

THE EVOLUTION OF THE SOIL MICROBIOTA UNDER THE INFLUENCE OF WINTER WHEAT CROP IN THE NORTH-EASTERN REGION OF MOLDOVA

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Abstract

Soil microbial diversity is important to sustainable agriculture because microbes mediate many processes that support agricultural production. During four growing seasons of the winter wheat crop (2019-2022), the microbiota of the soil was analyzed, following its evolution during this period of time. Each year, soil samples were taken at two key times for the wheat crop: the beginning of stem elongation (BBCH 32–34) and the end of flowering (BBCH 68–70). The aim of this study was to determine and compare communities of bacteria and fungi occurring in the rhizosphere of winter wheat. The analyzed parameters included: abundance of Gram-positive and Gram-negative bacteria, abundance and genera composition of filamentous fungi. The frequency and composition of soil microbiota were examined in 4-years cultivation of winter wheat. The total number of Gram-negative bacteria was significantly higher compared to the total number of Gram-positive bacteria and filamentous fungi. The dominant groups of fungi were the genera *Penicillium* and *Aspergillus*, which accounted for more than 50% of the total number of fungal colonies identified.

Key words: winter wheat, soil microbiota, bacterial communities, soil microflora

Winter wheat (*Triticum aestivum* L.) is a high-yielding hexaploid grass of the *Poaceae* family and is one of the world's staple foods (Asplung L. *et al*, 2010).

Wheat has accompanied human for over 10 thousand years and evolved to its present form through continuous selection and breeding, being one of the oldest cultivated plants, and its "domestication" is closely associated with the emergence of agriculture, having since then a major importance for human consumption. The domestication of wheat revolutionized the evolution of human culture and is responsible for the advances made by human civilization.

The latest statistical data show that worldwide wheat is cultivated on an area of over 220 million hectares, being the most cultivated cereal crop. In Romania, the area cultivated with wheat is approximately 2.2 million ha, being the second most important cultivated crop after maize.

The wheat crop is also important due to the influence it has on the soil microbiota, since the cultivation technology applied to winter wheat influences the soil microbiota (Kornilowicz-Kowalska T. *et al*, 2020; Harris J.A., 2003).

The number and diversity of microbial communities in soil ecosystems have a complex

relationship with the intensity of human intervention and are influenced by environmental, edaphic and management factors (Marschner P. *et al*, 2004, 2003). Soil microorganisms play essential roles in the amelioration of soil structure, regulation of organic matter decomposition, nutrient cycling, and degradation of hazardous substances (Dobrovol'skaya T.G., *et al*, 2015; Harris J.A, 2009).

The main purpose of the present research was to evaluate the influence winter wheat crop on soil microbiota abundance and composition.

MATERIAL AND METHOD

Soils in this study were sampled from northeast region of Moldavia, Romania.

Soil samples were collected from chernozem soil type cultivated with winter wheat under conventional agricultural conditions (synthetic pesticides and fertilizers were used).

The soil samples were taken from a depth interval of 7 – 10 cm from around the roots and then samples were processed by grinding and homogenization in a sterile mortar. Soil dilutions were prepared according to the method of successive dilutions and plating was done in Petri plates, by the incorporation in medium.

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For an easy identification of colonies, we have used different culture mediums, specific to each systematic group. Thus, for determining the total number of microorganisms, we have used the simple PDA (potato-dextrose-agar) medium, for determining the number of Gram-positive bacteria (G+), we have used the PDA with streptomycin (35 mg·L⁻¹) medium and for determining the number of filamentous fungi, we have used the PDA with rose-bengal (35 mg·L⁻¹) medium (Lipșa F.D. *et al*, 2020, 2018, Ulea E. *et al*, 2017).

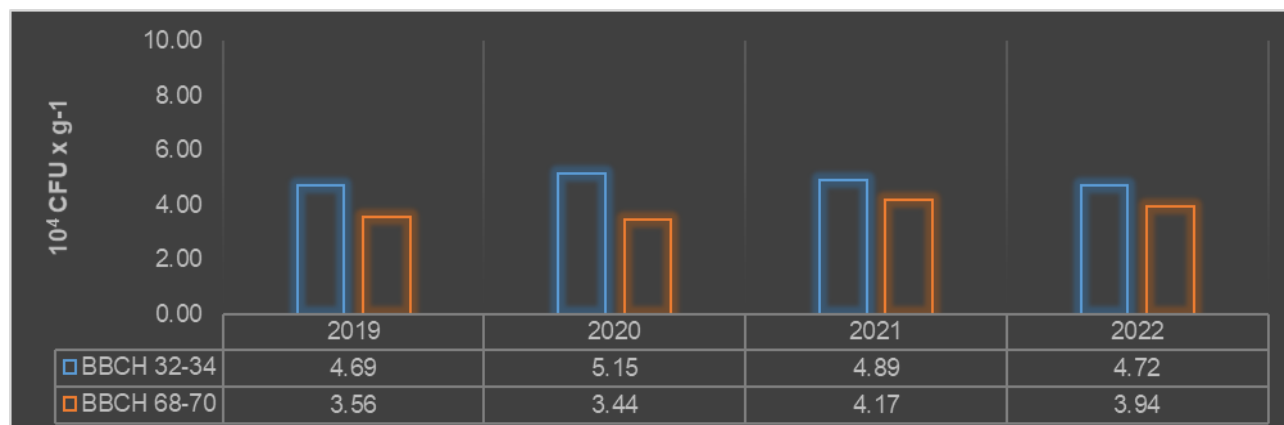
The number of bacterial colonies was determined at 24 hours after incorporation in medium and the fungus colonies at 5 days. The experiment was conducted with a threefold

