

INFLUENCE OF CROP SPECIES ON SOIL MICROBIAL ABUNDANCE AND DIVERSITY

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Abstract

*Agricultural crop species can influence the soil microbial population through the influence of root exudates, which compounds released by plant roots into the soil. Some crop species exude compounds that are more beneficial for certain groups of microbes, while others exude compounds that inhibit the growth of certain groups. Additionally, different crop species have different root systems, which can affect the physical structure of the soil and the availability of water and nutrients, which in turn can influence the soil microbial population. Moreover, crop species can also influence the soil microbial population by changing the soil pH and the level of organic matter. Crop species that produce large amounts of biomass can increase the amount of organic matter in the soil, which can support a greater diversity of microorganisms. Maize and soybean are some of the most important agricultural crop globally. Both corn and soybean are important crop species for the global food system and also for the economy of many countries, including Romania. A study was conducted to determine the composition of the soil microbial community in the rhizosphere of maize (*Zea mays L.*) and soybean (*Glycine max (L.) Merrill.*) at different phenological stages and to determine whether the two crops influence the microbiological abundance and diversity in the soil.*

Key words: soil microbial communities, maize, soybean.

INTRODUCTION

Global demand for food is currently projected to increase by approximately 70% by 2050 due to the need to feed the ever-growing human population (Wise, 2013; FAO, 2009). However, under current conditions, it is highly unlikely that agricultural practices will be able to fulfil this requirement, faced with continued land degradation, declining soil fertility, climate change and increased frequency of extreme weather events. In addition to these changes, the agricultural sector also faces important problems due to: a) economic growth, such as high production costs (i.e. the increase in the prices of agricultural inputs: seeds, fertilizers, pesticides, fuel, etc.) which leads to the registration of a low margins of profitability, b) the social situation, the lack of labor, the demands of the population for good quality food and without residues of chemical substances; c) environmental issues, avoiding or minimizing soil, water and air pollution; d) political, such as the ecological agreement of the European Union, through the application of which the concomitant promotion of crop

productivity, but also environmental sustainability is desired (Knez et al., 2022; Igliński et al., 2022). To deal with these situations, knowledge of soil microbiota and its role can complement conventional agricultural practices to ensure sustainable food supply (Tian et al., 2020).

Microorganisms play an essential role in sustaining life on Earth (Turner et al., 2013). However, so far we have been able to understand very little about the microorganisms that colonize environments such as ocean water, the atmosphere, soils, etc. Techniques based on the growth of microorganisms isolated from various environments on culture media have allowed them to be studied in detail. The exploitation of microorganisms associated with cultivated plants represents one of the solutions that is shown to be among the most promising in the long term, facing the challenges encountered on the road to ensuring food security, while also supporting the sustainability of the environment (Singh et al., 2020). Microorganisms are responsible for many processes that take place in the soil. As a result of these processes, nutrients, arrive in

forms accessible to plants, plant health can be promoted by means of microorganisms. Microorganisms also help plants improve resistance to biotic and abiotic stress (Tkacz et al., 2020).

The microbiota represents the totality of microorganisms that are naturally present in a given environment that is studied together with the host and the surrounding environment, such as the plant-soil complex. Microorganisms are in a very close relationship with the environment in which they live, but the changes that occur in the environment are able to produce changes on the microbiota.

Crops, widely grown in fields, for human consumption, animal feed, raw materials for various industries (i.e. wheat, corn, rice, soybean) have gone through important changes in cultivation technologies, along with industrialization and development of human society, due to the increased use of agricultural machinery, chemical fertilizers and pesticides. For these reasons, the composition of the microbiota associated with crop plants has been strongly modified (Lamb et al., 2011). Increasing the productivity of agricultural crops has been an objective pursued throughout the last centuries, but the current situation, represented by globalization, rising prices and climate change, requires finding solutions that allow the production of more food in an ecologically and socially sustainable way (Chaparro et al., 2012). To achieve this goal, agriculture must be based on the relationships that are established between plants and microorganisms, aiming to promote soil microbial diversity.

