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Changes in the Structure of Myco- and Microbiocenosis of Soil with Use of Fungi and Bacteria Strains Immobilized on Biochar as an Example of Ecosystem Maintenance Services

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ABSTRACT

During recent decades the importance of ecosystems management services and maintenance have became paramount. We have proposed and implemented the technology of mycocenosis regulation of the rhizosphere of walnut nursery plants. Biotechnology involves inoculation before planting the roots of tree plants with the mycorrhizal drug Mikovital, which contains strains of the fungus *Tuber melanosporum* VS 1223 and the ascomycete *Vitasergia svidasoma*. Also it was applied in the combination with Florabacillin, which contains live cells of the bacterium *Bacillus subtilis*. The introduction of the mycorrhizal remedy into the rhizosphere of the walnut contributed to the change of such ecological characteristics of mycocenoses as the length and biomass of the fungal mycelium and the number of spores. To increase the efficiency of mycorrhization, an immobilization medium biochar with fungi and bacteria was used (Mikovital + Florabacillin + biochar). After use of this soil improvement complex in the rhizosphere of plants, the length of fungal mycelium increased by 8–10 times, and biomass by 5–7 times, in comparison with the rhizosphere of walnut on the control plot. Based on the dominance indices we had seen a signifficant increase in species diversity, which confirms the positive effect of immobilization of fungal cells and bacteria on the biochar. Optimum concentrations of 0.2% biochar additive to the Mikovital. It also have reduced the number of pathogens in the rhizobiome of the treated plants. The created harmoniously functioning ecosystem of the nut nursery as the result of above experiments will provide further self-regulation of this local natural ecosystem, as evidenced by the development of plants, their appearance and growth, as well as previous studies in the hazelnut garden. Mechanisms for bioregulation of soil and plant ecosystems have effectively included the use of man-made technology to stimulate natural mechanisms.

Keywords: ecosystem maintenance service, bioregulation of soil ecosystems, soil improvement, mycorrhization, immobilization of cells, biochar, mikovital, *Vitasergia svidasoma*.

INTRODUCTION

All the services that nature provides to humans are ecosystem services. There are four main categories of ecosystem services, namely - supply, cultural and social services, regulation and ecosystem maintenance services. We will focus on the last two, as their harmonious development supports the services that ecosystems provide to humans. Ecosystem maintenance services are key in avoidance of a complete degradation of the ecosystem at all levels. We have worked in this field when we studied the levels of ecosystem degradation in the application of intensive tillage, in the extraction of minerals and the destruction of the surface layer of the soil. [Kopiy & Oliferchuk, 2016; Kopiy et al., 2016, 2017; Nazarovets et al., 2017].

Previously we have studied the reaction-response of soil mycobiota to the use of regenerative land use technologies in different ways of applying the mycorrhizal drug Mikovital to the growth and development of soybeans, barley and hazelnuts [Oliferchuk & Fedorovych, 2019, 2021]. It was found that the mycoflora of the rhizosphere of mycorrhizal and non-mycorrhizal plants differed significantly in species composition. The results of the analysis of the peculiarities of the formation of mycological structure in the studied ecotopes indicate an increase in the Shannon coefficient, ie the growth of biodiversity of soil micromycetes in the area where mycorrhizal plants were planted. The Simpson index also decreased, which indicated which indicated a decline in dominant species that are potential pathogens, namely species of *Fusarium, Trichothecium, Phoma, Alternaria*, some species of the genus *Penicillium* [Oliferchuk & Fedorovych, 2021].

Ecosystem maintenance is possible by harmonizing two processes: the creation of such conditions in the soil environment that would most effectively affect the composition and functions of living organisms, and in turn, form and maintain the environment itself. An ecosystem that is functioning harmoniously will influence all processes: climate regulation, soils and ensuring self-regulation of local natural ecosystems. For doing this, should be used mechanisms of bioregulation of soil and plant ecosystems, which include the use of technologies created by humans in order to stimulate effectively natural mechanisms. Only after all the processes of ecosystem regulation and maintenance have been carried out can be provided the supply, cultural and social services of the ecosystem.

It is proved that intensive land use methods significantly affect the movement of mineral components not only within the soil profile, but also between the soil, atmosphere and hydrosphere. The main processes are leaching of components into groundwater, greenhouse gas emissions into the atmosphere and mineralization of organic matter [Hester et al., 1996; Ju et al., 2006; Cameron et al., 2013; Watanabe et al., 2018]. All this leads to deterioration of the soil and deterioration of the quality of the environment. Therefore, various methods of nutrient retention in soils are currently being discussed in the scientific literature. One such method is the application of biochar to agricultural soils. The use of biochar improves the physical, chemical and biological properties of soils [Glaser et al., 2002; Juriga & Simansky, 2019], which has an indirect effect on increasing yields [Liu et al., 2013].

