Effect of Inoculation of Earthworms and Soil Microorganisms on Soil Structure and Productivity of Alfalfa

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Abstract

The paper deals with exploration the influence of earthworms and soil microorganisms on soil structure and productivity of alfalfa. The observation was carried out on Chromic-Vertic Luvisols (fine texture) from Sofia field (Bulgaria) in pot experiment. The inoculations were made with three ecological groups of lumbrids: anecic (*Lumbricus terrestris*), epigeic (*Eisenia fethida*) and endogeic (*Aporrectodea rosea*, *Aporrectodea caliginosa* and *Octolasion lacteum*) earthworms. The inoculation with soil microorganisms was provided with nitrogen-fixing bacteria from genus Rhizobium. The soil structure of studied treatments was characterized by indicators derived from the soil water retention curve and total porosity. The most pronounced effect of inoculations of earthworms and microorganisms was found in the volume of macropores which increased with depth while in the control variant it decreased with depth. The results showed that interaction between earthworms and soil microflora increased the biomass of alfalfa. Our study demonstrated that earthworms and soil microorganism have positive effect on the aeration of roots and yield of forage crops.

Key words: Alfalfa, Earthworms, Forage crops,Soil microorganisms, Soil structure.

MATERIAL AND METHOD

The experiment was carried out during 2017. Chromic-Vertic Luvisol was used in this study. Soil was collected from top layer (0-20 cm) of crop field from Chelopechene village, Sofia County. The soil was characterized with fine texture. Organic carbon content was 1.61 %, the available nitrogen content was 8.6 mg/kg, the available phosphorus content (P_2O_5) – 13.5 mg/100 g, the exchangeable potassium content (K_2O) – 23.0 mg/100g and the soil pH (H_2O) was 6.1. The soil was air-dried and sieved through a 2.0 mm sieve. The experiment was carried out using 10 L pots. The soil in each pot was mixed with 250g cow dung and, additionally, 250g cow dung were added in the center of the top soil. Three pots were prepared for each variant. All pots were arranged in the greenhouse.

The earthworms were collected from Sofia field by digging and hand sorting. Six earthworms were inoculated in each pot. The nitrogen-fixing bacteria *Rhizobium melliloti* strain 116 was used for alfalfa seed inoculation. The strain was obtained from soil microorganisms collection of the Soil microbiology Department of the Institute of Soil Science, Agrotechnologies and Plant Protection “N. Poushkarov”, Sofia. The experiment was designed with seven variants: 1) controls without inoculation, 2) inoculation with endogeic earthworms (*Aporrectodea rosea*, *Aporrectodea caliginosa* and *Octolasion lacteum*), 3) inoculation with anecic earthworms (*Lumbricus terrestris*), 4) inoculation with epigeic earthworms (*Eisenia fethida*), 5) mixed inoculation with the epigeic, endogeic and anecic earthworms, 6) mixed inoculation with the epigeic, endogeic and anecic earthworms and *Rhizobium melliloti* strain 116 and 7) inoculation with *Rhizobium melliloti* 116.

Fifty alfalfa seeds (cultivar Pleven 6) were planted in each pot. After the emergence of the fifth leaf, only 15 plants were kept per pot. The soil was irrigated daily to maintain the moisture content at approximately 60% of the soil field capacity. The shoots were collected, when the plants were at the blooming phase. The shoots were cut at the soil surface and the biomass was weighed. After that the shoots were dried with oven drying at 60^{o}C for 48h. At harvesting the roots were collected and their fresh and dry biomass was determined.

Vertically oriented cores were sampled at the end of the experiment at 0-5 cm and 10-20 cm soil depth in 100 cm^3 metal cylinders for determination of bulk density (ISO 11272:1998). Soil water retention at suction less than 33 kPa was determined using the undisturbed soil cores (100 cm^3) by a suction plate method similar to those proposed in ISO 11274: 1998. The drainage of the wetted samples at suction (P) 1, 5, 10, and 33 kPa (pF 1.0, 1.7, 2.0, and 2.5) was done by suction type apparatus (Shot filters G5 with diameters of pores 1.0-1.6 μm). Total porosity (P_t) was calculated using the measured bulk density and particle density 2.65 g.cm^{-3}. Volume of air filled pores at given suction P was calculated as the difference between soil total porosity P_t and the measured volume of water content (θ) retained at this suction. The effective pore diameter δ corresponding to P was calculated by Jurin’s formula: $\delta=4*\sigma/P$, where the surface tension is $\sigma=7.29*10^{-2}$ N m^{-1} and P is in Pa. The effective diameters of pores corresponding to suctions 1, 5, 10, and 33 kPa, are 300, 60, 30, and 10 μm, respectively.

