

Mycological and toxicological analysis of soil under the influence of crop cultivation technologies

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Abstract. The purpose of the research is to study the influence of various crop cultivation technologies on the structure of micromycete complexes and the level of microbial soil toxicosis. Extensive, organic and intensive crop cultivation technologies in a six-field crop rotation on soddy-podzolic gleyic soil for 2021–2022 have been studied. To study the structure of the micromycete complex the deep seeding method, the spatial occurrence index of species, the Syorensen-Chekanovsky similarity coefficient and the significance index were used. To determine the microbial toxicosis, the method of the initiated microbial community according to V.S. Guzev was used. According to the results of the experiments, fungi from the departments of *Zygomycota* and *Ascomycota* were discovered, in 2021 – 10 species, in 2022 – 16 species. In 2022, the largest number of micromycetes was recorded under cereals with extensive and intensive technologies, in 2021, a high number of fungi was recorded in variants with annual and perennial grasses using the same technologies. The greatest manifestation of microbial toxicosis was noted in 2021: the germinating ability of test crop seeds on control variants – 76.67-92.67%, when initiating a microbial community – 44.67-76.67%. The cultivation of grain crops by extensive and intensive technologies contributed to the greater manifestation of soil microbial toxicosis.

1 Introduction

The soil is daily affected by a wide range of chemical compounds coming from various sources [1]. Today, almost everywhere against the background of natural impacts on soil processes, anthropogenic ones are observed. Human economic activity is a powerful and intensive factor of influence on the soil.

Crop cultivation technologies, especially with the use of various types of fertilizers and chemical plant protection products affect fundamentally on the chemical, physico-chemical and biological properties of the soil, its water-air and thermal characteristics, can change the localization of microorganisms and energy material in the arable layer, as well as

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communities of soil micromycetes, rearrange and narrow their specific diversity, influence the intensity of microbiological processes, including toxicity, which ultimately leads to a decrease in the level of effective fertility.

Changes in soil mycocenoses should be assessed as negative. For example, the abundant development in the soils micromycetes of the genera *Aspergillus* and *Penicillium* in the literature is often associated with microbial toxicosis of soils. In addition, species of the genera *Alternaria*, *Cladosporium*, *Fusarium*, *Moniliella*, *Verticillium* have pronounced phytopathogenic properties. These data are in good agreement with the thesis about the redistribution of the degree of dominance between actively functioning microorganisms in the soil under the influence of various anthropogenic loads [2-5]. As a result of such changes, "microbiological soil fatigue" occurs and crop productivity decreases [6].

Therefore, the search for a combination of agricultural practices of crop cultivation that ensure the containment of negative impacts, the preservation and increase of soil fertility, obtaining high yields is of great importance.

Thus, the aim of the research was to study the influence of various crop cultivation technologies on the structure of micromycete complexes and the level of microbial soil toxicosis.

2 Materials and Methods

The experimental work was carried out in 2021-2022 in a two-factor stationary field test laid out on the experimental field of the FSBEI HE «Yaroslavl State Agrarian University» on soddy-medium podzolic medium loamy gleyic soil on a calcareous moraine.

The scheme of the field stationary two-factor (6 × 3) experiment and the conditions of the research site were set forth by us earlier [7].

Fertilization and soil treatment were carried out according to the descriptions given in the cultivation technologies.

Straw from previous grain crops was used according to variants and was covered with the first treatments for crops. Complex mineral fertilizer (azofoska NPK 16:16:16) was applied on variants with intensive technology of growing field crops in the norm N₆₀P₆₀K₆₀.

In 2021, the herbicide Deimos was used in options with intensive technology for growing grain crops at a rate of 0.25-0.3 l/ha, and in 2022, the herbicide Agritox at a rate of 1.0 l/ha. Spraying of crops was in the tillering phase of the crop before entering the tube in spring.

