The quantitative composition of micromycetes under cereal crops in chernozem soils in the Left-Bank Forest Steppe of Ukraine


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Introduction

The rhizosphere of plants is a dynamic environment in which there are many factors that determine the structure and composition of the microorganisms (Bruinsma et al., 2003; Kopylov, 2012). Over the recent decades, there has been a deterioration of the ecological balance between the organisms of the rhizosphere soil, driven by incorrect use of cultivation technology and by agro-climatic conditions (Persica et al., 2002). Therefore, the relevance and necessity of biological monitoring is increasingly confirmed and justified, namely the study of biological diversity and the processes and biological properties of soil, and is a complex microbiological system. According to Mishustin (1972), Patyka (2013) and Dernya-ryuk (2018), the content of microorganisms in 1 g of soil is billions of cells characterized by an extremely high diversity of species composition. However, according to Demirel (2005), soil microscopic fungi are mostly aerobes that inhabit the upper layers of the soil (0–20 cm), where their numbers can vary (from 50 to 350 and more CFU thousand/g of soil). In layers below 20 cm, the number of fungi is lower by 40–60%, due not only to lack of aeration, but also low content of organic matter (Polianskaya, 2012). Microbial groups mainly determine soil fertility, growth, and development of agricultural plants, participating in such important processes as the transformation of plant residues and humus formation, provision of plants with nutrients and the nutrient cycle (Pandey, 2018). Therefore, the presence of various groups of microorganisms, which differ in biological and biochemical specificity, in soil ecosystems determines their important role in soil processes (Shevchenko, 2006).

Soil microorganisms are an important component of agroecosystems, which due to physiological and genetic features respond quickly to changes in the quality of the soil environment. Each plant in the rhizosphere forms a specific composition of the microflora which depends on the phase of plant development and soil-climatic conditions. The objective of our study was the quantitative composition of ecological and trophic groups of rhizosphere soil micromycetes of different crops in chernozem soils in Left-Bank Forest Steppe of Ukraine. According to the results of research, it was determined that the rhizosphere soil under different crops – winter wheat, rye and oats in Chernihiv region – is characterized by the largest number of pedotrophic micromycetes. This indicates that the soil contains a sufficient amount of organic matter. The rhizosphere soils under winter wheat and spring barley in Kiev region were characterized by a larger number of pathogenic micromycetes and arylolytic and cellulolytic ecological-trophic groups. This indicates the intensive use of plant protection products. The rhizosphere soil under onions in experimental fields in Kharkiv region was characterized by a high number of the celluolytic group. This indicates the presence of cellulose-destroying microorganisms. According to the results of statistical analysis, it was found that the number of micromycetes in the rhizosphere soil of the studied varieties of crops was in direct correlation with the value of the hydrothermal coefficient (HTC) in the vegetation period. Weather conditions during the research vegetation period differed by agrometeorological indicators. The characteristic feature was a contrast of differences in air temperature and unequal distribution of rainfall, which affected the composition of the soil mycobiocenosis. The vegetation period of 2021 in Kyiv region was characterized by a sufficiently moist hydrothermal coefficient which increased to 1.81 while in Chernihiv, Kharkiv regions drought prevailed, but in some months the HTC increased to 1.52–1.54. It has been shown that the higher the HTC; the greater the number of micromycetes in all study regions.

Keywords: soil mycobiota; agroecosystem; number of micromycetes; hydrothermal coefficient; the root secretions of the plants; correlation coefficient.
acids, amino acids, peptides, alkaloids, glucosides, vitamins, phenolic substances, etc. (Pida et al., 2003; Temovy et al., 2018). The organic acids include malic, succinic, tartaric, citric, fumaric, oxalic and other acids. Root secretions, in turn, are a food substrate for other components of the soil biocenosis, in particular, micromycete fungi, which reproduce intensively in the root zone of plants (Ellanskaya et al., 2017). The rhizosphere of plants is a dynamic environment in which many factors determine the structure and composition of microorganisms that colonize it. Soil microflora is an obligate component of biocenosis, where interactions occur between plants and microorganisms: metabolism, their transformation, interaction of stressors (increase or decrease in nutrients in the soil) they can be used to assess the degree and pattern of soil contamination. Therefore, the objective of our study was the quantitative composition of ecological and trophic groups of rhizosphere soil micromycetes of different crops in chernozem soils of Left-Bank Forest Steppe of Ukraine.

