Biotechnology in Animal Husbandry 21 (5-6), p 175-179, 2005 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 633.31

THE NUMBER OF MICROORGANISMS IN THE SOIL UNDER DIFFERENT GRASS-LEGUMINOUS MIXTURES[1]

L. Mandić, D. Djukić, V. Stevović[2]

Abstract: The total number of microorganisms in the soil under grass-leguminous mixtures fertilized with different mineral nitrogen rates with or without the use of liming was investigated in the paper. The trial was set up on the humus-siliceous soil on Mt. Kopaonik during 2001. Microbiological analyses comprising the total number of microorganisms, ammonifiers and soil fungi were made towards the end of 2003.

The highest total number of microorganisms and number of ammonifiers were registered in the soil under the grass-leguminous mixture consisting of red clover, orchard grass, red fescue and timothy grass and the lowest one in the variant including the grasses mentioned with white clover. Higher nitrogen rates (100 kg ha^{-1}) had a depressive effect on the soil biological parameters studied, particularly in the variants without liming.

Key words: soil, grass-leguminous mixtures, microorganisms

Introduction

Sowing of grasses with leguminous plants is a precondition for producing good-quality forage. Accordingly, either with or without the application of mineral nitrogen, a higher biomass production with a better protein, carbohydrate and mineral composition may be obtained (*Stosić and Lazarević*, 1997). However, in order to maintain and improve the homeostatic soil capacity and total biogeocenosis composition, proper percentage shares of all the grass-leguminous mixture components and mineral fertilizers need to be determined.

Being in the biogeochemical sense the most active part of biogeocenosis with a very pronounced ability of hydrolysis, synthesis as well as accumulation and immobilization of different pollutants, microorganisms are essential indicators of soil biological productivity, environmental conditions and plant production quality (*Milosević et al*., 2004). According to the data by *Jose et al*. (2001), the mineralization ability and therefore the number of soil microorganisms is lower under individual grass and legume crops and far higher in the conditions of their joint growing. If inadequately used, nitrogen mineral fertilizers and some other anthropogenic activities may bring about lower soil biological productivity (quantitative and qualitative microorganism composition, plant yield), a lower quality of the products obtained as well as an array of environmental and health problems (*Djukić and Mandić*, 1997).

Liming and the application of organic and microbiological fertilizers, i.e. chemicals are precluding an adverse impact of increased mineral fertilizer rates and other potential xenobiotics (*Gostkowska et al*. 1998). This is particularly related to acid soils, which may be a limiting factor of the efficiency in growing forage plants.

Therefore, the paper was aimed at monitoring the number and the activity of the soil microorganisms under the conditions of applying mineral fertilizers and liming, which would pave the way for choosing the most suitable combinations of grass-legume mixtures in the particular growing conditions.

Material and Methods

The studies proceeded on the humus-siliceous soil of Mt. Kopaonik. The three-factorial experiment, following the randomized split block design with four replications, was set up in 2001.

1 Original scientific paper – Originalni naučni rad

2 dr Leka Mandić- Assistant Professor, dr Dragutin Djukić – Tenure professor, dr Vladeta Stevović – Assistant Professor, Faculty of Agronomy Čačak

Soil samples for microbiological analysis were taken in 2003 from the arable soil layer (35 cm deep). The experimental variants were as follows:

A – Mixtures: Red clover + GM (orchard grass, red fescue and timothy grass);
Bird's foot trefoil + GM (orchard grass, red fescue and timothy grass);
White clover + GM (orchard grass, red fescue and timothy grass);
Alsike clover + GM (orchard grass, red fescue and timothy grass).

B – Fertilization: $N_{60}P_{60}K_{60}$ (N_1);
$N_{100}P_{60}K_{60}$ (N_2).

C – Liming: no liming (-Ca);
liming (+Ca) – slaked lime at the rate of 2000 kg ha^{-1}.

The biological activity of the soil was determined through the total number of microorganisms (*Pochon and Tardieux*, 1962), number of ammonifiers (MPA) soil fungi (Chapek's agar – cit. *Govedarica and Jarak*, 1993).

