CROP SPECIES AND YEAR SEASONS AS DETERMINANTS OF MICROBIOTA STRUCTURE IN CHERNOZEM SOIL IN THE SOUTHEAST REGION OF MOLDAVIA, ROMANIA

Florin Daniel LIPŞA[1], Andreea Mihaela FLOREA[1], Andrei Mihai GAFENCU[1], Eugen ULEA[1]

e-mail: flipsa@uaiasi.ro

Abstract

In this study, we examined the diversity and abundance of microbial communities isolated from chernozem soil type in response to crop species and season from southeast region of Moldavia, Romania. Soil types, plant species, season, human activities and various land management regimes all have great impact on soil biology, but our knowledge of biodiversity of soil microorganisms is still very limited. Therefore, during 2018 we assessed the variation of microbial community in chernozem soil and its response to plant species (rapeseed, sunflower, sugar beet, wheat and maize) and season (spring, autumn). At each site, five replicate bulk samples were taken, consisting of 10 randomly collected sub-samples from the surface soil (10-15 cm horizon). The samples were transported to the laboratory, stored overnight at 4°C, air-dried at room temperature and sieved (2-mm mesh) prior to further use in the experiment. The influence of crop species and season on the total number of microorganisms ($CFU\ g^{-1}$), relationships between the main groups (bacteria and fungi) and the spectrum of filamentous fungi from our experiment were established. The abundance of the microbial community from all crop species were ranked as follows: wheat ($19.2\ x\ 10^6\ CFU\ g^{-1}$) > sunflower ($1.3\ x\ 10^6$ $CFU\ g^{-1}$) > maize ($1.1\ x\ 10^6\ CFU\ g^{-1}$) > sugar beet ($0.6\ x\ 10^6\ CFU\ g^{-1}$) > rapeseed ($0.4\ x\ 10^6\ CFU\ g^{-1}$). In case of yearly seasons, the microbial abundance decreased from spring to autumn. Occurrence and distribution of filamentous fungi isolated from soil samples provide new insights into ecology and niche specialization of several soil-borne species. Overall, genera composition of filamentous fungi from chernozem soil cultivated with different crop species was not very heterogeneous and most fungal genera were common to all location. Our results suggest that crop species and yearly seasons have a significant impact on microbial richness and diversity. Extensive use of xenobiotic compounds in agriculture will degrade soil microbial communities, because they affect directly microbial abundance and composition, and indirectly soil texture and fertility.

Key words: microbial community structure, chernozem soil, crop species, seasonal variation

Soil represents a habitat for many and various microorganisms that are important in recycling of plant nutrients, maintenance of soil structure, toxin removal, promoting plant growth, and suppressing soilborne plant diseases (Doran J.W. *et al*, 1996; Giller K.E. *et al*, 1998; Filip Z., 2002). Soil fertility is determined by its composition and properties (morphological, physical, chemical and biological), which have a strong impact on a range of processes influencing crop yield, including microbial diversity.

Microbial diversity (community structure) has been recommended as a biological indicator of soil quality, and describes the number of different genera/species and their relative abundance in a given community in a given habitat (Garbeva P. *et al*, 2008). 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 (Ulea E. *et al*, 2017). Lan G.Y. *et al* (2017) showed that seasonal changes explained the largest part (31.9%) of the total variance of bacterial community composition in soils from tropical region of Hainan (China). Soil bacteria were more sensitive than fungi to the fertilization practices (Ai C. *et al*, 2018). Their results suggest that different response patterns of soil bacteria and fungi to agricultural practices might have consequences for ecosystem function. Microbial community responses to alternative management may be indicative of soil quality change (Schutler M. *et al*, 2001). It is known that agricultural practices cause a loss to microbial biodiversity, and can lead to dramatic changes in bacterial compositions over a relatively short time period (Lan G.Y. *et al*, 2017). Once applied, agricultural chemicals influence directly or indirectly the agroecosystem structure and function (Joergensen R.G and Emmerling C., 2006; Lo C.C,

---

[1] "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Iasi

2010). In case of soil microbial communities, these chemicals affect their diversity, metabolic activities, reproduction and growth. In general, application of agrochemicals initially decreases the microorganisms number and activity, but as the chemical persists microorganisms develop tolerance/resistance and recolonize the soil.

The main purpose of the present research was to investigate how the structure of microbial communities from chernozem soil type is affected by crop species (rapeseed, sunflower, sugar beet, wheat and maize) and season (spring, autumn).