Statistical analyses of the study were conducted using SPSS software. Data on soil physical properties are presented as mean ± standard deviation. Means of plant biomass are compared by Least Significant Differences (LSD) test at p=0.05.

RESULTS AND DISCUSSION

Alfalfa is widely grown throughout the world as forage for cattle, and is most often harvested as hay, but can also be made into silage, grazed, or fed as greenchop. Alfalfa usually has the highest feeding value of all common hay crops (Nikolova et al., 1995). The productivity and nutritive value of alfalfa defined her as a leading perennial legume forage crop (Georgieva and Nikolova, 2012). It is used less frequently as pasture. When grown on soils where it is well-adapted, alfalfa is often the highest-yielding forage plant, but its primary benefit is the combination of high yield per hectare and high nutritional quality (Lenkov, 1973). Alfalfa is known for its tolerance to drought, heat, and cold and for the remarkable productivity and quality of its herbage. The plant is also valued for soil improvement and it is grown as a cover crop and as a green manure (Bratanov, 1987).

Earthworms are considered as ecosystems engineers with great impact on physical, chemical and biological properties of the soils (Lavelle et al., 2007). Availability of earthworms can increase soil aeration and drainage, whilst soil gut passage can improve soil crumb structure and lead to enhanced water holding capacity. The activity of earthworms has a decisive role in the formation of macro- and microaggregates (Six et al., 2005). The incorporation of organic material and its mixing with mineral soil can also lead to increased nutrient availability (Butt, 2011). Earthworms in a soil resulted in improved forage crop yields and a better quality of grasslands (Edwards et al., 1980).

Soil microorganisms influence plants and their growth may be limited, or promoted by the soil microorganisms (Turbe et al., 2010). Soil microorganisms are involved in many processes like: soil structure formation, decomposition of organic matter and the cycling of carbon, nitrogen, phosphorus, and sulphur (Van Elsas et al., 1997). In addition, microorganisms play key roles in promoting plant growth and in changes in vegetation (Doran et al., 1996).

RESULTS AND SUGGESTIONS

The results showed that the fresh and dry biomass of alfalfa increased in all variants with earthworms **(**Table 1). The highest values of biomass were registered in variants with anecic earthworms, mixed inoculation with the three ecological groups of earthworms and mixed inoculation with earthworms + *Rhizobium melliloti* 116 (treatment 3, 5 and 6). The inoculation with epigeic earthworms had slightly positive effect on the fresh and dry biomass of alfalfa. The single inoculation with *Rhizobium melliloti* 116 (treatment 7) had no significant effect on alfalfa biomass at the first blooming stage.

Table 1. Shoot biomass of alfalfa at the first cutting. Means with the same letter are not significantly different at p=0.05. LSD – Least Significant Difference

Treatment	Fresh biomass (g)	Dry biomass (g)
1. control	48.83 a	12.16 a
2. endogeic earthworms	55.86 bc	13.6 bc
3. anecic earthworms	57.6 cd	14.6 cd
4.epigeic earthworms	51.66 ab	12.43 ab
5. mixed inoculation with earthworms	59.00 c	15.3 d
6.mixed inoculation with earthworms + Rhizobium melliloti 116	61.66	15.5 d
7.Rhizobium meliloti 116	47.6 a	12.23 a
LSD $_{p=0.05}$	5.26	1.19

At the end of the experiment the fresh and dry biomass of alfalfa were nearly twice higher (Table 2). The results revealed positive effect of mixed inoculation with earthworms and nitrogen-fixing bacteria. The highest values of biomass were registered in variants with endogeic earthworms, anecic earthworms and mixed inoculation with the three ecological groups of earthworms + *Rhizobium mellilot*i 116 (treatments 2, 5 and 6). At this stage of the experiment *Rhizobium mellilot*i 116 showed positive effect on the alfalfa fresh and dry biomass. The inoculation with epigeic earthworms also increased the yield of alfalfa.