The separation of soil fungi was carried out by the deep sowing by diluting the soil suspension 1:1000 on Czapek's agar nutrient medium according to generally accepted methods [8]. The structure of micromycete complexes was characterized by the spatial occurrence of species. To assess the species diversity of the complexes the Syorensen-Chekanovsky similarity coefficient and the significance index were used [9,10]. To determine microbial toxicosis of the soil, the method of initiated microbial community (IMC) according to V.S. Guzev was used [10]. The yield of all field crops was taken into account by a continuous plot method with conversion to an absolute net production and standard grain moisture of 14% and green mass – 60%. The programs "DISANT" and "MicrosoftExcel" were used for statistical processing of experimental data.

3 Results

According to the results of the studies, micromycetes from the *Zygomycota* and *Ascomycota* Departments were found in the soil of the test area. In 2021, 10 species of soil fungi were discovered, and in 2022, the species diversity increased to 16.

The obtained data indicate that saprotrophic forms of fungi dominate in the soil. In 2021, the fungi of the genus *Aspergillus* were dominant which is not typical for soddy-podzolic soils. In 2022, the proportion of species of the genus *Penicillium*, more common for this type of soil, increased.

Within the genus *Aspergillus* there are various ecological groups, including saprotrophs that decompose polymers (cellulose) and various oligotrophic compounds. There are works that note the toxicity of species *A. flavus*, *A. fumigatus*, *A. niger* and *A. ustus* for soil invertebrates [11-14].

In our experiments, the genus *Aspergillus* was represented by species *A. flavus*, *A. niger*, *Aspergillus sp.1* and *Aspergillus sp.2*. In 2021, they dominated to one degree or another, more often *A. flavus*, whose significance index (SI) ranged from 0.3 to 2. *A. niger* dominated in some variants, with a significance index of 0.15-1.1; most often values were below 0.5 in *A. sp.1* and *A. sp.2*, the significance indices of which were 0.2-0.73, did not occupy a dominant position in the complexes. In 2022, the same representatives of this genus were discovered. However, the importance of species has changed. For example, *A. flavus* was recorded only under barley crops with underseeding of perennial grasses (SI 0.15-0.8) and sporadically under clover-timothy mixture (SI 0.83). *A. niger* was found on variants with cereals, perennial grasses of 1 year of use, and was practically absent in crops of perennial grasses of 2 year of use. Species *A. sp.1* and *A. sp.2* had a significance index of 0.15 to 0.5.

Species of the genus *Penicillium* in 2022 were found by us quite often and had wide values of the significance index 0.14-1.28. In the soil under the crops of perennial grasses of 1 year of use, they were dominant. At the same time, no clear dependence on crop was found. Under conditions of elevated temperatures during the growing period in 2021, penicilliums were found only under barley crops (SI of 0.52). Genus *Penicillium* includes various saprotrophs, parasitic and phytotoxic forms [15]. Penicilliums are typical cosmopolitans, although they are characteristic of the northern regions of the taiga zones of podzolic and soddy-podzolic soils.

The highest number of micromycetes in the soil layer of 0-10 cm in 2022 was observed under barley crops using both extensive and intensive technologies (Fig. 1). In the soil layer of 10-20 cm, both with extensive and intensive technologies, the highest numbers were under spring wheat crops.

Under the sowing of perennial grasses, the number of soil fungi was generally slightly lower than under cereal crops. In the case of comparing the number of micromycetes under the sowing of perennial grasses, it was higher throughout the entire plough-layer on the variants with perennial grasses of 1 year of use when using intensive cultivation technology, while with extensive technology it was higher only in the topsoil.

A comparison of the number of fungi obtained in 2022 with the data of the previous year showed that in the first year of the research the opposite picture was formed. In 2021, the minimum number of micromycetes was observed in the soil of the plough-layer under the sowings of spring barley with undersowing of perennial grasses. Higher numbers of fungi occurred in variants with annual and perennial grasses (Fig. 1).

In spring wheat crops the number of micromycetes varied greatly by layers. With the use of intensive cultivation technology of this crop the number of soil fungi increased more in the layer of 0-10 cm.

