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Effect of mineral and organic fertilization of alfalfa on some seed yield characteristics, root biomass accumulation and soil humus content

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Abstract: A field trial at the Institute of Forage Crops, Pleven, Bulgaria (2000-2003) was conducted to study the effect of mineral and organic fertilization on seed yield of alfalfa, root biomass accumulation and soil humus content. Alfalfa variety 'Victoria' was grown on a leached chernozem soil without irrigation. Rates of 70, 140 and 210 kg ha^{-1} mineral nitrogen (active ingredient) were tested as ammonium nitrate and well-matured cattle manure. It was found that mineral and manure fertilization at 140 and 210 kg ha^{-1} increased seed yield by 9.9% and 20.9% for mineral, and by 30.3% and 40.6% for manure. Seed yield was more stable under manure fertilization compared with mineral fertilization. Alfalfa accumulated between 2669 and 3098 kg ha^{-1} dry root mass after mineral fertilization and between 3310 and 3570 kg ha^{-1} after manure application. Additional root mass of 482 to 698 kg ha^{-1} was found to be accumulated for manure treatments compared to mineral fertilization. High ratios of nitrogen yields (192 - 216 kg ha^{-1}) and plant available nitrogen (77 - 86 kg ha^{-1}) have been obtained from the treatments with manure. The highest amount of humus remained in the soil after fertilization of alfalfa with manure at the rate of 210 kg ha^{-1}.
Key words: alfalfa, mineral fertilization, manure, seed yield, dry root mass, humus.

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Introduction

Agricultural production becomes imperative for establishing relationships between crop productivity and soil characteristics. A major component of sustainable agriculture is to sustain productivity and improve soil quality.

More effective use of sources for improving soil fertility, enhancing organic matter and recovering the nutrients have been encouraged (Jarvis and Barraclough 1991; Scholefield and Titchen 1995; Kusvuran *et al*. 2014).

In Bulgaria 100 million tons of fertile soil are lost annually. Each year 2.5 million tons of humus, 4 million tons of nitrogen and 3.5 million tons of phosphorus disappear from the soil as well.

Legumes enrich soil fertility due to their deep-reaching root systems, accumulation into soil and quick decomposition of root biomass (Stoddard *et al.* 2009; Luscher *et al*. 2014; Kusvuran *et al*. 2014).

Alfalfa (*Medicago sativa* L.) has long been recognized and valued as the "soil building" legume crop. This crop has the ability to accumulate significantly more nitrogen than other legumes through its deep rooting system and fix atmospheric N_2 from 40 to 80% of total plant nitrogen through biological nitrogen fixation (Heichel *et al*. 1981; Jarvis 2005). It can fix nitrogen on average 450 kg ha^{-1} per year (Vance 1978; Fishbeck *et al*. 1987; Heichel and Henjum 1991; Starchenkov and Kot's 1992).

Alfalfa has been widely planted in different areas worldwide on over 32 million hectares because of its forage, nutritional quality and high biomass producing values (Veronesi *et al*. 2010; Xie *et al*. 2013). It is one of the most important forage legume species in Bulgaria, and is considered the largest contributor to sustainable agriculture (Kertikova 2008). Alfalfa is important for increasing the fertility of the soil on which it is grown. This occurs because after each cutting a corresponding amount of the root system dies back and thus provides soil with organic matter that can be immediately degraded by soil micro organisms.

With the advent of the sustainable agriculture concept, the sustainability of soil productivity has now looked into more areas where the dependence on agrochemicals and fertilizers has sharply increased for crop production (Singh *et al*. 2012; Dwivedi 2014).

Increased attention is now being paid to enhance soil productivity through a balanced use of mineral fertilizers combined with organic sources (Singh *et al*. 2012; Dwivedi 2014).

The addition of organic materials of various origins to the soil improves soil quality as well plant productivity (Bulluck *et al*. 2002; Edmeades 2003; Celik *et al*. 2004).

The aim of this work was to study the effect of mineral and organic fertilization on some characteristics of seed yield of alfalfa, root biomass accumulation and humus content of soil.