Table 2. Shoot biomass of alfalfa at the second cutting. Means with the same letter are not significantly different at p=0.05. LSD – Least Significant Difference

Treatment	**Fresh biomass (g)**	**Dry biomass (g)**
1. control	87.07 a	17.93 a
2. endogeic earthworms	102.5 d	22.83 e
3. anecic earthworms	103.96 cd	22.4 de
4.epigeic earthworms	93.1 ab	21.1 cd
5. mixed inoculation with earthworms	93.9 abc	19.46 ab
6.mixed inoculation with earthworms + Rhizobium melliloti 116	102.03 bcd	20.8 bcd
7.Rhizobium meliloti 116	93 ab	20.3 bc
LSD $_{p=0.05}$	10.82	1.63

The fresh and dry roots biomass of the alfalfa at the end of the experiment is shown on Figure 1. No significant differences between the variants were found except in treatment 3. Anecic earthworms (variant 3) have slightly positive effect on the fresh root biomass of alfalfa. They create deep permanent vertical burrows in the soil, which improve the root growth.

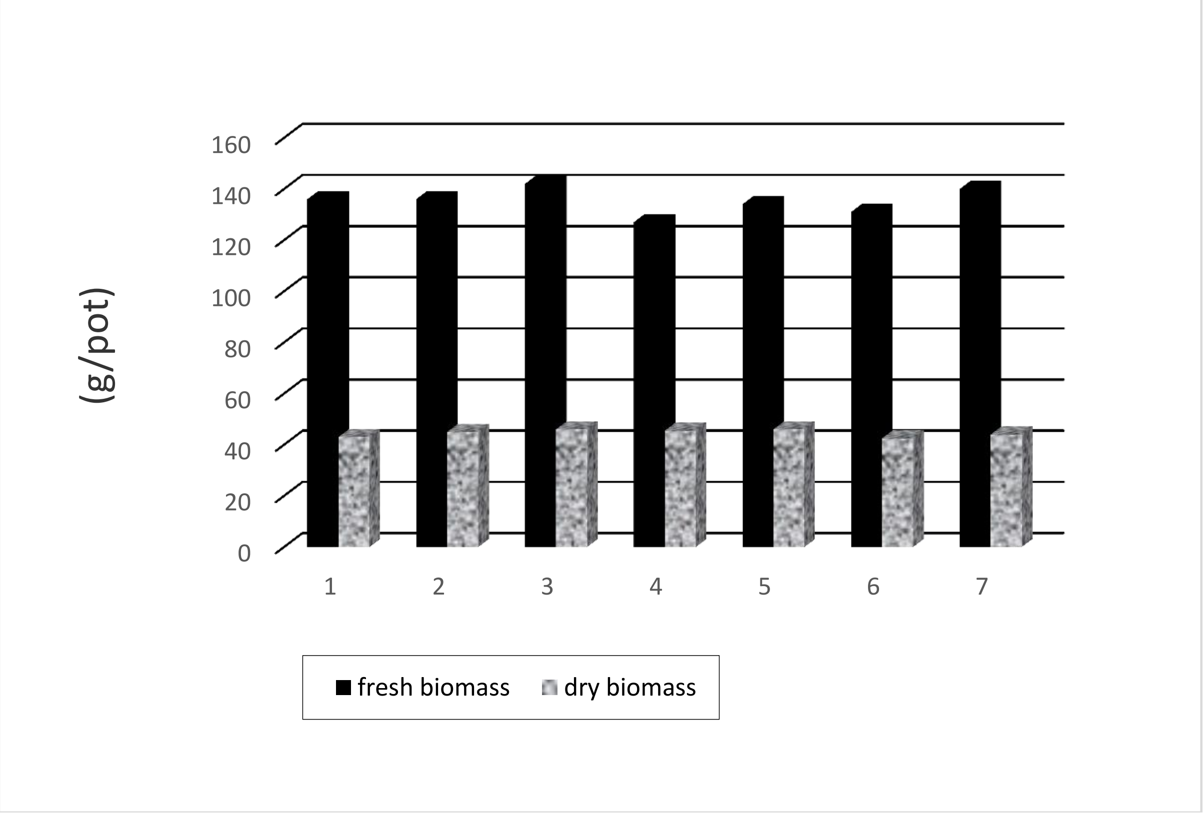

Figure 1. Root biomass of alfalfa. Legend: 1. control, 2. endogeic earthworms; 3. anecic earthworms; 4.epigeic earthworms; 5. mixed inoculation with earthworms; 6.mixed inoculation with earthworms + Rhizobium melliloti 116; 7. Rhizobium meliloti 116

As it was expected, the inoculations with earthworms had positive effect on the soil aeration in all treatments. This was expressed in bulk density decreasing and respectively in total porosity increasing in depth (Table 3). The relative decrease of soil bulk density at 10-20 cm depth was the highest (16% and 18%, respectively) in anecic earthworms and earthworms + *Rhizobium melliloti* 116 variants. The treatments with anecic earthworms (variant 3, 5, and 6) had slightly more compacted surface 0-5 cm soil layer and, respectively, the total porosity was 2-3% less than in the control (Table 3).

