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COMPARISON OF SOIL MICROBIOTA IN WHEAT AND BARLEY CROPS

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Abstract. *Agriculture represents a complex interface between plants and their associated microorganisms. In contemporary agriculture, special attention is given to environmentally friendly approaches, particularly in developing countries, to improve system sustainability while minimizing negative effects on the quality and quantity of production. Modern agricultural practices, such as extensive plowing, the use of harmful agrochemicals, and monoculture, have been shown to influence soil microbial community structure and sustainability. The diversity of microorganisms associated with plant roots is immense, numbering in the tens of thousands of species. This highly complex microbial community associated with plants is known as the plant's second genome, playing a crucial role in plant growth, development, and health. This study investigated differences in the abundance, composition, and diversity of microbial communities throughout the growing season in soils collected from fields cultivated with winter wheat and winter barley in the northeastern region of Moldova. The aim of this study was to (i) analyze the seasonal dynamics of microbial communities in the rhizosphere of winter wheat and winter barley and (ii) examine the structural changes occurring within microbial communities during the growing season. The obtained data show minor variations in the total number of bacteria and fungal colonies for wheat and barley during the growing season. Gram-negative bacteria are dominant, and among fungi, Penicillium spp. and Aspergillus spp. are the most common. Barley exhibited a slightly higher microbial diversity compared to wheat.*

Keywords*: soil microbiota, soil microbial diversity, winter wheat microbiota, barley microbiota*

INTRODUCTION

It is well known that the world's population is on an upward trajectory, approaching the nine billion mark. In this context, the pressure exerted by this population growth on agriculture, in order to increase production and ensure food for all the planet's inhabitants, is becoming increasingly acute. Agriculture, essential for global food security, faces not only higher production demands but also additional biotic and abiotic pressures. These are primarily driven by climate change, which negatively affects both agricultural yields and the quality of products. In particular, extreme phenomena such as droughts, floods, and high temperatures disrupt vegetation cycles, leading to increased production volatility and, consequently, greater risks to global food stability. In addition to these challenges, issues related to soil degradation, biodiversity loss, and limited water resources further exacerbate the situation (Agoussar and Yergeau, 2021).

The unprecedented pace of these changes facing agriculture today requires the adoption of innovative approaches to enhance the resilience of cultivated plants to stress factors. Among these approaches is the development and practical application of advanced biotechnologies, such as genetic engineering, to create crops that are more tolerant to drought, extreme temperatures, pathogens, and other stress factors (Gosal et al., 2009; Seid and Andualem, 2021; KhokharVoytas et al., 2023). Additionally, the implementation of sustainable agricultural practices, such as crop rotation, the use of organic fertilizers, and the reduction of pesticides and chemical fertilizers, plays a crucial role in soil and water conservation, as well as in reducing pollution and preserving biodiversity (Shah et al., 2021; Gamage et al., 2023).

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In addition to the use of advanced biotechnologies and sustainable agricultural practices, another essential aspect of enhancing crop resilience is the role of soil microorganisms (Umesha, 2018). These microorganisms play a crucial role in maintaining soil health, improving nutrient uptake by plants, and enhancing their resistance to stress factors (Alori et al., 2017; Sahu et al., 2017; Kumar and Verma, 2019). Beneficial microorganisms, such as nitrogen-fixing bacteria and mycorrhizal fungi, contribute to the natural fertility of the soil, thereby reducing dependence on chemical fertilizers and promoting more sustainable agriculture (Igiehon and Babalola, 2018; Wahab et al., 2023).

All these processes involving microorganisms play a crucial role in agricultural production. Although there is extensive theoretical knowledge about how microbial communities can be modified, implementing these principles in practice remains a major challenge due to the complexity of interactions between microorganisms (Lynch et al., 2004; Miller et al., 2018; Agoussar and Yergeau 2021).

Studies on microorganisms present in the rhizosphere of crops show that crop plants have a significant impact on the microbial diversity associated with them (Garbeva et al., 2008). Additionally, some studies have indicated that within the same species, the effect of cultivated genotypes on root and rhizosphere microbial communities varies over time, depending on the phenological stage of the crop (Chialva et al., 2021; Quiza et al., 2023). These shifts in microbial communities are linked to the plant's developmental stages and are associated with changes in the composition and concentration of plant exudates as the plant grows (Chen et al., 2019; Zhao et al., 2021).