Materials and methods

The experiment was carried out in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria (2000-2003) with alfalfa variety 'Victoria' on leached chernozem soil subtype without irrigation. Plot size was 10 m^2. The treatments were studied using 4 replications.

Nitrogen as ammonium nitrate was applied at rates of 70, 140 and 210 kg ha^{-1} as mineral and organic form. Phosphorous was applied as triple super phosphate at 300 kg ha^{-1} in all treatments. Potassium was applied as potassium chloride at 150 kg ha^{-1} in all treatments. Corresponding amounts of nutrients were applied as very well matured cattle manure. The following scheme was used (the rates are in active ingredient): $N_0P_{300}K_{150}$ – control; $N_{70}P_{300}K_{150}$ - N applied as mineral fertilizer; $N_{140}P_{300}K_{150}$ - N applied as mineral fertilizer; $N_{210}P_{300}K_{150}$ - N applied as mineral fertilizer; $N_{70}P_{300}K_{150}$ - N applied as manure; $N_{140}P_{300}K_{150}$ - N applied as manure; $N_{210}P_{300}K_{150}$ - N applied as manure.

Seed yield (kg ha^{-1}) was obtained from the second cut of alfalfa in the second, third and fourth years of growing (2001-2003). Sustainable yield index (SYI) developed by Singh *et al*. (1990) was used for assessment:

SYI (Sustainable yield index) = (Ym-Sd)/Ymax, where:

Ym – mean yield

Sd – standard deviation

Ymax – maximum yield

Generative stems weight/vegetative stems weight ratio was calculated after analyzing 25 stems of plants from each replication.

Soil samples from soil profile were taken at a depth of 40 cm, and root mass was washed with tap water (Beck *et al*. 1993). Dry root mass (kg ha^{-1}) was recorded as calculated from fresh mass and % dry matter (dried at 60^0C); N in dry root mass yield (kg ha^{-1}) was calculated by multiplying dry root mass yield by % N content. Plant available N (PAN) (kg ha^{-1}) was calculated according to Sullivan and Andrews (2012):

PAN (plant available nitrogen) (kg ha^{-1}) = Total N (kg ha^{-1}) x 0.4

0.4 = 40% Nitrogen release (accepted estimation).

Soil samples were taken at the end of the experiment and analyzed for humus content (%), humus composition (organic C, extracted with 0.1M $Na_4P_2O_7$+0.1M NaOH - total, in humic acids and in fulvic acids).

The soil and manure nutrients were determined by Page *et al*. (1982). The soil used had the following agrochemical characteristics: total C, 2.8; total N, 0.23; P (P_2O_5), 5.3 mg 100 g^{-1} soil; K (K_2O), 48.4 mg 100 g^{-1} soil; pH (H_2O) 7.05; NH_4^+-N, 11.2 mg N kg^{-1} soil; NO_3-⁻N, 8.8 mg N/kg^{-1} soil. The manure used had the following agrochemical characteristics on a dry basis: total C, 15.0%; total N, 0.72%; C:N, 20.8; ashes, 25%; NH_4^+-N, 42.8 mg N kg^{-1} dry manure; NO_3- N, 541.4 mg N kg^{-1} dry manure; total mineral N, 584.2 mg N kg^{-1} dry manure and very high maturity index (NO3-N to NH4-N = 12.6); P (P_2O_5), 45.3 mg 100 g^{-1} soil dry manure; K (K_2O), 78.3 mg 100 g^{-1} g dry manure; pH (H_2O), 6.7; humic acid, 8.55% dry matter; fulvic acid, 0.01% dry matter.

The concentrations of mineral nitrogen were estimated on the basis of available amounts of ammonium and nitrate nitrogen in the soil and those in the mineral fertilizer.

When applying manure, we accepted the mineralization percentages on the basis of total nitrogen as being more accurate, because the data on the total mineralization of nitrogen over a period of 5 years was 46% of the initial nitrogen in the manure. On that basis, we applied 20.7 t manure/ha corresponding to 70 kg mineral N ha^{-1}; 41.5 t manure corresponding to 140 kg mineral N ha^{-1} and 62.1 t manure corresponding to 210 kg mineral N ha^{-1}. The amount of humic acids applied to the soil with the manure was as follows: with the rate of 70 kg N ha^{-1} - 5.985 kg ha^{-1}, with the rate of 140 kg N ha^{-1} - 11.970 kg ha^{-1} and with the rate of 210 kg N ha^{-1} - 17.955 kg ha^{-1}.

