DYNAMICS OF SOIL BACTERIAL COMMUNITIES UNDER WINTER WHEAT IN THE NORTH-EAST PART OF ROMANIA

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Abstract

Agricultural soil microbial communities are influenced by a multitude of factors, including seasonality and local environmental conditions, management practices and their interaction, and cultivated species. Seasonality can result in variations in temperature, precipitation, soil moisture, and solar radiation, all of which drive rates of soil microbial metabolism and respiration, as well as taxonomic composition. Agricultural crop species can significantly impact the soil microbial population through the release of root exudates. Root exudates are a diverse array of organic compounds that are actively secreted by plant roots into the surrounding soil. These compounds include sugars, organic acids, amino acids, vitamins, enzymes, and other secondary metabolites. Root exudates serve various functions, including providing a carbon source for soil microorganisms, facilitating nutrient uptake by plants, and mediating plant-microbe interactions. This study investigates the dynamics in bacterial communities within the rhizosphere of winter wheat across different growth stages and geographical regions in north-eastern Romania. Soil samples were collected from two locations in Iasi County, one location in Botosani County, one location in Vaslui County, and one location in Galati County. Soil sampling occurred three times during the winter wheat growing season: in the fall of 2022, in April 2023, and in May 2023, covering three phenological stages (emergence, stem elongation, and flowering). The results obtained indicated differences between the wheat crop's phenophase and between locations; however, statistical analysis of the data demonstrated that these differences were not statistically significant.

Key words: soil microbiome, soil microbial community, winter wheat.

INTRODUCTION

In the year 2022, according to available statistical data, wheat was cultivated on approximately 219 million hectares of land worldwide, making it the most cultivated crop globally. Since 1961, the global wheat-growing area has fluctuated between 200 and 240 million hectares. The cultivated wheat area peaked around 1980 and has slowly declined to its current level (Shiferaw et al., 2013). Despite the relative stability of the wheat-growing area (including a modest decrease over the past half-century), the increase in global wheat production is attributed to consistent yield improvements. Yields have steadily increased from a global average of just over 1 tonne/ha in the early 1960s to 3.6 tons/ha currently (Roy et al., 2006). In Romania, the average wheat yield is over 4 tons/ha (Gafencu, 2019). Wheat is cultivated in over 120 countries across Africa, South, Central and North America, Asia, Europe, and Oceania, making it a vital crop for both emerging economies and developed countries.

Global demand for wheat has been uninterrupted and continues to rise steadily since its cultivation began, projected to increase by 60% by 2050, as wheat serves as a staple food for approximately 3 billion people, over a third of whom are among the poorest, and provides about one-fifth of the calories and proteins in the human diet. Wheat production is essential for global food security and political stability, which is why wheat is the most cultivated plant in the world (Erenstein et al., 2022).

To ensure sustainable and efficient wheat production, it is essential to understand and manage the health of the soil in which this crop is grown. Soil microorganisms play a crucial role for soil and plant health, and understanding them is essential for optimizing agricultural production. Therefore, knowledge of the soil microorganisms in which wheat is cultivated is not only useful but also indispensable for ensuring sustainable, efficient, and climate-resilient agricultural production. Research and

understanding of soil microbiota can significantly contribute to improving agricultural practices and promoting global food security.

The main purpose of this research was to investigate the abundance and diversity of bacterial communities in the rhizosphere of winter wheat cultivated in five locations in north-eastern Romania, as well as to examine the changes occurring in these microbial communities throughout the wheat growing season.

MATERIALS AND METHODS

For this study, soil was sampled from five different locations in the northeastern region of Romania (Figure 1). Soil samples were collected from two locations in Iași County, one location in Botoșani County, one location in Vaslui County, and one location in Galați County.