Some studies show that the type and rate of plant residue incorporation into the soil significantly influence the structure and diversity of microbial communities. Specifically, the incorporation of plant residues from corn crop led to an increase in copiotrophic bacteria, which thrive in nutrient-rich environments. In contrast, plant residues from wheat crop favored the development of oligotrophic bacteria, which are adapted to resource-limited conditions (Su, 2020). Other studies show that bacterial diversity in soil cultivated with winter wheat can be correlated with soil carbon and nitrogen fractions, while fungal richness can be correlated with partial nitrogen mineralization and microbial biomass of carbon and nitrogen (Fu et al., 2019). Additionally, some studies show that the type of management (conventional vs. organic) used in wheat cultivation has implications for microbial abundance and community function, with differences in soil microbial abundance and activity likely affecting crop yields and nitrogen uptake (Tautges, et al., 2016).

In the case of barley, some studies have followed the changes that occur in the bacterial communities in the rhizosphere under the influence of different phosphate sources, helping to understand the ecology of the soil microbiome cultivated with barley and the interactions between plants and microorganisms (Cardinale et al.,2019). Other studies have analyzed bacterial communities in the rhizosphere of some barley varieties, noting that approximately the same species of bacteria were identified in most varieties, but differences were observed in the abundance of the identified species (Zhang et al., 2023).

To fully understand the implications that microorganisms have in different agricultural processes, a first step is to know the structure and abundance of microbial communities in the soils we cultivate. In this regard, the purpose of the presented works is to (i) analyze the seasonal dynamics of microbial communities in the rhizosphere of winter wheat and winter barley and (ii) examine the structural changes that occur in microbial communities during the growing season.

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MATERIAL AND METHODS

The research sites were established at the Agralmixt S.A. farm in Andrieseni village, Iasi county, northeastern Romania (47°34'9" N, 27°20'38" E), at an altitude of 60 meters above sea level. The region has a temperate continental climate, with a multiannual average air temperature of 9.5°C and an annual precipitation of 520 mm (Gafencu et al., 2023).

During the study period (September 2023 – August 2024), the average air temperature increased to 13.52°C, potentially impacting local ecological and agricultural processes, including crop growth and water requirements. Temperature fluctuations were notable (figure 1), with the lowest recorded in January (-18.12°C) and the highest in July (46.95°C).

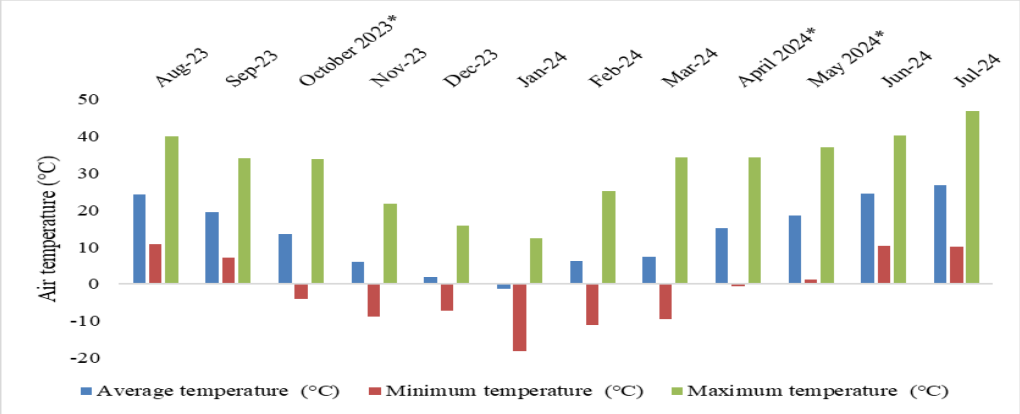

Figure 1. Monthly air temperature (average, minimum, and maximum) recorded during the winter wheat and winter barley vegetation period in 2024
at the Agralmixt S.A. farm, weather station 00001CED

Precipitation varied throughout the year, peaking in June (117.4 mm) and reaching its lowest in August (12.0 mm), with a total annual precipitation of 515.5 mm (figure 2).

