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INFLUENCE OF BIOCHAR AND MANURE FERTILIZATION ON THE MICROBIOLOGICAL ACTIVITY OF AGRICULTURAL SOIL

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Abstract

Fertilization with biochar (carbonized wood chips) and compost (well decomposed cow manure) was carried out on the following crops: zucchini (Cucurbita pepo), broccoli (Brassica oleracea), broad bean (Vicia faba) and leeks (Allium ampeloprasum). The introduction of organic fertilizers increases the development of all groups of microorganisms studied most strongly in zucchini, followed by broccoli and less in cultures of broad bean and leek. In zucchini, the application standalone of manure and biochar has a more microbial-stimulating effect, while in broccoli, broad bean and leek, the combined fertilizing variants increase the activity of soil microorganisms to a greater extent. As sampling days increase after application of organic fertilizers the biogenicity decreases, more noticeably after 200 days of application. The highest percentage in the composition of the total microflora is occupied by the ammonifying bacteria (non-spore-forming bacteria and bacilli), and the least represented are micromycetes and actinomycetes.

***Key words**: biochar, manure, crops, soil microorganisms.*

INTRODUCTION

Organic fertilization improves the development and activity of soil microorganisms, which helps the soil fertility to be improved by using of harmless ameliorants. A number of authors have investigated the organic fertilization effects on microbial comunities development and their activity in the soil (Li et al., 2004; Dinesh et al., 2010; Qiu et al., 2012; Muñoz et al., 2015). Partanen et al. (2010) has found that Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Deinococcus-Thermus bacteria and more than 2,000 different filotypes have participated in the composting process. Moulds and actinomycetes have been developing in both mesophilic and thermophilic composting phases, in the amount of 0.01 to 1 million on a gram of compost (Kowalik, 2015). The biochar is a carbon-rich product obtained by biomass heating in a closed container with little or no air available (Lehmann and Joseph, 2009).

In present study well decomposed cow manure and biochar from wood chips was used.

The manure contains a high amount of organic carbon, well stored with total N and medium stored with P and K, the values of the mobile forms being approximately equal to the reported total amounts. The ratio of ammonium to nitrate forms indicates that the mineralization process is not fully complete. The pH reaction of manure is slightly alkaline.

While the pH reaction in the analysed sample from biochar is highly alkaline. It contains a large amount of carbon, which confirms the ability of biochar to deposit carbon into the soil, reducing its release into the atmosphere. The mineral content of NPK is minimal. The presence of $CaCO_3$ is one of the causes of the highly alkaline reaction of the substrate.

Reducing the amount of green and brown plant materials and addition of rabbit fertilizer slows the development of microorganisms in the beginning of experiment, but in the following period they have been activated, and non-spore-forming bacteria have a significant role in the composting process, followed by the bacilli (Malcheva et al., 2018). The rabbit manure inclusion to compostable plant residues leads to an increase of soil nutrients upon matured compost application and, consequently, to an increase soil microbial activity (Malcheva et al., 2019). Micromycetes and bacteria exhibit different dynamics in the composition of soil microbiocenosis during mulching (Baldrian et al., 2012; Prewitt et al., 2014). It has been established that upon organic fertilizers using (Yankova et al., 2016) the fertilized soils show a greater amount of total microflora and higher activity of the microorganisms compared to the non-organic fertilized control sample. Non-spore-forming bacteria, followed by actinomycetes and bacilli occupy the major percentage share of the microbiocenosis composition.

Applying biochar leads to soil fertility and agricultural productivity augmentation, as well as it provides protection against certain plant diseases. Its application significantly improves soil quality (Petrova et al., 2019). It has been reported that soil microbial biomass and activity increase when it is used, especially when biochar was prepared at a lower temperature (350°C) and it was applied in the early stages of plant development (Luo et al., 2010). Higher temperature biochar production has eliminated potential substrates for germs. Produced at 700°C, the biochars, regardless of the raw material source, do not increase soil microbial biomass or activity (Zhang et al., 2014a). Sagrilo et al. (2014) demonstrate that large soil biochar additions significantly increase CO_2 emissions, while lower fertilization norms do not significantly affect emissions. Fabbri et al. (2012) investigated the degree of mineralization of the soil organic matter upon 20 types of biochar applying, depending on their chemical composition, and it has been found that biochar with higher concentrations of proteins and sugars (of incomplete transformation by pyrolysis) have increased the mineralization rate in its highest degree. In contrast, the biochar produced at higher-temperature result in lower CO_2 emissions. The soil conditions can strongly interact with the impact of biochar on soil biological activity (Blackwell et al., 2010). In arable soils, that are often fertilized and altered, the biochar long-term effect on the soil microbiota depends on the soil tillage. The properties of biochar must be considered in conjunction with soil conditions in order to be made properly design of its positive soil impact (Hardy et al., 2019).

