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Research Article

The Effect of Specific Soil Microorganisms on Soil Organic Matter and Stabilization of Quality Parameters under Drought Conditions

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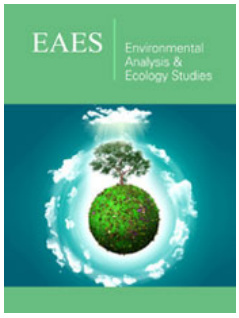

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Abstract

Soil chemical, biological and physical properties play important role in soil quality and are related with increasing OM, plant nutrient content and availability, soil microbiologcal activity. New generation of soil amendments with specific soil microorganizms are great interest worldwide. Field experiments were carried out in 2018-2019 in Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. The aim was to determine the effect of bio-products with *Trichoderma reesei*, *Acinetobacter calcoaceticus* and *Bacillus megaterium* on soil SOC, soil respiration and microbial biodiversity on loamy *Cambisol*. Under dry meteorological conditions, bio-products with *Trichoderma reesei*, *Acinetobacter calcoaceticus* and *Bacillus megaterium* caused increase in SOC content, C/N ratio, Humic/Fulvic acids ratio, increased soil respiration and microbial biodiversity. We concluded that use of mixture of three products: *Trichoderma reesei* + *Acinetobacter calcoaceticus* + *Bacillus megaterium* are the most promising bio-amendment under changing climate conditions.

Keywords: Soil organic carbon; PGPR; PGPF; AWCD

Abbreviations: SOC: Soil Organic Carbon; PGPR: Plant Growth-Promoting Rhizobacteria; PGPF: Plant Growth-Promoting Fungi; AWCD: Average Well Colour Development; R - Richness

Introduction

Soil is essentially a slow renewable resource with a high degree of degradation and a very low rate of regeneration [1]. Intensive agriculture leads to soil erosion, depletion of organic matter and other nutrients which results to permanent soil degradation and productivity losses [2]. To maintain optimal yields and less depletion of the soil, it is necessary to protect soil from losses of organic carbon and biodiversity, micronutrient imbalance, acidity, and salinity [3]. The input of exogenous organic matter, such as straw or livestock manure, can enrich soil with the necessary elements and retard SOM decomposition. Soil quality parameters can be determined by analysis of soil chemical, biological and physical properties together [2,3]. To determine soil quality indicators such as particulate organic matter, active C, total N, microbial biomass, biological activities, enzymes, soil pH, cation exchange capacity, salinity, bulk density, amino sugar, and soil aggregation should be known [2,4]. Soil microorganisms consists largely of primary decomposers that mineralize organic materials and release nutrients and energy for plants by enzyme-facilitated metabolic systems [2,4,5]. Plant growth and yield in natural environments depend on a many interaction between plant roots, bacteria and fungi [4,6]. In the sense of decomposition of organic residues microbial communities can be broken down

into fungal and bacterial groups, and research indicates that these groups functioning differently in the decomposition process [7,8].

Bacterial decomposition pathways support high turnover rates of easily available substances, fungal-dominated decomposition pathways are slower and includes complex organic materials [4,7,8]. For this day it is known that main plant-beneficial *Rhizosphere Microorganisms* are *Mycorrhiza, Rhizobia Bacteria*, PGPR such as *Pseudomonas spp., Bacillus spp.* or *Azospirillum spp.,* and PGPF such as *Trichoderma spp.* and non-pathogenic *Fusarium spp.* Strains [5,8,9]. PGPR and PGPF colonize plant roots and are responsible of plant defence not only against various foliar pathogens but also leaf-feeding insects [8,9]. The study was aimed to determine the influence of specific soil microorganisms (PGPR and PGPF individually and in a complex) on SOC, soil respiration and biodiversity on loamy *Cambisol*.

