow degradation is determined by changes that take place in plant living conditions and in the structure of vegetation. For a long-term period no elementary management measures were applied on permanent meadows in Romania, estimating that they could get efficient yields without technological inputs. The organic fertilization has a special significance for permanent meadows if their soils show some unfavourable chemical characteristics. The investigations carried out until today have demonstrated the positive effects of reasonably applied manure on grasslands. Within this context, the main aim of our study was to improve the productivity of natural grasslands by finding economically efficient solutions that respect their sustainable use and the conservation of biodiversity [1; 17; 31]. On the other hand, the productivity and fodder quality are influenced by the floristic composition, morphological characteristics of plants, grassland management, vegetation stage at harvest and level of fertilization [1; 4; 8; 34].

To accomplish the objectives of these studies we have conducted an experiment in the Cosna region, in four repetitions blocks with 20 sq. meter randomized plots on *Nardus stricta* L.

grasslands, situated at an altitude of 840 m, on districambosol with 1.36 mg/100 g soil PAL and 38.1 mg/100 g soil KAL [13].

The forage obtained from these grasslands is mainly used to feed dairy cows. The influence of manure has been analysed, and applied each year or every two years at rates of 20-50 t ha⁻¹ (table 6). The manure with a content of 0.42% total N, 0.19% $_{P2O5}$ and 0.27% K_2O was applied by hand, early in spring, at the beginning of grass growth. The Kjeldahl method was used for the determination of crude protein, the Weende method for the determination of crude fiber, the photometrical method for the determination of total phosphorus, ash was determined by ignition, whereas the nitrogen nutrition index (NNI) was determined by the method developed by Lemaire et al. (1989): NNI= $100 \times N/4.8 \times (DM)^{-0.32}$, where N: plant nitrogen content (%), DM: dry mater production (t ha⁻¹). All fodder analyses have been performed on samples taken from the first harvest cycle, based on the average values of the years 2009-2010. The vegetation was studied using the method Braun-Blanquét. For floristic data were calculated the mean abundance-dominance (ADm). Data regarding the sharwe of economic groups, species number and Shannon Index (SI) were processed by analysis of variance.

The use of 20-50 t ha⁻¹ manure accounted for, alongside the climatic factors, a significant yield increase, especially when applying 30-50 t ha⁻¹. At these rates, the DM yield recorded a significant increase, compared with the control variant. Considering the average of the two years, the control variant recorded values of 1.77 t ha⁻¹, whereas by fertilization, we obtained yields of 3.29-5.53 t ha⁻¹ DM, at rates of 30-50 t ha⁻¹, applied on a yearly basis, and 2.86-3.33 t ha⁻¹ DM at the same rates, applied once every 2 years, respectively (Table 6).

Manusayata	2009	2010	Average of	2009-2010
Manure rate	t ha ⁻¹	t ha ⁻¹	t ha ⁻¹	%
Unfertilized control	1.25	2.30	1.77	100
20 t ha ⁻¹ , every year	2.55*	2.40	2.48ns	140
30 t ha ⁻¹ , every year	2.34*	4.23**	3.29**	186
40 t ha ⁻¹ , every year	3.59***	4.74***	4.17***	236
50 t ha ⁻¹ , every year	4.73***	6.32***	5.53***	312
20 t ha ⁻¹ , every 2 years	2.28 ns	2.92 ns	2.60 ns	147
30 t ha ⁻¹ , every 2 years	2.59*	3.13 ns	2.86*	162
40 t ha ⁻¹ , every 2 years	1.78	4.14**	2.96*	167
50 t ha ⁻¹ , every 2 years	2.39*	4.28**	3.33**	188

 $*=P \le 0.05$; $**=P \le 0.01$; $***=P \le 0.001$; ns= not significant

Table 6. Influence of organic fertilization on the yield (t ha⁻¹ DM) of *Nardus stricta* grasslands from the Carpathian Mountains of Romania [34].