According to Xu et al. (2014) studies, the use of biochar for fertilization increases plant growth, soil pH, total carbon and nitrogen forms, C/N ratio and cations exchange capacity. Their results show that its application significantly increases the diversity and alters the relative abundance of some microbes which are associated with the carbon and nitrogen cycle. Nitrification and denitrification processes are stimulated while reducing N_2O emissions overall. According to these authors, the use of biochar will reduce the soil acidification caused by the nitrogen fertilizers using, as well as reduce hothouse gas emissions. Other authors have also argued for the ecological role of biochar applying for fertilization. Song et al. (2017) have found that wheat straw biochar significantly reduce the toxicity of polycyclic aromatic hydrocarbons (PAHs), it helps to preserve bacterial diversity in PAH contaminated soil, and it significantly affects the structure of the bacterial community after 12 weeks of biochar use, and the effects depend on the type of biochar - the reproduction of rare bacterial genera (relative abundance of 0.01-1%) in the investigated soil increases. Therefore, the use of wheat straw biochar can reduce the environmental risks of PAH and it helps in soil microbial ecology.

Chintala et al. (2014) have established that upon applying corn biochar there is a negative effect on the activity of some soil enzymes - esterase (fluorescein diacetate hydrolysis), dehydrogenase, β-glucosidase and protease. According to them, the biochar materials used are highly hydrophobic, with a high fragrance, regardless of the biomass raw materials and the pyrolytic process. Wang et al. (2015) have investigated the combined effect of different biochar amounts (0, 0.5, 1.0, 2.5 and 5.0% by mass) on enzyme activities and the microbial community in soil. Increased addition of corn biochar leads to a significant increase in soil organic carbon, total nitrogen and digestible forms of potassium and calcium. According to their opinion, nitrogen and calcium are dominant factors affecting the activity of soil enzymes. The activities of soil extracellular enzymes involved in carbon (C) and sulfur (S) cycles (except for β-xylosidase) suggest smaller amounts of biochar (0.5% by mass) to increase the activity of enzymes in the soil. However, the activity of 1-leucine aminopeptidase and urease involved in the nitrogen cycle increases with the biochar amount raising. The total phospholipid fatty acids content and the relative abundance of bacteria decrease significantly with the biochar amount added. The relative abundance of fungus in the soil with added urea is significantly higher than in other treated soils, and the abundance of actinomycetes does not show a clear response from the biochar addition. The microbial community composition changes are mainly related to the organic carbon content and the total nitrogen content with a significant negative correlation. Therefore, the effect of biochar addition on soil enzymes and the composition of the microbial community are highly variable. Liao et al. (2016) have found in their investigation that fertilization with cotton straw biochar enhances soil microbial biomass, gram-positive and gram-negative bacteria, actinomycetes and enzyme activity associated with C and N transformation. The use of rice straw biochar may improve nutrient status in soil (increase in pH, C, N, K, P readings) and affect the structure of the microbial community - the composition of soil bacteria consists mainly of proteobacteria, actinobacteria and acidobacteria (Gao et al., 2017). These authors have found that by increasing amounts of biochar applied, the proteobacteria and acidobacteria increased, while actinobacteria decreased. With respect to the fungal population, Ascomycota species decrease, and Zygomycota and Basidiomycota species increase with increasing biochar. Pursuant to a study by Zhang et al. (2014b), the microbial biomass ratio of C:N is significantly increased upon biochar applying and it causes a less extreme environment for microorganisms throughout the winter wheat development season. Application of palm core and rice husk biochar in soils increases pH, moisture, organic carbon, microbial C and N biomass, activity of β-glucosidase and xylanase enzymes (Simarani et al., 2018). The synergistic effect of biochar and addition of nutrients to the composition of the microbial community increases the content of carbon and nitrogen, the amount of soil microorganisms and the index of dehydrogenase activity in the soil (Mierzwa-Hersztek et al., 2019).