Biochar favourably affects soil fertility and productivity and may protect plants against infections [Nigussie et al., 2012]. Introduced into soil, it may contribute to increased water capacity of the site and lead to decrease in soil acidification [Karhu et al., 2011]. Characteristic properties of biochars include capacity for retention and transfer of nutrients resulting in their increased availability to plants and improved properties of soils [Hossain et al., 2011; Harvey et al.,2012] Biochars are reported to have good potential for removing heavy metals from aqueous solutions and reducing their mobility in water [Zhou et al., 2013; Wang et al., 2014].

Also, the introduction of biochar into the soil has many environmental benefits, including waste reduction, carbon sequestration, protection of water resources [Dias et al., 2010, Igaz et al., 2018]. Biocarbon can interact with microorganisms, soil mineral components, dissolved organic and inorganic compounds, roots, root exudates and gases [Grossman et al., 2010]. The beneficial effect of biochar on soil properties has led to its use for soil fertilization and reclamation [Beesley et al., 2011, Hale et al., 2013]. In this paper, we propose an approach that was applied to the ecosystem of the walnut orchard, where it was possible to investigate in detail the response of mycobiota to the applied technologies of bioregulation.

MATERIAL AND METHODS

We will demonstrate the concept described in the paper (Fig. 1) on local ecosystems of the nut nursery in Dnipropetrovsk region.

General concept and field experiments

The Dnipro nursery was established in 2015 on the territory of the Dnipropetrovsk region of the Apostolov district, in the village of Novoukrainske. The main activity is growing walnut seedlings. The area is 3.8 hectares and has meadow-chernozem deep-saline soils. In 2015, the first starter cuttings and mother trees were laid, as well as breeding samples for research. The whole garden is fertilized with cow and pork manure in the amount of 6 t/ha. Seedlings were subsequently fertilized with mineral fertilizers NPK 20-20-20. In 2018, some plants were replaced by new 2-year-old plants using the technology of regenerative land use in three variants of the experiment: inoculated with Mikovital (1), Mikovital + Florabacillin (2) and Mikovital + Florabacillin immobilized on biochar 3). We will consider two variants of the field experiment (1) and (3) and compare it with the control, because the indicators using the variant Mikovital and Mikovital + Florabacillin slightly differed from the studied indicators in laboratory experiments.

The proposed technology involves inoculation of tree plant roots with the mycorrhizal growth promoter Mikovital before planting, which contains strains of the fungus *Tuber melanosporum* VS 1223 and ascomycete (*Vitasergia svidasoma*, BioProject PRJNA807518), as well as in combination with Mikovital application of Florabacillin (*Bacillus subtilis*). The fungi *T. melanosporum* VS 1223 and (*Vitasergia svidasoma*) form a symbiotic relationship with the nut, forming mycorrhizae, *B. subtilis* bacteria are phytopathogens, and promote efficient mycorrhization by reducing competition with soil deuteromycetes. All species have been used as a means of biocontrol when planting different plant species [Oliferchuk et al., 2006; Oliferchuk & Fedorovych, 2019, 2021]. For immobilization of fungal and bacterial cells, the biochar of BM Sorbent LLC was used - a high-quality technical sorbent made of FSC Certified Wood. Figure 1 shows the structure of the biochar, Fig. 2 - biomass of fungi and bacteria immobilized on biochar.

Here we have studied the effects of mycorrhization of walnut trees before planting by soaking the roots of walnut before planting in the following variants of field experiments: Mikovital in one version of the experiment Mikovital + Florabacillin + biochar in the second. Control