Materials and Methods

Site and soil description

The field experiments were conducted in 2018-2019 at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (55°23′50′′ N and 23°51′40′′ E). The prevailing soil is *Endocalcari-Epihypogleyic Cambisol* (CMg-n-w-can). According to composition of soil aggregates - 52-54% sand (2,0-0,05mm), 29-32% silt (0,05-0,002mm) and 14-19% clay (<0,002mm) - soil was loam. Research scheme and product applications described in Table 1. During 2018-2019 where were grown two crops: winter wheat (*Triticum aestivum L.*) cv. Ada and spring barley (*Hordeum vulgare L.*) cv. Luokė. A field experiment was set up in three replications. The gross plot size and net harvested area of each individual treatment was 30,0 and 22,0m^2, respectively.

Table 1: Research design.

Treatments	Abbreviations	Application of Bioproducts	
		After harvesting - for decomposition of organic residues	After germination BBCH12-15
Control 1	K1	-	-
Control 2	K2	ammonium nitrate	-
Trichoderma reesei	TR	*Trichoderma reesei*	-
Acinetobacter calcoaceticus	AC	-	*Acinetobacter calcoaceticus*
Bacillus megaterium	BM	-	*Bacillus megaterium*
Trichoderma reesei+Acinetobacter calcoaceticus + Bacillus megaterium	TR+AC+BM	*Trichoderma reesei*	*Acinetobacter calcoaceticus+Bacillus megaterium*

*Used doses: *Trichoderma reesei* - 100mL/ha, *Bacillus megaterium* - 100mL/ha, Acinetobacter calcoaceticus - 100mL/ha, ammonium nitrate - 20kg N/t residues.

Soil sampling and analysis

Total nitrogen (N_{tot}) and total carbon (C_{tot} for estimation of C/N ratio) were analysed by the dry combustion method using a CNS autoanalyser Vario EL III (Elementar Analy-sensystem GmbH, Germany) [10]. Closed chamber method was applied to quantify total (autotrophic + heterotrophic) soil surface net CO_2 exchange rate (NCER). CO_2 fluxes were measured five times during each crop growing season, each measuring was made between 10a.m. and 12a.m. using a portable infrared CO_2 analyser (IRGA) attached to a data logger (LcSRS-1000; ADC BioScientific Ltd, UK). In each treatment, the collar was inserted to 7.0cm soil depth; the chamber hood was placed on the collar for 5min. until results were recorded in the data logger. Measurements were made with three replications. Expression of soil surface net CO_2 exchange rate:

$$NCER=u(-\Delta c),$$

Where u is molar flow of air per square metre of soil (mol m^{-2}s^{-1}) and Δc is difference in CO_2 concentration through soil hood, dilution corrected (µmol mol^{-1}).

Humic acid fulvic acids were determined according "Agricultural Chemical Analysis, Method 5.4. Cabi Publishing, 2002", gravimetric and spectroscopic methods. Substrate utilization potential as average well colour development (AWCD) and R index of the soil microbial community were determined according to the community level physiological profiles method using Biolog EcoPlates [11].

Result and Discussion

Soil organic carbon

According to the SOC content, the soil of experimental fields could be classified as having low humus content. At the beginning of the experiment SOC ranged from 0,91 to 0,93%. During 2018 - 2019 seasons (Figure 1) average SOC content was higher in all treatments with microorganisms, compared to K1 and K2. In 2018, the highest SOC concentration was in treatment 3 (*Trichoderma reesei*). It was 9,1% higher than in K1 and 6,7% higher than in K2. In 2019 highest SOC was in treatment 5 (*Bacillus megaterium*), i.e., 7,0% higher compared with K1, and 5,8% compared to K2.