There is a need to be further evaluated both the positive and the negative long-term effects of biochar on soil quality and crop productivity. Biochar may have a positive or negative effect on beneficial microorganisms in the soil, for which should be taken into account the temperature of pyrolysis when BC is prepared (Ajema, 2018). The researches on the long-term effects of biochar are insufficient (Maestrini et al., 2014; Sagrilo et al., 2014), although the biochar has existed in soil for centuries (Singh et al., 2012). New tools in molecular biology allow the identification of specific microbial groups associated with the presence of biochar, or because of the inherent ability to decompose biochar or specific microhabitats provided by the biochar porosity (Hardy et al., 2019).

The purpose of this research has been intended to investigate the effect of biochar of wood chips and cow manure alone and in combination on the soils microbiological activity with tested crops: zucchini (*Cucurbita pepo*), broccoli (*Brassica oleracea*), broad beans (*Vicia faba*) and leek (*Allium ampeloprasum*).

MATERIALS AND METHODS

The experiment has been carried out at the Vrazhdebna Training Center - Sofia on alluvial meadow soil (*Fluvisol*), on an area of 150 m^2 with an irrigation rate of 50 l/m^2. After the soil pre-treatment, the experimental plots were resized and on 31.03.2017 biochar (BC) was put in - BC (from pyrolysed wood chips) and well decomposed cow manure according to the following scheme:

► Variant 1 – Control (without ameliorants);
► Variant 2 - Manure 4 t/decare;
► Variant3 - Biochar 500 kg/decare;
► Variant4 - Manure 4 t/decare + Biochar 250 kg/decare;
► Variant 5 – Manure 4 t/decare + Biochar 500 kg/decare;
► Variant 6 - Manure 4 t/decare + Biochar 750 kg/decare.

The variants have been applied to the following crops: zucchini, broccoli, broad beans and leek. Sampling of soil samples has been carried out in sterile paper bags at a depth of 0-30 cm, at the maturity stage of the crop development – 80 Day After Incorporation of Ameliorants (DAIA) (zucchini), 102 DAIA (zucchini), 200 DAIA (broccoli), 445 DAIA (broad beans) and

557 DAIA (leek). A control, non-fertilized soil sample has been also examined for each piece. For microbiological analysis, the limit dilution method, solid medium culture (ordinary agar for non-spore bacteria and bacilli; Czapek-Dox agar for molds and bacteria absorbing mineral nitrogen; Actinomycete isolation agar for actinomycetes and bacteria absorbing mineral nitrogen), cultivation and determination of colony forming units (CFU) in 1 g of absolutely dry substrate. Statistical data processing involves the calculation of an average of three repetitions and a coefficient of variation (C.V.).

RESULTS AND DISCUSSIONS

The microbiological analysis for soil samples in zucchini culture (before the beginning of harvesting - 80 days after application of ameliorants) is presented in Table 1.

Table 1. Qualitative composition and amount of soil microorganisms under zucchini crop at the beginning of harvest (x 10^3 CFU/g abs. dry substrate); ± C.V.

Variant	Total microflora	Non-spore-for-ming bacte-ria	Bacilli	Micro-mycetes	Bacteria absorbed min. N	Coefficient of minerali-zation
V1	3200	2600± 0.314	200± 0.816	400± 0.408	6800	2.43
V2	26200	22200± 0.074	3000± 0.544	1000± 1.633	16800	0.67
V3	15600	11600± 0.141	3600± 0.227	400± 0.816	17200	1.13
V4	7420	4600± 0.177	1800± 0.454	1020± 0.800	18000	2.81
V5	12200	8400± 0.194	3200± 0.255	600± 0.544	17800	1.54
V6	10800	9600± 0.085	1200± 1.361	0	18800	1.74

The lowest microbial number, i.e. degree of microbial population or soil biogenicity has been established in the control soil (V1). This was observed both in terms of the overall microflora and the quantitative development of the individual microbial groups studied - non-spore-forming bacteria, bacilli, micromycetes (molds), bacteria absorbing mineral forms of nitrogen.

