DYNAMICS OF THE NUMBER OF MICROORGANISMS AND DEHYDROGENASE ACTIVITY IN THE RHIZOSPHERE OF MAIZE IN LONG–TERM MONOCULTURE

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Abstract: *Microbial activity plays an important role in regulating soil fertility. Microbiological processes in soil have been measured using several parameters, such as microbial biomass, respiration, and enzymatic activities. Enzyme activity is essential in both mineralisation and transformation of organic C and plant nutrients. The aim of this study was to investigate dynamics of the number of microorganisms and dehydrogenase activity in the rhizosphere of maize in long–term monoculture. The study was conducted in the multi–year stationary field experiment at the Institute of Field and Vegetable Crops, Novi Sad at Rimski Šančevi. The study treatments were: control variant - maize in monoculture without fertilizer or organic fertilizers; NPK – maize in monoculture, fertilized only with mineral fertilizers; NPK + manure – maize grown in monoculture, with application of manure and mineral fertilizers; NPK + crop residue – maize grown in monoculture, with plowing crop residues (maize) and the application of mineral fertilizers. Soil samples were taken for microbiological analyses at two dates. The number of microorganisms was determined by the dilution method on agarized selective medium. Total number of microorganisms was determined by the dilution method on agarized soil extract and number of ammonifiers on MPA medium. Nitrogen–free medium was used for determination of free N-fixing bacteria and the method of fertile drops for Azotobacter. Actinomycetes were determined on a synthetic medium and the number of fungi on Czapek-Dox medium. The number of P-mobilizing bacteria was done in glucose-asparagine agar and the number of cellulolytic microorganisms in Waksman-Carey medium. Dehydrogenase activity was determined spectrophotometrically. The highest number of azotobacter and fungi was obtained on variant NPK. The highest number of cellulolytic bacteria and actinomycetes was on variant manure + NPK and crop residue + NPK on first date of sampling. On second date of sampling we obtained higher total number of microorganisms, number of azotobacter, amonifiers and fungi. The dehydrogenase activity was higher at the second date of sampling, too, which is in correlation with increase number of most examined groups of microorganisms.*

Key words**:** *maize, microorganisms, long-term experiment, monoculture*

INTRODUCTION

Soil microorganisms have been recognized for their important role in residue decomposition, nutrient cycling and crop production. Understanding the shifts of microbial community structure and composition following long–term organic and inorganic fertilizer amendments may lead to development of better management practices for agroecosystems (DOLFING et al., 2004; POTTHOFF et al. 2006; SHEN et al., 2010).

Microbial activity plays an important role in regulating soil fertility. Indeed, the microbiological processes taking place in soil are at the centre of many ecological functions (NANNIPIERI et al., 1990) since microbiological activity is related to soil structure, soil fertility, and the transformation of soil organic matter – SOM (LADD et al., 1996). Microbiological processes in soil have been measured using several parameters, such as microbial biomass, respiration, and enzymatic activities (GARCIA et al., 1994). Enzyme activity is essential in both mineralisation and transformation of organic C and plant nutrients.

Organic and inorganic fertilizers are used primarily to increase nutrient availability to plants; however, they can affect the population, composition, and function of soil microorganisms (MARSCHNER et al., 2003). Organic fertilizers usually increase soil microbial biomass (KAUR et al., 2005; MASTO et al., 2006), CO_2 evolution (AJWA and TABATABAI, 1994), and enzyme activities (BALEZENTIENE and KLIMAS, 2009). Inorganic fertilizers had relatively less effect on soil microbial biomass and activities than organic fertilizers (PLAZA et al., 2004).

In the context of soil fertility management, long–term fertilizer experiments (LTFEs) are valuable assets for determining yield trends, changes in nutrient dynamics and balances, assessing soil quality and system sustainability. Trends in soil fertility changes in many of the short–term (BLAISE et al., 2005; JOVANOVIĆ et al., 2007) or LTFEs have been reported from samples obtained at the beginning or at the end of the cropping sequence (HATI et al., 2006). In contrast, information on the biological processes, such as soil enzymatic activities, which mediate nutrients cycling and influence their acquisition during active crop growth stages, is limited (MANDAL et al., 2007). Since microbial processes are dynamic, patterns of temporal fluctuation during crop growth are of great importance in relation to the nutrient supplying capacity of the ecosystem and the crop demands.

The aim of this study was to investigate dynamics of the number of microorganisms and dehydrogenase activity in the rhizosphere of maize in long–term monoculture.

