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EFFECT OF NITROGEN FERTILIZATION ON MICROBIAL PROPERTIES OF MEADOW SOIL

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ABSTRACT

The aim of this study was to determine the numerical depot of some micro-organisms colonizing grassland soil under the influence of varying nitrogen fertilization. The research has begun in 1993 on the gley soil created from strong clay and implemented the following combinations of fertilizer: "0", PK, PK + 55 kg N, PK + 110 kg N, PK + 220 kg N. Soil samples for quantitative microbiological tests were taken during the growing season 2006 i.e.: spring (III decade of April), and after each regrowth harvest (III decade of May, and I decade of July and II decade of September). Samples were taken from a depth of 0-15 cm, from all combinations in an amount of about 0.5 kg. Then, after shaking 10g of soil samples were diluted in the range from 10^{-1} to 10^{-8}. In order to know the number of selected microbial groups, inoculation was performed by standard methods. The most favorable for the development of the tested soil microorganisms was the sustainable grassland fertilization with phosphorus and potassium, and with a nitrogen dose of 110 kg N•ha^{-1}. The mineral fertilization of the permanent grassland reduced the number of bacteria and mould, and stimulated the growth of yeasts, which is a negative phenomenon for biological processes occurring in meadow soil.

Key words: nitrogen fertilization, number of bacteria, mould, yeast.

INTRODUCTION

In nature, soil and water are the main habitat of living organisms that occupy about 25% of the total biomass of the earth (Myrold and Posavatz, 2007). Researches of Smyk (1992) and Barabasz et al. (2002) about biological life of soil, clearly indicate that microorganisms are the primary determinant of soil fertility. In grassland, as a result of its specific nature (durability, biodiversity) each year leaves a considerable amount of plant mass, which after the death is a rich source of nutrients for such organisms. Research on sustainability, biodiversity and productivity of grassland ecosystems (Barabasz et al., 1999) indicate a big influence of the applied treatments on the biological life of soil. Cooperation of microorganisms and higher plants leads to the emergence of some kind of equilibrium in biocenotical systems of soil environment. The state developed in this way often enables the use of chemicals in a further intensification of agriculture (Doran et al., 1996).

Researches conducted on the microbiological activity of soil are quite numerous and multifaceted. They clearly underline, but do not explain the effect of chemicals irreplaceable in the plant production, such as chemical fertilizers, on the properties in question (Harrison et al., 2007; Wielgosz et al., 2004; Števliková et al., 2003; Wang et al., 2009). The aim of this study was to determine the numerical depot of some micro-organisms colonizing grassland soil under the influence of various levels of nitrogen fertilization.

MATERIAL AND METHODS

Samples of soil were collected from the plots located in the Agricultural Experimental Station in AP Siedlce. The plots with surface 15 m^2 (3 x 5) are objects of static fertilizer experiment conducted at a sustainable mineral meadow. The research began in 1993 on the gley soil created from strong clay and implemented the following fertilizer combinations:

1. "0";
2. PK;
3. PK + 55 kilograms N;
4. PK + 110 kilograms N;
5. PK + 220 kilograms N.

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Phosphorus and potassium fertilization were uniform: 80 kg P_2O_5•ha^{-1} applied once in spring in the form of triple superphosphate, 120 kg K_2O•ha^{-1} in three equal doses for 40 kg at each regrowth in the form of potassium salt. Nitrogen fertilization (i.e. 55, 110 and 220 kg N•ha^{-1}) were divided into three equal doses and applied for each regrowth in the form of ammonium nitrate.

Soil samples for quantitative microbiological tests were taken during the growing season 2006 i.e: spring (III decade of April), and after each regrowth harvest (III decade of May, and I decade of July and II decade of September). Samples were taken from a depth of 0-15 cm, from all combinations in an amount of about 0.5 kg. Then, after shaking 10g of soil samples were diluted in the range from 10^{-1} to 10^{-8}. In order to know the number of selected microbial groups, inoculation was performed by standard methods using the medium:

- Nutrient agar for total number of bacteria by incubation 2-3 days at temperature 37°C;
- Saburouda agar for yeast by incubation 5-7 days at 28°C;
- Fungiphil agar for moulds by incubation 5-7 days at 28°C.

The study was performed in the Microbiology Department. The obtained results were analyzed statistically by analysis of variance and the significance established at ≤ 0.05 level.

RESULTS AND DISCUSSIONS

The summary results from all of the vegetation period suggest the existence of changes in the number of designated microorganisms under the influence of the applied fertilizer (Figure 1). Generally it can be concluded that, in the analyzed material, the largest group of soil microorganisms are bacteria and moulds. Taking into account the impact of a combination of applied fertilizer on the abundance of different groups of microorganisms, results showed that fertilization with phosphorus and potassium only engender the most favorable conditions for the development of mold. The introduction to this set of 110 kg N•ha^{-1} positively influenced bacterial development, and the use of the highest doses of nitrogen i.e. 220 kg•ha^{-1} increased the abundance of yeasts.