Table 1

<table>
<thead>
<tr>
<th>Institution</th>
<th>Region</th>
<th>Type of soil</th>
<th>Hydrothermal coefficient (HTC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skvya Research Station of Organic Production of NAAS</td>
<td>Kyiv</td>
<td>chernozem soils deep low-humus slightly leached medium loam</td>
<td>1.72</td>
</tr>
<tr>
<td>Nosiv Selection Research Station Institute of Vegetable and Melon Growing NAAS</td>
<td>Chernihiv</td>
<td>chernozem soils deep low-humus leached</td>
<td>0.63</td>
</tr>
<tr>
<td>Kharkiv chernozem soils</td>
<td>Kharkiv</td>
<td>chernozem soils medium-strong and low-power leached, medium loam</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Note: HTC ≥ 1 – sufficient moisture; HTC = 0.8–1.0 – moderate hydration; HTC = 0.6–0.7 – insufficient hydration.

From the results of the calculation of HTC, which are presented in Table 1, it can be concluded that the vegetation period of 2021 in Kyiv Region was characterized by sufficient moisture (HTC 1.81), while drought prevailed in Chernihiv and Kharkiv Regions. However, in some months, the HTC increased to 1.31 in Chernihiv Region and to 1.53 in Kharkiv Region. Adverse weather conditions such as drought or waterlogging are crucial factors for changes in trophic links between micromycetes and other components of the microbiocenosis. Hydrothermal conditions influenced the quantitative composition of ecological and trophic groups of micromycetes in the studied soils.

Materials and methods

We studied the quantitative composition of the soil micromycetes under different crops: winter wheat of three varieties (Podolyanka, Knyazhna, Yuvivata 60), two oat varieties (Sviadok, Ternber), spring barley of two varieties (Sebastian, Helio), rye of two varieties (Synthetic, Donor) and onions of four varieties (Tkachenkivska, Mavka, Liabhryk, Armpoa). The study was conducted in 2021 on chernozem soils in the Left-bank Forest Steppe of Ukraine, namely in the fields of the Skvya Research Station of Organic Production (Kyiv regions), Nosiv Selection Research Station (Chernihiv region) and the Institute of Vegetable and Melon Growing (Kharkiv region). Soil sampling for the laboratory analysis was carried out in accordance with the state standard of Ukraine 7847 (2015). To determine the amount of microorganisms in the soil from the study area from 3 to 7 individual samples weighing 100–200 g were selected and averaged soil sample was prepared. Soil samples were taken in three phases of ontogenesis for cereals (fillering, flowering and ripening) and onion (3 true leaves, bulb development, and ripening). Weather conditions during the vegetation period of the research differed by agrometeorological parameters. To characterize the hydrothermal conditions, the hydrothermal coefficient of Selyaninov (2000) was calculated for the territories, which determined the total amount of rainfall in the growing period.

The data of regional meteorological stations (Kyiv, Chernihiv, Kharkiv regions) was used to study the influence of weather conditions on the number of soil mycobiomes under different crops (Table 1).

Fig. 1. Micromycetes of the main ecological-trophic groups on standard growth media:

- a – amylolytic – starch-ammonia agar (SAA),
- b – cellulolytic – Hutchinson’s and Clayton’s,
- c – oligotrophic – starvation agar (SA),
- d – pathogenic micromycetes – Chapek’s medium,
- e – pedotrophic – soil agar (SA),
- f – humate-forming – humate medium (HE),
- g – cellulolytic – Hutchinson’s and Clayton’s,
- h – amylolytic – starch-ammonia agar (SAA),
- i – oligotrophic – starvation agar (SA),
- j – humate-forming – humate medium (HA)
To determine the number of amylolytic, oligotrophic, pedotrophic, humified microorganisms and micromycetes, we used the method of deep inoculation. Using a sterile pipette, we transferred 1 cm³ of the suspension to a Petri dish. Then, molten agar that had been cooled to 45–50 °C was poured into the Petri dish and the inoculum was carefully mixed with the cell suspension in a circular motion. The number of parallel-inoculated Petri dishes should be no less than 3. For the study, we used dilutions of 10⁻³, 10⁻⁵, 10⁻⁷. Petri dishes were incubated at 28 °C for 3–7 days depending on the growth rate of microorganisms (Zvyagintsev, 1991; Markov et al., 2012).