The biogenicity of fertilized soils with manure and biochar is increased more than 8 times compared to the control (non-fertilized) soil. The addition of nutrients to the soil obviously improves the conditions for the development and multiplication of soil microorganisms, with microbes imported with the organic fertilizers themselves likely to have a significant role for increase in soil biogenicity. For the investigation phase, the microbial mineralization activity did not reach that of the control soils- the rate of the organics decompose was lower at 80 days after fertilization (except for variant 4 - manure 4t/decare + BC 250 kg/decare).

In a comparative aspect for imported fertilizer variants (Variants 2-7), the total microflora has the highest amount of manure fertilization at 4t/decare (Variant 2) and the lowest for combined manure fertilization 4 t/decare + BC 250 kg/decare (Variant 4). However, it has been found that in Variant 4, the rate of the organics decompose is the highest, including for control soils with a mineralization factor of 2.81. It is proved by the fact that the lower amount of microorganisms does not always implies their lower activity.

As per other variants of the combined fertilization experiment (Variants 5 and 6), it was observed that at the same amount of manure, by increasing the amount of biochar, the amount of microorganisms also increases.

The investigations also show differences in the composition of the microbiocenosis in the different variants of the experimental scheme. A regrouping of bacilli and micromycetes was observed - the amount of bacilli was higher in fertilized variants than in the control. Whereas in the non-fertilized variant, micromycetes occupy a higher percentage (13% in V1) in the composition of the total microflora than in the other variants.

The highest percentage in the composition of the total microflora in all investigated sites is occupied by non-spore-forming bacteria (62-89%), followed by bacilli in fertilized variants (11-26%), i.e. the group of ammonification that are involved in the initial stages of organic matter decompose. Their amount is highest in soil fertilized only with manure (V1) or only with biochar (V3) and lower in the combined fertilization with both products (Variants 4, 5 and 6). In all variants of the experiment, the amount of non-spore-forming bacteria increased from 1.8 (V4) to 8.5 (V2) times compared to the control untreated soil. This is also the trend in the development of bacillary microflora. The number of this group of microbes has an even more pronounced upward trend of development - it exceeds the same in the control samples of 6 times in V6 to 18 times in V3.

The microflora in the composition of the microflora is the least micromycetes, which are even missing in the combined variant with the highest concentration of biochar (V6). Obviously the highest amount of biochar (750 kg/decare) used in combination with manure inhibits their development.

The fertilization increases the development of bacteria that absorb mineral nitrogen - their amount is increased up to 3 times compared to the control sample. The highest is the amount at V6 and V4, where the coefficient of mineralization is also highest. Combined manure fertilization and biochar fertilization have been found to increase the activity of this group of microorganisms against samples using either manure or biochar alone.

The results for the quantity and quality composition of soil microorganisms in the zucchini culture at the end of harvest (102 days after application of ameliorants) are presented in the Table 2.

Table 2. Qualitative composition and amount of soil microorganisms under zucchini crop at the end of harvest (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Micro-mycetes	Bacteria absorbed min. N	Coeffi-cient of minera-lization
V 1	30800	16000± 1.617	14200± 0.575	600± 0.272	62400	2.07
V 2	165400	147200± 0.022	16000± 0.612	2200± 0.742	59200	0.36
V 3	183400	169600± 0.019	13200± 0.680	600± 0.816	19200	0.11
V 4	174000	168000± 0.097	5800± 0.282	200± 1.633	185600	1.07
V 5	34600	25600± 0.128	7400± 0.221	1600± 1.531	160000	4.85
V 6	32400	24000± 0.340	6800± 0.240	1600± 1.021	168000	5.45

The fertilization increases the biogenicity of all the variants tested. It was highest after the biochar adding only (V3 - BC 500 kg/decare) and lowest in the control sample (V1), as the difference was 6 times. In the variant with the addition of both ameliorants - Manure 4 t/decare + BC 250 kg/decare (V4), the total amount of microorganisms is 5.6 times higher than the control sample. The only use of manure (4 t/decare - V2) increased biogenicity by 5.4 times towards the control samples. However, by increasing the amount of biochar from 250 kg/decare to 750 kg/decare in combination with 4 t/decare of manure (V6), the total amount of micro-organisms decreases, as this decrease is smooth upon increasing from 250 kg/decare to 500 kg/decare biochar (1 time) and it is significant when 750 kg/decare biochar is added - 5 times. The introduction of carbonized plant residues as soil improver (biochar) after a certain amount results to delay the development of the microorganisms - their quantity, but not to slow their activity - the rate of degradation of the organic matter is highest in the variants with the lowest amount of total microflora. The bacteria that absorb mineral nitrogen develop most actively during fertilization with V4, V6 and V5, which causes higher values of the mineralization coefficient in these variants.

