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# RHIZOSPHERE MICROBIOTA PROFILES ACROSS DIFFERENT WINTER WHEAT CULTIVARS

**Andrei-Mihai GAFENCU, Andreea-Mihaela FLOREA, Florin-Daniel LIPȘA, Eugen ULEA**

“Ion Ionescu de la Brad” Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: andrei.gafencu@iuls.ro

***Abstract***

*Global changes, driven by climate change and the growing human population, have necessitated the development of innovative solutions to maintain agricultural productivity and quality. This has often led to the adoption of intensive agricultural practices, which significantly alter soil properties, including its physical, chemical, and biological characteristics. Soil microbiota are key mediators of essential soil processes, climate regulation, and plant health, influencing nutrient cycling, organic matter decomposition, and overall soil fertility. Plant roots serve as the primary source of nutrients for soil microbiota, releasing organic exudates into the soil. These exudates create a dynamic rhizosphere environment that attracts microorganisms, which, in turn, contribute to plant health by providing nutrients, releasing hormones, and neutralizing toxins. This study aimed to analyse the rhizosphere microbiome associated with three winter wheat (Triticum aestivum L.) cultivars Glosa, PG102, and Miranda. Soil samples were collected during different growth stages to assess the composition and variation of bacterial and fungal communities. Microbiological analyses revealed temporal and cultivar-dependent shifts in microbial abundance. The total number of bacteria was highest in autumn, decreased during winter, and increased again towards the flowering stage. Gram-positive bacteria followed a similar trend, with minor cultivar-specific deviations. Fungal community composition also varied over time. These results suggest that microbial communities are influenced by both environmental conditions and wheat genotype, highlighting the potential role of cultivar selection in shaping rhizosphere microbiota.*

***Key words***: *microbiota community structure, soil microbiota, winter wheat, rhizosphere.*

## INTRODUCTION

Soil represents a complex natural system composed of consolidated mineral and organic components, located on the surface of the lithosphere (Bardgett, 2005). It serves as the primary medium for terrestrial vegetation development and supports the diversity of organisms that carry out their biological and ecological cycles within it (Hillel, 2007). The properties and characteristics of soil are the result of biotic and abiotic components and the interactions between these components (Kumar et al., 2020). At the soil level, the zone where some of the most important processes take place is the rhizosphere. The term rhizosphere was first introduced by Hiltner in 1904 to define the region of soil directly influenced by plant roots and their associated microbial communities (Berg & Smalla, 2009). Due to the significant role of plant-microorganism interactions in the rhizosphere, understanding the factors that shape microbial communities in this habitat is essential (Kent & Triplett, 2002). These factors include root exudates, soil composition, moisture, temperature, biotic interactions, and the specific plant species cultivated, all of which contribute to determining the structure, diversity, and functionality of microbial populations (Huang et al., 2014; Hawkes et al., 2007; Brimecombe et al., 2000). The plant species plays a crucial role, as different species produce distinct root exudates and create unique microenvironments that influence microbial community composition and activity (Hernández-Cáceres et al., 2022). It has been demonstrated that rhizosphere microorganisms directly influence plant growth by producing phytohormones (e.g., auxins, gibberellins) and enhancing nutrient uptake (Kurepin, 2014).

Winter wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, playing a crucial role in global food security. Different winter wheat cultivars are selected based on environmental tolerance, disease resistance, yield, and grain quality. While genetic improvement of winter wheat cultivars often focuses on visible traits, their impact on the rhizosphere microbiome remains less explored. In wheat crops, microbial interactions play a crucial role in improving plant health and productivity. Beneficial microbes facilitate the solubilisation of essential nutrients such as phosphorus and nitrogen, making them more available to wheat roots. Additionally, certain microorganisms contribute to stress tolerance by protecting plants from pathogens and environmental stressors, such as drought and soil salinity. Understanding and harnessing these microbial communities can lead to more sustainable wheat production, reducing dependence on chemical fertilizers and promoting healthier soil ecosystems (Mahoney et al., 2017). The aim of this study is to compare the rhizosphere microbiome profiles associated with different winter wheat cultivars (Glosa, PG102, Miranda). By analysing the diversity and composition of microbial communities, the study seeks to identify specific variations that may be influenced by plant genotype.

