Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2018, 8(1), 859-864 doi: 10.15421/2017_285

ORIGINAL ARTICLE

The structure of micromycetes communities in crop rotations with sunflower

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Received: 10.02.2018 Accepted: 11.03.2018

Research results of the of sunflower crop aftereffect on the structure of communities of soil micromycetes are presented. The species structure of the mycobiota of various links of the crop rotation, determination of favorable conditions for existence and functioning of micromycetes-decomposers providing of faster mineralization of plant sunflower residues was set out. Differences in the reaction of mycocenoses to the factor of sunflower plant residues have been revealed depending on the ways of soil tillage and vegetative crop. It was determined that the determinative effect of sunflower residues appeared as an integral part of the complex of stress factors that block the development of the dominant species in the mycocenoses. When factors are applying, preference in forming communities is given to species tolerant to stress as well as species concentrated on the plant residues.
Key words*:* sunflower; plant residues; tillage; predecessor crop; rotation; micromycetes community; mycocenose

Introduction

The main direction of agrarian production development in near future is its biologization through the introduction of adaptive and environmental technologies. The monitoring and measures aimed at ensuring the stability of edaphic microflora as the main factor of soil-forming processes, ensuring the mineral balance of soil and its phytosanitary condition take an important place in this process. Soil fungi can form parasitic, associative or symbiotic relationships with higher plants, and their selection can activate or vice versa to block the activity of other participants in the cenosis (Kopylov, 2012). The total mass of mycromycetes in the biologically active part of the soil varies from 1 to 2,5 t / ha, and the flow of mineral elements passing through the microbial group during the growing season significantly exceeds the amount of mineral elements assimilated by plants (Zvyagintsev et al., 2005; Belyuchenko, 2016). In natural communities the stability of soil microflora is maintained due to relatively uniform rates of "supply" to the soil of plant residues. The natural plant communities with a balanced species composition are characterized by a stable complex of soil microflora. The intensity of the processes of "root replacement" or the renewal of the underground organs mass in meadow cenoses is about 30% per year, and the process of supplying to the soil of the seasonal-dying residues of above-ground part of plants lasts more than an year. (Alekhin, 1986). These characteristics determines the expressive tendency of micromycetes populations to the sod soil layer and the relative stability of seasonal and structural variations in their numbers. In agrocenoses, where one-species crop plays the role of edifier, the structure of the soil microbiota is less stable (Svistova, 2003).

The dynamics of development and the number of individual types of fungi is determined by both abiotic and anthropogenic factors: tillage, the number and forms of plant residues of the predecessor crop as well as root exudates of the vegetative species (Gransee, Wittenmayer, 2000; Mandiã et al., 2004; Ðukiã et al., 2007; Kriaučiūnienė et al. 2008; Kolesnikova, Voronin, 2011; Belyuchenko, 2016; Tshovrebov et al., 2017). The influence of these factors may lead to changes in the types of dominant species, ranks of abundance and other parameters of the soil microcoenose structure. In some cases the formation of specific functional rows of micromycetes is possible, including toxigenic or pathogenic to cultivated plants (Svistova, 2004; Makusheva et al., 2010; Maryina-Chermnykh et al., 2012; Kopylov, 2012).

Sunflower is a specific crop in Ukraine occupying from 10 to 30% of arable land. One of the main problems in its cultivation is the prolonged process of stubble residues decomposition, which is an habitat for the phytopathogenic microflora and the factor of the existence of a high infectious background. The walls of the epidermis cells and the mechanical tissues of the plant stems at the end of the vegetation include the high content of lignin, and the seed pericarp is almost entirely composed of this substance, characterized by increased resistance to biological decomposition. Depending on the crop rotation scheme and the environmental conditions the "plume" of the influence of sunflower on the soil microflora can be traced within 1-3 years. First of all, this is revealed in the high and relatively stable number of microscopic fungi; the plant residues are the nutrient substrate for them. In the decomposition of plant residues, microorganisms of different groups participate, but micromycetes are the main destructors of cellulose and lignin. The duration of final mineralization processes of plant residues under the field conditions is determined by the complex of abiotic factors (temperature and humidity of the soil, its density and mechanical composition, the ratio of carbon and nitrogen of the decomposed substrate, structure and activity of the soil microflora (Abiven et al., 2005; Varnaite, Raudoniene, 2008; Novikov, 2012). At present, the microbiological activity of the soil in sunflower rotation and its consequences are practically not investigated. The study of the interrelation of agrotechnical measures and phytopathogenic potential of the arable soil at the level of individual agrocenoses are extremely important and topical. The aim of our investigations was to study the species structure of the mycobiota of various links of the crop rotation, to determine favorable conditions for existence and functioning of micromycetes-decomposers capable providing of faster mineralization of plant sunflower residues.