The organic fertilization of *Nardus stricta* L. grasslands, with moderate rates of 20–30 t ha⁻¹ manure, has determined the increase in the CP (crude protein) content by 45.9 g kg⁻¹ DM,

compared with the unfertilized control variant. The rates of 40-50 t ha⁻¹ diminished the percentage of dominant species and the increase of CP yield with 246.2-422.8 kg ha⁻¹ when manure was added once a year and 189.0-243.2 kg ha⁻¹, when manure was added every 2 years, respectively, in comparison with the control variant (Table 7). The ash content increased in all fertilized soils, varying between 71.0–83.1 g kg⁻¹ DM, compared to merely 61.2 g kg⁻¹ DM at the control variant. The crude fiber content (CF) was the highest at the control variant (285.3 g kg⁻¹ DM) and the lowest at the variant fertilized with 50 t ha-1, applied once every 2 years, of 228.3 g kg⁻¹ DM. Phosphorus, an important element in animal nutrition, recorded an increase from 1.4 g kg⁻¹ DM at the control to 2.2 g kg⁻¹ DM with the use 50 t ha⁻¹ manure, applied once every 2 years (table 2). The NNI presented values comprised between 25-53, thus, indicating a deficiency in nitrogen nutrition.

Manure rate	t ha ⁻¹ DM	СР	Ash	CF	P _{total}	Kg ha ⁻¹ CP	NNI
Unfertilized control	1.77	62.6	61.2	285.3	1.41	110.8	25
20 t ha ⁻¹ , every year	2.48	88.2***	71.0	264.2	1.92*	218.7	39
30 t ha ⁻¹ , every year	3.29**	108.5***	83.1	270.4	2.05*	357.0	53
40 t ha ⁻¹ , every year	4.17***	97.9***	78.2	258.1	2.13**	408.2	52
50 tg ha ⁻¹ , every year	5.53***	96.5***	81.6	253.6	2.04*	533.6	55
20 t ha ⁻¹ , every 2 years	2.60	82.8***	79.0	247.5	1.86*	215.3	37
30 t ha ⁻¹ , every 2 years	2.86*	92.2***	77.5	241.6	2.17*	263.7	43
40 t ha ⁻¹ , every 2 years	2.96*	101.3***	80.7	230.5	1.95*	299.8	48
50 t ha ⁻¹ , every 2 years	3.33**	106.3***	79.2	228.3	2.22**	354.0	52

^{*} P≤0.05; ** P≤0.01; *** P≤0.001

CP=crude protein, CF=crude fiber, P_{total}= total phosphorus, NNI= nitrogen nutrition index

Table 7. Influence of organic fertilization on yield (t ha⁻¹ DM) and NNI and CP quantity (Kg ha⁻¹) and on chemical composition of the fodder obtained from *Nardus stricta* grasslands (g kg⁻¹ DM), mean 2009-2010 [34].

The organic fertilization on permanent grasslands has resulted in some changes in the canopy structure, both in terms of the number of species as well as in their percentage in the vegetal canopy [4; 8; 16; 22; 24; 34]. Thus, the number of species has increased from 18 at the control variant to 25-31 at fertilization rates, while the percentage of *Nardus stricta* L. species plunged from 70% at the control to 14-33% in the case of the fertilized experiments. Moreover, the legume species increased by 5-28% (Table 8a).

Species number increased towards the control, for all fertilization variants. Shannon weaver index (SI) was compared to the control with the value between 1.07 and 2.52 (Table 8b).

Biodiversity has become one of the main concerns of our world, because modern farming, forestry and meadow culture focussed, in these latter years, on developing methods and proceedings for achieving high productions, without being interested in the quality of produces or environment health. Among the factors threatening biodiversity, one enlists human

activities, high pressures on natural resources, division, change or even destruction of habitats, excessive use of pesticides, chemical fertilizers etc [36]. Nowadays, many specialists are concerned with adapting the technologies of fodder production to the new economic and ecological requirements, whilst the maintaining of biodiversity occupies an important place [3; 5; 9; 10; 14; 25; 35].