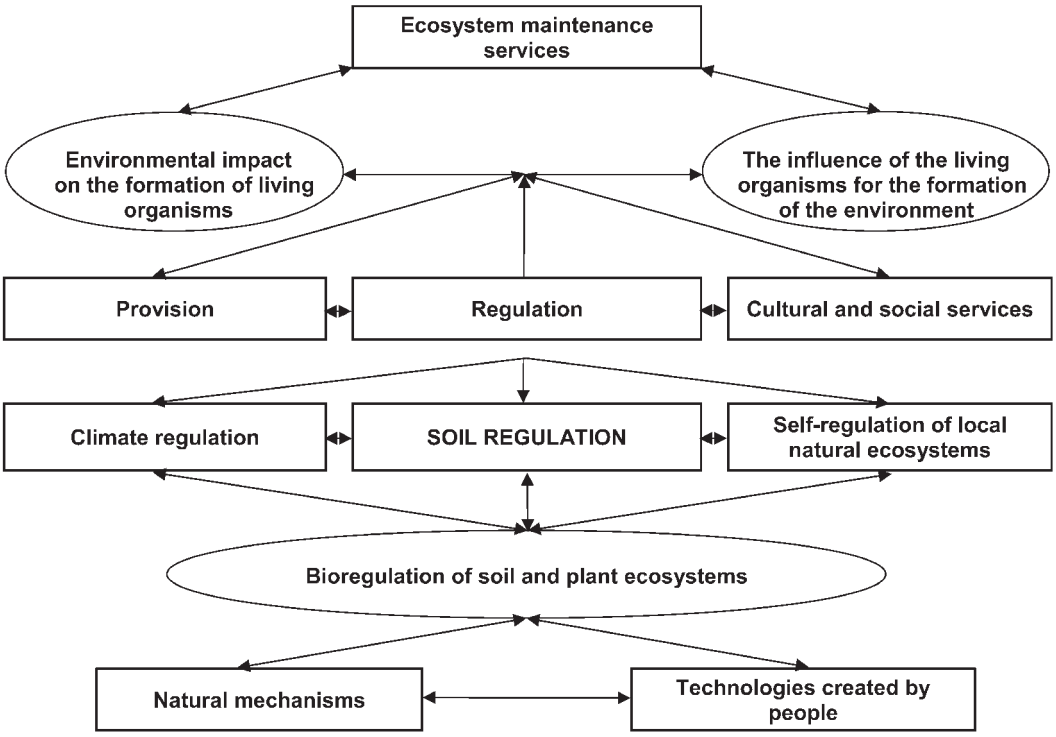

Figure 1. The role of ecosystem maintenance services in the functioning of ecosystem services

experiment was done without inoculation of roots. Soil samples were taken in spring, summer and autumn. A total of 57 soil samples were taken.

Isolation and identification of micromycetes

Isolation of micromycetes, their identification and statistical processing of the obtained results were carried out according to standard methods [Al-Askar et al., 2010]. Identification of micromycetes was performed after inoculation of fungal suspensions on nutrient media Chapek and agar. To isolate mycorrhizal symbionts used a buffer medium of the following composition (g per 1 liter of water): glucose – 8; K_2HPO_4 – 0.3; KH_2PO_4 – 0.9: $MgSO_4$ – traces, asparagine – 0.01. These fungi need growth substances for germination, so we used drugs similar to Symbiont 1 and Symbiont 2, which were developed in the 70–80s of the last century by Fania Geltzer. She developed a method of isolating endophytes to obtain their pure cultures from any plant, even those that were not considered mycotrophic [Geltser & Ignatev, 1989].

The analysis of the structure of micromycete groups was performed by calculating the values of the ecological indices of Berger-Parker, Shannon, Pisl and Simpson.

In addition to isolating pure cultures of deuteromycetes and endophytes, sequence analysis of soil samples was performed, where tree roots were inoculated with Mikovital, and where planting was performed without inoculation.

Sequence analysis of soil samples

DNA was isolated from rhizosphere soil samples using the following technique.

Metagenomic DNA extraction protocol

A modified protocol was used for isolation metagenomic DNA from soil [Devi et al., 2015]. 1 g soil sample and 2 g sterile glass powder (grinded laboratory glassware (borosilicate) using a pestle and mortar, sterilize by autoclaving at 121 °C for 15 min) were grinded for 5 min by a pestle and mortar. A grinded soil sample and glass powder was mixed by pipetting with 5 ml DNA extraction buffer [100 mM Tris, 100 mM EDTA, 1.5M NaCl (pH 8)], and transferred into a 2 ml tubes. The mixture was incubated in water bath at 65 °C for 10 min with invert mixing after every 2 min. The tubes were centrifuged at 12000 g for 5 min to collect 500 µl of the supernatant to a fresh 2 ml eppendorf tubes. The metagenomic DNA was precipitated by adding 100 µl of 3M sodium acetate (pH 5.2) along with 400 µl isopropanol and incubated at -20 °C in a deep freezer. After 20 min, the metagenomic DNA was pelleted by centrifugation at 12000g for 10 min, washed once with 70% (v/v) ethanol. The dried pellet was dissolved in 100 µl of TE buffer (pH 8). EZ-10 Spin Column DNA Gel Extraction Kit was used for purification of metagenomic DNA from humic acid.

Assessment of yield and purity of metagenomic DNA

An equal volume (2 µl) of metagenomic DNA extracts was loaded on to 1% (w/v) agarose gel along with 2 µl of Quick-Load Purple 1 kb Plus DNA Ladder (NEB). After electrophoresis, gel was stained with ethidium bromide and the bands were visualized using Syngene GeneGenius Bio Imaging System.