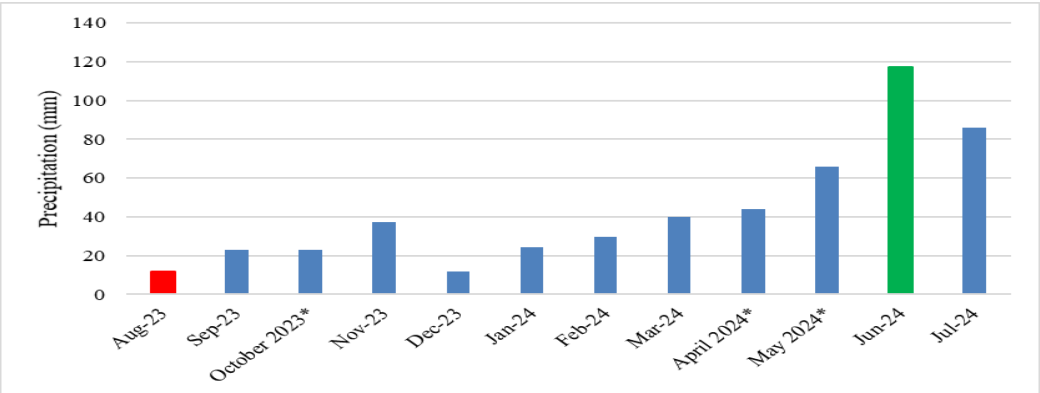

Figure 2. Atmospheric precipitation recorded during the winter wheat and winter barley vegetation period in 2024 at the Agralmixt S.A. farm, weather station 00001CED

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Soil samples were collected from chernozem soil cultivated with winter wheat and winter barley. Conventional agricultural practices were applied to both crops, including the use of chemical fertilizers and pesticides.

Soil sampling was conducted three times during the vegetation period of the wheat and barley crops. The first sampling took place in the fall of 2023, after crop establishment. The second and third samplings were carried out in the spring of 2024, in April and May. In this study, the analyzed cultivars included the Glosa wheat variety and the Cardinal barley variety. Within each analyzed plot, soil samples were collected separately for each crop from approximately 20 randomly selected points. Sampling was performed from the root zone of the plants at a depth of about 8 cm. Strict aseptic measures were followed during soil collection to prevent contamination, using sterile tools and containers. After sampling, the soil samples were transported to the Microbiology Laboratory of the Faculty of Agriculture at the Iasi University of Life Sciences.

The samples were stored overnight at 4°C, dried at room temperature the following day, and then sieved before microbiological analysis. 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 used, applying serial dilutions before inoculation.

In this study, Potato Dextrose Agar (Scharlau, Spain, 39 g/L) was used as the culture medium. To determine the total number of bacteria, standard Potato Dextrose Agar (PDA) was used, while the enumeration of Gram-positive bacteria was performed using PDA supplemented with streptomycin (35 mg/L). Filamentous fungi were quantified using PDA medium supplemented with Rose Bengal (35 mg/L) to inhibit the proliferation of fast-growing molds (Smith and Dawson, 1944).

For each sample, the successive dilution method was applied. To analyze bacterial and fungal populations, 1 mL of the suspensions diluted at 10^{-3} and 10^{-4}, respectively, was transferred to Petri dishes, followed by the addition of 17 mL of culture medium at 48°C. The contents were homogenized using circular movements before solidification. The Petri dishes were then incubated at 28°C. After 24 hours, the number of bacterial colonies was determined for both PDA and streptomycin-supplemented PDA media using the Scan® 1200 automatic colony counter. The total bacterial count per gram of soil was calculated by multiplying the obtained values by the inverse of the dilution factor, and results were expressed as CFU×10^5 g^{-1} dry soil.

Filamentous fungi were evaluated after a five-day incubation period, and identification was performed based on morphological characteristics (Figure 3), in accordance with established taxonomic references (Gilman, 1957; Barnett, 1960; Ellis and Ellis, 1985; Seifert and Gams, 2011; Guarro et al., 2012). Fungal isolates that failed to produce spores within this timeframe and could not be identified were classified as “Other species.”