Legend: Location No. 1: Stefanesti, Botosani; Location No. 2: Andrieseni, Iasi; Location No. 3: Ezareni, Iasi; Location No. 4: Codaesti, Vaslui; Location No. 5: Slobozia-Conachi, Galati
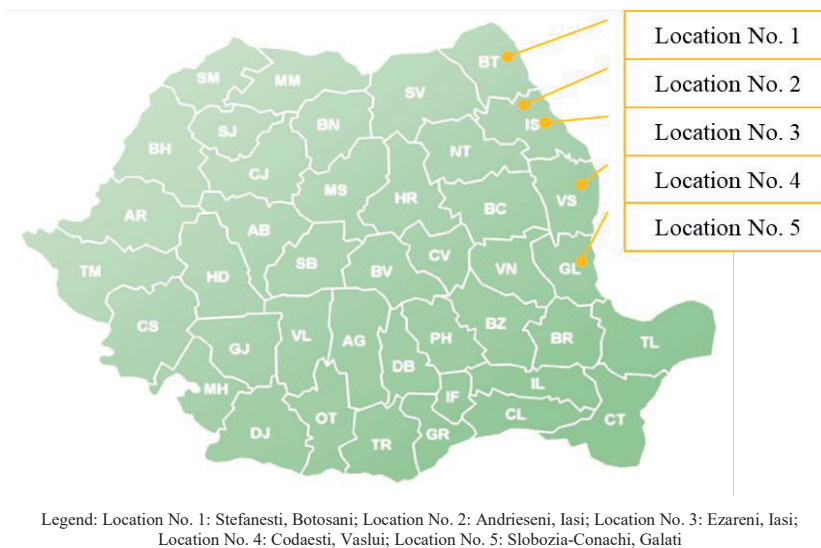
Figure 1. The locations where soil samples were collected

Soil sampling was conducted three times during the winter wheat growing season: [1] the first sample was taken in the fall of 2022, after crop sowing and emergence of wheat plants, [2] the second sample was taken in April 2023, and [3] the third sample was taken in May 2023, during wheat flowering.

Soil samples were collected from the rhizosphere of wheat plants, at a depth of 7-10 cm. From each wheat-cultivated plot in each of the 5 locations, an average sample was taken, consisting of 10 initial probes.

Soil sampling was conducted following all aseptic conditions to avoid soil contamination during collection. Soil samples were collected in paper bags and transported to the Microbiology Laboratory of the Faculty of Agriculture at Iasi University of Life Sciences for further investigation. Once in the microbiology laboratory, the soil samples were homogenized and ground. The organic matter present in the soil samples was removed using sterile tweezers.

For determining the total number of colony-forming units (CFUs) in the soil, the serial dilution method was used, and cultivation was performed on Potato Dextrose Agar (PDA) medium (Sarchlau, Spain, 39 g l^{-1}), in two different compositions: classic, and with Streptomycin. The antibiotic streptomycin (35 mg·L^{-1}) was used to control the reproduction of Gram-negative bacteria (Gafencu et al., 2021).

Serial dilutions were prepared in sterile water with a dilution factor of 10 (dilutions 10^{-1}, 10^{-2}, ..., 10^{-6}). This method generated a series of dilutions in which the number of microorganisms decreased. To prepare these dilutions, 9 mL of double-distilled sterilized water at 120°C for 30 minutes was distributed

into sterile tubes with a capacity of 15 mL. Subsequently, 1 g of soil was weighed on a sterile watch glass and introduced into the first dilution tube. After vigorous shaking for five minutes, a 10^{-1} (1/10) dilution was obtained. Successive dilutions, such as 1/100 (10^{-2}), were then prepared by transferring 1 mL of suspension from the previous dilution to another test tube containing 9 mL of sterile water. This process was repeated to obtain further dilutions.