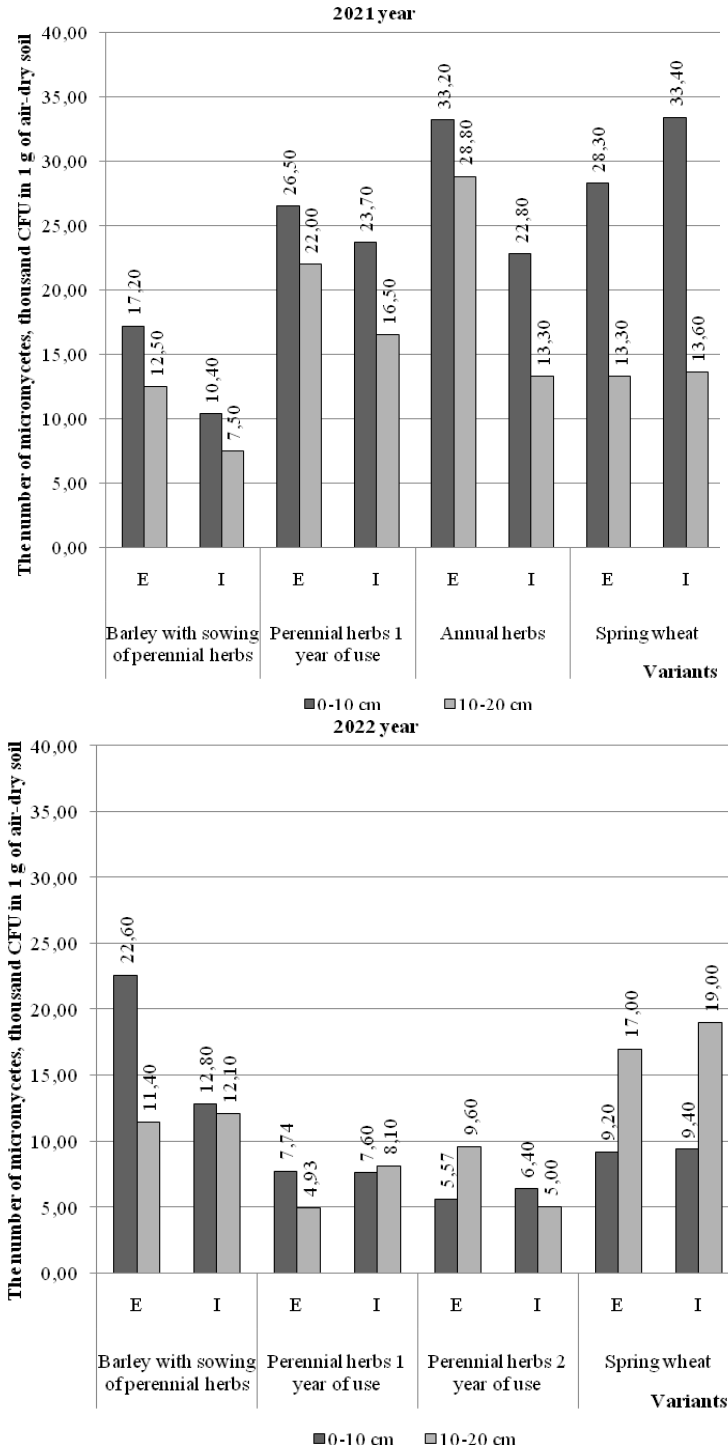


Fig. 1. The number of micromycetes in the soil under various crops of crop rotation in 2021–2022, thousand CFU in 1 g of air-dry soil.

When cultivating other crops – barley, annual and perennial grasses in the variants of intensive technology, the number of soil micromycetes was lower than in the extensive technology. The analysis of variance showed that, on average, for the factors under study, the noted influence of the applied technologies was significant only for the substrate.

In general, it should be noted that there was a decrease in the number of micromycetes in the experimental variants in 2022 compared to 2021.

One of the informative indicators for assessing the state of micromycete complexes is the spatial frequency of occurrence.