Current research shows the importance of plant-microorganisms relationships on the productivity and health of agricultural crops (Howard et al., 2020; Berg & Smalla, 2009; Morrissey et al., 2004).

As discussed above, the agricultural sector must be aware of the role that soil microorganisms have on agricultural crops, which is why the present study aims to present the influence that two of the most important and cultivated agricultural crops (corn and soybean) have on the abundance and diversity of bacteria and filamentous fungi in the soil.

MATERIALS AND METHODS

To highlight the microbiological activity carried out at the soil level, in the case of corn and soybean crops, and to determine the total number of bacteria (Gram-positive bacteria and Gram-negative bacteria) and filamentous fungi per gram of soil, the plating into nutritive media method was used.

The soil that was microbiologically analyzed in this study was taken from the northeastern region of Moldova, Romania. In the studied area, the climate is temperate continental. The average annual air temperature is $\sim 9.5^{\circ}\text{C}$, with extremes between 40.0°C and -35.0°C . The sum of the average annual precipitation is ~ 520 mm. Significant deviations from the long-term average of precipitation and temperature have been observed over the past 10 years. The soil texture is primary clay loam, and the natural vegetation of the site is silvo-steppe (Gafencu, 2019).

The analyzed soil type was represented by chernozem cultivated with corn and soybean under conventional farming conditions, using synthetic fertilizers and chemical pesticides.

Soil samples were collected for each cultivated species, 6 times during the vegetation period, between May and October 2022.

For each individual crop, 10 points were randomly chosen on the surface of the plot so that the entire surface was covered. Before each sampling, organic debris present on the soil surface was removed. Soil samples were taken from a depth of approximately 7-10 cm, using sterile instruments. Soil samples were collected in paper bags and transported to the microbiology laboratory for further analysis. The samples were kept overnight at 4°C , and the next day they were left to dry at room temperature. Any organic residue was removed from the soil mass, and then the soil was homogenized using a mortar and pestle (sterilized in advance to avoid contamination of the soil samples with other microorganisms).

After the preparation of soil samples for microbiological analyses, the total number of bacterial colony-forming units (CFU) was determined by serial dilutions and plating in nutritive media.

One gram of dry soil was introduced into a first tube (sterile 15 ml Falcon tube) containing 9 ml of sterile water and homogenized very well

(stirred for 5 minutes using a MX-RD-E Vertical Rotator), obtaining the dilution 10^{-1} . Then, from the 10^{-1} dilution, one millilitre of the suspension was transferred using a sterile Pasteur pipette into a second tube, thus obtaining the 10^{-2} dilution. After a series of successive dilutions, dilutions from 10^{-2} to 10^{-6} were prepared. From each dilution obtained, one millilitre of the suspension was spread on nutrient media to evaluate the number of bacteria and filamentous fungi. For an easy identification of the colonies, different culture media were used, specific for each group of microorganisms. Thus, to determine the total number of bacteria, simple PDA medium (Potato Dextrose Agar) was used, to determine the number of Gram-positive bacteria (G+), was used PDA medium with the addition of streptomycin, 35 mg L^{-1} , added after sterilization when the temperature of the medium was around $46\text{--}48^\circ\text{C}$, and to determine the number of filamentous fungi, the PDA medium with the addition of Rose Bengal, 33 mg L^{-1} was used. After inoculation the Petri dishes were incubated in the thermostat at a temperature of 28°C . The number of bacterial colonies was determined after 24 hours, and the fungal colonies after 120 hours. The determination of the number of bacterial colonies was performed using an Interscience Colony Counter Scan 1200 automatic colony counter.

The data obtained in the experiments were statistically evaluated using the SPSS program (IBM SPSS Statistics 26). and the result with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSIONS

The analysis of microbiota from the soil samples showed that there are differences in the abundance and structure of the microbial communities in the soil, being influenced by both, the crop plant and the phenophase in which the plants are found (seasonal variation). Similar results were obtained by other authors (Jat et al., 2021; Longley et al., 2020; Peralta et al., 2018), their results indicate that the growth stage and management system have significant effects on the corn and soybean soil microbiome.