## MATERIALS AND METHODS

The research sites were established at the Agralmixt S.A. farm, located in north-eastern Romania, Iasi County, Andrieseni village (47°34’9” N, 27°20’38” E), at an altitude of 60 meters above sea level. The region experiences a temperate continental climate, characterized by an average annual air temperature of 9.5°C and a total annual precipitation of 520 mm (Gafencu et al., 2023a).

During the studied period (September 2023 - August 2024), the average air temperature was 13.52°C, indicating an increase compared to the region's multiannual average. This average temperature can significantly impact ecological and agricultural processes in the area, directly affecting the growth rate of crops and other agricultural activities. Additionally, temperature fluctuations throughout the year, especially during the warmer months, may influence precipitation patterns and the water requirements of plants (Table 1). The data shows significant temperature fluctuations throughout the winter wheat vegetation period. January recorded the lowest temperatures (-18.12°C), while July had the highest (46.95°C). Precipitation was highest in June (117.4 mm) and lowest in August (12.0 mm), with a total annual precipitation of 515.5 mm.

Table 1. Monthly air temperature (average, minimum, and maximum) and atmospheric precipitation recorded during the winter wheat vegetation period in 2024 at the S.C. Agralmixt S.A. farm, weather station 00001CED

| Month | Average temperature (°C) | Minimum temperature (°C) | Maximum temperature (°C) | | Atmospheric precipitation (mm) |
|---|---|---|---|---|---|
| August 2023 | 24.29 | 10.75 | 39.89 | | **12.0** |
| _September 2023*_ | _19.46_ | _7.16_ | _33.98_ | | _23.2_ |
| October 2023 | 13.60 | -4.02 | 33.87 | | 23.0 |
| November 2023 | 6.07 | -8.78 | 21.74 | | 37.4 |
| December 2023 | 1.89 | -7.32 | 15.83 | | **12.0** |
| January 2024 | **-1.31** | **-18.12** | 12.44 | | 24.4 |
| February 2024 | 6.19 | -11.22 | 25.08 | | 29.8 |
| March 2024 | 7.29 | -9.42 | 34.23 | | 40.0 |
| _April 2024*_ | _15.03_ | _-0.71_ | _34.31_ | | _43.8_ |
| _May 2024*_ | _18.62_ | _1.24_ | _37.06_ | | _65.8_ |
| June 2024 | 24.47 | 10.31 | 40.12 | | **117.4** |
| July 2024 | **26.68** | 10.19 | **46.95** | | 86.2 |
| | | | | | |
| **Annual average (°C)**<br>**Total sum (mm)** | **13.52** | | | | **515.5** |

*Months in which soil samples were collected

Soil samples were collected from chernozem soil cultivated with winter wheat under conventional agricultural practices, including the use of synthetic pesticides and fertilizers. Soil sampling was conducted three times during the winter wheat growing season: [1] the first sample was taken in the fall of 2023, after crop sowing and emergence of wheat plants, [2] the second sample was taken in April 2024, and [3] the third sample was taken in May 2024, during wheat flowering. In this study, three winter wheat varieties were examined: Glosa, PG102, and Miranda, each with its unique agronomic traits. Glosa is a widely used variety, prized for its high yield potential and strong disease resistance, making it suitable for a wide range of environmental conditions. PG102 is known for its adaptability to various climates and its resilience to environmental stresses such as drought and extreme temperatures. Miranda stands out for its exceptional grain quality and durability, often selected for its superior performance across different soil types and climatic conditions. Within each analysed plot, soil samples were collected separately for each tree cultivar (Glosa, PG102, and Miranda) from 20 randomly selected points. The soil was sampled from around the plant roots at a depth of approximately 7-8 cm (Figure 1). After collection, samples were transported to the Microbiology Laboratory at the Iași University of Life Sciences, where they were stored overnight at 4°C, dried at room temperature, and sieved before microbiological analysis.