Materials and methods

Experiments to determine the effect of sunflower residues on the abundance and structure of micromycetes cenoses were carried out for 2015-17 in the Institute of Agriculture of the North-East of Ukraine in grain-falow-tilled crop rotation. Soil cover – chernozem (black soil). Soil samples for analysis were taken from the top layer (0-25 cm) by agrochemical boom. The number and species composition were determined on the Chapek nutritional media with sucrose.

The incubation temperature was 30 °C. Counting of micromycetes colonies was carried out for 7-10 days. The number of microorganisms was expressed in colony units (CFU) per 1 g of dry soil. Identification of species was carried out on the basis of a combination of cultural and morphological traits in accordance with the help of identifiers corresponding to each group of micromycetes and using the database **(**Pidoplichko, 1977; Sutton, 2001; Watanabe, 2002; Domsh et al., 2007). As a criterions for micromycetes evaluation, the total number of fungi and their species composition was used. The occurrence rate was chosen to characterize fungi and identify of dominant, subdominant and rare species. The similarity of the mycocenoses of different variants of the experiment was carried out with the Sorensen index. Berger-Parker index and Shannon index were used for degree of dominance and diversity species assessment. Investigation of the influence of the plant residue of sunflower on the soil micro-complex was carried out on a gradient with the selection of such factors as: predecessor crop, way of tillage and vegetative crop according to the scheme (Table 1).

Table 1. Scheme of experiment

Variant, N	Predecessor crop	Way of tillage	Vegetative crop	Factor
Control	Spring barley	2-times disking	fallow	
2	Sunflower	2-times disking	fallow	predecessor crop
3	Sunflower	plowing	fallow	predecessor crop, tillage
4	Sunflower	2-times disking	corn	predecessor crop,
5	Sunflower	2-times disking	winter wheat	vegetative crop

* In the second year of research all the plots were sown with spring barley

Results and discussion

Agrophytheoses is a system of anthropogenic origin where artificially created biotic groups are characterized by environmental instability. This determines the high degree of its internal variability on the one hand and dependence on the action of abiotic factors on the other. The most dynamic indicator of populations of soil fungi is their concentration in the upper layer of soil. According to Bilai (1984) and Senchakova, Svistova (2009) the range of indicators of the soil fungi concentration in the phytocoenoses of the Forest-Steppe zone varies from 28 to 40 × 10^3 CFU / g soil. In the experiment, the value of the indicator varied from 17 to 44 × 10^3 CFU / g, which indicates significant differences in the conditions for the formation of soil myco-complexes, depending on the predecessor, tillage and vegetative crop. The maximum amount of micromycetes in the experiment - 44 × 10^3 CFU / g was marked in the control. In our opinion, the factors supporting the high level of micromycetes were the surface tillage, the predominance in rotation of crops with a fibrous root system (winter and spring grains) and the availability of decomposing their residues by fungi-destructors. The obtained data coincide with the results of other authors researches, who underlined the increasing of fungal microflora in agrocenoses compared with the virgin and fallow soils (Svistova, 2003; Belyuchenko, 2016). The isolation of the certain factor influence - the predecessor (sunflower crop) and tillage - indicates that the replacement of the "nutritive substrate", the localization character of roots and plants residues led to the gradual decrease in the number of micromycetes to 39 x 10^3 CFU / g in variant 2 and up to 32 x 10^3 CFU / g in variant 3. Comparison of these data with the control (minus 11 and minus 27%, respectively) indicates that significant changes in the concentration of soil fungi are revealed only in cases of complex effect on the microbiocenoses of several stress factors (Fig.1)

The results obtained on plots with corn crop (variant 4) and winter wheat (variant N 5) were more complicated to comment. The average values of the micromycetes number in these variants were 17.3 × 10^3 CFU / g and 41 × 103 CFU / g.

As the dominant stressful factor of influence on the soil microcenoses in the variant with corn should be considered the drying of the upper soil layer due to the evaporation and absorption of water by the root system of vegetative plants. On the contrary, the stabilizing effect on soil cenoses on plots with winter wheat was observed.