A close examination of the microbiota in the corn and soybean rhizosphere shows us the presence of a high variability in the abundance activity and structure of the microbial communities.

The results show us that the crop influences the total number of bacteria in the soil (Figure 1). Even if plots cultivated with corn and soybean are found in the same area, the soil has the same physical and chemical properties, under the influence of the cultivated plant, changes occur at the level of the soil microbiome.

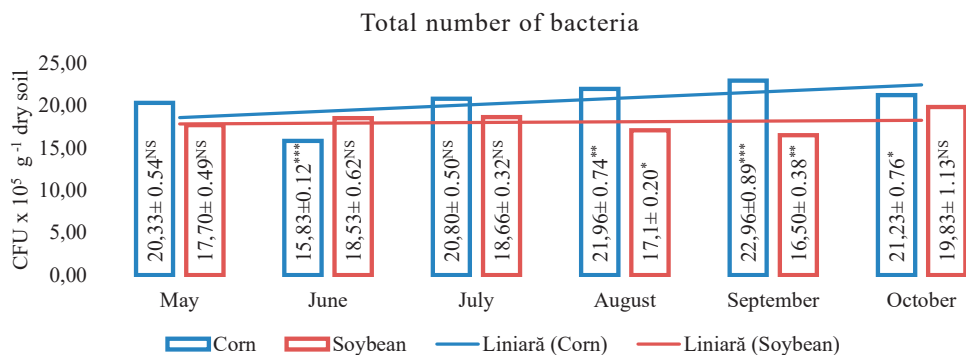


Figure 1. Evolution of total soil bacteria during the vegetation period of corn and soybean crops [NS = not significant; *not significant; **significant; ***distinctly significant]

The results show that in the case of the corn crop, the total number of bacteria increased from May to October. The highest values were recorded in September, when a count of $22.96 \pm 0.89 \text{ CFU} \times 10^5 \text{ g}^{-1} \text{ dry soil}$ was recorded. If in the case of the corn crop the trend is upward,

in terms of the total number of bacteria, in the soybean crop it can be observed that the trend was linear. Even though from May to October different values were recorded at each time when samples were taken, it can be seen that the values obtained were somewhat similar.

Gram-negative bacteria (G^-) represent the most important group of microorganisms in the studied soil. This particular group of bacteria represents about 70-75% of the total number of microorganisms that can be cultivated on the culture medium. In the case of Gram-negative (G^-) bacteria, the numerical evolution was similar to that recorded for the total number of bacteria. In the case of the soil taken from the corn crop, it can be observed that from May to

October, during the studied period, the number of Gram-negative bacteria (G^-) followed an upward direction. The highest value was obtained in September, 21.47 ± 1.07 CFU $\times 10^5$ g $^{-1}$ dry soil. In soybean crop, the numerical density of Gram-negative bacteria (G^-) remained unchanged throughout the vegetation period, except for October, when the highest value was recorded, 18.30 ± 1.12 CFU $\times 10^5$ g $^{-1}$ dry soil (Figure 2).

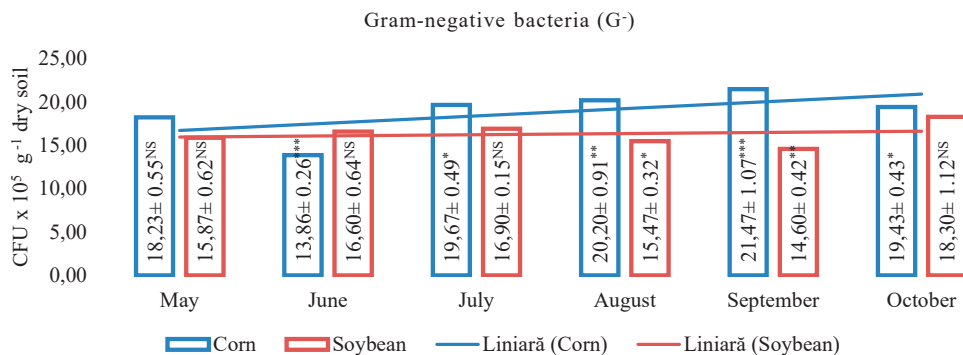


Figure 2. Evolution of total soil Gram-negative bacteria (G^-) during the vegetation period of corn and soybean crops [NS = not significant; *not significant; **significant; ***distinctly significant]