1 mL of suspension from each dilution was plated on Petri plates, and after an incubation period of 24 hours at 28°C, bacterial colonies (Figure 2) were counted using an automatic colony counter (Scan® 1200, Interscience, France). To determine the number of bacteria in one gram of soil, the number of colonies that developed on the Petri plate was multiplied by the inverse value of the dilution (Gafencu & Ulea, 2023).
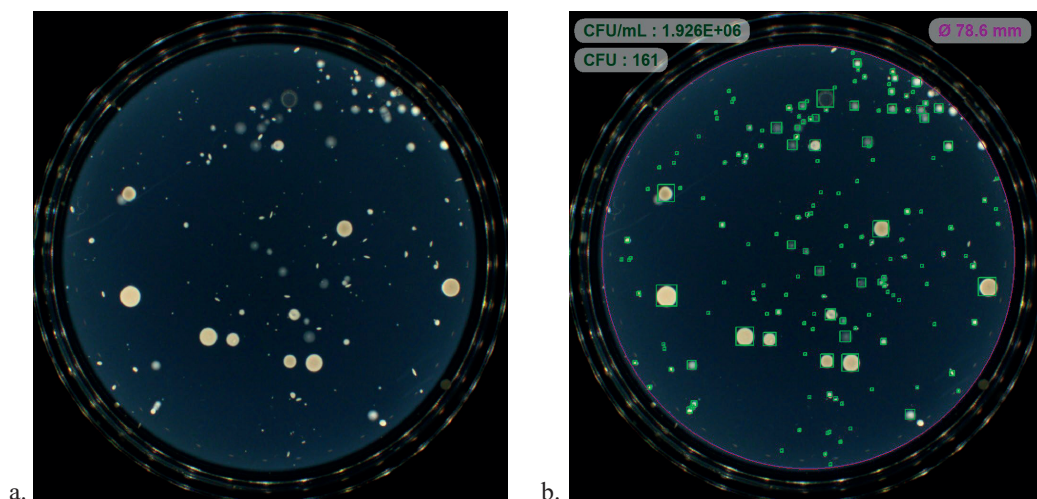

a.
b.

Figure 2. Bacterial Colonies on Potato Dextrose Agar media: using the Petri plate as a counting method
(a - before counting, b - after counting with Scan® 1200, Interscience, France)

The counting result was correlated with the dilution used, and the final result was expressed in colony-forming units (CFUs) per 1 gram of soil (Gafencu et al., 2023).

Statistical analysis of the data obtained in the experiments was performed using the SPSS program (IBM SPSS Statistics 26) for Windows. For the statistical analysis of the data, a benchmark represented by the average value was utilized for comparison with the obtained values. Additionally, mean values were determined for each sampling moment, including the total number of bacteria, Gram-positive bacteria, and Gram-negative bacteria.

RESULTS AND DISCUSSIONS

In all five locations, the winter wheat crop was preceded by sunflower crop. In all location, the winter wheat cultivar Glosa was cultivated. Soil cultivation practices were similar across all locations, with land preparation occurring after

sunflower harvesting. Plant residues were shredded, followed by scarification, and then preparation of the seedbed for the wheat crop. Chemical fertilizers were applied in moderate amounts at all locations. Additionally, pesticides were applied in all locations to control weeds, pests, and diseases.

The soil serves as a vital habitat for plants as well as for soil microorganisms. Plants influence the composition and dynamics of microbial communities present in the rhizosphere, and recent studies have evaluated the factors that influence the composition of these root-associated communities (Berg & Smalla, 2009). Such studies are important because the structure of rhizosphere microbial communities can significantly affect nutrient availability for plants, organic matter decomposition, phytopathogen activity, and so on (Chapparo et al., 2012; Howard et al., 2020). Studies on soil microbiota are essential for understanding how plants shape these communities and for

evaluating the impact of these microorganisms on plant health and soil fertility (Lamb et al., 2011; Tkacz et al., 2020). For a detailed analysis of the variations in the total number of bacteria within the winter wheat rhizosphere, across different seasons and different locations, we can closely examine the dynamics and differences among the recorded values (Figure 3).
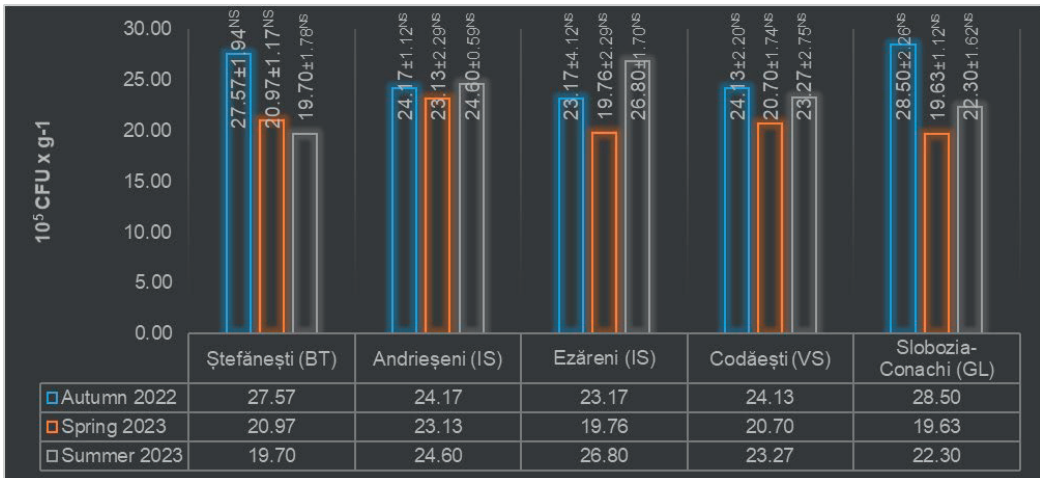