Table 3. Bulk density and soil porosity at the end of the experiment

Treatment	**Depth, (cm)**	**Soil moisture (%)**	**Bulk density ($g.cm^{-3}$)**	**Total porosity (%v/v)**
1. control	0-5	15.4	1.12	57.8
	10-20	17.4	1.20	54.8
2. endogeic earthworms	0-5	14.8±0.2	1.15 ±0.03	56.7±1.3
	10-20	14.1	1.06	59.9
3. anecic earthworm	0-5	17.8±2.0	1.17 ±0.03	55.8±1.2
	10-20	13.7	1.00	62.2
4. epigeic earthworms	0-5	13.9±1.2	1.14 ±0.02	56.9±0.7
	10-20	12.8	1.08	59.3
5. mixed inoculation with earthworms	0-5	17.2±0.5	1.19 ±0.02	54.9±0.8
	10-20	13.9	1.10	58.4
6. mixed inoculation with earthworms + Rhizobium melliloti 116	0-5	17.1±1.4	1.18 ±0.05	55.4±1.9
	10-20	15.6	0.99	62.8
7. Rhizobium melliloti 116	0-5	13.5±0.7	1.10 ±0.02	58.4±0.7
	10-20	12.4	1.10	58.6

The experiment set up explained the homogeneity of water retention properties in depth in the control variant. (Table 4) The endogeic earthworms (variant 2), mixed inoculation with earthworms + *Rhizobium melliloti* 116 (variant 6), and inoculation with *Rhizobium melliloti* (variant 7) did not increase the water retention at suctions less than 33 kPa. The treatments with anecic earthworms increased the water content hold at -33 kPa in the surface 0-5 cm by near 3%w/w. Slight increase (by 1%w/w) was observed also in the treatments with epigeic earthworms (variant 4) and mixed inoculation with earthworms (variant 5) (Table 4). The increased water retention capacity can be explained with initiation of microaggregate formation within worm casts in the surface layer.

Table 4. Soil water retention (W, %w/w) at different potential (kPa) and depth of the soil.

treatment	Sampling depth ,cm	- 1 kPa	- 5 kPa	-10 kPa	-33 kPa
1. control	0-5	26.0	23.0	21.5	21.4
	10-20	25.7	22.1	20.8	20.1
2. endogeic earthworms	0-5	26.9±1.2	22.9±0.6	21.4±0.6	20.9±0.4
	10-20	26.4	22.9	21.2	20.3
3. anecic earthworms	0-5	29.5±2.9	26.0±2.9	24.3±2.3	24.0±2.2
	10-20	27.6	23.5	21.6	20.4
4. epigeic earthworms	0-5	28.3±1.7	24.5±1.8	22.9±1.8	22.3±1.8
	10-20	25.0	22.2	20.9	20.7
5. mixed inoculation with earthworms	0-5	28.6±1.2	24.3±1.1	22.7±0.8	22.2±0.7
	10-20	27.0	23.1	21.2	20.7
6. mixed inoculation with earthworms + Rhizobium melliloti 116	0-5	25.5±1.7	22.0±1.2	20.5±1.1	19.8±1.2
	10-20	26.5	22.4	20.7	20.3
7. Rhizobium melliloti 116	0-5	27.4±0.1	23.2±0.2	21.4±0.4	20.7±0.2
	10-20	26.4	21.8	19.6	16.5

The amount of air-filled soil pores increased in 10-20 cm soil depth in all studied variants of inoculations (Table 5). It can be concluded that the increase of total porosity in depth was on the account of the increasing of large macropores (Table 5). The increase of soil aeration capacity (volume of pores with diameter greater than 60 μm) in depth is highest (10%vol. and 12%vol.) at the variants with inoculation of anecic earthworms and mixed inoculation with earthwormss (Figure 2). The improvement of soil aeration status allowed the roots of alfalfa to reach more nutrients and water in the soil which resulted in the highest biomass formation (Tables 1 and 2) found in this experiment.