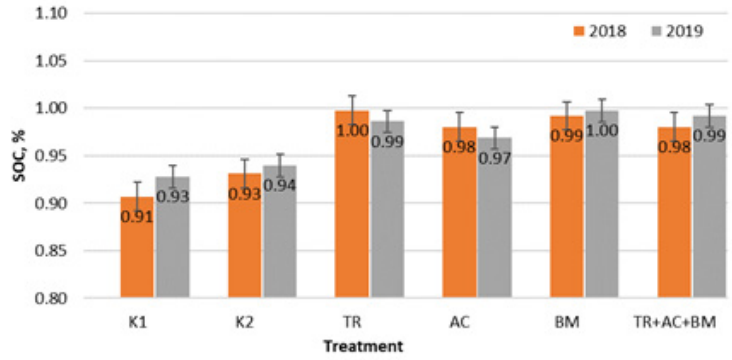

Figure 1: Average SOC concentration data during 2018-2019 field experiment.

The decomposition intensity of organic matter is best determined by the C/N ratio. In 2018 period C/N ratio (Figure 2) was 10,2% higher in treatment 6 with *Trichoderma reesei*, *Acinetobacter calcoaceticus*, *Bacillus megaterium* compared to K1 and 9,3% with K2. In 2019 period C/N ratio (Figure 2) was highest in treatment 6 it was 4,5% higher than K1 and 6,8% than K2. An increased C/N ratio during experiment period means that the ratio of mineralization / humification processes has changed.

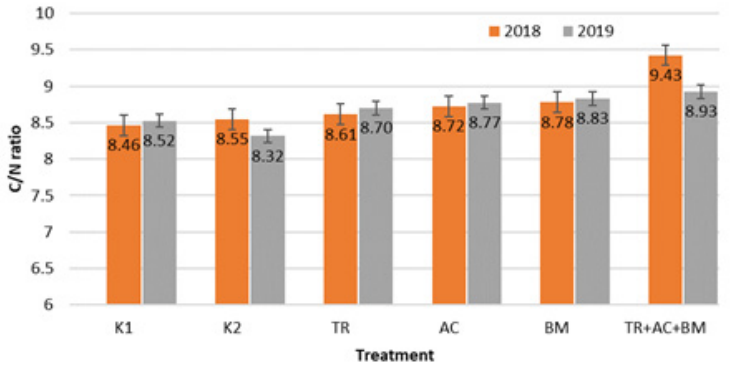

Figure 2: Average C/N ratio during 2018-2019 field experiment.

Humic/fulvic acid ratio

Soil humus, especially its stable fractions, is formed slowly, so this indicator does not fully reflect the humification processes of embedded organic matter. The high humus content and low humic/fulvic acid ratio indicate that this humus is not long-lasting and the humification processes in such soils are weak. Humic /fulvic acid ratio was detected in 2019. Highest HA/FA (Figure 3) ratio was in treatment 6 - 51.5% higher than K1 and 48.5 % than K2.

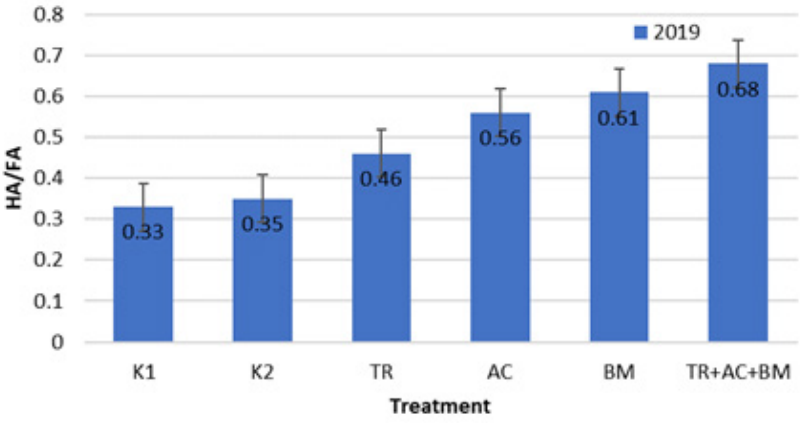

Figure 3: Average humic/fulvic acid ratio in 2019.