MATERIAL AND METHOD

Soils in this study were sampled from southeast region of Moldavia, Romania. Coordinates for the coverage area extended from latitude 44.935 N to 45.282 N and from longitude 27.082 E to 27.322 E. At each site, five replicate bulk samples were taken, consisting of 10 randomly collected sub-samples from the surface soil (10-15 cm horizon). Approximately 1000 g of soil was collected in sterile plastic bags from each site and any organic residue was removed from the surface before sampling.

Soil samples were collected from chernozem soil type cultivated with different plant species (rapeseed, sunflower, sugar beet, wheat and maize) under conventional agricultural conditions (synthetic pesticides and fertilizers were used). Microbial counts in the soil samples were recorded in spring (May) and autumn (September) 2018. The altitude ranges from 25 to 99 meters above sea level.

The samples were transported to the laboratory, stored overnight at 4°C, air-dried at room temperature and sieved (2-mm mesh) prior to further use in the experiment. Soils pH was determined with a glass electrode in a 1:2.5 soil to water ratio and the values covered a range between 8.1 and 8.4.

The total numbers of bacteria of colony forming units (CFUs) were determined by serial dilution and plating into nutritive media. One gram of soil was mixed with 9 mL sterile water (dilution 10^{-1}) and then 1 mL of the dilution 10^{-1} was poured into 9 mL sterile water (dilution 10^{-2}). After a successive tenfold dilution series, 10^{-2} to 10^{-6} dilution were prepared. Aliquots (1 mL) of 10^{-2} to 10^{-6} dilution were spread on nutritive media for assessing the total number of bacteria (Lipsa F.D. and Ulea E., 2018)

Average numbers of colony forming units in 1 g of dry weight soil ($CFU \cdot g^{-1}$) was determined using the plate counting method (Bressan M. *et al*, 2015), on potato dextrose agar medium (PDA) in different compositions: classic, with streptomycin and rose-bengal stain. Streptomycin antibiotic (35 mg·L^{-1}) was used to control the reproduction of Gram negative bacteria and rose-bengal stain was used to limit the growth of fast-growing moulds (e.g. *Rhizopus* spp., *Trichoderma* spp.). Czapek-Dox agar media was used for filamentous fungi identification. Light microscopy (1000x magnification) was used to determine the colonial features and the morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on fungal material mounted in lactophenol by slide culture technique. Fungi were identified to genus level based on morphological and physiological characteristics following the works provided by Ellis (1971, 1997), De Hoog et al. (2000), Barnett and Hunter (1999).

The number of bacterial colonies was determined at 24 hours 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. Microbiological media plates were prepared using Masterclave 09 plate maker and an aliquot portion of 15mL of media was poured using APS 320 automated Petri plate filler (AES Laboratoire, France).

The data obtained in the experiments were statistically evaluated with SPSS 16.0 for Windows and the results with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSIONS

Analyzing the ratio between the main groups of microorganisms found in the soil during the observation time, we found differences among seasonal variation and crop species. Bacterial and fungal counts in the soil samples were recorded in spring (May) and autumn (September) 2018. The highest counts of total bacteria were recorded throughout the spring seasons, whereas the lowest count was found throughout the autumn seasons (*figure 1*). The dynamics of the bacterial community from all crop species were ranked as follows: spring (22.6 x 106 CFU g^{-1}) > autumn (15.8 x 106 CFU g^{-1}). These results are in line with the previous studies and the favourable conditions (temperature, soil moisture) during spring (Bouskill N.J. *et al*, 2013; Francioli D. *et al*, 2014; Valverde A. *et al*, 2014) account for them. The recorded data showed significant seasonal variations (spring vs. autumn) (p<0.05).

Our findings about different crop species and season fluctuation showed that the bacterial richness, assessed through the plate counting method, was ranked wheat > sunflower > maize > sugar beet > rapeseed. Application of xenobiotic compounds on soils covered with annual crops determined an increase or a decline in bacterial richness as direct response to the degree of habitat disturbance. Organic fertilization of agricultural soils yielded distinct community structures with