Experimental data were statistically processed using SPSS software program.

Agrometeorological conditions (Table 1) in the first year were favorable for the initial development of plants. However, a prolonged drought period occurred in May and June. Scarce amounts of precipitation hindered plant development, and the root system was formed in extremely dry soil.

During the second and third years, agrometeorological conditions for alfalfa development were favorable (there was a drought period in August for the second year only).

In the fourth year, in late April to mid-May, a long drought occurred, precipitations were uneven, and average daily temperatures were low.

Table 1. Agrometeorological conditions for May - September (2000-2003)

Months	Average temperature, °C				Sum of precipitation, l m^{-2}			
	2000	2001	2002	2003	2000	2001	2002	2003
May	18.6	17.6	19.5	20.5	10.5	50.5	32.0	74.5
June	22.4	20.3	21.9	23.9	24.4	79.5	133.0	12.8
July	25.0	24.6	23.1	23.7	54.3	45.3	165.0	49.7
August	25.2	25.2	21.6	25.5	8.0	13.1	152.0	1.4
September	17.5	19.0	17.1	17.7	65.6	64.9	70.0	67.6

Results and Discussion

The effective management of agricultural practices is crucial to achieving a balance between ever increasing food demands and environmental protection.

The fertilizer rates used in this study were justified given that humic acids are strong biostimulants and can compensate for an insufficient nutrient supply in the soil.

The highest seed yield was obtained during the second year of alfalfa growing (the first one for seeds) (Figure 1). This result is in agreement with the biological characteristic of the crop. The lowest rates of fertilizers (both mineral fertilizer and manure) had a negative effect on seed yield which decreased by 18.0% for mineral fertilizer and by 27.5% for manure compared to the control. When using fertilizers at the highest rates tested (210 kg ha^{-1}), seed yield was 12.0% higher for mineral fertilizer and 20.5% higher for manure compared to the control.

During the next (third) year of growing (the second one for seeds), seed yield increased with increasing rates of both fertilizers, and the increase was more significant when manure was used. The phosphorous requirements of alfalfa are enhanced when seeds are formed (Sheaffer *et al*. 1971).

We assume that manuring increased phosphorous concentration in the soil. Manure has a high phosphatase activity as compared to the soil; it increased mineralization and phosphorous availability for the plants (Kostov 1997). Kahiluoto *et al*. (2015) suggested that phosphorus was more plant-available from manure than from the chemical fertilizer.

Seed yield was half reduced as compared to the previous year for the control, by about 30% for mineral fertilizer and by about 20% for manure. During the last year, with increasing rates of fertilization seed yield increased significantly more for the higher rates for both types of fertilizers.

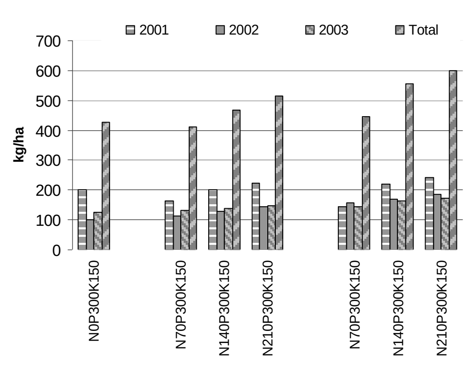

Figure 1. Seed yield of alfalfa (2001–2003)

Data on total seed yield for the three years showed that it was on the level of the control for the lowest rates of both types of fertilizers. Seed yield was the highest for the rate of 210 kg N ha^{-1} applied as manure. The comparison between the variants showed a 8.3 to 16.3% increase in seed yield for manure variants as compared to mineral fertilized ones.

The sustainable yield index was lowest for control plants (Figure 2). Sustainable yield index values showed that seed yield of alfalfa crop was more stable under organic fertilization compared with mineral fertilization.