The data obtained were processed using the method of the variance analysis of the three-factorial experiment with the significance testing of the differences performed using the LSD test.

Results and Discussion

Statistical analysis of the data on the effect of different nitrogen rates and soil liming used under the grass-leguminous mixtures pointed at a significant impact of the factors under consideration on the soil microbiological activity studied.

As a general indicator of the soil biogenity, the total number of bacteria was the highest in the soil treated with the mixture of red clover, orchard grass, red fescue and timothy grass (tab.1). Somewhat lower number of the bacteria was recorded with Alsike clover and the grass mixture, and the lowest one with white clover + grass mixture. Similar results were obtained by other authors growing red clover and various grasses on the acid-reaction soils (*Gil et. al*., 2001), i.e. red clover and other legumes in the variants with and without liming (*Jarak et al*., 2004).

Table 1.
The total number of microorganisms in the soil under grass-leguminous mixtures ($10^6 g^{-1}$ absolutely dry soil)

A		Red clover + GM		Birds foot trefoil + GM		White clover + GM		Alsike clover + GM		$\overline{X}$
B		**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**	
C	**+Ca**	34	29	24	18	20	17	27	20	**23.63**
	-Ca	21	18	22	13	23	11	24	19	**18.87**
$\overline{X}$		**25.5**		**19.25**		**17.75**		**22.50**		
$\overline{X}$	N_1	**24.37**								
	N_2	**18.13**								

LSD

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	2.36	1.67	1.67	3.36	3.36	2.36	4.75
0.01	3.19	2.25	2.25	4.54	4.54	3.19	6.35

GM – grass mixture

Higher nitrogen rates gave rise to a pronounced decrease in the number of microorganisms, particularly in the variants without liming. According to *Jemcev and Djukic* (2000), adverse effects of high nitrogen rates could be attributed to the changes in the soil microorganism complex structure, that is, to the activation of toxigenic microorganisms and to an onset of the soil microbe toxicosis. According to *Gostkowska et al.* (1998), a decrease in the depressive effect of high nitrogen rates, in the variants with liming is attributed to an improvement of agrochemical soil properties, particularly as regards a reduction in the

content of mobile aluminium and manganese forms that in acid soils may have a toxic effect on soil microorganisms.

Statistical analysis of the number of ammonifying microorganisms points at an absence of significant differences between the variants of red and Alsike clovers grown jointly in mixtures with the grasses mentioned (tab. 2). Their number in the other two variants is much lower. The most frequently cited explanations regarding the difference in the number and mineralization ability of the soil under legumes and grass-leguminous mixtures refer to the nature of the residues – low C:N, lignin:N, (lignin + polyphenol):N ratios etc. (Fox et al., 1990). Compared with the rest of the biological parameters studied, the ammonifiers exhibited the highest sensitivity to the higher nitrogen rates (*Mandic et al*., 2001). The same data presumed that the initially used lower nitrogen rates for fertilizing legumes might favour the number and activity of soil ammonifiers. However, soon after, at the rate of 80 kg ha^{-1}, there occurred a pronounced sensitivity both in terms of the number of these microorganisms and of the activity of the proteolytic enzymes responsible for the transformation of organic nitrogen compounds in the soil.

Table 2. The number of ammonifiers in the soil under grass-leguminous mixtures, (10^5 g^{-1} absolutely dry soil)

A		Red clover + GM		Birds foot trefoil + GM		White clover + GM		Alsike clover + GM		$\overline{X}$
B		**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**	
C	**+Ca**	81	77	75	62	71	60	85	70	**72.63**
	-Ca	69	63	70	60	70	56	69	58	**64.37**
$\overline{X}$		**72.50**		**66.75**		**64.25**		**70.50**		
$\overline{X}$	N_1	**73.75**								
	N_2	**63.25**								

LSD

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	3.85	2.73	2.73	5.45	5.45	3.85	7.71
0.01	5.19	3.68	3.68	7.34	7.34	5.19	10.39