Species				Plan	t ADm¹ c	legree '	%			
Species	V ₁	V ₂	V ₃	V ₄	V ₅	V_6	V ₇	V ₈	V_9^2	
Agrostis capillaries	+	5 _	3	2	1	+	+	/+ 🗆	5	
Anthoxanthum odoratum	-	4	+	-	+	3	6	4	5	
Briza media	+	6	6	5	6	8	7	8	10	
Cynosurus cristatus	-	-	-	-	-	3	+	-	-	
Dactilys glomerata	-	-	-	-	3	-	-	-	-	
Festuca pratensis	-	+	10	6	2	-	-	2	-	
Festuca rubra	+	1	+	3	3	+	5	5	3	
Nardus stricta	70	32	15	14	15	41	32	33	31	
Phleum pretense	+	7	2	-	-	-	-	-	2	
Grasses	70	55	36	30	30	55	50	52	56	
Lotus corniculatus	-	18	13	2	3	5	5	5	+	
Trifolium pretense	+	10	5	3	5	3	4	3	5	
Trifolium repens	-	+	+	-	+	2	3	2	+	
Legumes	0	28	18	5	8	10	12	10	5	
Achillea millefolium	+	3	12	35	40	20	9	6	5	
Ajuga reptans	+	+	+	+	+	+	+	+	+	
Alchemilla xanthochlora	6	2	6	2	6	3	6	3	6	
Chrysanthemum leucanthemum	2	3		-	-	-	-	7		
Campanula obietina	FC.	7	+	+	7#	J.	+-	2	4	
Centaurea cyanus	-	-	+	-	-	-	-	-	-	
Cerastium semidecandrum	1	+	+	+	+	+	5	3	+	
Cruciata glabra	2	2	3	+	3	+	+	3	3	
Fragaria vesca	-	-	+	+	+	+	+	+	+	
Hyeracium pilosella	3	2	3	+	-	+	-	+	+	
Hypericum maculatum	2	+	1	3	2	2	4	6	6	
Leucanthemum vulgare	-	-	+	-	-	-	-	-	+	
 Luzula multiflora	-	-	-	-	-	+	+	-	-	

		Plant ADm¹ degree %								
Species		$\overline{V_1}$	V ₂	V ₃	V_4	V_5	V ₆	V ₇	V ₈	V ₉ ²
Lychnis flos-cuculi		-	-	+	+	+	+	-	-	-
Prunella vulgaris		+	+	+	+	-	+	+	-	+
Polygala amarelle		-	-	-	-	-	-	+	-	-
Polygala vulgaris		-	-	- /	-	+ _	+	-	-	-
Plantago lanceolata		-	7	7)(+	3	+	+	2	+
Potentila ternate	5	5	4	5	+	4	5		2	2
Rumex acetosa		-	-	-	-	- []	+	-	+	-
Rumex acetosella		-	1	+	-	+	1	+	-	-
Ranunculus acer		1	-	+	2	2	1	+	2	+
Taraxacum officinale		2	0	2	+	+	3	4	4	-
Thymus pulegioides		2	-	2	-	-	+	+	+	-
Tragopogon pratensis		-	+	-	-	-	+	+	-	+
Veronica chamaedrys		1	+	12	+	-	-	10	5	1
Veronica officinalis		1	-	-	23	2	-	-	-	7
Viola tricolor		2	+	+	+	-	+	+	+	5
Forbs		30	17	46	65	62	35	38	38	39
Number of species		18	25	30	25	26	31	28	26	27
Shannon Index (SI)		1.07	2.27*	2.52*	1.93	2.15*	1.96	2.28*	2.50*	2.41*

¹ ADm – mean abundance-dominance;

Table 8. Influence of organic fertilization on the evolution of the vegetal canopy, [34].