PCR amplification of 16S and ITS2 rRNA was performed with primers (v1-4). Amplicon sequencing libraries on the Illumina platform were created using the NEBNext® DNA Library Prep Kit. The library preparation products were analyzed for size distribution using an Agilent 2100 bioanalyzer and quantified by real-time PCR. Sequencing was performed on the Illumina MiSeq platform (2x250bp) Processing of 16S and ITS2 results Sequencing was performed according to the data processing method. Random selection of 5,000 readings from a one-way fq sample is compared with the Nt database. We have identified 10 species in the rhizosphere of the walnut that carry the greatest load in the ecosystem. Variations in the sequence of the 16S gene were used to characterize the microbial and fungal communities of the rhizosphere of the walnut soil in the Dnipro nursery. The significance of the structure of differences of microbial communities between groups was checked using the methods of T-test, MetaStat, LEfSe, Anosim and MRPP. The effect of changes in environmental factors (in this case, inoculation of plant roots with a stimulator of mycorrhiza formation) gave CCA / RDA analysis and correlation analysis. In order to study the composition of the microbial community in each sample, operational taxonomic units (OTUs) were obtained by clustering with an identity of 97% on the effective tags of all samples, and then identified.

RESULTS AND DISCUSSION

The number of micromycetes

Soil samples were taken from the rhizosphere of walnut in autumn, at the end of the growing season. Soil samples from the rhizosphere of the walnut inoculated with Mikovital and soil from the rhizosphere of the non-inoculated nut were compared. With use of the drug Mikovital there was an increase in the number of micromycetes in 3–10 times compared with the control sample. During the immobilization of fungi that are part of Mikovital and the strain *Bacillus subtilis*, which is part of Florabacillin on the biochar, the number of fungal propagules in one gram of soil increased by 2.5–3.5 times compared to the control sample. This is confirmed by the high and medium degree of connectivity (r = -0.63–0.88) between the number of micromycetes and the use of biotechnology with the introduction of strains of bioregulators, as well as high and medium degree of connectivity (r = -0.78–0.98) with using strains of bioregulators immobilized on the biochar (Fig. 2, 3).

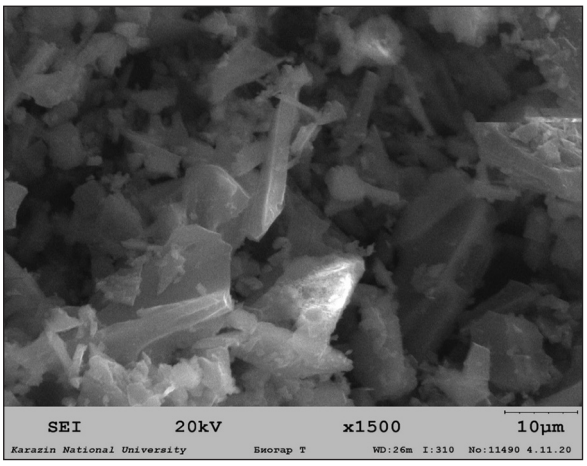

Figure 2. Electronic photography of the biochar

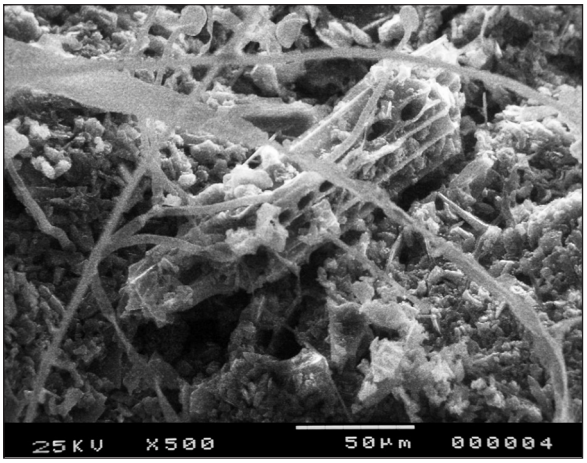

Figure 3. Electronic photo of a biochar inhabited by bacteria and fungi

Biomass of fungal mycelium and spores of micromycetes

The introduction of mycorrhizal preparation into the walnut rhizosphere (Table 1) manifested itself in changes in such ecological characteristics of mycocenoses as the length and biomass of fungal mycelium and the number and biomass of spores, which may indicate the influence of various factors on the formation of microscopic fungal populations in edaphotopes, described also in the works of Marfenina OE [Marfenina, 2005].