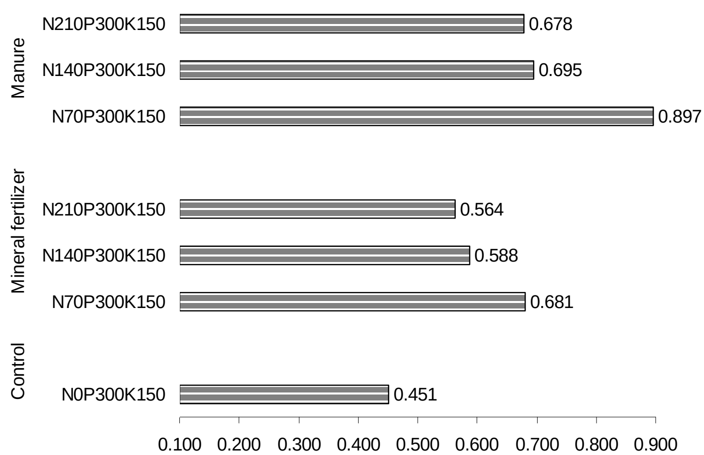

Figure 2. Sustainable yield index (SYI) for alfalfa grown for seeds

During the first year of seed yielding alfalfa, the generative/vegetative stems weight ratio was higher in variants with manure applied at the rates of 140 and 210 kg ha^{-1} (Figure 3).

High humidity during the second experimental year stimulated the vegetative development of alfalfa and as a result new vegetative stems were formed in the cut for seeds. It was the reason for the values of the generative/vegetative stems weight ratio. Plants diverted the nutrients for the formation of a new cut, thus affecting seed formation, and seed yield during this year was low.

The generative/vegetative stems weight ratio was higher in the next i.e. third year. Due to unfavorable agrometeorological conditions for pollination, seed yield was low.

The correlation between seed yield and the generative/vegetative stems weight ratio was found to be very high (r= 0.9515).

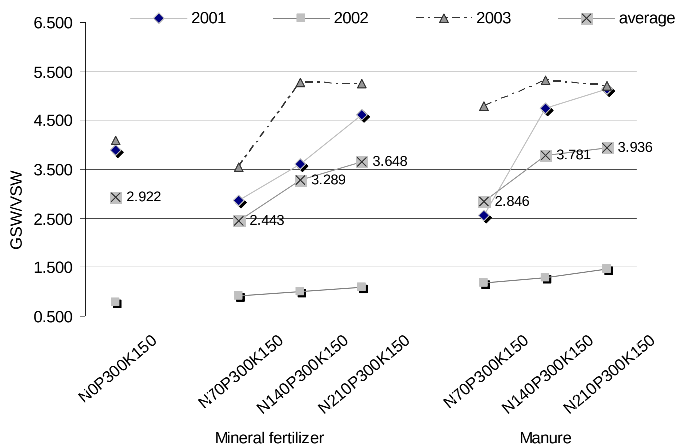

Figure 3. Generative stems weight/vegetative stems weight ratio

An important agronomic characteristic is root biomass formation. Campbell *et al*. (1997) found that 70% of the root mass of alfalfa is to the depth of 40 cm. The research related to this aspect is more difficult (Hakl *et al*. 2007, 2011, 2012). Different amounts of dry root mass of alfalfa were found in field trials - 4200 kg ha^{-1} (Sergeeva 1955), 4830 kg ha^{-1} (Campbell *et al*. 1997). A significantly lower amount i.e. 928 - 1556 kg ha^{-1} dry root mass accumulated in alfalfa after 5 years of growing, as reported by Biederbec *et al*. (2005).

Dry root mass of alfalfa in our study varied from 2669 kg ha^{-1} to 3570 kg ha^{-1} (Table 2). For treatments with mineral fertilizer at the rate of 140 kg ha^{-1}, dry root mass was higher than the control by 11.0%. The highest rate of mineral fertilizer had a depressing effect on root mass formation; consequently, root mass was less than in the control.