Previous research, done in different climatic and managerial conditions proved that there is a relationship between biodiversity and pastures productivity. The latter is influenced by the soils fertility, chemical reaction, and usage, intensity of grazing, altitude, amount and distribution rainfalls [7; 12; 18; 19; 23; 31].

The management applied on oligotrophic grasslands from Garda de Sus (Apuseni Mountain) is a traditional one. The maintenance activities ar only manually performed, among them the fertilization with stable manure being the most important one [26]. The grassland type of the untreated witness is Agrostis capillaris L Festuca rubra L The productivity of the respective meadows is very low, situation wich explains one of the reasons for the abandonment of oligotrophic grasslands in the area.

The low yield can be explained through the reduced quantities of rainfall from spring and through the reduced trophicity of the soil. The species diversity ot the studied phytocenosis is medium, and the number of species ranges from 20 up 24.

 $^{^{2}}$ V_{1} is control, V_{2} - V_{9} are the manure rates applied; *= P<0.05, ** = P<0.1, ***= P<0.01

The floristic structure of the trated variants is significantly correlated with the general cover. The administration of technological inouts produces a considerable decrease of the phyto-diversity, especially in case of the variants treated with larger quantities of fertilizers [27].

3.3. Vinassa used as a fertilizer on Festuca valesiaca grassland

The objective of this study was to identify new opportunities to improve the permanent grassland, using "vinassa" as a fertilizer. The experiment was conducted on a permanent pasture of *Festuca valesiaca*, located on a land with a slope of 7-11% in 2000. The soil was of cambic chernozem type with loam-clay texture, low leachated with pH values from 6.5 to 7.1. The content in mobile phosphorus (P_{AL}) was of 28-46 ppm, mobile potassium (K_{AL}) of 333-400 ppm, the humus of 3.22-4.85% and total nitrogen (N_t) of 0.6-1.25, in the layer of 0-20 cm. The administration of the by-product was made after the dilution with water in 1:5 ratio and the way of using lawn was that of a grassland.

During the three years of study, there was a steady increase in dry matter production (Table 9). The meadow of *Festuca valesiaca* positively reacted to the use of "vinassa" by-product in normal vegetation conditions. The exception was 2002, when due to bad weather conditions, mainly water shortages in spring, the productions recorded a slight decrease in comparison with 2000.

Thus, in 2000, the production, in variants with total application of the sub-product in spring, ranged from 3.35 t ha⁻¹ DM in the control sample and 3.99 t ha⁻¹ DM, to 7 t ha⁻¹ "vinassa". In 2000, in the variants with fractional application of fertilizers, the productions ranged from 3.07 t ha⁻¹ in the control samples and 4.15 t ha⁻¹ at 5 t ha⁻¹. 2001 was characterized by increased lawn productivity in both applications of vinassa, with increased production for the variants of full implementation in spring when the average productions were 5.97 t ha⁻¹ in the control sample and 10.86 t ha⁻¹ at 7 t ha⁻¹ "vinassa".

In 2002 the recorded productions were lower than in 2001, due to less favorable weather conditions. In 2002 we noticed that the uneven distribution of rainfall during the vegetation period, that is heavy rains in the second half of the year, positively influenced the production increases, especially in the variants with fractional application.

The analysis of the average productions obtained in the three years shows that "vinassa" sub-product had a positive effect on the productivity of meadows, but also the results were conditioned by the climatic factors. In comparison to the control sample, in the case of vinassa with total application in spring, the production increased between 23 and 69% (2 t ha-"vinassa" and 7 t ha-"vinassa"), being statistically ensured, while the fractional application yielded smaller increases of 13-39% (2 t ha-"vinassa" and 7 t ha-"vinassa").

Using the "vinassa" sub-product as nitrogen-potassium fertilizer on permanent meadows of Festuca valesiaca L. determines production increase, statistically ensured of 23-69% (2 t ha⁻¹ "vinassa" and 7 t ha⁻¹ "vinassa") at total application in spring and 13-46% in fractionated administration (2 t ha⁻¹ "vinassa").