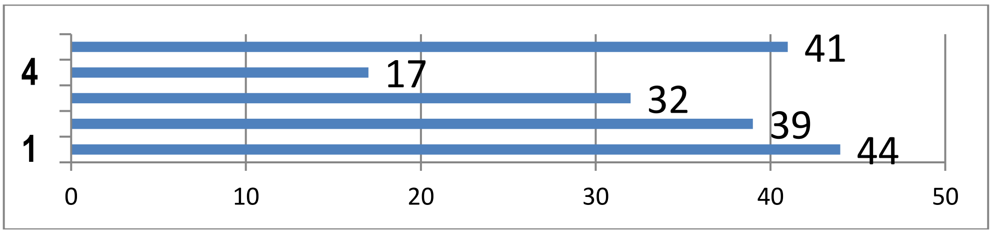

Fig. 1. Dynamics of the micromycetes number in rotation (2015-2017), 10^3 CFU/g of soil.

Greater information on the dynamics of fungal communities in agrocenoses depending on the character of predecessor crop residues, tillage way and vegetative crop was ensured by analysis of species composition with the allocation of dominant, subdominant, secondary (minor) and rare species (Table 2). In the plots that have not been influenced by sunflower for last 4 years (control) 32 species of mycomycetes have been allocated. Saprotrophic complex of dominant and subdominant species formed by *Penicillum, Fusarium, Aspergillus, Chaetomium,* and *Mucor.* The strong adaptive capacity of the *Penicillium* and *Aspergillus* genus both to abiotic environmental factors and nutrient substrates led to their domination in the structure of this cenoses. At the same time, the presence of the *Chaetomium* genus among the dominant species indicates to the high level of fungistasis in this variant. The change in the type of the main nutrient substrate of micromycetes in variant 2 did not cause significant changes in the species composition of soil microbiota. The micromycetes communities were represented by the sufficiently high number of saprotroph and antagonist types (*Penicillum, Aspergillus, Chaetomium*) that inhibited the development of the pathogenic microflora, in general preserving the rank community structure which was inherent to control. Phytopathogenic species were found as secondary (*Alternaria, Cladosporium herbarum*) or rare (*Verticillum dahlia*) ones, which indicates to the light deterioration of the phytosanitary soil state.

The plowing and processing of the above-ground sunflower parts to the depth of 20-24 cm in variant N 3 resulted in a significant restructuring of micromycetes communities. The turning over of the upper soil layer changed the vertical structure as well as the spatial localization of the micocenoces of the pedosphere. The species, characterized by rapid growth and the ability to quickly absorb readily available carbohydrate substratum (in particular members of the Fusarium genus), which were inherent in the lower soil layers, appeared closer to its surface, where the seasonal increase in their number was limited by the level of capillary moisture. On the contrary, cellulose-destroying micromycetes characterized by slower growth were concentrated in places of plant residues accumulation.

The presence of destructive processes in this variant is evidenced by the transition to the subdominant rank of *Trichoderma viride, Cladosporium herbarum, Acremonium strictum* as well as the number reducing of rare species. Secondary (minor) and rare species form a reserve of cenoses, which provides the adaptation of mycobiotic to vectorized changes in environmental conditions (Safonov, 2013). This structure type of mycocenoses can not be stable, despite the dominant position of micromycetes that mineralize plant residues (Voronin, Kolesnikova, 2012).

The most obvious appearance of change in the mycoconioses structure was noted in variant 4. Stress situation in this plot was caused by drying of the upper soil layer because of evaporation for the first half of the growing season and water absorbtion by the root system of corn in July-August, which blocked the seasonal processes of mycobiota development. Under these conditions, the formative component of the mycocenoses species colonized the plant residues of sunflower and species resistant or well adapted to stress were.

The similar phenomenon known as "concentration of dominance" was accompanied by statistically significant decrease in the total number of micromycetes and decrease (up to 13 species) of species diversity with the prevalence of *Mucor, Alternaria* and *Fusarium* genera. The transition to the dominant group of the last genera illustrates the tendency to form the complex of phytopathogenic microflora. This is also confirmed by the transition of the *Ulocladium consortiale* species to the subdominants group as well as the decrease in the number of minor and rare species.