MATERIAL AND METHODS

The study was conducted in the multi–year stationary field experiment at the Institute of Field and Vegetable Crops, Novi Sad at Rimski Šančevi. The field is located on a calcareous chernozem on loess terrace. The experimental design was a randomized, complete block design (split-plot design experiment) with four replications. The study treatments were:

- Ø – control variant (maize in monoculture without fertilizer or organic fertilizers);
- Monoculture: NPK – maize in monoculture, fertilized only with mineral fertilizers;
- Monoculture: NPK + manure – maize grown in monoculture, with application of manure and mineral fertilizers;
- Monoculture: NPK + crop residue – maize grown in monoculture, with plowing crop residues (maize) and the application of mineral fertilizers;

Application of mineral fertilizer – NPK (for variants with mineral fertilizers) was conducted in the fall, with 60 kg ha^{-1} N, P_2O_5 and K_2O (fertilizer N: P: K – 15:15:15) and in the spring, using 60 kg ha^{-1} nitrogen (urea fertilizer, 46% N). The variants of the experiment which have organic fertilizer–manure is applied every other year in the amount of 25 t ha^{-1}. The experiment was grown with maize hybrid NS 6010.

Soil samples were taken for microbiological analyses at two dates (10 July and 5 October). Total number of microorganisms was determined by the dilution method on agarized soil extract and number of ammonifiers on MPA medium (dilution 10^6) (POCHON and TARDIEUX, 1962). Nitrogen–free medium was used for determination of free N-fixing bacteria (dilution 10^6) and the method of fertile drops for *Azotobacter* (dilution 10^2) (ANDERSON, 1965). Actinomycetes were determined on a synthetic medium and the number of fungi on Czapek-Dox medium (dilution 10^4). The number of P-mobilizing bacteria was done in glucose-asparagine agar (10^4) and the number of cellulolytic microorganisms in Waksman-Carey medium (dilution 10^5). All microbiological analyses were performed in three replications and the average number of microorganisms was calculated at 1.0 g absolutely dry soil (JARAK and DJURIĆ, 2004). Dehydrogenase activity was determined spectrophotometrically by the method of THALMANN (1968) and expressed in μg TPF/1 g of soil.

RESULTS AND DISCUSSIONS

Microbial diversity in soils is influenced by different factors including anthropogenic activities. Microbial communities are known to respond to organic matter amendments with increased activity and growth, which affects soil processes, including nitrogen (N) mineralisation (STENBERG, 1999). The interactions involve root exudates, which shape the structure and enhance the activity of microbial communities, and the nutrients released by microorganisms, which affect plant growth. Root exudates comprise a wide range of substances including sugars, amino acids, siderophores and enzymes. The structure of rhizosphere microbial communities is the result of complex interactions between plant genotype and fertilization. Root exudates from maize are composed of 65% sugars, 33% organic acids and 2% amino acids and fertilization modifies the composition of root exudates, leading to increased bacterial biomass and different bacterial community structure (BAUDOIN et al., 2003).

The analysis of experimental data obtained (Table 1) showed that a significant number of microorganisms is dependent on the applied system of fertilization (A) and the sampling date (beginning and end of growing season) (B).

Table 1.

Dynamics of number of microorganisms and dehydrogenase activity in the rhizosphere of maize monoculture

Group of microorganisms	Treatments											
	Control			NPK			NPK + manure			NPK + crop residue		
	Sampling		Average	Sampling		Average	Sampling		Average	Sampling		Average
	I	II		I	II		I	II		I	II	
Azobacter	66.26**b**	88.66**ab**	77.46**B**	129.81**a**	160.14**a**	144.97**A**	66**b**	130.82**a**	98.41**B**	63.25**a**	96.56**ab**	79.91**B**
Ammonifiers	109.60	120.92	115.26	105.57	125.49	115.53	106.48	130.63	118.55	73.92	177.04	125.48
Total number of microorganisms	123.17	182.96	153.07**AB**	129.38	193.06	161.22**AB**	103.12	158.68	130.9**AB**	102.63	138.57	120.6**B**
Fungi	10.17**b**	11.41**b**	10.79**B**	14.17**b**	20.94**a**	17.56**A**	14.85**ab**	13.97**b**	14.41**AB**	10.64**b**	11.86**b**	11.25**B**
Actinomycetes	33.09**ab**	37.16**ab**	35.13	38.98**ab**	40.21**a**	39,59	21.26**ab**	20.66**ab**	20.96	28.9**ab**	11.34**a**	20.12
P–*mobilizing bacteria*	148.04**bc**	217.26**a**	182.65	126.98**c**	176.55**abc**	151.76	140.87**bc**	178.24**abc**	159.54	142.93**bc**	195.92**ab**	169.43
Cellulolytic bacteria	9.06**ab**	6.60**b**	7.83	8.27**ab**	7.77**b**	8.02	16.21**a**	10.94**ab**	13.58	9.46**ab**	5.93**b**	7.69
Cellulolytic fungi	5.99	4.80	5.40	6.50	5.40	5.95	7.13	6.06	6.60	3.61	4.75	4.18
Cellulolytic actinomycetes	26.56**a**	3.59**cb**	15.08	15.32**ab**	7.02**cb**	11.17	18.67**a**	3.03**cb**	10.85	17.24**a**	2.39**c**	9.81
Dehydrogenase activity	248	218	233	183	231	207	170	188	179	174	221	197