higher richness and diversity (Ge Y. *et al*, 2008). The intensity of human intervention, the environmental and edaphic factors from each location could have a specific impact on the results.
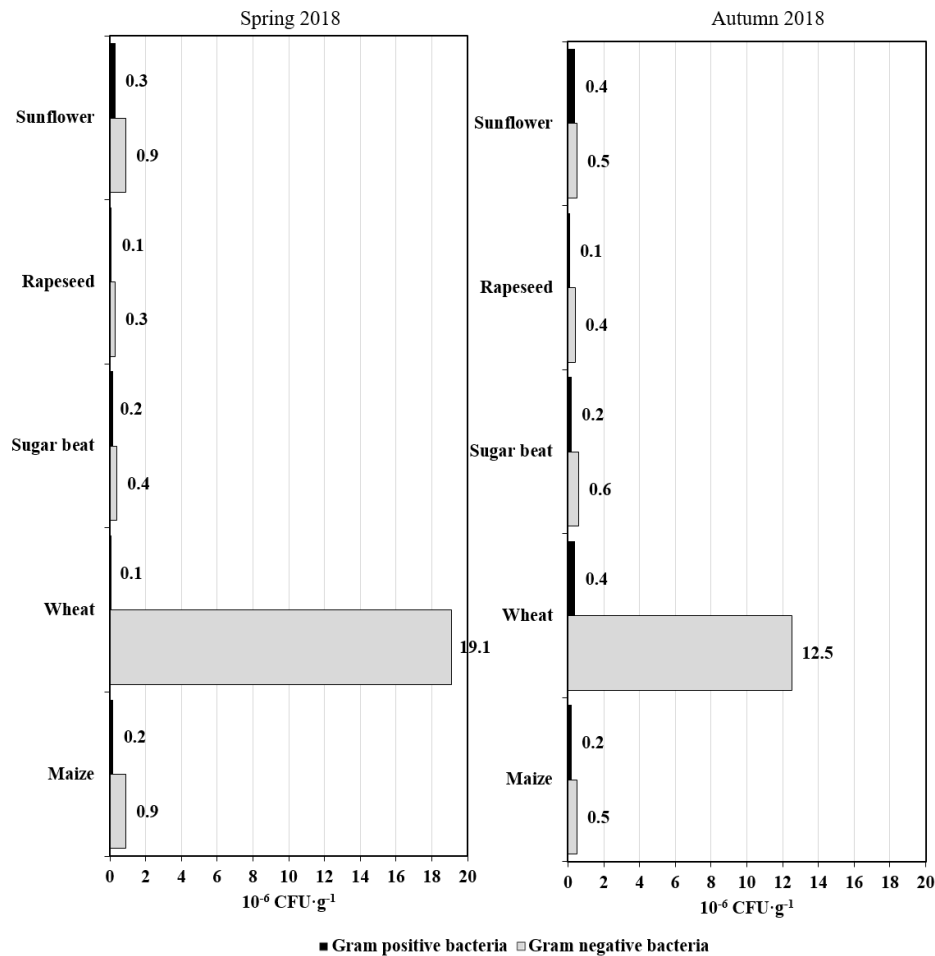

Figure 1 **Evolution of total soil bacteria as influenced by seasonal fluctuations and crop species**

The community structure in 2018 was dominated by Gram-negative bacteria (G-) during both seasons and a possible explanation is the low amount of precipitation recorded in the region of Moldavia. These results show that the environmental factors and physicochemical properties of soil from each location could have a specific impact on soil microbial communities and ecological function (Bissett A. *et al*, 2013).

During spring, in case of wheat, G- bacteria represent 99.4%, while in case of rapeseed G- bacteria represent only 77.0%, from total number of microorganism. The numbers of micromycetes ranged from a minimum value of 0.2 (wheat) to 13.2% (rapeseed). The average amounts of viable Gram-positive bacteria (G+) during the investigation period in the chernozem soil type