RESULTS AND DISCUSSIONS

The cultivation technology was relatively similar for both plant species. *Brassica napus* served as the preceding crop. Following its harvest, soil preparation involved shredding of plant residues, scarification, and seedbed preparation. Chemical fertilizers were applied in moderate amounts to ensure adequate nutrient availability. In addition, during the vegetation period, pesticides were employed to prevent and control pathogens, pests, and weeds, thereby promoting the optimal development of the wheat and barley crops.

The analysis of microbiological data highlights differences in soil bacterial density between wheat and barley crops. Across all three determinations, total bacterial counts were

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higher in soils under barley than under wheat. In autumn 2023, the most pronounced difference was observed 18.20±1.37×10^5 CFU/g soil for barley versus 16.23±1.39×10^5 CFU/g soil for wheat. In the spring analyses, bacterial densities remained greater in barley soils (16.03±1.69 and 17.27±1.07 × 10^5 CFU/g soil) than in wheat soils (15.93±0.74 and 16.43±1.33×10^5 CFU/g soil), although the disparities were less marked (Figure 1).

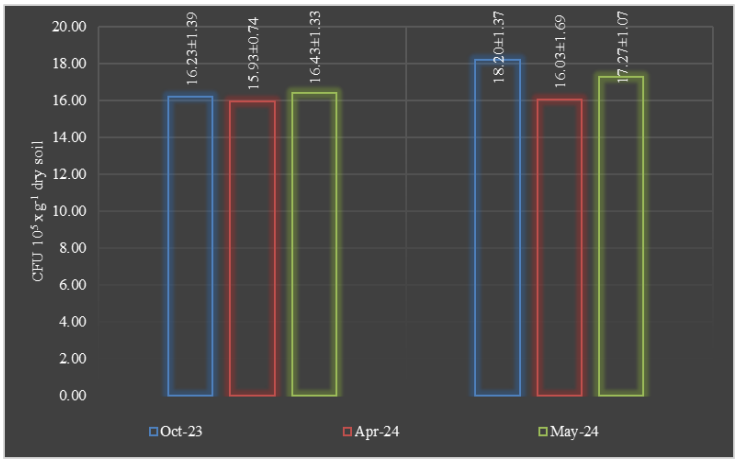

Figure 1. Evolution of the total number of bacteria during the growing season

The dynamics of Gram-positive bacteria densities in soils under wheat and barley cultivation exhibited a slight temporal fluctuation (Figure 2). In autumn 2023, counts were 1.80±0.15×10^5 CFU/g soil in wheat plots and 1.70±0.15×10^5 CFU/g soil in barley plots. By April 2024, densities peaked at 1.87±0.12×10^5 CFU/g soil for wheat and 1.67±0.09×10^5 CFU/g soil for barley. A subsequent decline was observed in May 2024, with values of 1.60±0.10×10^5 CFU/g soil and 1.50±0.20×10^5 CFU/g soil for wheat and barley, respectively.

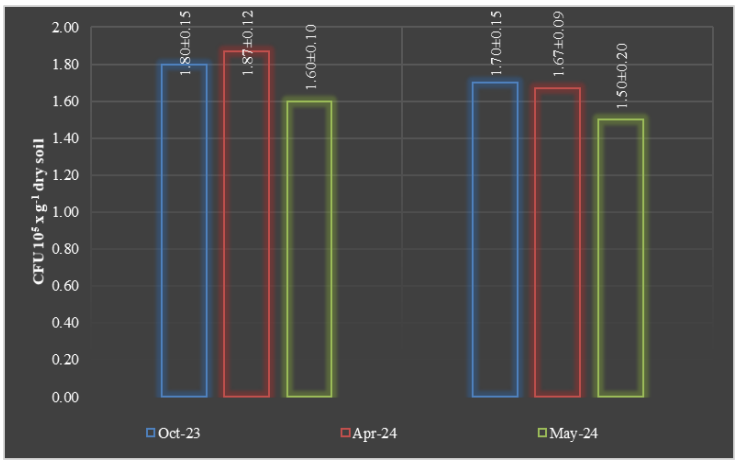

Figure 2. Evolution of Gram-positive bacteria during the growing season

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The dynamics of Gram-negative bacteria densities in soils under wheat and barley cultivation varied throughout the monitoring period (Figure 3). In autumn 2023, the highest bacterial densities were observed in barley soils ($16.50\pm1.40\times10^5$ CFU/g soil), compared to wheat soils ($14.43\pm1.36\times10^5$ CFU/g soil). By April 2024, the densities in wheat and barley soils were relatively similar, with $14.07\pm0.63\times10^5$ CFU/g soil for wheat and $14.37\pm1.62\times10^5$ CFU/g soil for barley. In May 2024, a slight increase in bacterial density was observed in both soils, with barley soils showing higher counts ($15.77\pm1.04\times10^5$ CFU/g soil) compared to wheat soils ($14.83\pm1.33\times10^5$ CFU/g soil).