Dry root mass accumulation in manure treatments varied between 3310 and 3570 kg ha^{-1}. It was significantly higher as compared to the control for the rate of 140 kg ha^{-1}, where the highest amount of dry root mass was observed. There was an increase of 482 to 698 kg ha^{-1} in root mass for manure treatments compared to the mineral ones. Our results are in good agreement with the findings of Raza *et al*. (2009) for dry root biomass of alfalfa in organically managed fields at Raasdorf, Eastern Austria.

By accumulating root mass into the soil alfalfa contributed to enhancing the soil fertility (Kusvuran *et al*. 2014). Dubach and Russelle (1994) concluded that decaying alfalfa roots are a good source of plant available nitrogen. They determined that 15.36 kg N ha^{-1} was available from decaying alfalfa roots (for comparison purposes - 3.47 kg N ha^{-1} was available from decaying birdsfoot trefoil roots), with alfalfa roots adding a significantly higher amount of nitrogen to the agro-ecosystem as compared to other legumes.

Table 2. Dry root mass accumulation of alfalfa grown for seed for three years

Treatments	Dry root mass	To control	To mineral fertilizer	Additional to the mineral fertilizer
	kg ha^{-1}	%		kg ha^{-1}
$N_0P_{300}K_{150}$	2792±248	-	-	-
Mineral fertilizer				
$N_{70}P_{300}K_{150}$	2828±292	+1.3	-	-
$N_{140}P_{300}K_{150}$	3098±719	+11.0	-	-
$N_{210}P_{300}K_{150}$	2669±361	-4.4	-	-
Manure				
$N_{70}P_{300}K_{150}$	3310±716	+18.6	+17.1	482
$N_{140}P_{300}K_{150}$	3570±220	+27.9	+15.2	471
$N_{210}P_{300}K_{150}$	3367±830	+20.6	+26.2	698
Max	3570			
Min	2669			
SE (P=0.05)	260			

±, STDEV

In this study, the amount between 171 and 185 kg ha^{-1} was found for N in dry root mass yield under mineral fertilization (Table 3).

High ratios of nitrogen yields were obtained from the treatments with manure (from 192 kg ha^{-1} to 216 kg ha^{-1}). The highest amount of root mass was accumulated after manure application at the rate of 140 kg ha^{-1}. Nitrogen in the root mass yield in manure-treated variants was 12.3 to 24.9% higher than nitrogen in the root mass yield in variants with mineral fertilization.

Appropriate results have been provided in view of N in alfalfa yield replenishing organic matter in the soil and having a serious contribution to soil quality improvement. Biederbec *et al*. (2005) found that N supply to soils increases soil organic matter and improves soil structure, and a considerable amount of nitrogen is released from roots into the soil. According to Kusvuran *et al*. (2014), the amount of nitrogen accumulated in roots from alfalfa is observed to be higher than that observed for other species.

Table 3. N in dry root mass of alfalfa after growing for seeds

Treatments	N in dry root mass	Increase to control	To mineral fertilizer	Additional to the mineral fertilizer
	kg ha^{-1}	%		kg ha^{-1}
$N_0P_{300}K_{150}$	163±3.5	-	-	-
Mineral fertilizer				
$N_{70}P_{300}K_{150}$	171±5.5	+5.2	-	-
$N_{140}P_{300}K_{150}$	185±13.9	+13.4	-	-
$N_{210}P_{300}K_{150}$	167±14.8	+2.5	-	-
Manure				
$N_{70}P_{300}K_{150}$	192±17.88	+18.1	+12.3	21
$N_{140}P_{300}K_{150}$	216±26.5	+32.7	+16.9	31
$N_{210}P_{300}K_{150}$	209±30.0	+28.0	+24.9	41
Max	216			
Min	163			
SE (P=0.05)	7.8			

±, STDEV

Plant available nitrogen (Figure 4) varied from 65 to 86 kg ha^{-1} and followed the tendency for N in dry root mass yield. Manure treated plants gave 8.4 to 16.6 kg ha^{-1} more plant available nitrogen compared to mineral treatment.