In 2022, under barley crops with extensive technology, *Penicillium sp.1* dominated in the fungal complex, *A. flavus* and *P. sp.2* were classified to the group of frequent occurrence. Species of *Rhizopus sp.*, *A. niger*, *Phialophora sp.*, *Trichoderma viride* were among the rare. When using intensive technology the same type of penicillium dominated, *A. niger* has moved into the group of frequent occurrence.

In 2021, the complex looked different due to the dominance of species *A. flavus* and the often found *Mucor hiemalis*. The group of rare species included *A. niger*, *Penicillium sp.*, *Cladosporium herbarum*, and others.

Under the sowings of perennial grasses of 1 year of use *Penicillium sp.2* occupied the dominant position, *Rhizopus sp.*, *A. niger*, *A. flavus*, *Phoma sp.*, *Trichoderma viride* are classified as rare in turn. In the previous year of the study, *A. flavus* was dominant, *Mucorhiemalis* and *A. sp.1* were included in the group of frequently occurring species. Only *A. niger* was classified as rare species.

The fungi complex under the clover-timothy mixture for 2 years of use has been depleted. In addition to the dominant *P. sp.2*, the smallest number of rare species was found here compared to the complex under other crops.

The same species dominated in the soil under spring wheat crops as under barley. Completely different fungi were included in the group of frequently occurring ones *A. niger* and *Rhizopus sp.* In 2021, the complex under spring wheat was represented by several types of aspergillus, Mucorales, trichoderma and cladosporium were rarely found.

In the complexes of micromycetes under the vetch-oat mixture in 2021, as well as under other crops, *A. flavus* dominated, other types of aspergillus and Mucorales were often found.

Most of the biomass of microbial cenosis of soils is made up of micromycetes (86-94%), which mainly determine the direction and activity of toxicological processes in the soil. Microorganisms forming phytotoxic substances began to be studied relatively recently, but their widespread distribution and a large role in causing soil toxicosis indicate how important the problem of their study is [16]. Such works are of particular importance in connection with the intensification of agriculture.

The level of microbial toxicosis was assessed according to the gradation: a low degree was assigned to variants when the germination of test culture seeds was 76% and higher, an average degree - if the germination fit into the parameters of 50-75%, a high degree – if the germination was 49% and lower, respectively [17].

Germination of test culture seeds showed that the greatest manifestation of microbial toxicosis was in 2021 (Table 1). Thus, the germination of test culture seeds on control variants in the soil layer of 0-20 cm varied within 76.67-92.67%, which corresponds to an average and low level of a sign or absence of toxicosis. The initiated microbial community increased soil toxicosis to a medium and high degree (the germination of radish seeds was 44.67-76.67%, which confirmed the influence of soil microflora on the development of soil toxicity).

Table 1. Assessment of microbial toxicosis of soil under various technologies of field crops cultivation by germination of test culture seeds (radish) in the soil layer 0-20 cm, %.

| Experiment Options | | IMC Option | 2021 | | 2022 | |
|--|---------------------------------------|------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Factor A. Crop rotation crop | Factor B. Crop cultivation technology | | Germination of seeds, % | Degree of Soil Toxicity | Germination of seeds, % | Degree of Soil Toxicity |
| Barley with undersowing of perennial grasses (2021) – perennial grasses for 1 year of use (2022) | Extensive | control | 92.67 | Missing | 81.33 | Low |
| | | starch | 49.33 | High | 84.67 | Low |
| | Organic | control | 92.67 | Missing | 84.67 | Low |
| | | starch | 70.00 | Average | 81.33 | Low |
| | Intensive | control | 76.67 | Average | 82.67 | Low |
| | | starch | 44.67 | High | 92.00 | Missing |
| Perennial grasses for 1 year of use (2021) – perennial grasses for 2 years of use (2022) | Extensive | control | 91.33 | Missing | 78.00 | Low |
| | | starch | 58.00 | Average | 86.00 | Low |
| | Organic | control | 88.00 | Low | 91.33 | Missing |
| | | starch | 76.00 | Low | 84.67 | Low |
| | Intensive | control | 92.00 | Missing | 91.33 | Missing |
| | | starch | 55.33 | Average | 93.33 | Missing |
| Annual grasses (2021) – spring wheat (2022) | Extensive | control | 85.33 | Low | 80.00 | Low |
| | | starch | 61.33 | Average | 90.67 | Missing |
| | Organic | control | 90.00 | Missing | 74.33 | Average |
| | | starch | 58.67 | Average | 80.67 | Low |
| | Intensive | control | 86.67 | Low | 90.00 | Missing |
| | | starch | 47.33 | High | 92.67 | Missing |
| Spring wheat (2021) – barley with undersowing of perennial grasses (2022) | Extensive | control | 92.67 | Missing | 89.33 | Low |
| | | starch | 46.67 | High | 83.33 | Low |
| | Organic | control | 92.00 | Missing | 76.00 | Low |
| | | starch | 53.33 | Average | 78.67 | Low |
| | Intensive | control | 84.67 | Low | 84.67 | Low |
| | | starch | 67.33 | Average | 83.33 | Low |