Period	Variant	2000	2001	2002	Average 2000	-2002
	Variant	t ha ⁻¹	t ha ⁻¹	t ha ⁻¹	t ha ⁻¹	%
	1	3.35	5.97	4.94	4.75	100
	2	3.36	8.06	6.65	6.02*	123
	3	3.44	8.39	7.04	6.29*	132
a ₁	4	3.69	8.54	7.11	6.45*	136
	5	3.59	9.24	8.47	7.10**	149
	6	3.76	10.07	9.48	7.77***	164
	7	3.99	10.86	9.17	8.01***	169
Average		3.93	8.92	7.95	6.93	146
	1	3.07	5.97	5.17	4.74	100
	2	3.24	5.49	7.40	<i>5.38</i> ns	113
	3	3.47	5.59	7.94	<i>5.67</i> ns	120
a_2	4	3.60	5.63	8.06	<i>5.76</i> ns	122
	5	4.15	5.86	8.47	<i>6.16</i> ns	130
	6	3.80	6.08	9.48	6.45*	136
	7	3.90	6.16	9.67	6.58*	139
Average		3.63	6.18	8.28	6.03	127

 $^{*=}P \le 0.05$; $**=P \le 0.01$; $***=P \le 0.001$; ns= not significant

Table 9. Influence of vinassa on production of dry matter a meadow of Festuca valesiaca [32].

4. Conclusions

Organic farming is a dynamic sector in Romania recording an upward trend, both in the vegetal and animal production sector in recent years.

One of the essential conditions for the development of organic farming is to promote the concept of organic farming so as to make the consumer aware of the benefits of organic food consumption.

The fertilization of mountain grasslands with organic fertilizers leads to an improvement in terms biodiversity, productivity and quality.

Fertilization with manure contributed to the improvement of the botanical structure by increasing the percent of grasses, thus disfavouring the leguminous plants. The used management, characterized in time by low inputs and stability, has contributed to obtaining rather high productions and conservation of the biodiversity of the meadows from Romania.

The highest biodiversity was found in the grassland from Pojorata, covered with 45 species of Agrostis capillaris + Festuca rubra, compared to 40 species found in the grassland from Ezareni, covered with Festuca valesiaca.

The fertilization of *Nardus stricta* grasslands with 20-50 Mg ha-1 manure influenced the yield increase by 40-212% and brought along important changes in the chemical composition of fodder, improving its quality significantly, by increasing the CP content from 62.6 g kg⁻¹ DM (control) to 108.5 (30 Mg ha⁻¹ manure, applied once every 2 years); the total phosphorus from 1.41 to 2.22 g kg⁻¹ DM and ash from 61.2 to 83.1 g kg⁻¹, and by diminishing the CF content from 285.3 to 228.3 g kg⁻¹ DM, thus increasing fodder digestibility.

The application of 20-50 Mg ha⁻¹ manure determined important changes in the flower composition as well, by lowering the percentage of *Nardus stricta* species from 70% to 14-33% and increasing the percentage of legumes (*Lotus corniculatus, Trifolium pratense* and *Trifolium repens*) and forbs.

The administration of "vinassa" sub-product in doses of 4-7 t ha⁻¹ by 1:5 dilution with water does not determine spectacular increases in the production of DM.

In quantitative terms we noticed that, when fractioning, the vinassa production was lower than in variants with total application in spring, this being also caused by less favorable climatic conditions.

The period of vinassa application, the dosage used and the climatic conditions affect the productivity of permanent meadows. The best results were obtained when using doses of 6-7 t ha⁻¹ "vinassa", with total application in spring (7.65 - 8.01 t ha⁻¹ DM).

The results presented in this study, on land considered to be regionally representative of large parts of the Romania, indicated that fertilization treatments were able to maintain a high diversity of species. Production was influenced by climatic conditions, fertilizer application rate and the combination fertilizers applied. Using a low input-based management system can be a solution that will lead to higher yields and contribute to biodiversity conservation.

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