Gram-positive bacteria (G^+) represent the particular group of microorganisms that was the least represented during the study (Figure 3). The results show that the numerical density of Gram-positive bacteria (G^+) was not influenced by the plant crop. In both cases,

corn and soybean, the trend was the same. The numerical density of Gram-positive bacteria (G^+) decreased from May until October. The highest values, 1.93 ± 0.03 and 1.90 ± 0.06 CFU $\times 10^5$ g $^{-1}$, respectively, were recorded in the soybean crop, in July and September.

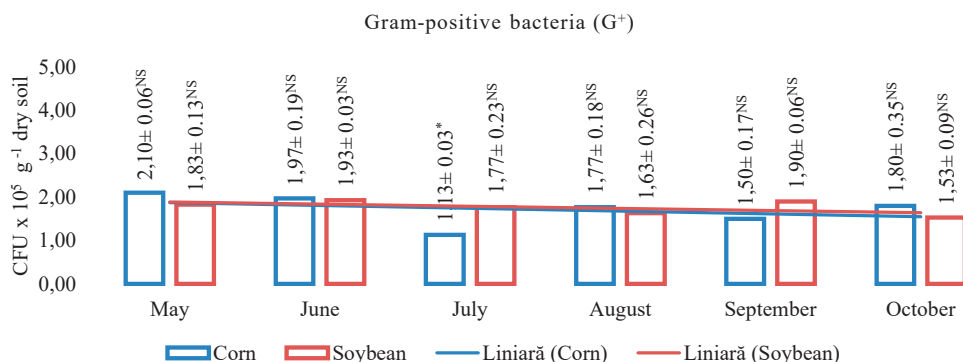


Figure 3. Evolution of total soil Gram-positive bacteria (G^+) during the vegetation period of corn and soybean crops [NS = not significant; *not significant; **significant; ***distinctly significant]

The results obtained from the microbiological analysis regarding the abundance and structure of the communities of filamentous fungi in the soil indicate the presence of saprophytic and

parasitic fungal genera. The determined fungal genera and their structure are shown in Figure 4.