In 2022, according to the to the experimental variants under study, the germination of test culture seeds in the soil layer of 0-20 cm varied on the control variants - 74.33–91.33%, on the induced variants - 80.67–93.33%, which corresponds to a low degree of microbial toxicosis or its absence.

Analysis of the data obtained as a result of measuring the length of the seedling and root of the test culture showed that the values were recorded similarly to germination in both 2021 and 2022.

The productivity of crops is one of the main indicators determining the effectiveness of various cultivation technologies (Table 2)

Table 2. Crop yieldin centners of feed units/ha.

| Cultivation technology | 2021 | | 2022 | |
|------------------------|--|-----------|--------------------------------------|-----------|
| | Crop | c.f.u./ha | Crop | c.f.u./ha |
| Extensive | Barley with undersowing of perennial grasses | 11.32 | Perennial grasses for 1 year of use | 29.89 |
| Organic | | 10.60 | | 31.62 |
| Intensive | | 15.21 | | 46.44 |
| Extensive | Perennial grasses For 1 year of use | – | Perennial grasses for 2 years of use | 47.75 |
| Organic | | – | | 56.94 |
| Intensive | | – | | 56.94 |
| Extensive | Annual grasses | 14.02 | Spring wheat | 10.56 |

| Cultivation technology | 2021 | | 2022 | |
|--------------------------------|--------------|-----------|--|-----------|
| | Crop | c.f.u./ha | Crop | c.f.u./ha |
| Organic | Spring wheat | 12.78 | Barley with undersowing of perennial grasses | 9.65 |
| Intensive | | 18.65 | | 11.02 |
| Extensive | | 7.99 | | 18.25 |
| Organic | | 8.42 | | 8.08 |
| Intensive | | 15.03 | | 21.06 |
| HCP ₀₅ for Factor A | | 8.61 | X | 40.52 |
| HCP ₀₅ for Factor B | | 5.45 | X | 24.89 |

When studying the yield of crops under study, it was found that it depended on the prevailing meteorological conditions of the year, cultivation technology.

Considering the influence of the studied cultivation technologies, it can be noted that all the studied crops formed the largest collection of feed units per unit area when they were cultivated using intensive technology with the use of fertilizers and plant protection products in the prevailing weather and climatic conditions of the study years. But the changes were in the nature of a trend.

However, the calculation of economic efficiency showed that the lowest cost of production was noted during the cultivation of crops using organic technology – 709.39 rubles. / cf.u. and the highest net income – 701.64 rubles per 1 ha, as well as the most profitable – 159.06% compared with extensive and intensive technologies, where net income and profitability in the cultivation of grain crops turned out to be negative.

4 Discussion

Analyzing the taxonomic composition of micromycetes of the test area over two years of research, it can be noted that in the first year of research, the greatest species diversity was observed under the sowing of spring wheat, the smallest – under spring barley with the undersowing of perennial grasses, in both cases using extensive technology. Species of the genus *Aspergillus* have been massively developed, which we associate to a certain extent with the elevated temperatures of the soil sampling period. In the second year of research, a large number of micromycete species were found in the soil not only under wheat, but also barley, both with extensive and intensive technologies. The maximum species diversity was observed in the substrate when applying mineral fertilizers for perennial grasses (9 species).