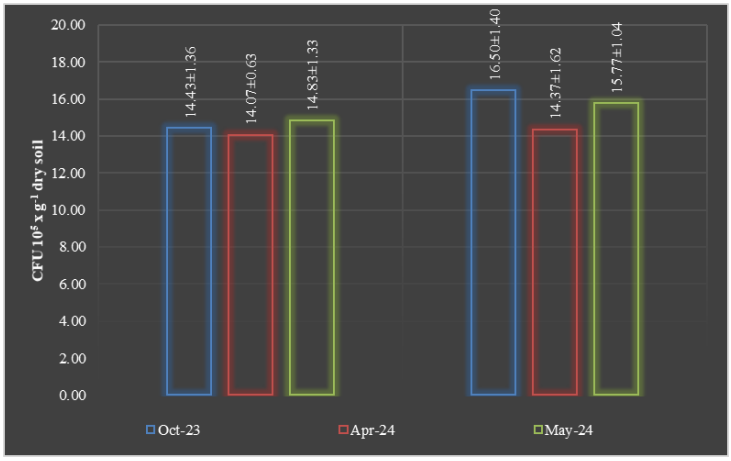

Figure 3. Evolution of Gram-negative bacteria during the growing season

The relative abundance of fungal genera in soils cultivated with wheat and barley, expressed as percentages, highlights distinct community profiles influenced by crop type and season (Tabel 1).

In autumn 2023, *Penicillium* spp. dominated both wheat (49%) and barley soils (37%), followed by *Aspergillus* spp. (25% in wheat, 30% in barley). Barley soils showed higher proportions of *Fusarium* spp. (6%) and *Rhizopus* spp. (4%) compared to wheat.

By April 2024, *Penicillium* spp. remained the dominant genus in both crops, increasing to 55% in barley and 48% in wheat. Wheat soils had relatively balanced proportions of *Aspergillus* spp. (27%) and other genera like *Chaetomium* spp. and *Alternaria* spp. (~5%), while barley soils showed greater presence of *Chaetomium* spp. (7%).

In May 2024, although *Penicillium* spp. continued to be predominant, its proportion slightly decreased in wheat (40%) and barley (41%) soils. Wheat soils exhibited increased diversity, with notable contributions from *Trichoderma* spp. (8%) and *Chaetomium* spp. (7%), whereas *Alternaria* spp. and *Fusarium* spp. were more prominent in barley (6% each).