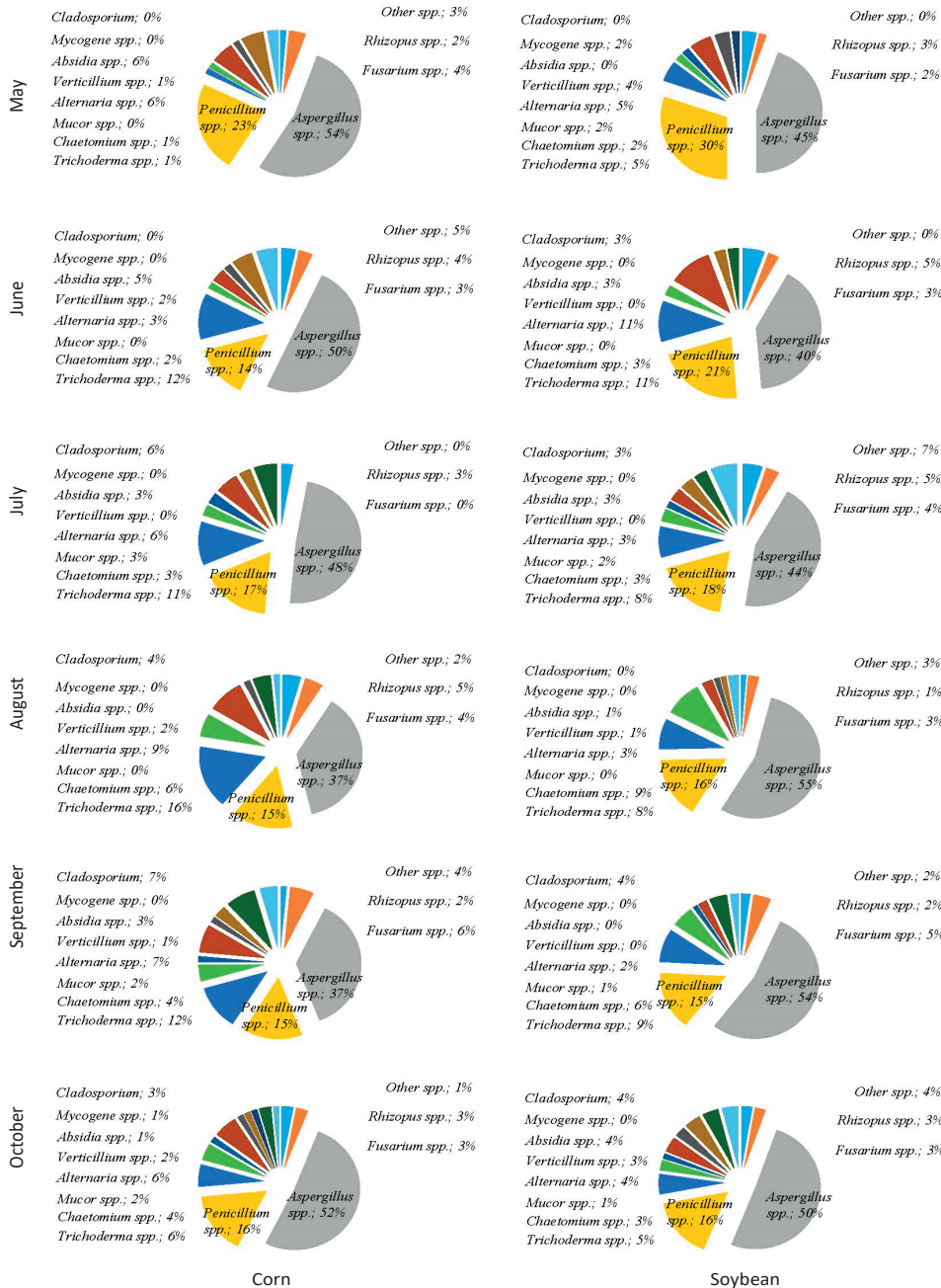


Figure 4. Frequency of isolated filamentous fungi during the vegetation period of corn and soybean crops

As a result of the research, we observed that the number of isolated filamentous fungi genera were different from one culture to another and, in the case of the same culture, throughout the vegetation period. The species of filamentous fungi culturable on artificial medium belonged

to 12 genera of micromycetes: *Cladosporium*, *Mycogone*, *Absidia*, *Verticillium*, *Alternaria*, *Mucor*, *Chaetomium*, *Trichoderma*, *Rhizopus*, *Fusarium*, *Penicillium* and *Aspergillus*. In addition to these genera that were identified based on morphological characters, other

filamentous fungi were also developed, in the case of which the identification could not be 100% certain, which is why they were included in a separate group, *Other* spp.

Among the micromycetes determined, in all cases, the genus *Aspergillus* stood out due to the large number of colonies it formed on the culture medium, the isolation of this genus being represented by a rate between 37 and 55% of the total genera identified. Another genus of micromycetes frequently identified was represented by *Penicillium*, with a rate between 14 and 30%. The genus *Fusarium* was also frequently isolated, this could be related to the fact that on the plots analyzed, maize monoculture was practiced for a long period of time.

CONCLUSIONS

Our observations on the total number of microorganisms/g (CFU g⁻¹ dry soil) in the sampled soils showed differences in the microbiological activity carried out in the rhizosphere of corn and soybean crops. Also, during the vegetation period, the abundance and structure of microbial communities experienced changes, generally showing a positive trend. Finally, in the soil layer (up to a depth of 10 cm) intense biological activity takes place due to the presence of microorganisms (Gram-positive and Gram-negative bacteria and micromycetes).

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