Table 5. Size (effective diameter in μm) distribution of air-filled soil pores (%vol.)

treatment	Sampling depth, cm	>300 μm	>60 μm	>30 μm	>10 μm
1. control	0-5	28.7	32.1	33.7	33.9
	10-20	23.9	28.2	29.8	30.7
2. endogeic earthworms	0-5	25.8±3.6	30.4±2.7	32.2±2.7	32.7±2.4
	10-20	31.8	35.6	37.4	38.3
3. anecic earthworm	0-5	21.3±1.3	25.3±1.3	27.3±0.7	27.7±0.7
	10-20	34.5	38.6	40.5	41.7
4. epigeic earthworms	0-5	24.6±0.6	29.0±0.9	30.9±0.9	31.4±0.9
	10-20	32.3	35.4	36.8	37.0
5. mixed inoculation with earthworms	0-5	20.8±2.8	25.9±2.7	27.8±2.3	28.4±2.1
	10-20	28.5	32.9	35.0	35.6
6. mixed inoculation with earthworms + Rhizobium melliloti 116	0-5	25.3±5.2	29.4±4.5	31.1±4.2	31.9±4.4
	10-20	36.6	40.6	42.3	42.8
7. Rhizobium melliloti 116	0-5	28.2±1.2	32.8±0.9	34.8±0.7	35.6±0.8
	10-20	29.7	34.8	37.2	40.5

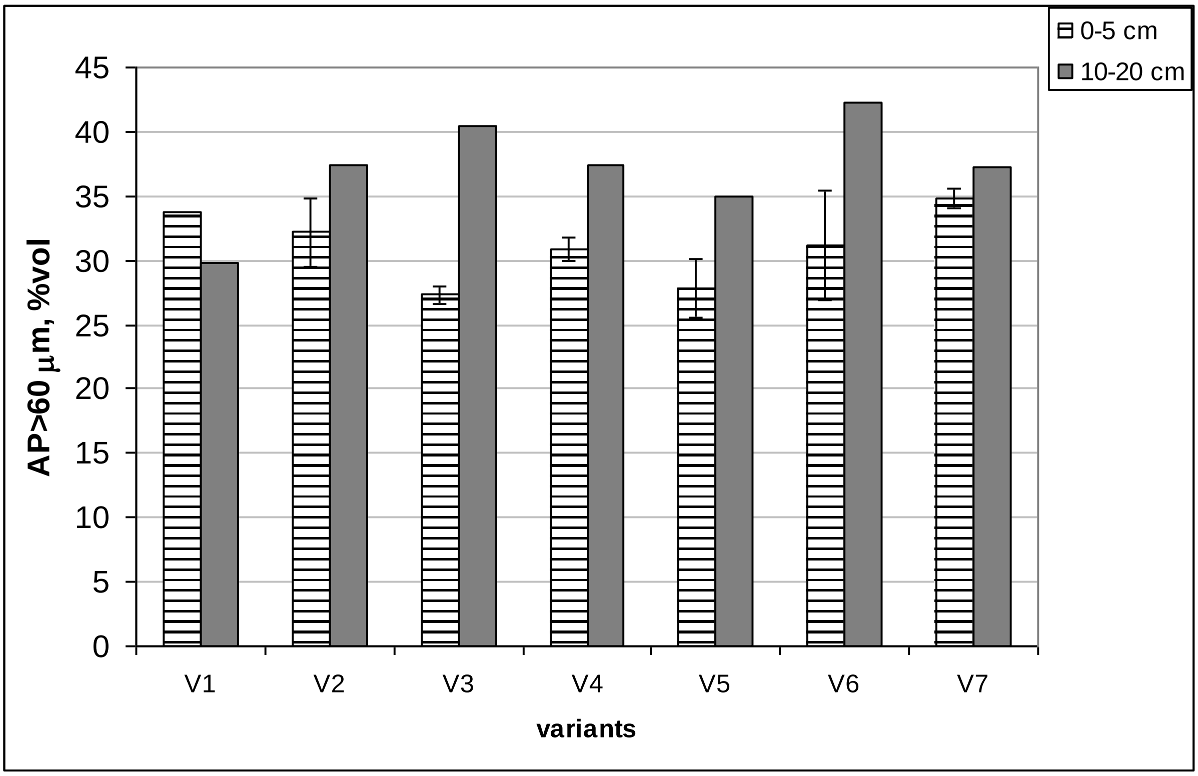

Figure 2. Soil aeration capacity (volume of soil pores>60 μm).

In conclusion, the inoculation of soil with earthworms and nitrogen-fixing bacteria in conditions of a pot experiment with alfalfa increased the crop productivity and improved the soil aeration status. The treatments had positive effect on soil aeration capacity in depth. Results obtained suggested that earthworm inoculations could be successfully used in practices to improve soil structure and productivity of alfalfa.

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