Soil surface net CO_2 exchange rate

During experiment period, the mean soil NCER (Figure 4) was highest in treatment No. 6 (*Trichoderma reesei + Acinetobacter calcoaceticus + Bacillus megaterium*). In 2018, in this treatment, the NCER was higher by 26,5%, compared to both K1 and K2. In 2019, the mean NCER was 23,7% higher than in K1 and 33,7% higher than in K2.

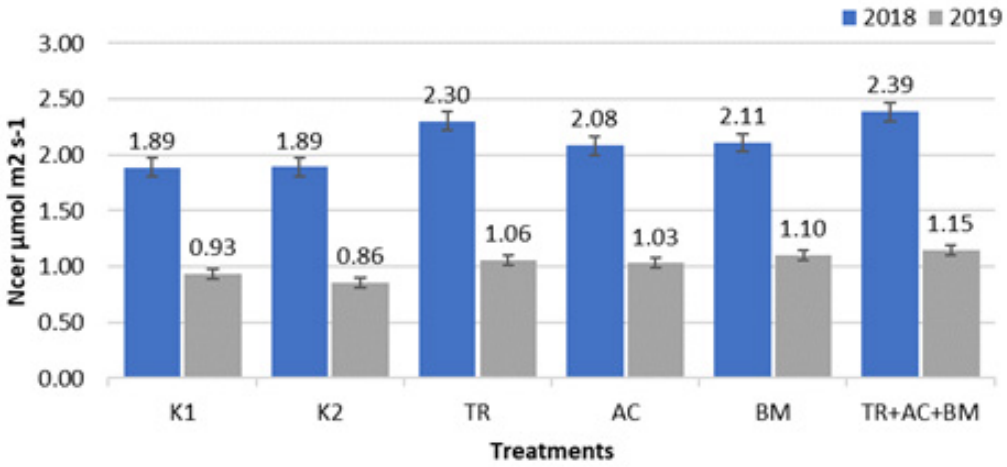

Figure 4: Average soil net CO_2 exchange rate.

To compare the strategy of substrate consumption by the soil microbiomes, the matters contained in a Biolog EcoPlate were classified into 6 main groups: carbohydrates, carboxylic acids, polymers, amino acids, amines and amides and miscellaneous. Data revealed that in both dry experimental years dominated carboxylic acids. In 2018 they consist 28-32%, in 2019 - 28-31% from all substrate C sources (Figure 5 & 6). Carbohydrates amounted 21-23% and 25-28%, amino acids 19-20% and 17-20%, polymers 14-16%, amines 4-6% and 4-5%, and miscellaneous 8% and 5-8%,respectively.