While preparing the dilution, the test sample for the control was inoculated in 1 mL of water in a Petri dish with the addition to the growth medium. Also, the agar was poured to the control dish. Both control dishes were inoculated under the same conditions as samples of soil dilutions. At the end of the incubation period, agar should have no visual signs of microorganisms growth. Cellulolytic microorganisms were inoculated as follows: from each dilution of the soil suspension, starting from the highest, we took 1 cm³ using a sterile pipette and transferred it to the liquid medium. The number of parallel-inoculated tubes of a certain dilution should be at least 3. The inoculated test tubes were incubated in a thermostat at 28 °C for 3–4 weeks.

For the control and experimental samples, we calculated mean arithmetic values and standard deviations. If the coefficient variation value exceeded 15%, even for one of the samples, the experiment was repeated. When counting colonies, we chose reproductions not exceeding 50–150.

The number of colonies that had grown was counted using a SCAN-40000 automatic counter (Interscience, France). In cases of higher numbers of colonies and their even spread, the bottom of the Petri dish was divided into 4 or more equal sectors to count the number of colonies in two or three sectors (but not less than 1/3 of the dish surface), the arithmetic mean of the colonies was determined and multiplied by the total number of sectors per dish. The number of microorganisms per 1 g of dry soil in CFU was calculated according to the technique (Mirkhink, 1988).

The number of cellulolytic microorganisms was determined by calculation of the most operative amount (MOA) of cells in units of volume of the original substrate according to the McCready table. The results were expressed in colony-forming units (CFU) in 1 g the studied soil sample (Zvyagintsev, 1991).

The isolates of microscopic fungi were identified to genus and species using a DINO-2000 biological microscope. Phytopathogenic fungi were identified according to the guides (Pitt, 2009; Watanabe, 2010; Guarro, 2012; Colin et al., 2013; Koval et al., 2016). The Latin names of fungi are consistent with the Fungal Databases Nomenclature and Species Banks (www.mycobank.org).

We performed single-factor analysis of variance (ANOVA) and Turkey test. The difference between the control and experimental parameters was considered significant when the probability of the difference was P < 0.05. We determined the correlation dependence (Kaminsky et al., 2011) between the number of micromycetes in the rhizosphere soil and the value of the HTC.

Results

On the research fields of the Nosiv Selection Research Station (Chernihiv Region), in the rhizosphere of winter wheat, oats and rye, we observed an increase in the number of all ecological-trophic groups in the flowering phase when the hydrothermal coefficient was 1.31, indicating a drought (Fig. 2).

During the ontogenesis of the plants, the largest group was pedotrophic micromycetes, ranging from 14.20 to 9.11 × 10⁶ CFU/g of soil. The number of pedotrophic micromycetes was found in soil under winter wheat, where their number reached 19.10 × 10⁶ CFU/g of soil and the lowest number was under rye sowing – 10.31 × 10⁶ CFU/g of soil. Therefore, we believe that the quantitative composition of the microorganisms may be influenced by metabolites of agricultural crops. At the same time, the cellulolytic groups of micromycetes significantly increased, ranging from 0.22 to 1.91 × 10⁷ CFU/g of soil. This indicates the presence of cellulose-destroying microorganisms that are able to decompose cellulose more intensively. The largest number of cellulolytic micromycetes was characterized by rhizosphere soil under rye, equaling 1.91 × 10⁷ CFU/g of soil, the smallest number was under oats – 1.11 × 10⁶ CFU/g of soil. We determined that the most active cellulolytic microorganisms were Penicillium, Stachybotrys, Aspergillus, Cladosporium, Fusarium, which are able to decompose specific substances and do not require a large amount of nutrients, thus providing an opportunity for the development of other micromycetes. Thus, pathogenic micromycetes in the rhizosphere soil under crops increased in the direction oats-wheat-rye, where their number ranged 6.21 to 8.12 × 10⁶ CFU/g of soil. Also, we found a small number of amylolytic groups under rye, their number was the highest and amounted to 3.81 × 10⁶ CFU/g of soil, indicating the presence of freely hydrolyzed organic substances. Thus, the number of basic ecological-trophic groups the micromycetes under the different crops differed significantly, the greatest diversity of amylolytic, cellulolytic and pathogenic micromycetes was seen in soil under rye. The largest number pedotrophic micromycetes was in soil under winter wheat.