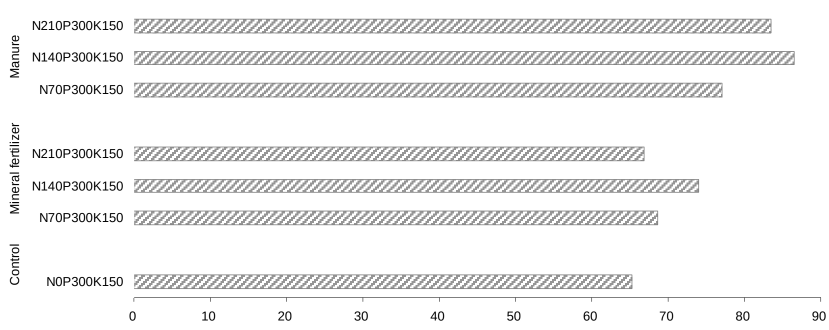

Figure 4. Plant available nitrogen (PAN)

By fixing the atmospheric N_2, alfalfa adds humus to the soil (Biederbec *et al*. 2005).

In our study (Table 4), the soil humus content increased under mineral fertilization at the low rate only (by 7.6%). Manure treatment led to an increase in humus content with increasing rates tested (from 17.4 to 62.9%). The highest amount of humus remained in the soil after fertilization of alfalfa with manure at the rate of 210 kg ha^{-1}.

Table 4. Humus content and humus composition of leached chernozem soil after mineral fertilization and manure treatment of alfalfa grown for seeds

Treatments	Humus	Organic C	Organic C, extr. with 0.1M $Na_4P_2O_7$+0.1M NaOH % to the soil			Ch/Cf
	%		total	in humic acids	in fulvic acids	
$N_0P_{300}K_{150}$	2.28	1.32	0.18	0.17	1.06	1.32
Mineral fertilizer						
$N_{70}P_{300}K_{150}$	2.44	1.42	0.36	0.18	0.18	1.00
$N_{140}P_{300}K_{150}$	2.24	1.30	0.36	0.18	0.18	1.00
$N_{210}P_{300}K_{150}$	2.14	1.24	0.31	0.18	0.13	1.38
Manure						
$N_{70}P_{300}K_{150}$	2.68	1.55	0.46	0.37	0.09	4.11
$N_{140}P_{300}K_{150}$	3.01	1.75	0.59	0.51	0.08	6.38
$N_{210}P_{300}K_{150}$	3.71	2.15	0.64	0.37	0.27	1.37
SE (P=0.05)	0.21	0.12	0.04	0.05	0.02	0.79

Soil organic matter is an important source of nutrients for plant growth that needs to be maintained for agricultural sustainability (Herencia *et al*. 2007). Carbon and nitrogen content are characteristics showing the soil quality. Plants can translocate 20-50% of total fixed carbon to their roots (Lynch and Whipps 1990; Kuzyakov and Domanski 2000). With maximizing root biomass production, C inputs into the soil increased (Kwabiah *et al*. 2005). Root biomass contains approximately 40% C and about 18% of the root C for year could be converted in humus (Barber 1979; Kwabiah *et al*. 2005).

Tiwari *et al*. (2002) have also reported that the inclusion of manure in the fertilization schedule improves the organic carbon status and available N, P, K and S in soil. The use of organic manure enhances the content of organic carbon in the soil more than the application of the same amount of nutrients as inorganic fertilizers (Gregorich *et al*. 2001). A study in the US (Follet 2001) estimates that the application of manure in the soil would result in a sequestration at the rate of 200-500 kg C ha^{-1} year^{-1}. It is important to note that alfalfa forms the humic acid type of humus which is much more stable, and stimulation effect on seed yield is higher as compared with the fulvic acid type of humus (Table 4).

Organic C content in our study followed the tendency for humus accumulation in the soil. Probably, the need for more nitrogen for the formation of more cuts when alfalfa was grown for forage and the removal of nitrogen from this agro-ecosystem requires more nitrogen mineralization of organic nitrogen containing compounds of the soil.

One of the most biochemically active elements in humus is humic acid. Due to the biochemically active nature of humic acid and its ability to form both soluble and insoluble complexes with various metals, minerals and organics, nutrients are mobilized in forms that plants can accept. Once more, the biochemically active nature of humic acid works to enhance root mass.