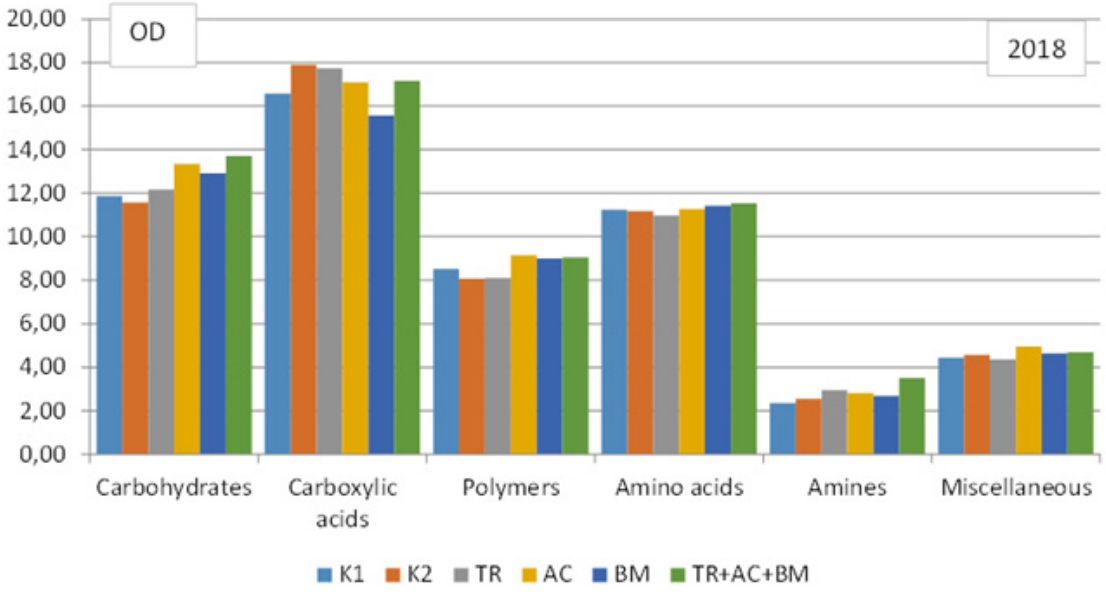

Figure 5: Level of consumption of substrates by microbial communities of soil, in 2018.

In first dry year, influence of soil microbiological amendments on C sources of soil substrate was insignificant (Figure 5). In next dry year, all products caused significantly higher OD values of each substrate category, compared ti K1 and K2. Most significant effect revealed combined application TR+AC+BM (Figure 6). The high level of carbohydrates and carboxylic acids substrate consumption is probably connected with the complexity of these substrates chemical structure and they require less time for decomposition.

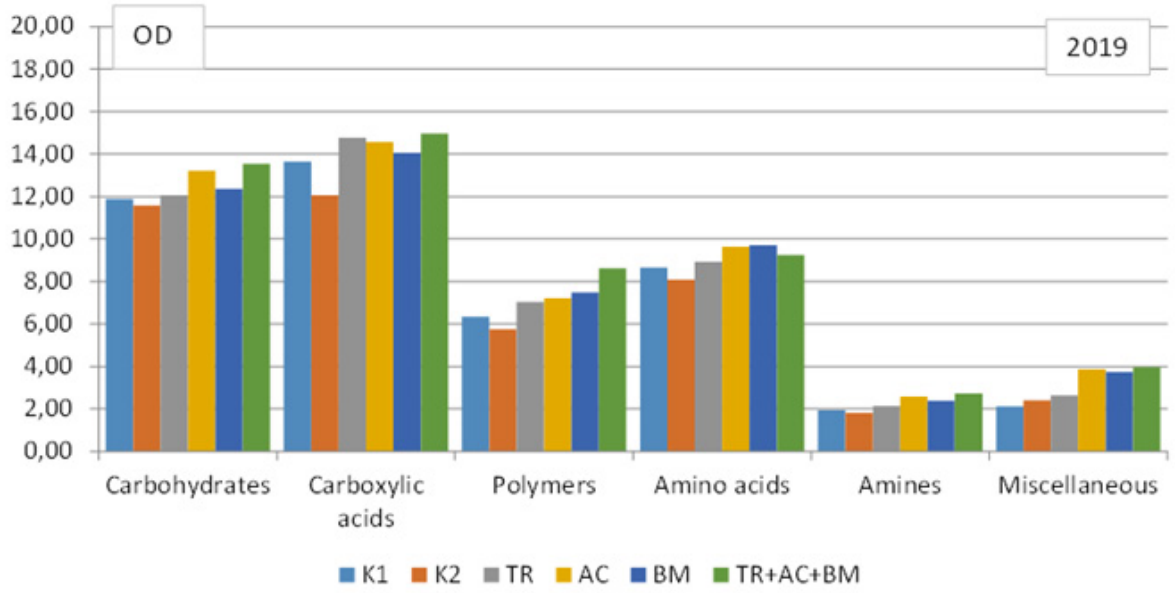

Figure 6: Level of consumption of substrates by microbial communities of soil, in 2019.