In the surface layer of soil 0–10 cm, the length and biomass of fungal mycelium increased 1.5 times with the use of both Mikovital (the first version of the experiment) and Mikovital + Florabacillin + biochar (the second version of the experiment), the number of spores increased by 0.5 and 1.2 times, respectively, and biomass – 4.5 in the first option, 5.5 times in the second option compared to the control soil (Table 1). At a depth of 20–40 cm, the length and biomass of fungal mycelium increased by 1.5 and 2.0 in the first variant of the experiment and 1.5 times in the second variant, and the number of spores and their biomass – in 0.8 and 2.0 and 0.7 and 1.8 times, respectively. The increase in the length and biomass of mycelium, as well as the number and biomass of spores in the surface layers of the soil is due to the fact that the surface layers of the soil after inoculation of plant roots and planting seedlings watered with a solution of drugs outside and mulched plants. In the rhizosphere of plants, the length of fungal mycelium increased 8 and 10 times, respectively, biomass - 5 and 7 times, due to the formation of mycorrhizal symbiosis, and the number of spores and their biomass increased by 4.5–5.5 and 5.7–7, 2 times.

It should be noted that the difference in the speed of formation of mycorrhizal symbiosis in plants inoculated with Mikovital and Mikovital + Florabacillin after 3.5 months was insignificant. Since mycorrhizal associations develop throughout the life of the plant, further studies for their evolution during longer time scale would be beneficial. The results of the correlation analysis showed a medium and high degree of association (r = -0.62–0.84)

Table 1. Length and biomass of fungal mycelium, the number of spores and their biomass in the soil, where Mikovital and Mikovital + Florabacillin + biochar were applied

Experiment options	Length of fungal mycelium, m / g	Biomass of fungal mycelium, mg / g	Number of spores, cl/g	Biomass spores, mg/g
Mikovital				
0–10 cm	213.12±2.15	0.34±0.03	237.60±1.20	0.540±0.003
20–40 cm	168.00±2.03	0.36±0.05	328.00±1.30	0.084±0.003
rhizosphere	465.84±0.24	0.60±0.03	441.00±1.30	0.550±0.003
Mikovital + Florabacillin + Biochar				
0–10 cm	225.79±2.15	0.18±0.03	237.60±1.20	0.360±0.003
20–40 cm	168.07±2.03	0.18±0.05	328.00±1.30	0.216±0.005
rhizosphere	582.30±0.24	0.84±0.03	558.60±1.20	0.720±0.003
Control (meadow black soil)				
0–10 cm	142.08±2.15	0.23±0.03	198.00±1.20	0.180±0.003
20–40 cm	112.05±2.03	0.18±0.05	164.00±1.30	0.120±0.005
rhizosphere	58.23±0.24	0.12±0.03	98.00±1.30	0.100±0.003

between the length of the fungal mycelium and the use of Mikovital and the use of Mikovital + Florabacillin + Biochar (r = -0.71–0.89).

Identification of soil microscopic fungi by morphological and cultural characteristics

From the soils of the studied sample areas of the nursery, 40 species from 15 genera of micromycetes and 2 species of ectomycorrhizal symbionts from the *Basidiomycota* division were isolated and identified (Table 2). The most represented were the genera *Penicillium* (12 species), *Aspergillus* (7 species), *Fuzarium* (5 species), *Acremonium*, *Mortierella*, *Rhizopus*, *Trichoderma* – 2 species each, *Humicola*, *Aureobasidium, Actinomucor, Alternaria, Trichocladium, Oidiodendron* one by one.

From the rhizosphere of plants in the control ecotope, the fungus *Colletotrichum graminicola* was isolated, which is a necrotroph, hemibiotroph, latent pathogen, but can act as an endophyte and saprotroph. The application of Mikovital into the rhizosphere of the researched plants reduced the incidence of *Colletotrichum graminicola* from 35% to 5%, and the immobilization on the biochar and the addition of the bacterium *B. subtilis* in the preparation of Florabacillin reduced its frequency of occurrence to 2%. A similar reaction to the use of Mikovital occurred in a number of pathogenic micromycetes. Thus, the dominance of *Aspergillus niger* decreased from 57% to 12% with Mikovital, and immobilization on biochar and the use of bacteria – up to 7%. The frequency of occurrence of *A. fumigatus* decreased from 63% to 9 and 7%, respectively, *Fusarium oxysporum* from 54% to 5 and 3%, respectively. Instead, there was an increase in the frequency of occurrence of species from the genus *Trichoderma* and the diversity of species in the genera *Penicillium* and *Aspergillus*. It is important to emphasize that only from the rhizosphere of plants treated with Mikovital and Mikovital + Florabacillin + biochar were isolated mycorrhizal basidiomycetes *Rhizopogon subcaeruliscens* and *Truncocolumella citrina* with an frequency of occurrence of 21–24% and 12–17%, respectively.