under cultivation with different crop species varied from 0.4% in case of wheat to 20.5% in case of soil cultivated with sunflower (*figure 2*). These results showed that the environmental factors (yearly seasons) and crop species could have a specific impact on soil microbial communities and ecological functions. Chen J. *et al* (2007) mentioned that the structure of microbial communities exhibited obvious variance after treatment with inorganic fertilizers and pesticides in different types of soils for a long period. Garbeva P. *et al* (2008) showed that in the same soil type the bacterial community depends on land use and management (p<0.05). Similarly, Francioli D. *et al* (2014) reported that different land usages and seasons strongly influenced the dynamics and the composition of the bacterial community.
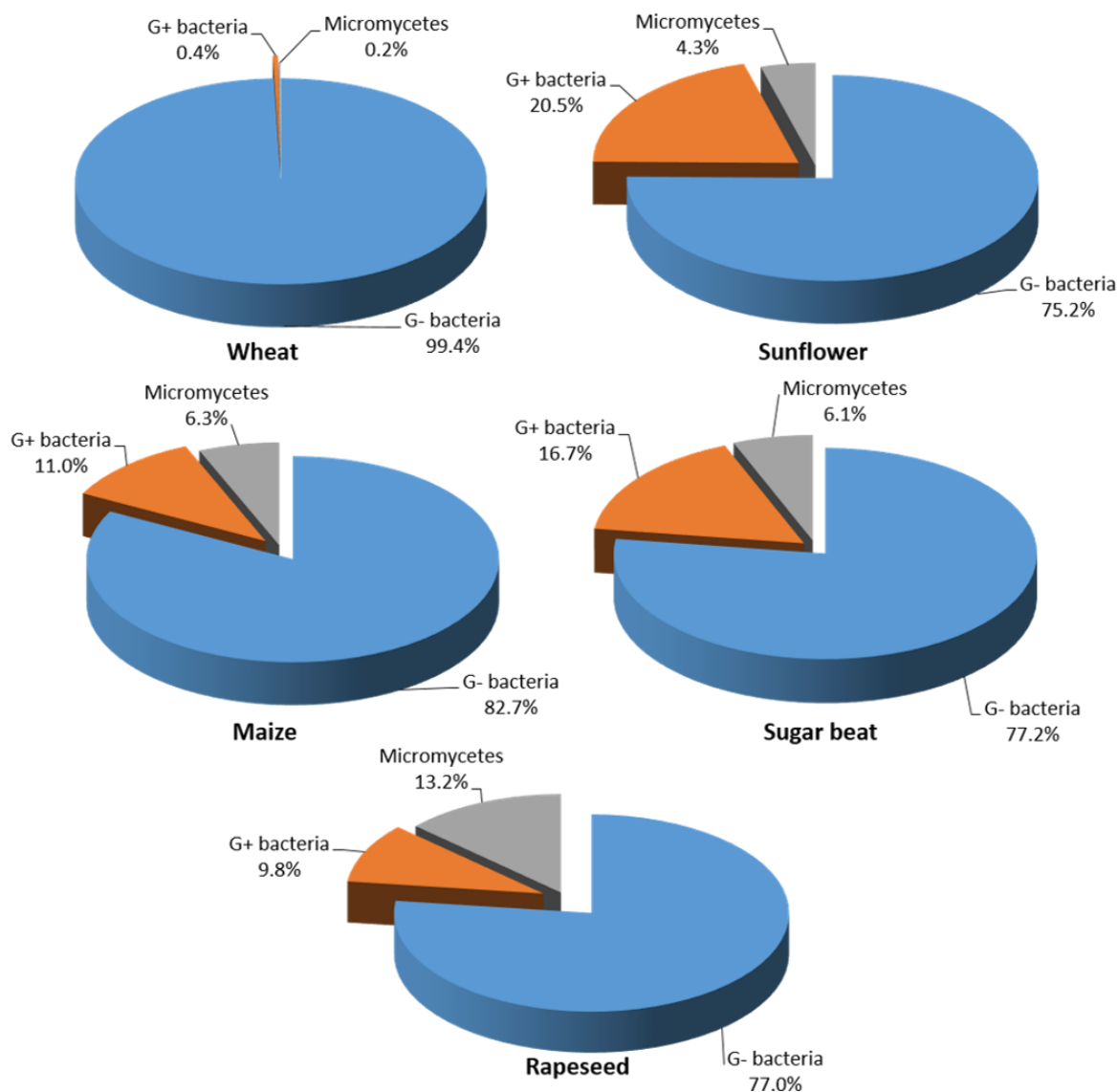
Figure 2 **Frequency of isolated microbiota from chernozem soil cultivated with different crop species**

We noticed that the number of isolated fungus genera in the all observed soil cultivated with different crop species were not identical, but in the same time the differences were not high. In general, soils under integrated management have a significantly reduced fungal richness compared to organic. In our study the fungal community composition was almost similar from spring to autumn and for all crop species cultivated on chernozem soils. The biodiversity of the isolated fungal genera is low, fact which indicated that the usage of inorganic fertilizers and pesticides reduce the microbial community composition. The isolated species belonging to six micromycetes genera: *Penicillium*, *Aspergillus*, *Fusarium Rhizopus*, *Trichoderma* and *Thielaviopsis,* Among the determined micromycetes in all the studied variants, we pointed out *Penicillium* genus, which was isolated at a rate comprised between 60.3 (maize) and 76.7% (wheat) of the total identified genera (*figure 3*).