As regards the humification processes accompanying the formation of humic acids, it was found that mineral fertilization did not change the amount of humic acids. This is an important ecological conclusion because seed yield was higher after mineral fertilization as compared to unfertilized control. Fertilization with manure significantly increased the amount of humic acids (105.6-183.3%).

A very low increase was found in the amount of fulvic acids after mineral fertilization, and it was relatively higher for manure application for higher rates only.

The predominating conception in the literature is to increase the level of soil organic matter and maintain high and stable yields through fertilization with particularly high rates of manure (30-130 t ha^{-1}) (Michel *et al*. 2002; Raviv *et al*. 2004). Use of organic resources is a feasible approach to overcome soil fertility constraints. Nitrogen applied with organic fertilizers did not cause toxicity of the soil, nor did it depress the biological fixation of free-living soil nitrogen fixators, because of the slow release of mineral nitrogen.

In view of the decline in organic matter content, reduction in soil fertility, and the rise in energy and nitrogen fertilizer costs over the past decades, the role of alfalfa as a builder of soil organic matter and a factor contributing to maintaining and conserving the agroecosystem will likely gain more ecological and economical importance in the future.

Conclusion

Mineral and manure fertilization at the rates of 140 and 210 kg ha^{-1} increased seed yield of alfalfa by 9.9 and 20.9% for mineral fertilizer, and by 30.3 and 40.6% for manure. Seed yield was more stable under manure fertilization compared with mineral treatment.

Dry root mass accumulation after mineral fertilization varied between 2669 and 3098 kg ha^{-1} and after manure between 3310 and 3570 kg ha^{-1}. Additional root mass i.e. 482 to 698 kg ha^{-1} was found to be accumulated for manure treatments compared to the mineral ones.

High ratios of nitrogen yields (from 192 to 216 kg ha^{-1}) and plant available nitrogen (from 77 to 86 kg ha^{-1}) were obtained from the treatments with manure.

With increasing rates of manure, humus content in the soil increased. The highest amount of humus that remained in the soil was after fertilization of alfalfa with manure at the rate of 210 kg ha^{-1}.

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UTICAJ MINERALNIH I ORGANSKIH HRANIVA KOD LUCERKE NA PRINOS SEMENA, BIOMASU KORENA I SADRŽAJ HUMUSA U ZEMLJIŠTU

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Rezime

Ogled je sproveden u Institutu za krmno bilje, Pleven, Bugarska (2000-2003) u cilju proučavanja uticaja mineralnih i organskih hraniva na prinos semena lucerke, akumulaciju biomase korena i sadržaja humusa u zemljištu. Sorta lucerke Viktorija je gajena na izluženom černozemu bez navodnjavanja. Doze mineralnog azota (aktivni sastojak) od 70, 140 i 210 kg ha^{-1} su testirane u vidu amonijum nitrata i dobro zgorelog stajnjaka. Utvrđeno je da mineralno i organsko đubrivo u dozama od 140 i 210 kg ha^{-1} povećava prinos semena za 9,9% i 20,9% za mineralna, a za 30,3% i 40,6% za organska đubriva. Prinos semena je bio stabilniji kod đubrenja stajnjakom u poređenju sa mineralnim. Lucerka je akumulirala između 2669 i 3098 kg ha^{-1} suve korenove masa posle mineralne ishrane i između 3310 i 3570 kg ha^{-1} posle primene stajnjaka. Dodatnih 482 do 698 kg ha^{-1} mase korena je akumulirano primenom mineralnih đubriva. Visoki odnosi azotnih prinosa (od 192 do 216 kg ha-1) i biljkama dostupng azota (od 77 do 86 kg ha-1) su dobijeni kod tretmana sa organskim đubrivom. Najviši iznos humusa ostao je u zemljištu nakon ishrane lucerke organskim đubrivom u dozi od 210 kg ha^{-1}.

Ključne reči: lucerka, mineralno đubrenje, stajnjak, prinos semena, masa suvog korena, humus.