repetition for each microbiological determination and the counts obtained were averaged.

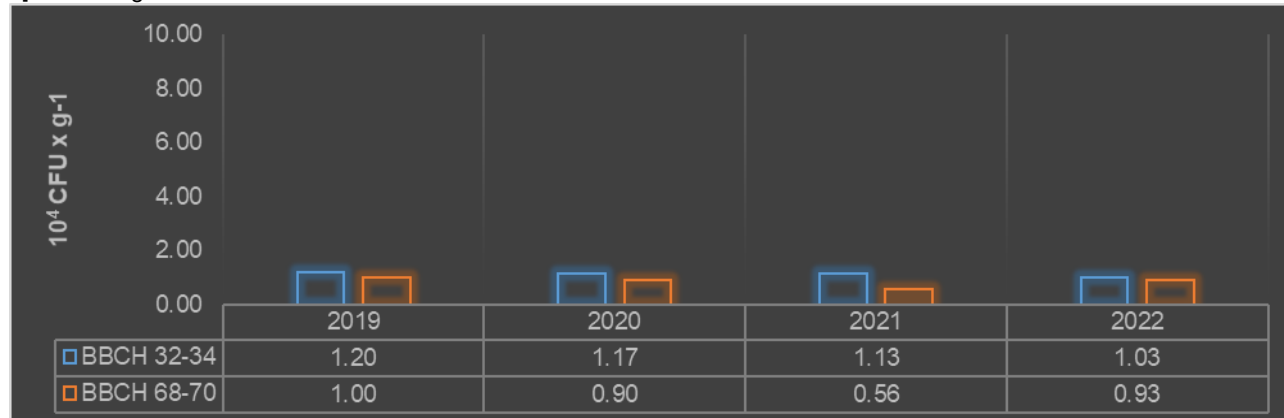
Fungi were identified to genus level based on morphological and physiological characteristics following the works provided by Ellis M.B. (1985), Barnett H.L. and Hunter B.B. (1972).

RESULTS AND DISCUSSIONS

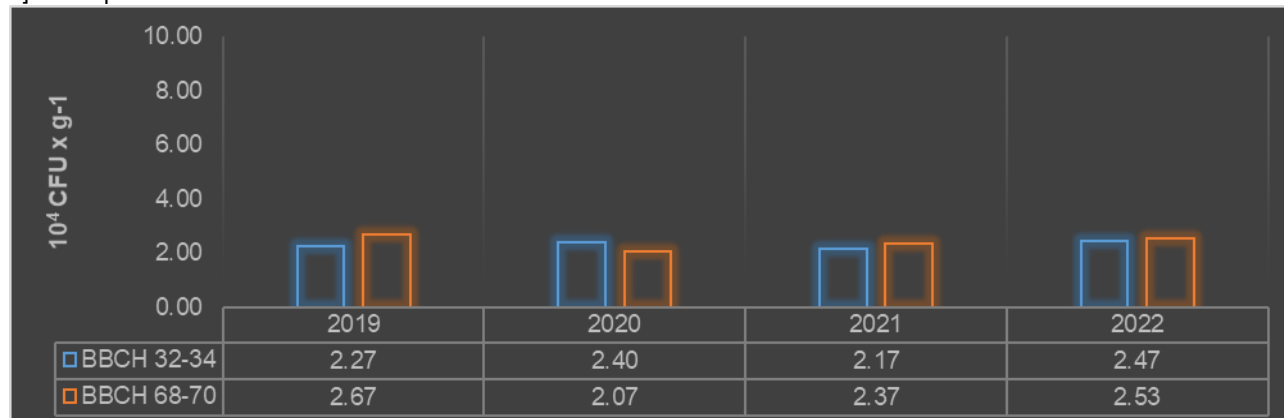
After comparing the sets of results obtained in the period 2019-2022, variations in the abundance and dynamics of Gram-positive and Gram-negative bacteria are observed (*figure 1*). The group of bacteria best represented during the research period was Gram negative bacteria.