Also, the studies were conducted based on the stationary field experiments in the Skvyra Research Station of Organic Production (Kiev Region). The number of micromycetes of the main ecological-trophic groups in the soil was determined during the ontogenesis of different agricultural crops: winter wheat of Podolyanka variety and spring barley varieties Sebastian and Helios.

During the ontogenesis of the plants, we observed increase in the number of all ecological-trophic groups in the flowering phase, when the hydrothermal coefficient equaled 1.81, which indicates high moisture. In the ripening phase, the system reached climax – a stable balanced state, and the number of micromycetes did not increase, the hydrothermal coefficient was 0.83, indicating sufficient moisture (Fig. 3).

In all ontogenesis phases, in the rhizosphere soil under the spring barley and winter wheat, we observed a significant increase in the number of pathogenic micromycetes, ranging 9.21 to 14.41 × 10⁶ CFU/g of soil. The composition of rhizosphere micromycetes is important for the formation of plant productivity, since they can have beneficial, neutral, or harmful connections with roots. Also, a high number of pedotrophic groups of micromycetes was under spring barley, their number reached 9.01 × 10⁶ CFU/g of soil, under winter wheat – 9.51 × 10⁶ CFU/g of soil. This indicates that soil contains a sufficient amount of organic matter.

There was a significant increase in groups of micromycetes that grow slowly and begin to develop after all easily available carbohydrates are used: Trichoderma, Fusarium, Aspergillus, Penicillium, which can decompose specific human substances. During the ontogenesis of barley, in the rhizosphere soil, their number ranged 4.41 to 5.61 ×10⁶ CFU/g of soil, while under winter wheat it was 7.11 × 10⁶ CFU/g of soil on average. Also, during the ontogenesis of winter wheat and spring barley, a small number of the mycobionts of rhizosphere soil was observed for the following groups: amylolytic 2.12 × 10⁶ CFU/g of soil, cellulolytic 1.51 × 10⁶ CFU/g of soil, which perform degradation of cellulose-containing substrates in presence of enzymes. They do not require a large number of nutrients, but thus provide an opportunity for the development of other mycorrhizomes that absorb products of decomposition. Thus the rhizosphere soil under spring barley crops was characterized by a larger number of pathogenic micromycetes, as well as amylolytic and cellulolytic ecological-trophic groups. At the same time, the rhizosphere soil under winter wheat was characterized by a larger number of the pedotrophic and humid-forming ecological-trophic groups of micromycetes.

Based on the stationary field experiments at the Research Station of the Institute of Vegetable and Melon Growing (Kharkiv Region), we determined the number of soil micromycetes of the main ecological-trophic groups during the ontogenesis of different varieties of onion.

During the growing season, we observed an increase in the number of all ecological-trophic groups in the phase of bulb development, when the hydrothermal coefficient was 1.53, indicating high moisture level. In the ripening phase, the number of micromycetes almost did not increase, the hydrothermal coefficient was 0.31, indicating severe drought in this period of plant development (Fig. 4).
Fig. 2. The number of microorganisms in rhizosphere of soil under various agricultural crops during ontogenesis: a – tillering, b – flowering, c – ripening (x ± SD, Tukey test, n = 5); letters a – c indicate statistically significant (P < 0.05) differences in the number of microorganisms within a group.