Variety of micromycete groups in the rhizosphere of walnut

The greatest diversity of microscopic fungi species had been observed in the surface layer of soil under the mulch, from which 25 species of microscopic fungi were isolated and identified. The group was dominated (60–72%) by *Penicillium decumbens, P. cyclopium Mortierella izabelina, Rhizopus nigricans, Trichoderma harsianum,* typical common species (33–53%) include *Alternaria alternata, Penicillium notatum phylum Aspergillus,* and *Trichoderma viride* (Table 2). From a depth of 20–40 cm, 18 species of fungi were identified. Typical common species of this ecotope (30–50%) are *Pennicillum citrinum* and *Rhizopus stolonifer*.

Pathogens and toxin-forming agents *A. fumigatus, A. niger, F. oxysporum, Colletotrichum graminicola* were identified in the rhizosphere of non-inoculated nut as typical and frequent species (50–63%). After processing the roots of walnut

Table 2. The frequency of occurrence of soil micromycetes in the rhizosphere of walnut after treatment with Mikovital, Mikovital + Florabacilin + biochar and in the control soil sample (%); (-) - not found.

#	Name of the species	0–10 cm	20–40 cm	Mikovital, rhizosphere	Mikovital + Florabacilin + biochar, rhizosphere	CONTROL, rhizosphere
1	2	3	4	5	6	7
Zygomycota phylum						
1.	*Mortierella isabellina* Oudem	33	23	10	12	5
2.	*M. ramanniana* (Moller) Linnem.var ramanniana Evans	12	7	-	-	7
3.	*Rhizopus nigricans* Ehrenb	42	17	-	-	3
4.	*Rh. stolonifer* (Enrenb.) Vull.	15	50	-	-	-
5.	*Actinomucor elegans* (Eidam) C.Benj.& Hesselt	-	5	2	2	5
Ascomycota phylum						
6.	*Acremonium strictum* W. Gams.	-	7	5	7	-
7.	*Acremonium* sp.	4	2	12	10	4
8.	*Colletotrichum graminicola* G.W. Wilson	-	5	5	2	35
9.	*Trichocladium* sp.	-	7	2	3	21
10.	*Oidiodendron cereale* G.L. Barron	4	3	-	-	6
11.	*Stachybotrus chartarum* (Ehrenb.) S. Hughes	-	2	-	-	7
Deuteromycetes class						
Family *Moniliaceae*						
12.	*Penicillium lanosum* Westl.	5	11	7	10	-
13.	*P. notatum* Westl.	23	15	12	12	2
14.	*P. decumbens* Thom	-	5	9	7	-
15.	*P. chrizogenum* Thom	5	-	5	8	-
16.	*P. corylophylum* Dierckx, R.P.	12	2	11	12	-
17.	*P. citrinum* Thom	15	32	5	7	-
18.	*P. digitatum* Sacc.	-	7	8	7	-
19.	*P. lanosum* Westl.	7	-	5	9	-
20.	*P. drevi-compactum* Dierckx	-	3	5	11	-
21.	*P. cyclopium* Westl.	38	21	-	-	-
22.	*P. rubrum* Stoll	-	-	5	7	-
23.	*P. tardum* Thom	3	-	10	12	-
24.	*Aspergillus niger* v. Tiegh	-	2	12	7	57
25.	*A. fumigatus* Fres.	2	-	9	5	63
26.	*A. flavus* Lk. Ex Fr.	5	-	5	3	-
27.	*A. terreus* Thom	-	-	7	5	-
28.	*A. versicolor* (Vuill.) Tiraboschi	3	-	3	7	-
29.	*A. ustus* (Bain) Thom	25	-	7	11	-
30.	*A. sulphureus* (Fres.) Thom	-	-	3	9	-
31.	*Trichoderma harsianu*m Rifai	22	5	32	30	5
32.	*T. viride* Pers: Gray	12	7	34	27	2
Family *Tuberculariaceae*						
33.	*Fusarium oxysporum* Schlecht v.orthoceras (App.et Wr.) Bilai	14	18	5	3	54
34.	*F. culmorum* (Sm.) Sacc.	-	14	2	2	13
35.	*F. gibbosum* App. et. Wr.	7	9	-	-	3
36.	*F. moniliforme* Sheld.	-	7	-	-	5
37.	*F. lateritium* Ness	-	11	-	-	-
Family *Dematiaceae*						
38.	*Humicola grisea* Alf Egeberg Traan	-	-	7	12	-
39.	*Alternaria alternate* (Fr.)Keissl	12	-	8	7	3
40.	*Aureobasidium pullulans* (d By) Arnaud	15	5	5	5	2
Phylum *Basidiomycota*						
1.	*Rhizopogon subcaeruliscens* AHSm.	-	-	21	24	-
2.	*Truncocolumella citrina* Zeller	-	-	12	17	-