Principle Components Analysis (PCA) of average well colour development (AWCD) data revealed that during the first 4 days of the investigation average well colour development (AWCD) has grown in all samples selected and this index shows that microbial community metabolic activity in relation to carbon substrates under analysis is high. In 2018, in the 4th day of incubation, the highest AWCD index was found in treatment No.6 - it was 21-22% higher than in K1 and K2. Meanwhile, difference from controls K1 and K2 in treatments 3-5 amounted only 3-8%. In 2019, in the 4th day of incubation, the highest AWCD index also was found in treatment No.6 - it was 22-28% higher than in K1 and K2 treatments. Difference from controls K1 and K2 in treatments 3-5 reached only 8-13% (Figure 7).

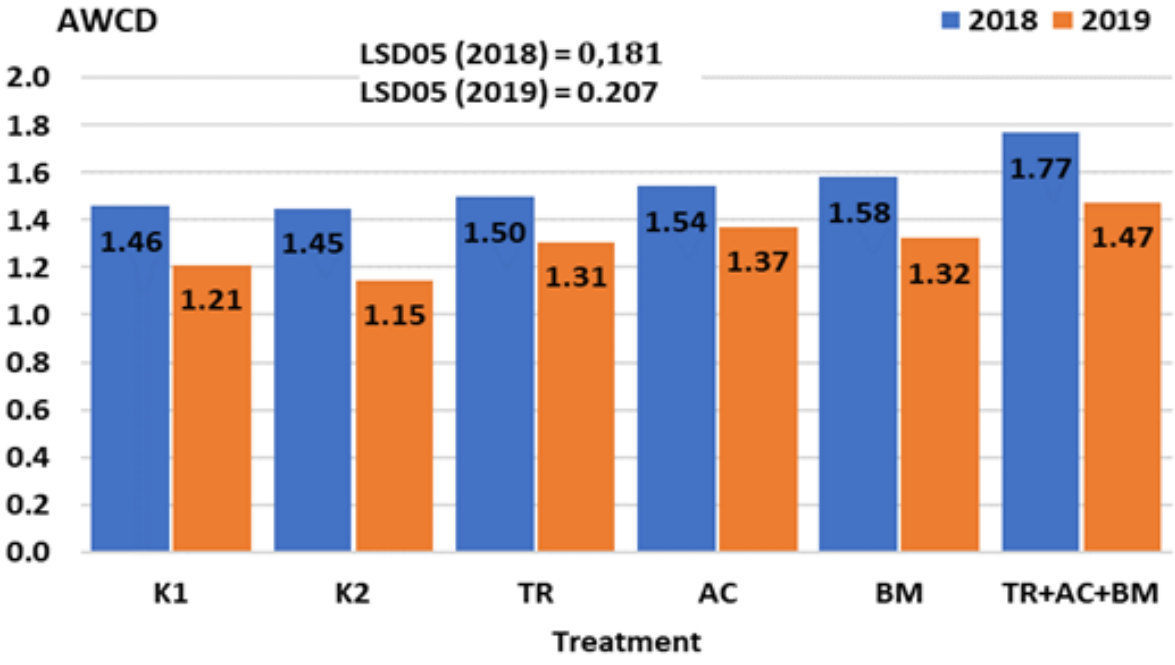

Figure 7: Influence of microbiological amendments on AWCD.

The richness (R) index showed that the microbial diversity varied with the type of soil management and generally increased with the combined application of microbiological products TR+AC+BM (Figure 8). Microbial richness varied significantly ($P < 0.05$) in both experimental years. In 2018 the highest R value was determined in treatment No. 6 and it was 4-5% higher than in K1 and K2 treatments. Meanwhile, differences from controls K1 and K2 and treatments No. 3-5 were insignificant. In 2019 the highest R value was determined in treatments No. 4 and No. 6. R index was 6-7% and 7-8% higher, respectively, than in K1 and K2. The correlation between AWCD and R indices was very high (r= 0.91-0.95). The increase in values of ACWD and R is likely related with COC increase.