Figure 1. Soil sampling in the rhizosphere of wheat plants

To determine the total number of bacteria in the soil, expressed as colony-forming units (CFU) per gram of dry soil, the plate culture method was employed, using serial dilutions before plating (Gafencu et al., 2024). The Potato Dextrose Agar (PDA) medium (Scharlau, Spain, 39 g/L) was used for bacterial cultivation (Gafencu et al., 2023b). To selectively enumerate Gram-positive bacteria, Streptomycin (35 mg/L) was incorporated into the PDA medium. The antibiotic was thoroughly mixed into the medium post-autoclaving (15 minutes at 121°C) at approximately 48°C. For filamentous fungi assessment, the PDA medium was supplemented with Rose Bengal (35 mg/L) to limit the spread of fast-growing moulds (Smith & Dawson, 1944).

For each sample, successive dilution methods were applied. To assess bacterial and fungal populations, 1 mL of suspension from the $10^{-3}$ and $10^{-4}$ dilutions, respectively, was transferred to a Petri dish, followed by the addition of 17 mL of culture medium at 48°C. The contents were homogenized using orbital movements before solidification. The Petri dishes were then incubated at 28°C (Gafencu et al., 2021). After 24 hours, bacterial colony number were determined for both PDA and Streptomycin-supplemented PDA media using the Scan® 1200 automatic colony counter (Figure 2). The total bacterial count per gram of soil was calculated by multiplying the obtained values by the inverse of the dilution factor. Results were expressed as CFU×$10^5$ $g^{-1}$ dry soil.

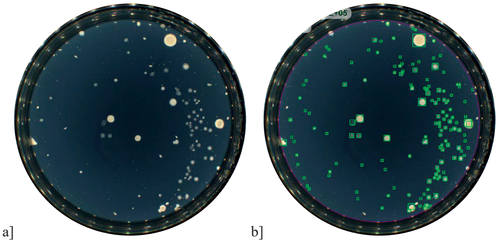


Figure 2. Determination of the number of bacterial colonies grown on the culture medium (a] before counting; b] after counting)

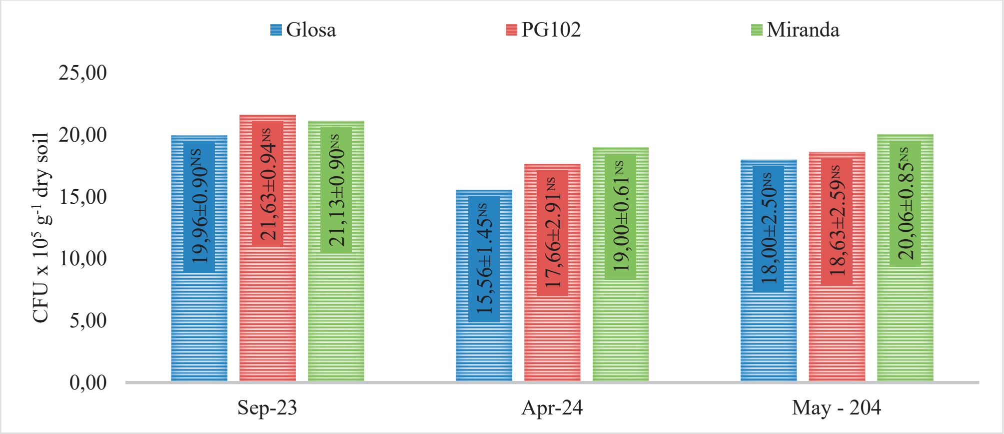
Filamentous fungi were evaluated after five days, and identification was based on morphological characteristics (Figure 3) following established taxonomic references (Gilman, 1957; Barnett, 1960; Ellis & Ellis, 1985; Seifert & Gams, 2011; Guarro et al., 2012). Fungal species that did not form spores within this period and could not be identified were categorized as "*Other species*".

Figure 3. Fungal colonies grown on PDA medium supplemented with Rose Bengal

Statistical analysis of the collected data was performed using IBM SPSS Statistics 26.