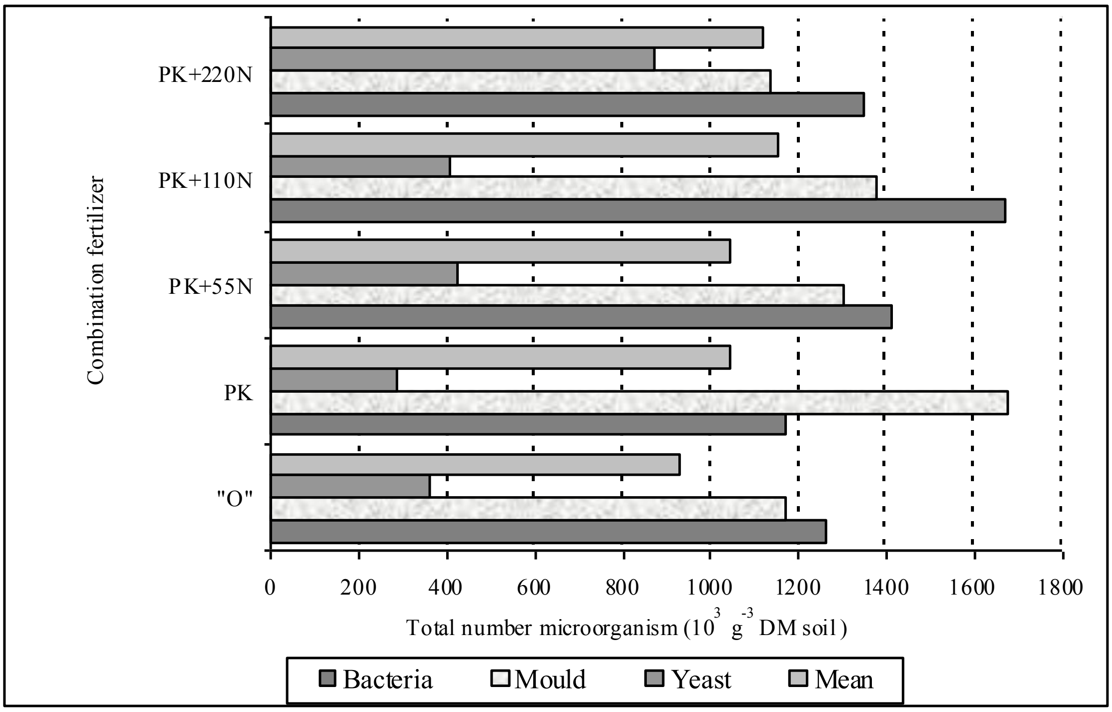

Figure 1. Some groups of microorganisms in soil (annual sum), depending on the applied fertilization

Taken into consideration the average numbers of designated microorganisms, results demonstrate that nitrogen fertilization positively affected the life of the analyzed meadow soil, but only to a level of 110 kg N•ha^{-1}, while increasing the dose of nitrogen to 220 kg•ha^{-1} is less recommended.

Similar conclusions on the basis of their research have made Smyk (1992), Myśków et al. (1996) and Barabasz et al. (1999). They found that nitrogen fertilization in a dose not exceeding 120 kg•ha^{-1} enhances the biological activity of soil, measured by the degree of microorganisms development, as well as by the direction and intensity of the respective processes. They argue that over fertilizing the soil with nitrogen promotes the formation of the so-called nitrosamines, substances that are highly toxic and adversely affecting the soil microorganisms.

A more detailed analysis of the obtained results presented in table 1, 2 and 3 also confirms what is known from the literature (Barabasz et al., 1999; Inselsbacher et al., 2010; Loiseau et al., 1994; Marschner et al., 2003; Song et al., 2007; Števliková et al., 2003), that mineral fertilization significantly differentiates the biology of the soil throughout the growing period. The numbers of bacteria (Table 1) underwent significant changes under the influence of nitrogen rate and of the sampling date of the soil material for microbiological analysis. The largest number of bacteria (600•10^{3}•g^{-1}D.M.) was found in the soil material collected after first harvest from the plots where phosphorus and potassium fertilization, and a dose of 110 kg N•ha^{-1} was applied. The numbers of bacteria in the control plots, where in the soil material was collected in spring showed the lowest number of 160•10^{3}•g^{-1}D.M.

Our results testify about the need of systematically supplementing nutrients in the soil especially on grassland, which are intensively utilized.