a) Gram-negative bacteria



b) Gram-positive bacteria



c) Filamentous fungi

Figure 1 Dynamics of soil microbiota (Gram-negative bacteria, Gram-positive bacteria, Filamentous fungi) in winter wheat crop soil during 2019-2022

Analyzing the abundance of micro-organisms in the soil at each of the two sampling times, it can be seen that in the case of bacteria, the values recorded were higher at the first sampling (BBCH 32-34), compared to the values recorded at the second sampling (BBCH 68-70). This can be explained by the presence of substances secreted by wheat roots in the first phases of vegetation that attract bacteria to the rhizosphere area.

Analyzing the numerical density of the bacterial population during the 4 years, it can be seen that some differences were recorded, but the values obtained show that the technology applied to the wheat crop did not produce major changes at the level of microbial communities in the soil.

The same situation, as in bacteria, was also observed in the case of filamentous fungi isolated from the soil.

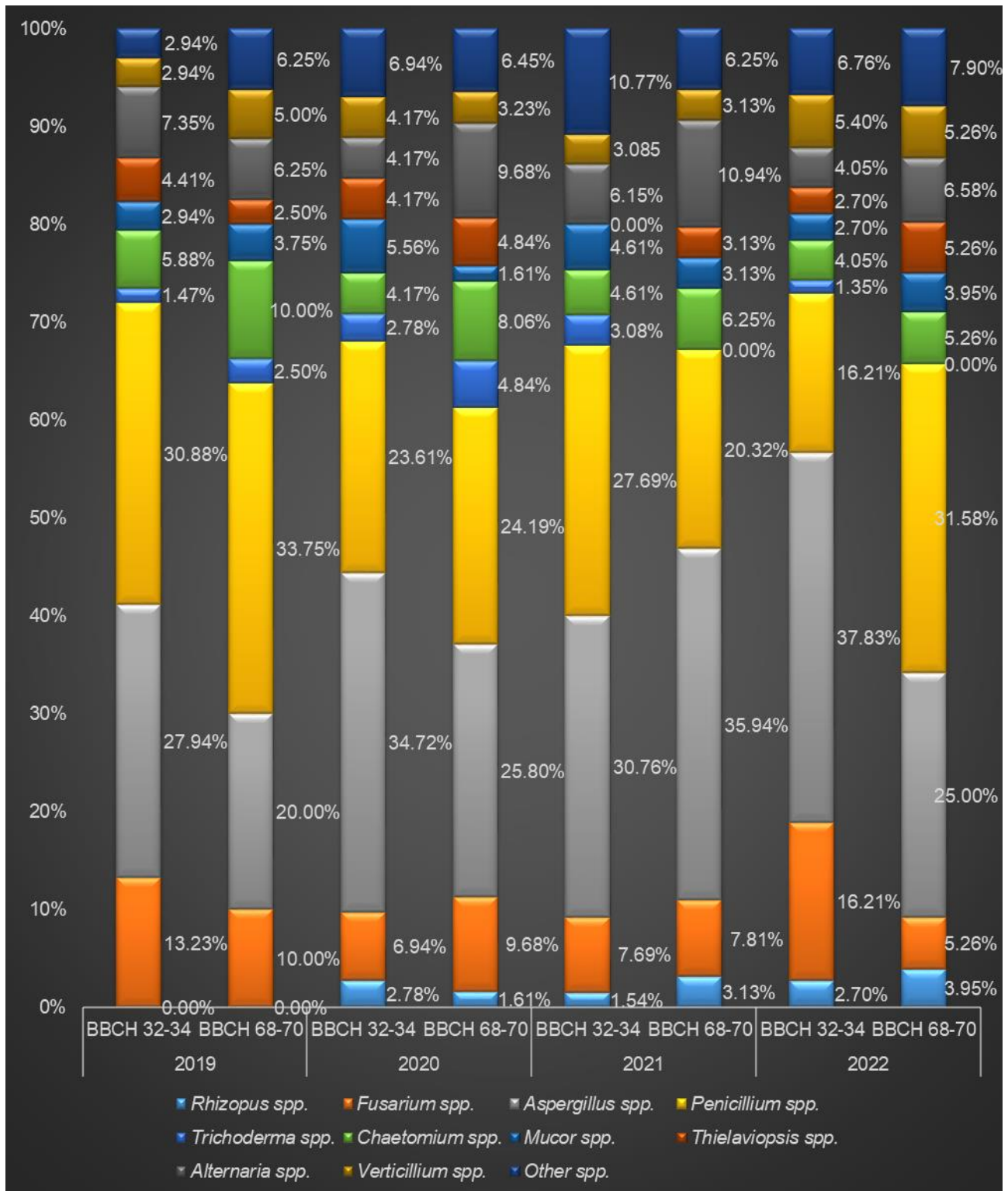


Figure 2 Frequency of filamentous fungi isolated from winter wheat crop soil during 2019-2022

In the case of filamentous fungi growing on the synthetic culture medium, ten distinct genera of fungi were identified during the studied period, to which are also added some unknown species (figures 2).