Figure 3. Comparison of total bacterial number colonizing the rhizosphere of winter wheat across locations and seasons

The results highlight that the highest values of the total number of bacteria were recorded in the autumn of 2022 in the rhizosphere of winter wheat cultivated in Slobozia-Conachi Galați and Ștefănești - Botoșani, with values of 28.50 ± 2.26 and 27.57 ± 1.94 CFU x 10^5 g^{-1} of dry soil, respectively. The lowest values were recorded in Ezăreni - Iași, with 23.17 ± 4.12 CFU x 10^5 g^{-1} of dry soil. The data indicate that there are differences between locations regarding the total number of bacteria, especially between Ștefănești - Botoșani and Ezăreni - Iași. However, the statistical interpretation of the results suggests that no significant differences were identified between locations or between the monitored seasons regarding the total number of bacteria.
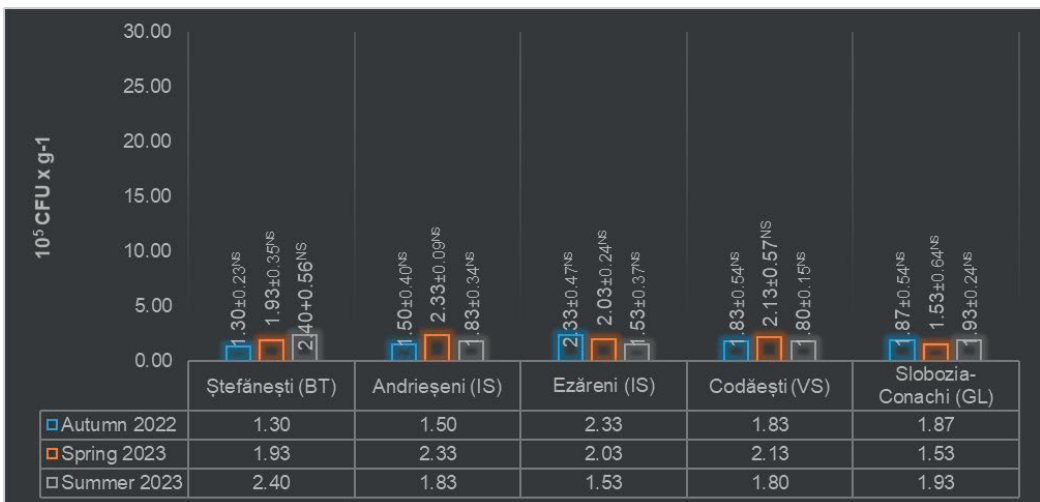

Figure 4. Comparison of Gram-positive bacteria colonizing rhizosphere of winter wheat across locations and seasons

The analysis of Gram-positive bacteria data (Figure 4) reveals important variations in their abundance across different locations (Ștefănești, Andrieșeni, Ezăreni, Codăești, and Slobozia-Conachi) over three seasons (Autumn 2022, Spring 2023 and Summer 2023).

The population size of Gram-positive bacteria exhibits the highest levels of abundance in winter wheat rhizosphere collected from Ezareni, especially in Autumn 2022 and Spring 2023. The abundance of Gram-positive bacteria from soil collected from other locations such as Slobozia-Conachi and Ștefănești show variations between seasons.