In addition to the bacteria, other organisms also live in the soil. According to Kumicki-Goldfinger (1994) and Horn (1997), the most important role among them is played by fungi.

According to Horn (1997) and Rezacova et al. (2007), fungi are a group of eukaryotic organisms, and thus are at a higher level of development than bacteria and actinomycetes. Among the fungi two groups of organisms are noteworthy. There are moulds and yeasts, which significantly differ functionally, but are in very close phylogenetic relationship. Moulds play an important role in the soil biology.

Table 1. Total number of bacteria (10^{3}.g^{-1}D.M. of soil) depending on the applied fertilization

Fertilization	The date of taking samples								Mean for fertilization
	Spring		After I cut		After II cut		After III cut		
0	160c	C	500a	B	240c	BC	360b	AB	315 B
PK	280ab	B	350a	C	260b	C	280ab	B	293 B
PK+55 kg N	360b	A	450a	B	200c	C	400ab	AB	353 AB
PK+110 kg N	360bc	A	600a	A	310c	AB	400b	AB	418 A
PK+220 kg N	200b	C	250b	D	400ab	A	500a	A	337 B
Mean for date	272b		430a		282b		388a		

Means in lines followed by the same letters create homogenous groups.
Means marked with the same capital letters in columns create homogenous groups.

The data in table 2 show that in the control plots (without fertilization), in samples taken in the spring, inhibited growth of mould (197•103 •g^{-1}D.M.) can be observed. This can be explained by the fact that the moulds are outstanding oxygeners (Gołębiewska, 1986) and on the unfertilized grassland turf is loosened (Smyk, 1992) and as a result of spring water retention, soil is less oxygenated.

Significant increase in the number of moulds in samples taken in spring was influenced by fertilization only with phosphorus and potassium (387•10^{3}•g^{-1}D.M.).

Intensive development of moulds in these plots may be the result of the impact of leguminous plants, which showed a larger percentage under this type of fertilization (Wielgosz et al., 2006). Moulds as saprophytes take an active part in the decomposition of dead plant material and make it available again for living organisms in the form of simple chemicals. The best foods for moulds are carbohydrates and ammonium salts, but most of them also contain the enzymes which dissolve the protein. By contrast, the products of mold metabolism are organic acids (Myśków et al. 1996). This is also confirmed by significantly higher average number of moulds in these plots for all over the study period (420•103•g^{-1}D.M.).

Wielgosz et al. (2004 and 2006) also reported about the stimulating influence of leguminous plants on the development of filamentous fungi, in comparison with the fallow soil.

Table 2. Total number of mould (10^3. g^{-1}D.M. of soil) depending on the applied fertilization

Fertilization	The date of taking samples								Mean for fertilization
	Spring		After I cut		After II cut		After III cut		
0	197d	C	247c	B	340b	A	387a	B	292BC
PK	387b	A	298c	A	417b	A	577a	A	420A
PK+55 kg N	287b	B	253b	B	383a	A	377a	B	325B
PK+110 kg N	297c	AB	197c	C	383b	A	500a	A	314B
PK+220 kg N	280b	B	247b	B	400a	A	207b	C	283C
Mean for date	290bc		248c		385a		410a		

Means in lines followed by the same letters create homogenous groups.
Means marked with the same capital letters in columns create homogenous groups.

Among the fungi, the second group of organisms includes the yeast. According to Gołębiewska (1986) in soil there is much less yeast than bacteria and moulds, because they dominate on fruits and other environments rich in sugars (Table 3).

Table 3. Total number of yeast (10^3.g^{-1}DM of soil) depending on the applied fertilization

Fertilization	The date of taking samples								Mean for fertilization
	Spring		After I cut		After II cut		After III cut		
0	50a	A	100ab	A	112b	B	97ab	A	90A
PK	23a	A	190b	B	43a	A	27a	A	70A
PK+55 kg N	33a	A	297b	C	57a	AB	33a	A	105A
PK+110 kg N	57a	A	283b	C	30a	A	33a	A	101A
PK+220 kg N	50a	A	193b	B	277c	C	353d	B	218B
Mean for date	43a		213a		104a		109a		

Means in lines followed by the same letters create homogenous groups.
Means marked with the same capital letters in columns create homogenous groups.

The lowest abundance of these microorganisms (average 70 10^3•g^{-1}D.M.) was found on plots fertilized only with phosphorus and potassium, but the introduction of nitrogen positively affected the development of these organisms.