Of the fungi identified, more than half were represented by species of the genera *Aspergillus* and *Penicillium*. In addition to these, species of the genus *Fusarium*, *Alternaria* and *Chaetomium* were frequently identified.

In some samples, the genera *Mucor*, *Rhizopus*, *Trichoderma*, *Verticillium*, and *Thielaviopsis* were also identified. In some cases, a few species could not be correctly identified based on morphological characteristics, which is why they were grouped in the *Other* spp. category.

CONCLUSIONS

The analysis of the results obtained during the experimental period shows that the biological activity of the main groups of microorganisms in the soil (Gram-negative bacteria, Gram-positive bacteria, Filamentous fungi) does not undergo important changes over time if the culture technology applied is well established and applied to the wheat crop.

The highest biological activity was recorded at the first sampling, when the wheat crop was in the BBCH 32-34 phenophase.

The observations made showed a very good activity of Gram-negative bacteria, which represented about 55-65% of the total microorganisms identified. Gram-positive bacteria represented about 15-20%, and filamentous fungi represented about 20-25% of the microorganisms identified.

These results show us that the conscious application of agricultural practices can support the balance of soil microbiota.

ACKNOWLEDGMENTS

This research was supported by the project "PROINVENT", Contract no. 62487/03.06.2022 - POCU/993/6/13 - Code 153299, financed by The Human Capital Operational Programme 2014-2020 (POCU), Romania.

REFERENCES

- Asplung L., Hagenblad J., Leino M.W., 2010** - *Re-evaluating the history of the wheat domestication gene NAM-B1 using historical plant material*. Journal of Archaeological Science. 37(9):2303-2307.
- Barnett H.L., Hunter B.B. 1972** - *Illustrated genera of imperfect fungi.*, (3rd ed).
- Dobrovolskaya T.G., Zvyagintsev D.G., Chernov I.Y., Golovchenko A.V., Zenova G.M., Lysak L.V., Manucharova N.A., Marfenina O.E., Polyanskaya L.M., Stepanov A.L., Umarov M.M., 2015** - *The role of microorganisms in the ecological functions of soils*. Eurasian Soil Sci. 48 (9), 959–967.
- Ellis M.B., Ellis J.P., 1985** - *Microfungi on Land Plants. An identification Handbook*. Macmillan Publishing Company New York.
- Harris J.A. 2003** - *Measurements of the soil microbial community for estimating the success of restoration*. European Journal of Soil Science, 54(4), 801-808.
- Harris J.A., 2009** - *Soil microbial communities and restoration ecology: facilitators or followers?* Science, 325(5940), 573-574.
- Kornilowicz-Kowalska T., Andruszczak S., Bohacz J., Kraska P., Mozejko M., Kwiecińska-Poppe E., 2022** - *The effect of tillage and no-tillage system on culturable fungal communities in the rhizosphere and soil of two spelt cultivars*. Applied Soil Ecology, 174, 104413.
- Lipșa F.D., Florea, A.M., Gafencu, A.M., Ulea E. 2020** - *Crop species and year seasons as determinants of microbiota structure in chernozem soil in the southeast region of Moldavia, Romania*. Lucrări Științifice, Seria Agronomie, 63(1), 101-106.
- Lipșa F.D., Gafencu A.M., Florea A.M., Filipov F., Ulea E. 2018** - *Responses of microbial communities in different soil types under various management regimes in the southeast region of Moldavia, Romania*. Lucrări Științifice, Seria Agronomie, 61(2), 15-20.
- Marschner P., Crowley D., Yang C.H., 2004** - *Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type*. Plant and soil, 261(1), 199-208.
- Marschner P., Kandeler E., Marschner B., 2003** - *Structure and function of the soil microbial community in a long-term fertilizer experiment*. Soil Biology and Biochemistry, 35(3), 453-461.
- Ulea E., Lipșa F.D., Bălău Mihaela Andreea, Filipov F., Morari E.C., 2017** - *Diversity of soil bacteria as indicators of soils pollution in Moldavia region, Romania*. Environmental Engineering and Management Journal 16(4):879-889.