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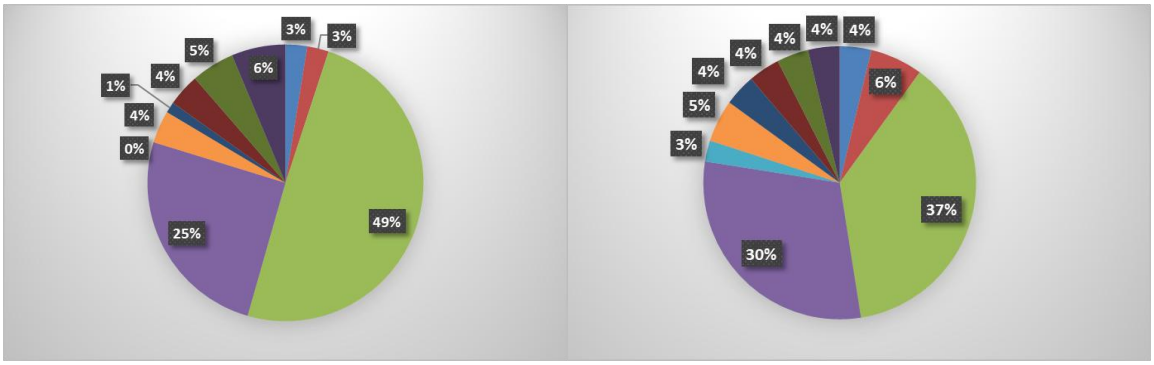
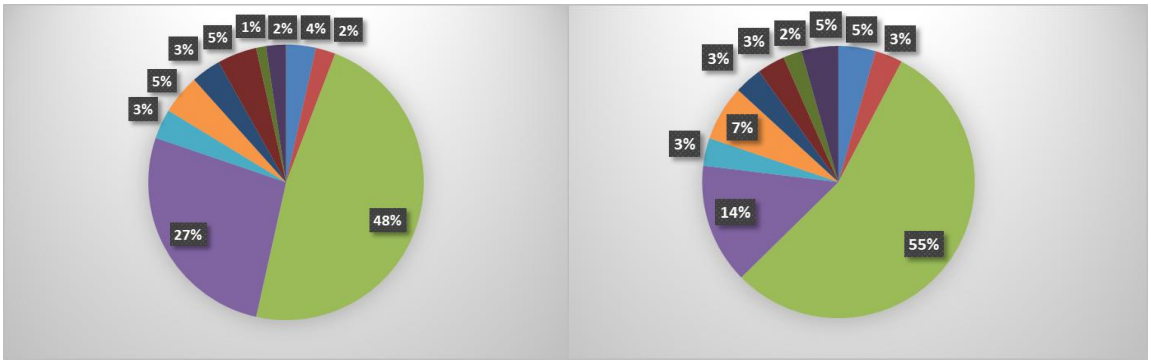


October 2023



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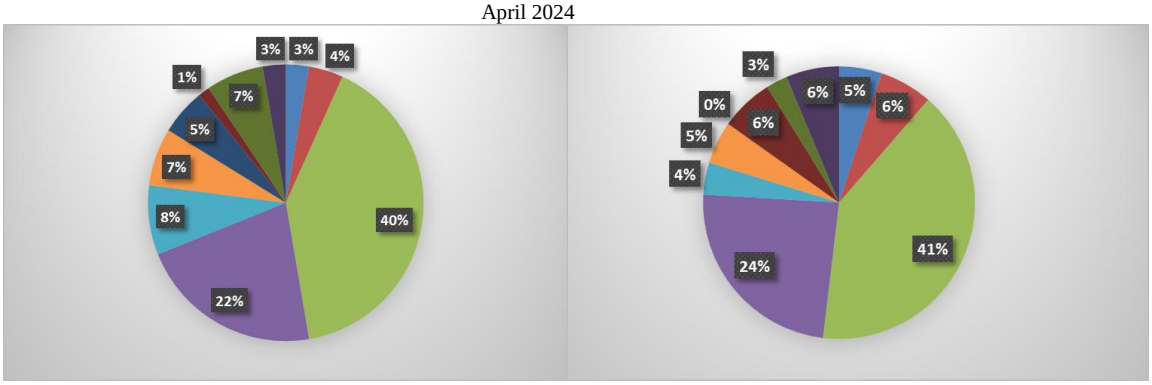

May 2024

Wheat **Barley**

Rhizopus spp. *Fusarium spp.* *Penicillium spp.* *Aspergillus spp.*
Trichoderma spp. *Chaetomium spp.* *Mucor spp.* *Alternaria spp.*
Verticillium spp. *Other species*

Figure 4. Changes in fungal communities during the growing season

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CONCLUSIONS

Based on the data obtained in this study, it can be concluded that crop type influences the structure and dynamics of soil microbial communities, both bacterial and fungal. Soils cultivated with barley generally exhibited a higher total number of bacteria compared to those cultivated with wheat, with the most notable differences observed in autumn.

Additionally, Gram-negative bacteria were slightly more abundant in barley soils, while Gram-positive bacteria showed only minor variations between the two crops.

Regarding fungal communities, *Penicillium* spp. was the dominant genus in all analyzed periods; however, barley soils supported greater fungal diversity, with higher values recorded for species such as *Fusarium* spp., *Rhizopus* spp., and *Alternaria* spp.

These findings highlight that, compared to wheat, barley promotes more intense microbial activity and a more diverse fungal community in the soil, which may have important implications for soil health and the sustainable management of crop systems.

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