The highest percentage in the composition of the total microflora is occupied by ammonify-cation non-spore-forming bacteria and bacilli, which initiate the processes of organic matter decomposition. The development of non-spore-forming bacteria follows the trends found in the total amount of micro-organisms analysis. While in bacilli only fertilization with manure V2 alone is higher than in control sample. The least represented in the composition of the total microflora are micromycetes, as the fertilization increasing the amount of molds by 3.7 times at V2 and 2.7 times at V5 and V6.

The microbiological analysis after fertilization with the same ameliorants in broccoli culture (200 days after application of ameliorants) is presented in Table 3.

Table 3. Qualitative composition and amount of soil microorganisms under broccoli crop (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Micro-myce-tes	Bacteria absorbed min. N	Coefficient of mineralization
V 1	47400	38400± 0.213	6800± 0.120	2200± 0.742	83200	1.84
V 2	67200	52800± 0.155	10400± 0.785	4000± 1.225	57600	0.91
V 3	50200	38400± 0.213	10000± 0.816	1800± 0.907	73600	1.52
V 4	85800	70400± 0.162	12800± 0.638	2600± 0.628	62400	0.75
V 5	172000	160000± 0.102	9000± 1.633	3000± 0.544	48000	0.28
V 6	81200	62400± 0.151	10800± 0.756	8000± 0.408	40000	0.55

It has been also established for this culture that the fertilization increases the total amount of microorganisms in all variants - 3.6 times (V5), 2 times (V4, V6), 1.4 times (V2), 1 time (V3) compared to control sample (V1). The results show that the use of the same enhancers and norms in broccoli increases the amount of microorganisms less than in the the zucchini experiment. Again, the highest percentage in the composition of the total microflora is occupied by non-spore-forming bacteria, followed by germs, and the least represented are micromycetes. In all researched groups, the fertilization increased the amount of microorganisms, with the exception of bacteria that absorb mineral nitrogen - their lower amount towards to the control sample determines a lower rate of mineralization of organic substances after fertilization.

The biogenicity decreased in the test broad beans culture (445 days after application of the amendments) after fertilization with the same amendments compared to the previous two cultures, but remained higher than the control sample, except for the use of V6 (Table 4):

Table 4. Qualitative composition and amount of soil microorganisms under broad bean crop (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria absorbed min.N	Coefficient of mineralization
V 1	4060	3100± 0.263	360± 0.454	240± 0.680	360± 0.454	5700	1.65
V 2	4240	3160± 1.034	620± 0.527	260± 0.628	200± 0.816	5640	1.49
V 3	4140	3200± 1.021	540± 0.605	220± 0.742	180± 0.907	5540	1.48
V 4	4200	3300± 0.495	500± 0.327	200± 0.816	200± 1.633	5700	1.50
V 5	4580	2700± 0.605	660± 0.247	240± 0.680	980± 0.333	5660	1.68
V 6	3940	2940± 0.278	660± 0.495	180± 0.907	160± 1.021	5720	1.59

The total amount of microorganisms is highest upon using manure 4 t/decare + BC 500 kg/decare (V5), but the values of the total microflora are close - 1 times higher in variants 2 to 5 than in the untreated control sample. The tendency for increased participation in the composition of the total ammonia microflora and lower presentation of actinomycetes and micromycetes persists, except for V5, where the amount of micromycetes is higher than that of bacilli. The activity of the micro-organisms after fertilization is close to that of the non-fertilized control sample, comparing the values of the mineralization coefficient.