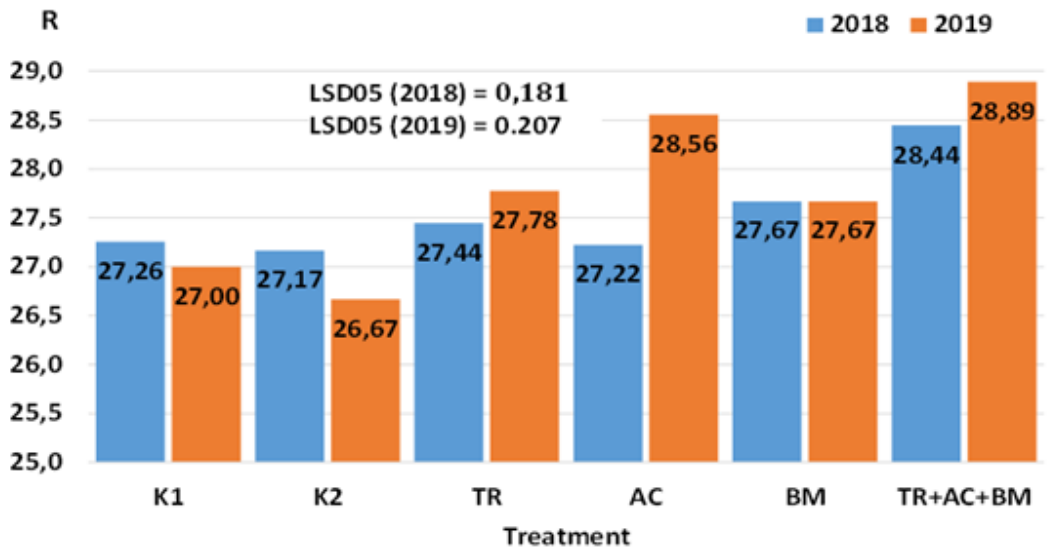

Figure 8: Influence of microbiological amendments on soil R index.

Climate change, production intensification conditioned great changes of soil environment. Therefore worldwide is increased interest in application of microbial amendments that provide natural benefit for soil and crops. Under commercial field conditions, microbial amendments can play an important role for environment and human health also [12].

Securing and improving soil quality is a central challenge in face of climate change. Considering the importance of micro-biota for soil ecosystem the exploitation of microbial activity could provide means to achieve soil improving. The application of microbial amendments could improve use of plant nutrients, reduce their release into soil and water and consequently reduce the negative effects on the environment [13].

Our results obtained are in line with other published data. We found positive effects of microbiological soil amendments on Cambisol properties. Bio-products with *Trichoderma reesei*, *Acinetobacter calcoaceticus* and *Bacillus megaterium* caused increase in SOC content, C/N ratio, Humic/Fulvic acids ratio and improved soil vitality by increasing soil respiration. We suppose that such results were governed by changes in soil physical properties. Pore structure, soil aggregate stability and water retention are under investigation in our experiment. Similar results were reported from sodic soils [14].

Bio-products act through a complex mechanisms including plant roots, nitrogen fixation, P-solubilization, etc [16]. Therefore, we think that microbiological product of new generation can be advantageous under intensive farming conditions in commercial agriculture.

Conclusion

1. Under drought conditions, bio-products with *Trichoderma reesei*, *Acinetobacter calcoaceticus* and *Bacillus megaterium* caused increase in SOC content, C/N ratio, Humic/Fulvic acids ratio.

2. All tested bio-products increased soil respiration. The highest respiration was observed in soil after application with mixture of three products: *Trichoderma reesei + Acinetobacter calcoaceticus + Bacillus megaterium*. It was 23.7-26.5% higher, compared to soil respiration in crop growing technologies without bio-products.

The highest AWCD index and R index was found in treatment after application with mixture of three products: *Trichoderma reesei + Acinetobacter calcoaceticus + Bacillus megaterium*.

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