In the soil sample prevailed from the area cultivated with maize was recorded the filamentous fungi maximum count (6.7×10^5 CFU g^{-1}), while the minimum (2.9×10^5 CFU g^{-1}) was found in case of wheat under conventional agricultural condition (data not shown).

The investigations conducted on the frequency and spectrum of micromycetes genera shown different values depending of soil types land use and management. The identification of fungi genera which activates in the rhizosphere area of arable land under agricultural rotation shows a relative small number of genera with *Penicillium* and *Aspergillus* as dominant genera in cases of typical chernozem cultivated with wheat, sunflower, maize, sugar beet and rapeseed, with more that 85.0% from all fungi. Highest fungal richness (six genera) was obtained under on the soil cultivated with sunflower (*Helianthus annuus*).

In very small ratio was present *Thielaviopsis* spp. and *Trichoderma* spp. (*figure 3*). Our findings about the influence of crop species on soil microbiota showed that the filamentous fungi richness was ranked maize> sunflower > rapeseed > sugar beet > wheat.
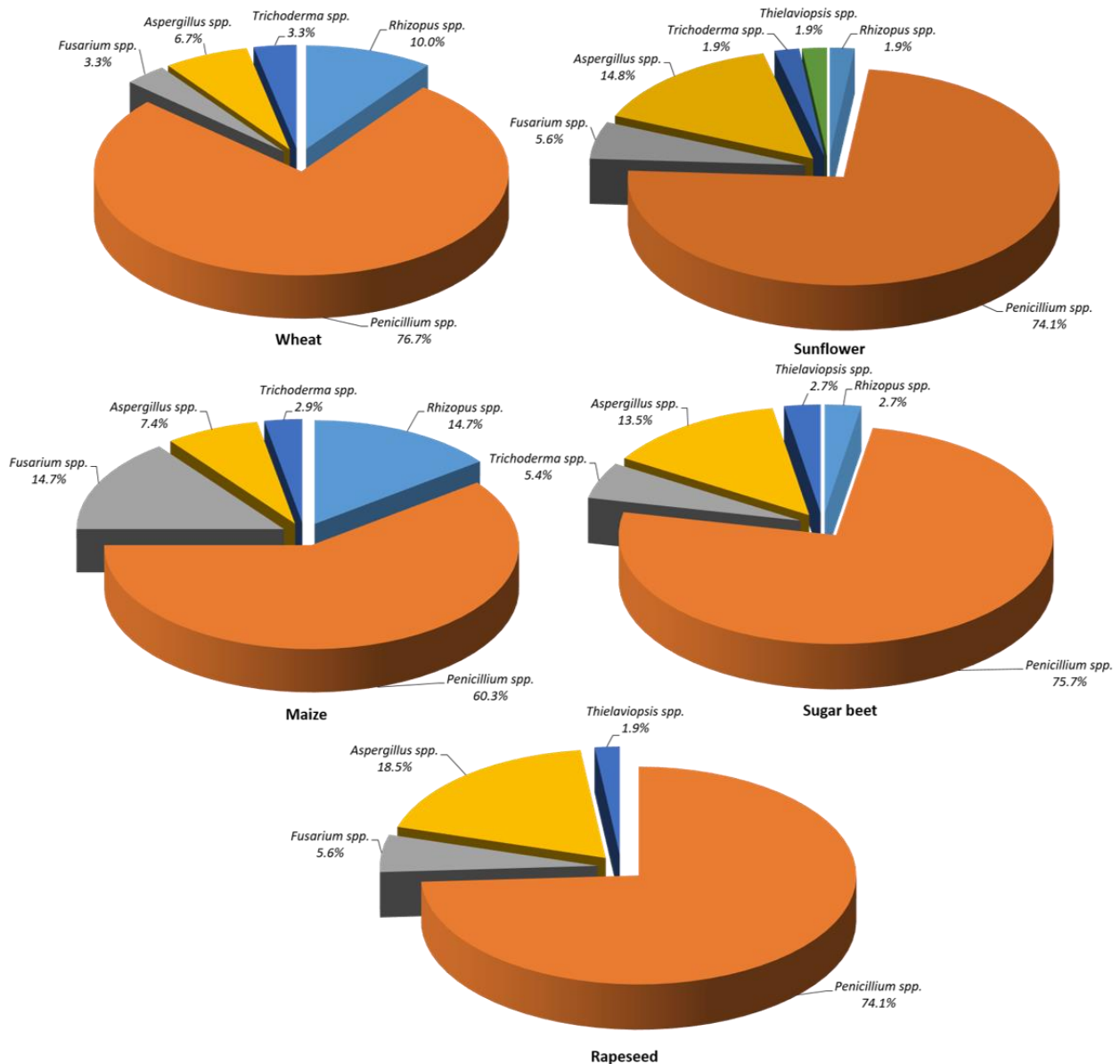