Their greatest abundance (average 218 103•g^{-1}D.M.) was recorded in soil material taken from the plots where the highest dose of nitrogen (220 kg•ha^{-1}) was applied. Indeed, nitrogen fertilization acidified the soil environment and, from studies of Wielgosz et al. (2006), results that it favors the development of these microorganisms. In the soil there is much less yeast than bacteria and molds. From a physiological point of view, yeasts, as well as moulds, are also heterotrophic organisms. But mainly they feed with simple sugars and do not have the capacity to decompose complex polysaccharides. In anaerobic condition they lead to alcoholic fermentation (Gołębiewska, 1986).

On the other hand, Jodełka et al. (2008), assessing the numbers of microorganisms in the grassland soil with foliar as well as with soil fertilization, proved that with the higher level of nitrogen fertilization applied to soil, the amount of yeast in the soil increased with the successive dates of sward harvesting.

Regarding the average value of these organisms in different periods, the largest number of bacteria and yeast was found in soil material collected after first harvest, but mould was dominant in the material collected from the soil after the harvest of III cut. Evolution of microbial biomass in soil under the influence of applied agronomic treatments (fertilization) provides information about current and potential state of biochemical activity and biological productivity of the meadow ecosystem (Figure 2).

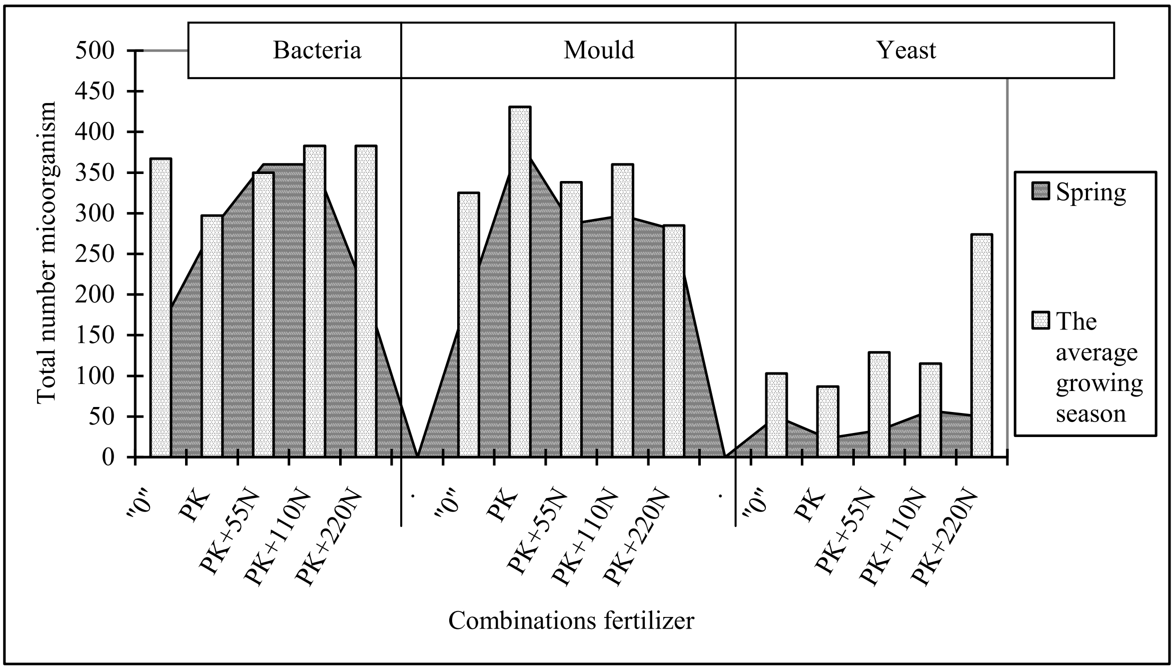

Figure 2. Comparison of total number of microorganisms (10^3.g $^{-1}$ D.M.), depending on the date of sample taking (spring and mean of three estimations during the vegetation season)

According to Doran et al. (1996) collaboration of microorganisms with higher plants leads to the emergence of some kind of equilibrium in biocenotical systems in soil environments, to which any new supply of the chemical substances interferes. Wielgosz et al. (2006), as well as Rezacova et al. (2007), affirmed that different chemical composition of root exudates of different plant species can modify soil microbial communities. These authors point out that the age and stage of plant development changes the character of secretions, which consequently has an impact on the populations of microorganisms.

CONCLUSIONS

The most favorable for the development of the tested soil microorganisms was the sustainable grassland fertilization with phosphorus and potassium, and with a nitrogen dose of 110 kg N•ha^{-1}. The mineral fertilization of the permanent grassland by the application of 220 kg N•ha^{-1} reduced the number of bacteria and moulds, and stimulated the growth of yeasts, which is a negative phenomenon for biological processes occurring in meadow soil. The largest number of bacteria and yeasts were found during the harvest of the first cut, but molds were more frequent after harvest of III cut.

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