GM – grass mixture

As opposed to the biological indicators previously mentioned, the number of soil fungi was of rather stable character in the variants with the clovers and grass mixtures studied, but it was far lower on the variant Bird's foot trefoil + grass mixture (tab. 3). The decrease in the soil fungi number may be associated with the HCN production in younger development stages of bird's foot trefoil, but investigations by most authors suggested that the concentration of the compound in the rhizosphere of different species of the genus *Lotus* was found to be insignificant (*Gerbrehiwot and Beuselinck*, 2001). Increased synthesis of certain chitinase isoforms in the root of bird's foot trefoil is positively correlated with the expressed antifungal effect and its resistance to many fungal diseases (*Heather et al*., 2003).

Table 3. The number of fungi in the soil under grass-leguminous mixtures, (10^5 g^{-1} absolutely dry soil)

A		Red clover + GM		Birds foot trefoil + GM		White clover + GM		Alsike clover/ + GM		$\overline{X}$
B		**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**	
C	**+Ca**	15	24	3	7	16	22	17	18	**15.25**
	-Ca	16	25	3	4	18	23	19	20	**16.00**
$\overline{X}$		**20.00**		**4.25**		**19.75**		**18.50**		
$\overline{X}$	N_1	**13.37**								
	N_2	**17.87**								

LSD

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	2.46	1.75	1.75	3.51	3.51	2.46	4.98
0.01	3.35	2.36	2.36	4.73	4.73	3.35	6.71

GM – grass mixture

The use of liming, on the whole, did not have a significant effect on the change of the number of this group of microorganisms. Since the soil fungi are a group of microorganisms with a highly developed enzymic system (*Sirskaja et al*. 1989), the stimulative effect of higher nitrogen rates, in all the variants investigated, was completely expected.

Conclusions

Based upon the results obtained the following conclusions may be drawn:

- the number of the soil microorganisms investigated depended on the type of grass-leguminous mixture, nitrogen rate and on liming used;
- the highest total number of microorganisms and the number of ammonifiers were registered in the soil under the grass-leguminous mixture comprised of red clover, orchard grass, red fescue and timothy grass, and the lowest one in the variant with the grasses mentioned and white clover;
- the lowest number of soil fungi was recorded in the soil under bird's foot trefoil and the grass mixture mentioned, and their number in the other variants was quite similar;
- higher nitrogen rates (100 kg ha^{-1}) displayed a depressive impact on the soil biological parameters, particularly in the variants without liming;
- the findings on the biological indicators of the soil for the agro-ecological region concerned denoted that the most optimal grass-legume mixture was that of red clover + orchard grass + red fescue + timothy grass with previously used liming and fertilizing with 60 kg N ha^{-1}.

BROJNOST ZEMLJIŠNIH MIKROORGANIZAMA POD RAZLIČITIM TRAVNO-LEGUMINOZNIM SMEŠAMA

L. Mandić, D. Đukić, V. Stevović

Rezime

U radu je proučavana brojnost mikroorganizama u zemljišta pod travno-leguminoznim smešama, đubrenim različitim dozama mineralnog azota, uz primenu kreča ili bez njega. Ogled je postavljen na humusno silikatnom zemljištu na Kopaoniku tokom 2001. godine. Mikrobiološke analize, koje su obuhvatile određivanje ukupne brojnosti mikroorganizama, brojnosti amonifikatora i zemljišnih gljiva rađene su krajem 2003. godine.

Najveća ukupna brojnost mikroorganizama, i brojnost amonifikatora konstatovana je u zemljištu pod travno-leguminoznom smešom koju su činili crvena detelina, ježevica, crveni vijuk i mačji rep, a najmanja na varijanti pomenutih trava sa belom detelinom. Veće doze azota (100 kg ha^{-1}) su depresivno delovale na proučavane biološke parametre zemljišta, naročito na varijantama bez kalcijizacije;

Ključne reči: zemljište, travno-leguminozne smeše, mikroorganizmi.

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