The highest number of azotobacter and fungi was obtained on NPK variant, on average for both date sampling. These results are opposite to results ĐORĐEVIĆ et al. (1993), which show negative effect of mineral fertilizer on number of azotobacter except in confirmation with manure and crop residues. However, in experiment ĐORĐEVIĆ et al. (1993) applied doses of fertilizer were 332 and 664 kg ha^{-1} NPK, but in our experiment they were 120 kg ha^{-1} NPK. There were no significant differences with other groups of microorganisms, on average for both date sampling. The highest number of P–mobilizing was obtained on second date of sampling on control variant and was significantly higher from all other variants. The highest number of cellulolytic bacteria was obtained on variant NPK + manure and cellulolytic actinomycetes on variant NPK + manure and NPK + crop residue (table 1). ZHONG et al. (2010) in examination the effect of mineral and organic fertilizers on microorganisms in soil concluded that the soil bacteria are more sensitive indicator of soil fertility than fungi.

In second date of sampling at the end of vegetation we obtained higher total number of azotobacters, ammonifiers and fungi, but number of actinomycetes and cellulolytic

microorganisms was higher on first date of sampling. We could explain that at the early growing season was more difficult available nutrients in the soil, and hence the greater number of actinomycetes and cellulolytic microorganisms. At the end of vegetation due to increased easy available nutrients, the total number of microorganisms in the rhizosphere increased. Dehydrogenase activity also rose slightly in the second sampling date, which is correlated with the increase of most examined groups of microorganisms. Dehydrogenase activity in the second period increases on the fertilized variants. The highest dehydrogenase activity on average for early period was in the control treatment and lowest in the variant NPK + manure application (table 1).

MANDIC et al. (2005) studied the impact of different types of fertilizers on the number of azotobacters in smonitsa seeded with maize, indicate that lower doses of nitrogen throughout the growing season stimulate azotobacters development. A similar effect was expressed by solid manure. Something rapid loss of active influence on azotobacter exhibited liquid manure. According to these authors the high doses of nitrogen repressed the number of microorganisms, especially in the early stages of vegetation and at low soil moisture period (mid-growing season). The toxic effect of high doses of nitrogen was significantly lower in rhizosphere, as well as in periods of increased humidity.

These changes in microbial metabolic diversity are associated with modifications in microbial community structure (ANDERSEN et al., 2010). This is important since the soil microbial pool will determine the rhizosphere microbial community (BERG and SMALLA, 2009). Thus, the application of organic fertilizer resulted in different microbial communities from those that develop after the application of inorganic fertilizer, although some of these effects have only been found in long–term experiments (TOLJANDER et al., 2008). Many authors have reported that organic fertilisation caused an increase in soil biological activity (MARINARI et al., 2000; MANDAL et al., 2007). The organic fertilisers supplied phosphate to the soil, giving a more balanced nutritional status than mineral fertiliser.

Soil enzyme activities respond much more quickly to the changes in soil management practices as compared to total soil organic matter (GOYAL et al., 1999). Dehydrogenase activity has been proposed as a measure of overall microbial activity (SAMUEL, 2010), since it is an intracellular enzyme related to oxidative phosphorylation processes. GARCIA et al. (1997) found that dehydrogenase activity is a good index of the soil microbial biomass in semiarid Mediterranean areas.

CONCLUSIONS

On average, for both dates of sampling, the highest number of azotobacter and fungi was obtained on variant NPK. The highest number of cellulolytic bacteria and actinomycetes was on variant manure + NPK and crop residue + NPK on first date of sampling. On second date of sampling we obtained higher total number of microorganisms, number of azotobacter, amonifiers and fungi. The dehydrogenase activity was higher at the second date of sampling, too, which is in correlation with increase number of most examined groups of microorganisms.

ACKNOWLEDGEMENTS

This study is part of the TR 031073 project financially supported by the Ministry of Education and Science of the Republic of Serbia.

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