## RESULTS AND DISCUSSIONS

The cultivation technology was similar for all three studied wheat varieties. The preceding crop was sunflower. After harvesting the sunflower crop, soil preparation included shredding plant residues, soil scarification, and seedbed preparation. Chemical fertilizers were applied in moderate amounts, ensuring good nutrient availability. Additionally, during the vegetation period, pesticides were used to prevent and control pathogens, pests, and weeds, thereby supporting the optimal development of the wheat crop. The results obtained from analysing the influence of wheat plants on the total number of bacteria in the soil show that in autumn, the total bacterial number reached its highest values. During winter, the total number of bacteria decreased, with the lowest values recorded in April. Towards the end of the vegetation period, the total number of bacteria increased but did not reach the value observed in autumn (Figure 4).



NS – $P > 0.05$ * – $P \leq 0.05$ ** – $P \leq 0.01$ *** – $P \leq 0.001$

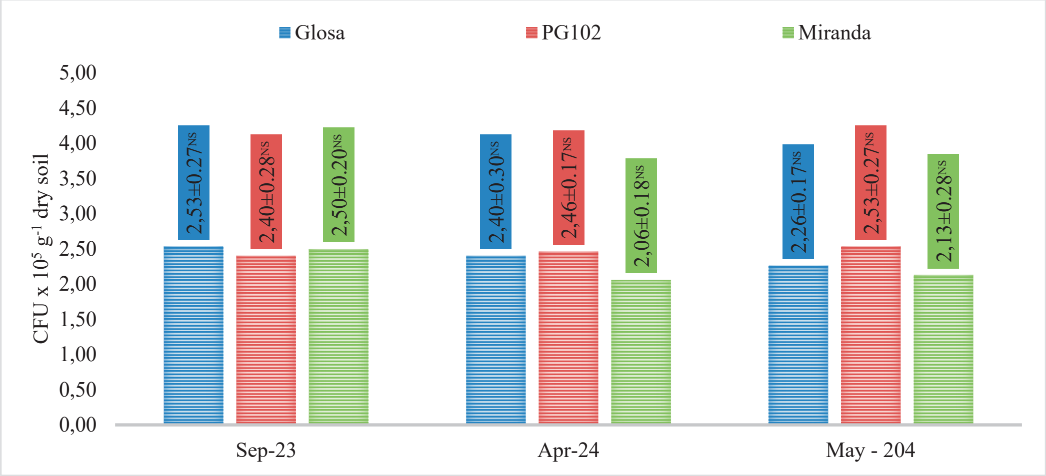
Figure 4. Total number of soil bacterial communities under the influence of winter wheat cultivar

This trend was observed in all three wheat cultivars studied. The highest total number of bacteria in the soil was observed for the PG102 wheat cultivar, in autumn, while the lowest values were recorded in the rhizosphere of the Glosa cultivar in April. For the Miranda cultivar, the total number of bacterial colonies in the soil showed the smallest differences across the three sampling periods.

Figure 5 presents the recorded results regarding the number of Gram-positive bacteria. A similar trend was observed in this particular bacterial group. The highest values were recorded in the fall of 2023. By April, the number of Gram-positive bacteria had slightly decreased, followed by an increase up to the wheat flowering stage.

For the Glosa cultivar, the number of Gram-positive bacteria decreased from 2.53±0.27 CFU × $10^5$ $g^{-1}$ dry soil in the fall to 2.40±0.30 CFU × $10^5$ $g^{-1}$ dry soil in April. However, by May, a further reduction was observed, reaching 2.26±0.17 CFU × $10^5$ $g^{-1}$ dry soil. In contrast, a different pattern was observed for the PG102 cultivar, where the number of Gram-positive bacteria in the soil increased from fall to May, rising from 2.40±0.28 CFU × $10^5$ $g^{-1}$ dry soil to 2.53±0.27 CFU × $10^5$ $g^{-1}$ dry soil.

By analysing the total number of bacteria and the number of Gram-positive bacteria in the rhizosphere of the studied wheat cultivars, it is evident that bacterial communities reached their peak after crop establishment, declined over the winter, and gradually increased until the flowering stage. However, when examining the Gram-positive bacterial community specifically, slight deviations from the general trend were observed, suggesting potential shifts in bacterial community structure within the wheat rhizosphere. These variations could be attributed to the influence of the specific cultivar studied.