These fluctuations suggest that factors influencing the growth of Gram-positive bacteria may vary over time, even within the same geographical area. Despite observed variations in the abundance of Gram-positive bacteria across locations and seasons, the statistical tests do not provide sufficient evidence to support the significance of these differences.

The analysis of data regarding Gram-negative bacteria (Figure 5) indicates that no statistically significant differences have been identified between the values recorded in different locations or across the monitored seasons.

The highest values of the Gram-negative bacteria were recorded in the autumn of 2022 in the rhizosphere of winter wheat cultivated in Slobozia-Conachi Galați and Ștefănești - Botoșani, with values of 26.63 ± 2.64 and 26.27 ± 1.71 CFU x 10^5 g^{-1} of dry soil, respectively.

The lowest values of the Gram-negative bacteria were recorded in the summer of 2023 in the rhizosphere of winter wheat cultivated in in Ștefănești - Botoșani, with 17.30 ± 1.30 CFU x 10^5 g^{-1} of dry soil.
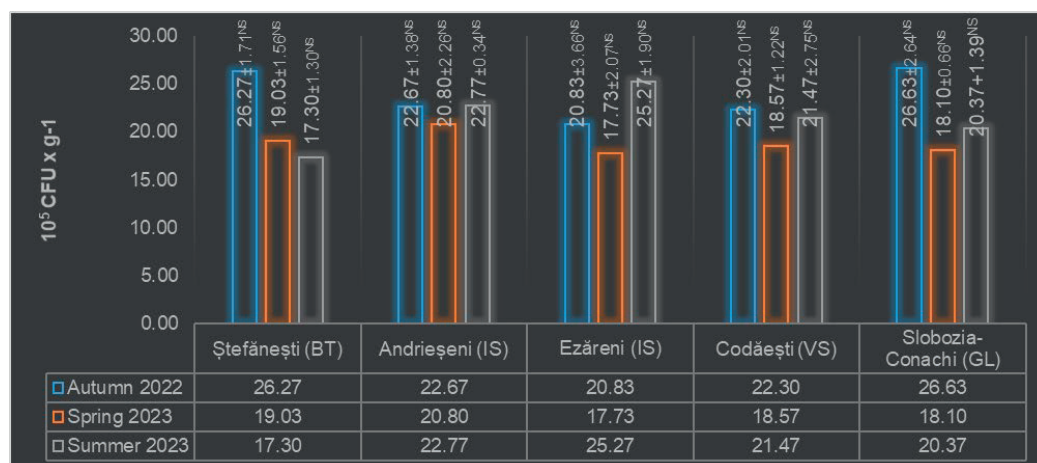

Figure 5. Comparison of Gram-negative bacteria colonizing rhizosphere of winter wheat across locations and seasons

	Ștefănești (BT)	Andrieșeni (IS)	Ezăreni (IS)	Codăești (VS)	Slobozia-Conachi (GL)
Autumn 2022	26.27	22.67	20.83	22.30	26.63
Spring 2023	19.03	20.80	17.73	18.57	18.10
Summer 2023	17.30	22.77	25.27	21.47	20.37

Although there are variations in the abundance of these bacteria across different locations and seasons, the statistical analysis does not provide sufficient evidence to support the significance of these differences.

CONCLUSIONS

Taking into account all the data provided in this study regarding the total number of bacteria, Gram-positive bacteria, and Gram-negative bacteria across various locations and seasons, several conclusions can be drawn: Firstly, while significant variations in bacterial abundance were observed across different locations and seasons, statistically, these differences are not assured, suggesting that the factors influencing these variations may be consistent across the entire study area. Secondly, soil collected from Ezăreni (IS) exhibited the most variation in bacterial abundance, often ranking among the highest or lowest depending on the season. Thirdly, the season played a significant role in bacterial dynamics, with some locations showing significant increases or decreases between the monitored seasons.

Overall, these results underscore the complexity of bacterial dynamics in various environments

and highlight the importance of further research to better understand microbial interactions and their influences in different ecosystems.

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