Fig. 3. The number of microorganisms of rhizosphere of soil under various agricultural crops during ontogenesis: a – tillering, b – flowering, c – ripening (x ± SD, Tukey test, n = 5); letters a – c indicate statistically significant (P < 0.05) differences in the number of microorganisms within a group.
It should be noted that despite the decrease in the hydrothermal coefficient, the cellulosolytic group of microorganisms increased by the end of the vegetation period. In the phase of ripening, the rhizosphere soil under the pungent varieties of onions (Lyubchyk, Tkachenkivska) contained a smaller number of cellulose-destroying micromycetes − 0.51 to 6.51 × 10^6 CFU/g of soil. At the same time, semi-pungent varieties of onions Mavka and Amphora accumulated cellulosolytic micromycetes, which amounted to 0.91−7.35 × 10^6 CFU/g of soil. By decomposing cellulose, cellulosolytic microorganisms exude certain enzymes into the environment, promoting the formation of humic substances from the products of cellulose breakdown, which are consumed by other representatives of soil biocenosis. A significant role in this process is played by fungi, including some saprotrophic genera: Trichoderma, Chaetomium, Dicoccom, Stachybotrys, Penicillium, Aspergillus, Alternaria. Therefore, a large amount of fungi that destroy cellulose leads to increased development of humate-forming and pedotrophic ecological-trophic groups. A small share of the mycobiota of rhizosphere soil under onions comprised amylolytic ecological-trophic group − 0.32–0.61 × 10^6 CFU/g of soil. In the presence of enzymes that group is able to decompose complex polysaccharides, which are often present in plants. Therefore, this group of micromycetes, similarly to cellulose-destroying fungi, can infect plants.

The highest development of pathogenic micromycetes was observed in the phase of bulb formation – 0.61 to 0.70 × 10^6 CFU/g of soil under pungent varieties of onions and 0.71–0.92 × 10^6 CFU/g of soil in rhizosphere soil under semi-pungent varieties of onions. That is due to sufficient humidity and optimal temperature (HTC – 1.53) for the development of the soil mycoflora.

At the end of vegetation, there was a decrease in the number of this ecological and trophic group – 0.45–0.58 × 10^6 CFU/g of soil. The exception was Lyubchyk variety: in the rhizosphere soil – despite the drought – we observed an increased number of micromycetes − 0.71 × 10^6 CFU/g of soil. Therefore, there is a reason to believe that root exudes of plants have a significant effect on spread of certain groups the microorganisms in the soil.

According to the results of research, the hydrothermal coefficient changed significantly during the vegetation period of plants in Chernihiv, Kharkiv and Kyiv Regions. High moisture supply in Kyiv Region was in April–May (HTC 1.72–1.81), in June (HTC – 1.31) in Chernihiv Region and in May in Kharkiv Region (HTC – 1.53). All those months were in the flowering phase (cereals) and the phase of bulb formation (onion). In those particular phases, we observed increase in the number of all ecological-trophic groups. Therefore, we determined a correlation between the number of micromycetes in the rhizosphere soil and the value of the HTC in the growing season of 2021 (Fig. 5).

The results presented in Figure 5 show that the value of the coefficient of determination was higher than 0.99 in all the experimental variants. The value of the Pearson correlation coefficient ranged 0.98 in the variant with Kharkiv region to 0.99 in the variant with Kyiv and Chernihiv Regions. This indicates increase in the number of all ecological-trophic groups depending on increase in the hydrothermal coefficient. In addition, the figure clearly shows that the higher the HTC, the greater the number of micromycetes in all studied regions.

**Discussions**

In the global context, the ecological problems of climate changes and their impact on the soil system and its microbiological component are some of the main topics of scientific researches (Demyanyuk et al., 2018).
In particular, soil scientists have proven that the natural potential of soil fertility is significantly influenced by the qualitative and quantitative composition of its microflora (Medkov et al., 2021). The number, biomass and taxonomic structure of the soil microbial complex depend on many factors (Kolodiazhnyi & Patyka, 2014). Microbial groups in soil are significant in the functioning of the agroecosystem. The effect of agrotechnical activity on the number of microorganisms of various ecological-trophic and physiological groups depends on the type of soil (Kurdysh, 2009), moisture (Banerjee et al., 2016a), presence and availability of nutrients (Banerjee et al., 2016b), which have been analysed as the main factors of influencing the diversity and the structure of soil microbiomes. The changes in the structure of microbial cenosis of soil in the process of change in hydrothermal parameters of the environment are being clarified by the work of scientists. Temperature and content of moisture in soil are the essential factors influencing soil biodiversity (Kurshbourn, 2006). Hydrothermal regime determines the type of vital activity of soil organisms, phytophobia, activity of biochemical processes in soil (Sussele et al., 2012; McDaniel et al., 2013; Brygadyrenko & Nazimov, 2015; Zazhurskyi et al., 2019).