NS – $P > 0.05$ * – $P \leq 0.05$ ** – $P \leq 0.01$ *** – $P \leq 0.001$

Figure 5. Number of Gram-positive soil bacterial communities under the influence of winter wheat cultivar

The diversity of fungi in the rhizosphere of the studied wheat cultivars exhibited variations in fungal community composition throughout the growing season, reflecting the influence of seasonal conditions and cultivar-specific factors (Figure 6).

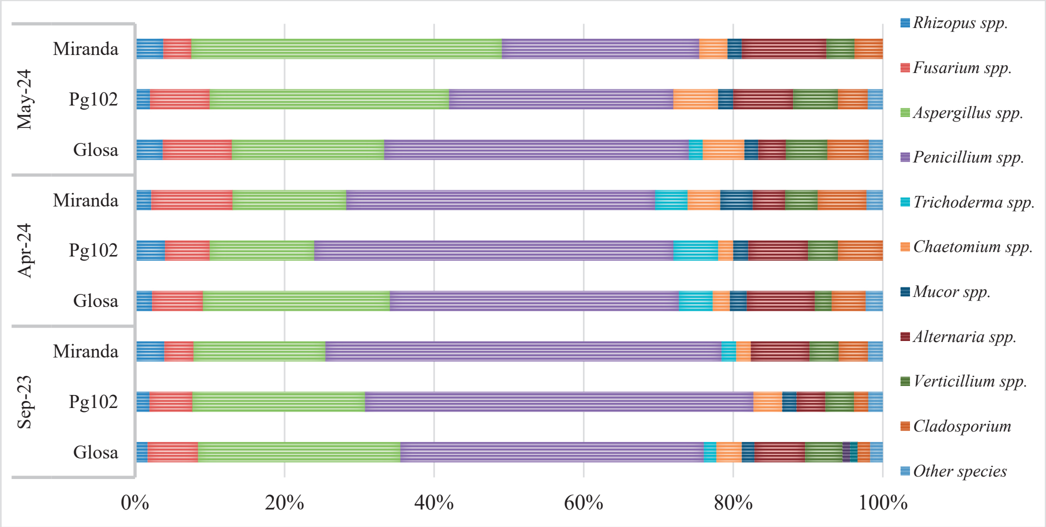


Figure 6. Results on the influence of winter wheat cultivar on the structure of fungal communities in the rhizosphere

Comparing the data from September 2023 to May 2024, notable changes were observed in the abundance of different fungal genera. *Aspergillus* spp. showed a significant increase in the Miranda cultivar (from 17.6% to 41.4%), suggesting a high adaptability to spring conditions. In contrast, *Penicillium* spp., which was dominant across all samples, decreased in Pg102 and Miranda, indicating possible shifts in microbial competition within the soil. *Trichoderma* spp., a genus known for its beneficial effects on plant health, was absent in Pg102 in both autumn and May but present in April, suggesting a seasonal influence or an association with wheat's developmental stage. Additionally, *Alternaria* spp. showed a significant increase in the Miranda cultivar (from 7.8% to 11.3%), indicating a potential preference for certain environmental conditions. These fluctuations in fungal communities suggest that the structure of the rhizosphere microbiome is influenced by both environmental factors and cultivar-specific characteristics, which may have implications for soil health and crop productivity.

## CONCLUSIONS

The study demonstrates that winter wheat cultivars influence the structure and dynamics of microbial communities in the rhizosphere. The total number of bacteria was highest after crop establishment, declined during winter, and recovered towards the end of the vegetation period. PG102 exhibited the highest bacterial abundance in autumn, while Glosa recorded the lowest values in April, suggesting cultivar-dependent variations in microbial support. The composition of Gram-positive bacteria showed minor fluctuations, indicating a relatively stable subpopulation within the broader microbial community. Fungal diversity exhibited seasonal changes, with increases in *Aspergillus* spp. and *Alternaria* spp. in Miranda, while *Penicillium* spp. declined in PG102 and Miranda, suggesting shifts in fungal competition and adaptation to environmental conditions. These results underline the importance of plant genotype in shaping soil microbial ecosystems, which can have implications for soil fertility, nutrient availability, and plant health.

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