Figure 3 **Taxonomical compositions of filamentous fungi from chernozem soil cultivated with different crop species**

CONCLUSIONS

All investigated microbial groups were found in all locations and number of the microorganisms was uneven by cultivated crop species. On average, abundance of the most studied microbial groups was the lowest in the chernozem soil cultivated with rapeseed, while the highest abundance was obtained in chernozem cultivated with wheat. In the soil sample prevailed from the area cultivated with maize was recorded the filamentous fungi maximum count, while the minimum was found in case of wheat under conventional agricultural condition.

The investigations conducted on the frequency and diversity of soil microbiota have a complex relationship with the intensity of human intervention and are influenced by environmental, edaphic and management factors.

Intensive and unmannered application of pesticides and inorganic fertilizers in agriculture will pollute the soil, water and air, and cause many human diseases. Also, they affect directly the microbial soil abundance and composition, and indirectly soil texture and fertility. These findings contribute significantly toward an understanding the changes in microbial biomass and structure as response to agricultural management practices.

REFERENCES

Ai C., Zhang S., Zhang X., Guo D., Zhou W., Huang S., 2018 - *Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation*. Geoderma, 319:156-166.

Bissett A., Brown M.V., Siciliano S.D., Thrall P.H., 2013 - *Microbial community responses to anthropogenically induced environmental change: towards a systems approach*. Ecology Letters, 16(s1):128-139.

Bouskill N.J., Lim H.C., Borglin S., Salve R., Wood T.E., Silver W.L., Brodie E.L., 2013 - *Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought*. The ISME journal 2:384-394.

Bressan M., Gattin I.T., Desaire S., Castel L., Gangneux C., Laval K., 2015 - *A rapid flow cytometry method to assess bacterial abundance in agricultural soil*. Applied Soil Ecology 88:60-68.

Chen J., Zhuang X., Xie H., Bai Z., Qi H., Zhang H., 2007 - *Associated impact of inorganic fertilizers and pesticides on microbial communities in soils*. World Journal of Microbiology and Biotechnology, 1:23-29.

Doran J.W., Sarrantonio M., Liebig M.A., 1996 - *Soil health and sustainability*. Advances in Agronomy 56:2–54.

Filip Z., 2002 - *International approach to assessing soil quality by ecologicaly - related biological paarmeters*. Agriculture, Ecosystems & Environment 88:689-712.

Francioli D., Ascher J., Ceccherini M.T., Pietramellara G., 2014 - *Land use and seasonal effects on a Mediterranean soil bacterial community*. Journal of soil science and plant nutrition 3:710-722.

Garbeva P. van Elsas J.D. van Veen J. A., 2008 - *Rhizosphere microbial community and its response to plant species and soil history*. Plant and Soil 302(1):19-32.

Ge Y., Zhang J.B., Zhang L.M., Yang M., He J.Z., 2008 - *Long-term fertilization regimes affect bacterial community structure and diversity of an agricultural soil in northern China*. Journal of Soils and Sediments 1:43-50.

Giller K.E., Witter E., Mcgrath S.P., 1998 - *Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils*. Soil Biology and Biochemistry 30:1389-1414.

Joergensen R.G., Emmerling C., 2006 - *Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils*. Journal of Plant Nutrition and Soil Science 169(3):295-309.

Lan G.Y., Li Y.W., Wu Z.X., Xie G.S., 2017 - *Soil bacterial diversity impacted by conversion of secondary forest to rubber or eucalyptus plantations—a case study of Hainan Island, south China*. Forest Science 6:87-93.

Lipsa F.D., Ulea E., 2018 - *Practicum de microbiologie alimentară*, Ed. Ion Ionescu de la Brad Iaşi. 163 p., ISBN 978-973-147-275-1.

Lo C.C., 2010 - *Effect of pesticides on soil microbial community*. Journal of Environmental Science and Health Part B 45(5):348-359.

Schutter M., Sandeno J., Dick R., 2001 - *Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping system*. Biology and Fertility of Soils 34:397-410.

Ulea E., Lipsa F.D, Balau M.A., 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.

Valverde A., Makhalanyane P., Cowan D.A., 2014 - *Contrasting assembly processes in a bacterial metacommunity along a desiccation gradient*. Frontiers in microbiology 5:1-8.