When determining the similarity coefficient of Syorensen-Chekanovsky in 2022, the greatest differences were noted in complexes of micromycetes under the sowings of wheat and perennial grasses of 2 years of use (31%). They were slightly smaller when comparing soil data under perennial grasses of 2 years of use and barley (43%). In 2021 studies, the coefficient values varied 65-92%, which indicates that the complexes of soil fungi were practically indistinguishable, which may be associated with the mass development of *aspergillus* in the soil of all variants.

When comparing two-year data, it was noted that crops influenced the structure of soil fungal complexes. The formation of these differences can be explained by the physiological and biochemical characteristics of plants, including different chemistry of rhizodeposites, and, accordingly, different substrate for the development of fungi.

The most pronounced microbiological toxicosis was observed for extensive (germination on control variants is 85.33-92.67%, on starch variants – 46.67-61.33%) and intensive (germination on control variants is 76.67-92.00%, on starch variants – 44.67-67.33%) cultivation technologies of all crops, which in our opinion is associated with the conditions that add up on these technologies provoking the development of toxicogenic forms of microorganisms, especially hydrolytics.

The induced microbial community on the soil sampled from plots using organic technology for cultivating field crops contributed to a decrease in the number of ungerminated seeds due to the application of organic fertilizers, which leads to a decrease in the number of phytopathogenic and toxigenic microorganisms in the soil. A similar opinion was noted by M.S. Sokolov and coauthors [18], as well as V.R. Slavkin [19].

In 2022, in the second year of the factors' action, the effects of microbial systems of both variants of the induced microbial community were equalized regardless of anthropogenic load, which is confirmed by the research of A.V. Kurakov [20].

Grain crops of crop rotation contributed to a greater manifestation of microbial toxicosis of the soil. Under the grasses the best conditions for the inactivation of toxins were created. Thus, it is necessary to take into account the detected phenomena in agronomic practice, since provoking an increase in pathogenic microflora can affect the development of such a negative phenomenon as soil "fatigue."

In general, the yield of field crops was at a low level, which could be due to unfavorable weather conditions of the growing season in both years of research.

Our calculations of the comparative economic efficiency of crop production using three technologies allow us to conclude on the economic efficiency of organic technology for crops. The reason for the higher economic indicators in this variant was lower costs per 1 hectare, compared to intensive ones, where high costs are associated with the purchase and application of mineral fertilizers, plant protection products, as well as with the harvesting and transportation of large volumes of products, which affected its cost.

In unfavorable agro-climatic conditions of the Yaroslavl region, the use of intensive grain production technologies is necessary to obtain the planned yields. Intensive production technology allows you to get more output from a unit of processed area and allows you to quickly recoup production costs. Nevertheless, in the modern world, food production in natural conditions is becoming a global competitive advantage. Organic products of agricultural production are the most environmentally friendly, are in high demand both within the region and beyond. Therefore, to make a profit using organic technology, it is necessary to use varieties and hybrids of crops resistant to adverse conditions, diseases and pests, assess agro-climatic conditions, apply environmentally friendly technologies for the preservation and reproduction of soil fertility, use modern multi-operational agricultural machinery, set the price of products produced using organic technology higher than usual, this is due to the need to cover higher costs for its production and sale.

5 Conclusion

In conclusion, it should be noted that in the conditions of field stationary test in 2021-2022 by studying the state of the soil fungi structure under crops using various technologies, it was revealed that the complexes are depleted, in some variants their structure is disturbed, phytopathogenic species are found, in connection with which monitoring studies of the state of agrocoenosis are necessary.

It has been established that crops, different in biology and cultivation technologies, differently affect the processes of formation of toxic properties of soddy-podzolic gleyic soil caused by microorganisms. Crops with high intensity of cultivation technologies (barley, spring wheat) increase microbial toxicosis of the soil to a greater extent than crops that do not require high doses of fertilizers and the use of pesticides (perennial and annual grasses).

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