Such trends, similar to those for broad beans, were also observed in the tested leek experiment - 557 days after the ameliorants introduction (Table 5):

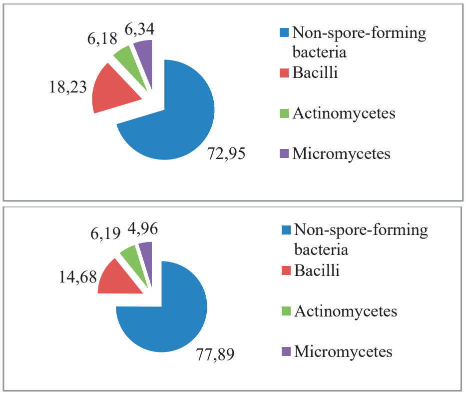
Table 5. Qualitative composition and amount of microorganisms under leek crop (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total Microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria absorbed min. N	Coefficient of mineralization
V 1	3720	2760± 0.296	580± 0.282	240± 1.361	140± 1.166	3600	1.08
V 2	4140	3200± 0.510	540± 0.302	260± 1.256	140± 0.583	4000	1.07
V 3	3880	2760± 0.171	620± 0.263	320± 0.510	180± 0.907	3900	1.15
V 4	4280	3000± 0.544	580± 0.563	340± 0.480	360± 0.907	3760	1.05
V 5	4040	3020± 0.541	600± 0.544	240± 0.000	180± 0.454	3580	0.99
V 6	4820	3760± 0.217	540± 0.907	360± 0.454	160± 0.510	4740	1.10

Similarly, the biogenicity for this crop increased after fertilization (about 1 time in all variants), but less than in the testing of zucchini and broccoli. The fertilization redistributes the relative participation of individual microbial groups in the composition of the microflora by degree of dominance, but in all variants of the experiment the composition of the common microflora is dominated by ammoniating bacteria (non-spore bacteria and bacilli), and micromycetes and actinomycetes are the least represented.

The biggest difference in the redistribution of individual groups of microorganisms towards the control has been established in zucchini crop 102 days after fertilization. In summary, the percentage of microorganisms in the composition of the total microflora in all experiments is presented in the Figure 1: A (control variants) and B (fertilized variants).

As a summarized trend for all crops and all variants, Figure 1 A and B established that fertilization increases the development of non-spore bacteria by 5% towards the control samples.



A.

B.

Figure 1. Percentage distribution of microorganisms under control samples (A) and fertilized variants (B)

While the bacilli group and the micromycetes group are respectively about 3% and 1% less represented after fertilization than in the control samples. The actinomycetes group shows close values in control samples and fertilized variants. Some authors also reported that the application of organic fertilizers, including and biochar in soils mainly increases the amount and diversity of bacteria in the composition of total microflora (Partanen et al., 2010; Xu et al., 2014; Yankova et al., 2016; Song et al., 2017). While other studies have indicated that increasing the biochar concentration inhibits bacterial reproduction, the development of micromycetes is activated, and the abundance of actinomycetes does not show a clear response from the addition of biochar (Wang et al., 2015). Gao et al. (2017) establish that with increasing amount of applied biochar, the development of proteobacteria and acidobacteria increased, while actinobacteria decreased. In their study, Liao et al. (2016) reported that fertilizing with biochar increases the amount of bacteria and actinomycetes. Therefore, the effect of biochar addition on the microbial community composition is highly variable.

CONCLUSIONS

Fertilization with manure and biochar increases the soil biogenicity. It is increased up to 8 times in zucchini, up to 4 times in broccoli and up to 1 time in broad beans and leek. Not only the different organic material as a crop type is relevant to this trend, but also the test time, humidity and nutritional status of the soils investigated. As sampling days increase after ameliorants applying, biogenicity decreases, more significantly after 200 days of applying.

As a summarized tendency for the zucchini crop, single applying of manure and biochar has a more microbial stimulating effect. For broccoli and broad bean, the introduction of Variant 5 (Manure 4 t/decare + BC 500 kg/decare) increases the total amount of micro-organisms to the highest extent. Only in leek, the addition of the highest amount of biochar - 750 kg/decare in combination with 4 t/decare of manure increases soil biogenicity the most.

The fertilization redistributes the relative participation of individual microbial groups in the common microflora composition by degree of control, but in all variants of the experiment ammoniating bacteria (non-spore bacteria and bacilli) prevail, and micromycetes and actinomycetes are the least represented.

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