Table 3. Indices of ecological diversity of micromycete groups of monitoring sites

Experiment options	Serensen coefficient	Berger-Parker index	Simpson index	Shannon index	Pisl index
0-10 см layer of soil	0.36	3.2	0.67	2.08	0.23
20-40 см layer of soil	0.27	2.5	0.59	1.94	0.30
Treatment with Mikovital, rhizosphere	0.46	5.9	0.31	4.98	0.56
Treatment with Mikovital + Florabacillin + biochar, rhizosphere	0.51	7.2	0.32	5.47	0.62
Control, rhizosphere	0.29	3.8	0.47	2.31	0.29

with Mikovital there were changes in the species composition of the cenosis. Thus, *T. viride, T. harsianum, P. notatum* dominated (60–65%), *M. isabelina, R. chrizogenum* belonged to the typical and frequent (33–53%). The ectomycorrhizal symbiont *Rhizopogon subcaeruliscens* (truffle from the *Basidiomycota* family) was isolated from the rhizosphere of inoculated plants. The introduction of Mikovital and Mikovital + Florabacilin + Biochar into the rhizosphere contributed to the increase of biodiversity of micromycete species. From this biocenosis species of *P. corylophylum, P. lanosum, P. rubrum, P. tardum, Humicola grizea,* ascomycete *Oidiodendron cereale* and another species of ectomycorrhizal symbiont *Truncocolumella citrina* were isolated.

Ecological and systematic analysis of walnut rhizosphere mycocenoses

The analysis of the structure of soil micromycete groups, in which Mikovital and Mikovital + Florabacillin + biochar were used, was carried out. Since the drugs were used in the rhizosphere of plants, we were interested in comparing them with the corresponding values of the same indicators in the rhizosphere. It turned out that the edaphotopes of these zones enrich the species diversity of microscopic fungi, as indicated by an increase of 1.55–1.89 times the values of the Berger-Parker index, 2.16–2.37 times the Shannon index and 1.93–2, 14 times the Pisl index and a decrease of 1.47–1.52 times the Simpson index (Table 3). Similar patterns of species composition were observed during all seasons. Indices of species diversity of micromycetes indicate that the most diverse species composition of mycobiota is observed when using the inoculation of Mikovital + Florabacillin + Biochar.

Molecular ecology methods in the rhizosphere microbiome of the walnut inoculated with Mikovital revealed 20 phylum of bacteria of 83 genera and 6 phylum of fungi of 100 genera of fungi, as well as unclassified sequences, the relative share of which in the microbiome was 3.04–7.8%. Analysis of the taxonomic structure of the bacterial 16S microbiome at the level of phylum showed that the three dominant clades – *Actinobacteriota* (26.41–68.20%), namely *Firmicutes* (5.93–15.32%) and *Proteobacteria* (3.29–8.50%). Analysis of the mycobiome structure (ITS) at the level of divisions with and without the use of mycorrhizal preparation showed the presence of *Ascomycota* (41.01–93.17%) that were dominant in both ecotopes, *Basidiomycota* (2.82–6.40%) and *Monerelomycota* (0.82–0.41%) (Table 2).

It was shown that the rhizosphere soil microbiome was more diverse under the conditions of plant inoculation with strains of *Tuber melanosporum VS1223* and *Vitasergia svidasoma* (Mikovital). Thus, the dominant, with a relative share in the total microbiome were *Thermoleophilla* – 8.44–21.81%, *Actinobacteria* – 12.16–31.48%, *Rubrobacteria* 5.81–14.99%. The *Frankiales* family dominated among bacteria of the *Actinobacteria* class – 5.65–14.6%, *Micrococcales* – 3.99–10.3%.

Subdominants among bacterial phylum were *Firmicutes* – 5.93–15.32%, *Cloroflexi* –3.04–7.86%, *Proteobacteria* – 3.29–8.50%, the least represented was the family *Bacteriodota* – 0.05–0.13%.

The microbiome of the studied soils presents species belonging to genera that play a key role in the formation of a healthy soil environment: for example, the genus *Geodermatophilus* – a family of actinobacteria in the order *Geodermatophiliales* have a complex life cycle and produce stable esterase enzymes [Normand et al., 2015]. They have the ability to withstand adverse environmental conditions such as ultraviolet light, ionizing radiation, desiccation, and heavy metals [Jin & Lee, 2013; del Carmen Montero-Calasanz et al., 2015]. This resistance to environmental hazards is a hallmark of

Terrabacteria, a phylogenetic group consisting of *Actinobacteria* and four other major eubacterial lines (*Firmicutes, Cyanobacteria, Chloroflexi*, and *Deinococcus*).