The micromycetes-subdominants were also represented in this variant by the *Penicillum, Aspergillus,* and *Botrydis* genera. In this group there were also species that are active cellulose utilizers (*Trichoderma viridie, Trichoderma lignorum* and *Cladosporium herbarum)*. The neutral factor which from our point of view, appeared under the influence of vegetative crop (corn) was the presence of species that did not occur in the control: *Trichoderma koningii Oudenx (Oudem*). The minimal impact of sunflower crop was fixed in variant 5.

The winter type of development of vegetative crop, the high degree of soil surface protection from heating and drying during growing season and high activity of the root system have created favorable conditions for the preservation of saprotrophic micromycetes associations with the dominance of *Penicillum glabrum Penicillum thomii, Aspergillus niger, Aspergillus flavus, Chaetomium globosum* and *Trichoderma lignorum*. In this case an important role of the rank of the dominant group is to maintain the anti-phytopathogenic potential of the environment.

The transition of the *Chaetomium globosum* species to the rank of dominants is evidence of increasing of fungistatic soil properties in this variant. At the same time, the wide representation in the microcenoce of the *Trichoderma lignorum, Trichoderma viride* and *Cladosporium herbarum* indicates to the active processes of biodegradation and mineralization of sunflower residues.

As it was noted by Kriaučiūnienė et al. (2008) the development of trichodermal fungi and the chemical roots composition of vegetative crop are the determinative factors limiting the development of phytopathogenic mycomycetes on plant residues. Under such conditions, the presence in microcoenoce phytopathogenic species of *Verticillum, Botrydis, Alternaria* in the minor or rare rank did not determine the level of its suppression.

Table 2. Species structure of the micromycetes complex depending on the tillage and predecessor crop.

Rank	Control	Sunflower+disking+ fallow	Plowing +fallow	Disking+ corn	Disking+winter wheat
Dominants	*Penicillium chrysogenum Thom* *P. cyclopium Westl* *P.glabrum (Wehmer) Westl.* *P. glaucum Link* *P. thomii Maire* *Fusarium oxysporum (Schlect.) Snyd. et Hans* *Aspergillus flavus Link* *A niger van Tiegh.*	*Aspergillus niger Tiegh.* *A. flavus Link* *Chaetomium globosum Kunze* *Fusarium oxysporum (Schlect.) Snyd. et Hans* *Penicillum glabrum (Wehmer) Westl.* *P. thomii Maire*	*Aspergillus niger Tiegh* *Fusarium oxysporum (Schlect.) Snyd. et Hans* *Fusarium solani Appel.* *Penicillum glabrum (Wehmer) Westl.*	*Alternaria alternata (Fr.) Keissl.* *A. tenuis Nees* *F usarium. solani Appel* *Mucor ssp. Mich*	*Aspergillus flavus Link* *A. nigerTiegh.* *Chaetomium globosum Kunze* *Penicillum glabrumWehmer* *P. glaucum Link* *Trichoderma lignorum(Tode) Harz*
Subdominants	*Acremonium strictum W.Gams* *Chaetomium globosum Kunze* *Mucor ssp. Mich* *Rhizopus stolonifer (Ehrenb.) Vuill* *Trichoderma viride Pers.*	*Mucor ssp. Mich* *Trichoderma viride Pers* *Rhizopus stolonifer (Ehrenb.) Vuill* *Ulocladium consortiale (Thom.) E.G.Simmons*	*Acremonium strictum W.Gams* *Cladosporium herbarum Link ex Fr.* *Trichoderma viride Pers*	*Aspergillus niger van Tiegh.* *Botrydis cinerea Pers.* *Cladosporium herbarum Link ex Fr.* *Penicillum glabrum (Wehmer) Westl.* *Trichoderma viride Pers.* *Trichoderma lignorum (Tode) Harz.* *Ulocladium consortiale (Thom.) E.G.Simmons*	*Cladosporium herbarum Link ex Fr.* *Mucor ssp. Mich.* *Rhizopus stolonifer (Ehrenb.) Vuill.* *Trichoderma viride Pers.*
Secondary (minor)	*Alternaria alternata (Fr.)* *Alternaria tenuis Nees* *Aspergillus fumigates Fresen.* *Botrydis cinerea Pers.* *Cladosporium herbarumh Link ex Fr.* *Gliocladium spp* *Fusarium solani (Mart) Appl. et Wr.* *Rhizopus microspores Tiegh* *Rhizopus oryzae Went et Prin* *Trichoderma harzianum Rifai* *Trichoderma lignorum (Tode) Harz*	*Acremonium strictum W.Gams* *Alternaria tenuis Nees* *Botrydis cinerea Pers* *Cladosporium herbarumh Link ex Fr.* *Gliocladium spp* *Rhisopus oryzae* Went & Prins. Trichoderma lignorum (Tode) Harz *Mortierella globosus Ficher*	*Alternaria alternata (Fr.)* *Botrydis cinerea Pers.* *Gliocladium spp* *Mucor ssp. Mich* *Penicillium variotii (Bainier) Sacc.* *Rhisopus oryzae Went et Prin.* *Verticillum dahliae Kleb.*	*Trichoderma koningii Oudenx (Oudem)*	*Acremonium strictum W.Gams.* *Alternaria alternata (Fr.)* *Botrydis cinerea Pers* *Fusarium oxysporum (Schlect.) Snyd. et Hans* *Fusarium solani Appel* *Phytium torulosum Coker (P. Patt)* *Rhizopus microspores Tiegh.* *Rhizopus oryzae Went et Prin,* *Ulocladium consortiale (Thom.) E.G.Simmons Ehrenb*
Rare	*Helmintosporuim sativum Pamm.* *Mortierella verticillata Linnem* *Mortierella globosus Ficher* *Paecilomyces variotii (Bainier) Sacc.* *Paecilomyces lilacinus (Thom) Samson* *Pythium torulosum Coker (P. Patt)* *Verticillum dahliae Kleb.* *Ulocladium consortiale (Thom.) E.G.Simmons Ehrenb.*	*Mortierella verticillata Linnem* *Paecilomyces variotii(Bainier) Sacc* *Phytium torulosum Coker (P. Patt)* *Verticillum dahliae Kleb* *Helmintosporuim sativum Pammel*	*Mortierella globosus Ficher* *Mortierella verticillata Linnem*	*Paecilomyces variotii (Bainier) Sacc*	*Helmintosporuim sativum Pamm.* *Paecilomyces lilacinus (Thom) Samson* *Verticillum dahliae Kleb.*