The root secretions of plants have a significant effect on the distribution of certain groups of microorganisms in the soil (Yang, 2000). Root secretions are known to account for about 20% of the total number of photosynthesis products in plants (Broeckling et al., 2008). The work of many scientists has shown that the composition of root exometabolites depends on the conditions and stage of plant development. Differences in the quantitative and qualitative composition of root secretions and in the trophic needs of microorganisms cause a significant effect on the growth of microflora of different taxonomic groups in the root zone, as well as their antagonistic activity (Cheng, 2015; Dragomir & Nicolae, 2015; Poliak & Sulharynycz, 2019). The microflora of the rhizosphere varies depending on the species and stage of development of plants. Among the microorganisms that can dissolve mineral phosphates, the most diverse were genera Penicillium and Streptomyces. In natural conditions, cellulose is transformed with the participation of groups of microorganisms. A significant role in this process is played by fungi, including saprotrophic representatives of genera Trichoderma, Chaetomium, Dicoccom, Stachybotrys, Penicillium and Aspergillus (Kurdysh, 2009; Roy, 2005).

We studied the number of the main ecological-trophic groups in agroecosystems of agricultural crops on the chernozem soils in the Left-Bank Forest Steppe of Ukraine. The results demonstrate that the structure of the soil microbiome is determined by biotic and abiotic factors. Thus, the composition of the microbiocoenosis of soil, content of microflora that is useful or phytopathogenic for agricultural plants, is a dynamic process in which significant factors are the hydrothermal coefficient during the growing season, soil type and root secretions of the agricultural plant. All this helps to better understand the relationship between plants and microorganisms which determines their role in microbial-plant associations and in parasite-host systems in nature.

**Conclusion**

During the ontogenesis of plants in Kyiv, Chernihiv, and Kharkiv regions, we observed increase in the number of all ecological and trophic groups in the flowering phase (cereals) and the phase of bulb formation (onions), when the hydrothermal coefficient equaled 1.31 to 1.81, which is high humidity. In the fields of the Nosivka Research Station (Chernihiv Region), the number of the main ecological-trophic groups of microorganisms under different crops varied significantly. The soil under rye, winter wheat, and oats was characterized by the greatest number of pedotrophic microorganisms. The rhizosphere soils in the Skvyra Research Station (Kyiv Region) under spring barley and winter wheat were characterized by the highest number of pathogenic microorganisms. In the fields of the Institute of Vegetable and Melon Growing (Kharkiv Region), the soil contained a high number of the cellulosolytic group of microorganisms, which despite the decrease in the hydrothermal coefficient grew until the end of the growing season. Exometabolites of agricultural plants (winter wheat, spring barley, oats, rye, onion) can influence the number of microorganisms in the soil by their physiological and biochemical substances. We determined that each plant species has a specific rhizosphere microbiome, depending on the plant species, plant development phase and soil-climatic conditions.

**Fig. 5.** Correlation between the number of micromycetes of soil rhizosphere under agricultural crops and the value of HTC in different regions: **a** – Chernihiv Region, **b** – Kyiv Region, **c** – Kharkiv Region

The root secretions of plants have a significant effect on the distribution of certain groups of microorganisms in the soil (Yang, 2000). Root secretions are known to account for about 20% of the total number of photosynthesis products in plants (Broeckling et al., 2008). The work of many scientists has shown that the composition of root exometabolites depends on the conditions and stage of plant development. Differences in the quantitative and qualitative composition of root secretions and in the trophic needs of microorganisms cause a significant effect on the growth of microflora of different taxonomic groups in the root zone, as well as their antagonistic activity (Cheng, 2015; Dragomir & Nicolae, 2015; Poliak & Sulharynycz, 2019). The microflora of the rhizosphere varies depending on the species and stage of development of plants. Among the microorganisms that can dissolve mineral phosphates, the most diverse were genera Penicillium and Streptomyces. In natural conditions, cellulose is transformed with the participation of groups of microorganisms. A significant role in this process is played by fungi, including saprotrophic representatives of genera Trichoderma, Chaetomium, Dicoccom, Stachybotrys, Penicillium and Aspergillus (Kurdysh, 2009; Roy, 2005).

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**References**