Nocardioides is a gram-positive, mesophilic and aerobic bacterial genus of the *Nocardioidaceae* family. The ability of *N. sinplex* to decompose a mixture of *Rubrobacter* is described. These actinobacteria have high resistance to the herbicides 2,4-D and 2,4,5-T and are indicators of extreme soil conditions. In the microbiome of the nursery, this genus occupies 5.1–14.9%.

Actinomycetes of the genus *Kriblella* 15% of the total number of microorganisms in the treated sample. It is known that *Kriblella*, actinomycetes that have a greater ability than other groups of organisms to synthesize biologically active substances [Anderson et al., 2000].

Actinobacteriota – are of great economic importance, as agriculture and forests depend on their contribution to soil systems. In the soil, they promote the decomposition of the organic matter of dead organisms *Angustibacter* - actinobacteria, some of which live in symbiosis with plants, fix nitrogen in exchange for vegetable sugars.

As it is known, correct bacterial community present in the soil lays foundation for successful mycorrhizal formation. The microbiome of the mycorrhized nut nursery contains *Marmoricola*, a gram-positive and chemoorganotrophic genus of bacteria from the *Nocardioidaceae* family, which actively colonizes plant roots and is a stimulator of mycorrhizal formation through the synthesis of biologically active substances [Tsavkelova et al., 2006]. On the territory treated with Mikovital and Mikovital+Florabacillin+biochar, representatives of bacterial genera that promote better plant development were found, for example:

Gemmatimonas is a group of bacteria, the number of which increases from tree mulching and biochar application, as they participate in the transformation of organic substances [Zhang et al., 2003].

Azotobacter - bacteria that are of great importance for nitrogen nutrition of plants, fixing nitrogen and turning it into ammonia. The genus *Nitrosospira* carries out the first stage of nitrification – oxidation of ammonium nitrogen to nitrites, oxidizes ammonia. *Nitrospira* – oxidizes nitrites in the second stage of nitrification.

The genus *Paenibacillus* is a representative of the rhizosphere microflora, some strains are endophytes, colonize plant tissues and have bactericidal and fungicidal effects [Grady et al., 2016].

The genus *Bacillus* and *Pseudomonas* are used as a means of biological control of plant diseases, since a large number of strains synthesize biologically active exometabolites: enzymes, pigments, polysaccharides, polyamines, etc.

The genus Comamonas is involved in natural biodegradation processes [Opota et al., 2014].

Among the genus *Proteobacteria* is *Alysoisphaera*, a proteobacterium that causes stem disease. *Pseudohrensia* is a group of bacteria that decompose organic remains and resist pathogens.

Udaeobacter - *«Candidatus Udaeobacter»*, belongs to the most common soil bacteria in the whole world. Representatives of *Udaeobacter* can use nutrients that are released as a result of the lysis of other soil microorganisms under the influence of antibiotics and thereby reduce the energetically expensive synthesis of necessary biomolecules.

In the studies, special attention was paid to the nut rhizosphere mycobiome, since the introduction of mycorrhizal species caused an impact on the rhizosphere mycobiota and the number and species composition of endophytes. A decrease in the level of pathogenic deuteromycetes was observed, and the number of mycorrhizae and species that contribute to humus formation increased. Species causing stem rot diseases were completely displaced from the rhizosphere (Table 2).

CONCLUSIONS

A practical example shows the role of ecosystem maintenance and regulation services for restoring the microbial and mycocenosis of the walnut rhizosphere.

The species composition and structure of micromycete communities in the rhizosphere of walnut seedlings planted on the territory of 3.8 ha on meadow black soil, deep-saline soils were studied, and the peculiarities of the dynamics of their number in connection with the use of the drug Mikovital were established. The effect of *Tuber melanosporum VS1223* and *Vitasergia svidasoma* strains, which are components of the Mikovital drug, on the species composition of soil deuteromycetes and its role as a bioregulator of walnut rhizosphere mycocenosis is shown and proven. Changes in the length of the fungal

mycelium, its biomass, the number of spores and their biomass in the studied edaphotope are shown. Based on the determination of the similarity coefficient of groups, indices of dominance and species diversity, it was proved that when using Mikovital and immobilizing fungi and bacteria in the Mikovital+Florabacillin+biochar complex, a significant reorganization of the mycocenosis is observed in comparison with the walnut rhizosphere soil in the control area. The effectiveness of the use of biochar for the immobilization of cultures of fungi and bacteria has been proven. Adding biochar to the preparation in the amount of 0.2% helps to reduce the content of pathogens and has a positive effect on the microflora of the nut rhizosphere.

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