The reflection of the quantitative and species composition of the mycomycetes in the plots of control and experiment was the indices of species similarity and diversity of mycromycetes communities (Table 3)

Table 3. Indices of species similarity and diversity of mycromycetes communities

Variant	Berger-Parker Index	Sørensen index	Shannon Index
Control	5.9	-	3.4
Sunflower+disking+ fallow	5.1	0.67	3.0
Plowing +fallow	2.7	0.58	2.4
Disking+ corn	1.6	0.44	2.1
Disking +winter wheat	5.8	0.69	3.5

The choice of the indexes aimed at comparing variants depending on the number of the dominant species (Berger Index), the shares of each species (including minor species) in the soil coenoses (Shannon Index), the species structure of soil cosenoses compared to the control plots (Sorensen coefficient). The most distinct differentiation of the experimental variants under the influence of such factors as changing in the nutrient medium, place of its location and the characteristics of the vegetative crop provided an analysis of the Berger index values. The high values of the index on the control plots as well as in the variant 2 and variant 5 are mainly due to the dynamics similarity of the species dominant of *Penicillum* genus to the dynamics number of mycosenoses in general. In this case, the value of the index on the plot with winter wheat crop – 5.8 is close to the control values and indicates to the attraction of the dominant species to the condition complex formed by the activity of the root system of cereal crops. Structural stability of micromycetes communities in these variants is also confirmed by the high values of the Sørensen index. Another situation was noted in variants with overlapping additional stress factors. Significantly decreasing of Berger-Parker index (occurring at variants 3 and 4) and the slight difference between the control and the other variants in the Shannon index, indicates to the temporary position as the dominant species of *Fusariumg* genus. The change of the dominant in this case can take place both at the expense of the species represented in the mycosenoses and species brought with sunflower residues.

Conclusions

It was found that in any of the variants (under experimental conditions), stubble sunflower residues were not the determining factor in changing the general structure and indicator complex of micromycetes inherent in the black soil of the Forest-Steppe zone. The "medium-forming role" of stubble sunflower residues can only revealed itself as a component of stress factor complex that blocks the development of dominant species which were typical for certain conditions (soils). When factors imposed, the preference obtained stress-tolerant species for the mycocenose formation as well as species concentrated on stubble sunflower residues.

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Citation:
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Ukrainian Journal of Ecology, 8(1), 859–864.