RESPONSES OF MICROBIAL COMMUNITIES IN DIFFERENT SOIL TYPES UNDER VARIOUS MANAGEMENT REGIMES IN THE SOUTHEAST REGION OF MOLDAVIA, ROMANIA

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Abstract

In this study, we examined the diversity and abundance of microbial population isolated from different soil types under different agricultural regimes (permanent grassland and arable land under agricultural rotation) from southeast region of Moldavia, Romania. Soil types, 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, in 2018 we assessed responses of soil microbial communities to various management regimes, and analyzed six soils showing different land use from distinct localities. 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 soil types, human activities and land use 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.
Occurrence and distribution of filamentous fungi isolated from these soils provide new insights into ecology and niche specialization of several soil-borne species. Overall, genera composition of filamentous fungi from different soil types was not very heterogeneous and many fungal genera were common to all location.
Our results suggest that land usage and soil management system 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, soil types, different agricultural regimes

The number and diversity of bacteria in soil ecosystems have a complex relationship with the intensity of human intervention and are influenced by environmental, edaphic and management factors (Ulea *et al*, 2017). Lan *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 *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 (Schutter *et al*, 2001). It is know that agricultural practices cause a loss to microbial biodiversity (Rodrigues *et al*, 2013), and can lead to dramatic changes in bacterial compositions over a relatively short time period (Lan *et al*, 2017). Once applied, agricultural chemicals influence directly or indirectly the agroecosystem structure and function (Joergensen and Emmerling, 2006; Lo, 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.

Given recent efforts to quantify soil health and its influence on ecosystem functioning, it is imperative that we develop a better understanding of the underlying mechanism driving soil communities composition and diversity (Van Horn *et al*, 2013). Despite their important role in soil systems, compositional and functional responses of microbial communities to different land use and management regimes are not fully understood.

The main aim of the present research was to investigate the soil microbial response to differences in soil type, land use and management. Other purpose of this research was to find

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connections between diversity and structure of microbiota from different agro-ecosystems and soil pollution.

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 27.082 E. At each site (Table 1), 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 six different soils types under different land regimes: arable land under agricultural rotation and permanent grassland (P). The six soil types (Florea *et al*, 2012) are classified in 4 classes according to World Reference Base for Soil Resources (WRB, 2015). In the organically managed arable farms, compost and animal green manures are permitted to build up the soil fertility, while in the conventionally managed soils synthetic pesticides and fertilizers are used. The altitude ranges from 25 to 99 meters above sea level (Table 1).

Table 1

Characteristic of the tested soils

Soil sample	Soil type	Soil class	Land use[a] and management[b]		Alt. m	pH value	GPS location Lat. N/Long. E
1	Typical chernozem	Chernisols	A	C	25	8.4	44.935 / 27.275
2	Calcaric chernozem		A	C	76	8.2	45.222 / 27.148
3	Cambic Phaeozem		A	O	99	7.4	45.282 / 27.082
4	Solonetz	Salsodisols	P	-	24	8.5	44.976 / 27.322
5	Arenosol	Protisols	A	C	44	8.4	45.160 / 27.220
6	Calcaric-Mollic Gleiosol	Hydrisols	P	O	87	7.6	45.275 / 27.089

[a]arable land under agricultural rotation (A) and permanent grassland (P)

[b]under conventional (C), organic (O) and unmanaged (-) agricultural condition.

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 7.4 (preluvosol) and 8.5 (solonetz).

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 (Lipşa and Ulea, 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 et al., 2015), on potato dextrose agar medium (PDA) in different compositions: classic, with streptomycin and rose-bengal stain. Streptomycin antibiotic (35 $mg \cdot L^{-1}$) 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 soil types, land use and management.

The best represented microorganism group for all soil types is that of Gram-negative bacteria (G-). The most abundant G- bacterial community was recorded in case of soil type cambic phaeozem cultivated with hay crop (lucerne, *Medicago sativa*) under organic agricultural system, while the smallest community was represented by soil type

solonetz (saline soil) covered with unmanaged permanent grassland. In case of cambic phaeozem cultivated with hay crop G- bacteria represent 96.9%, while in case of solonetz G- bacteria represent only 51.6%, from total number of microorganism. The numbers of micromycetes ranged from a minimum value of 0.4 (cambic phaeozem) to 34.8% (solonetz). The average amounts of viable Gram-positive bacteria during the investigation period in different soil types under different land use and management regimes varied from 2.7% in case of cambic phaeozem to 16.7% in case of typical chernozem (*figure 1*).

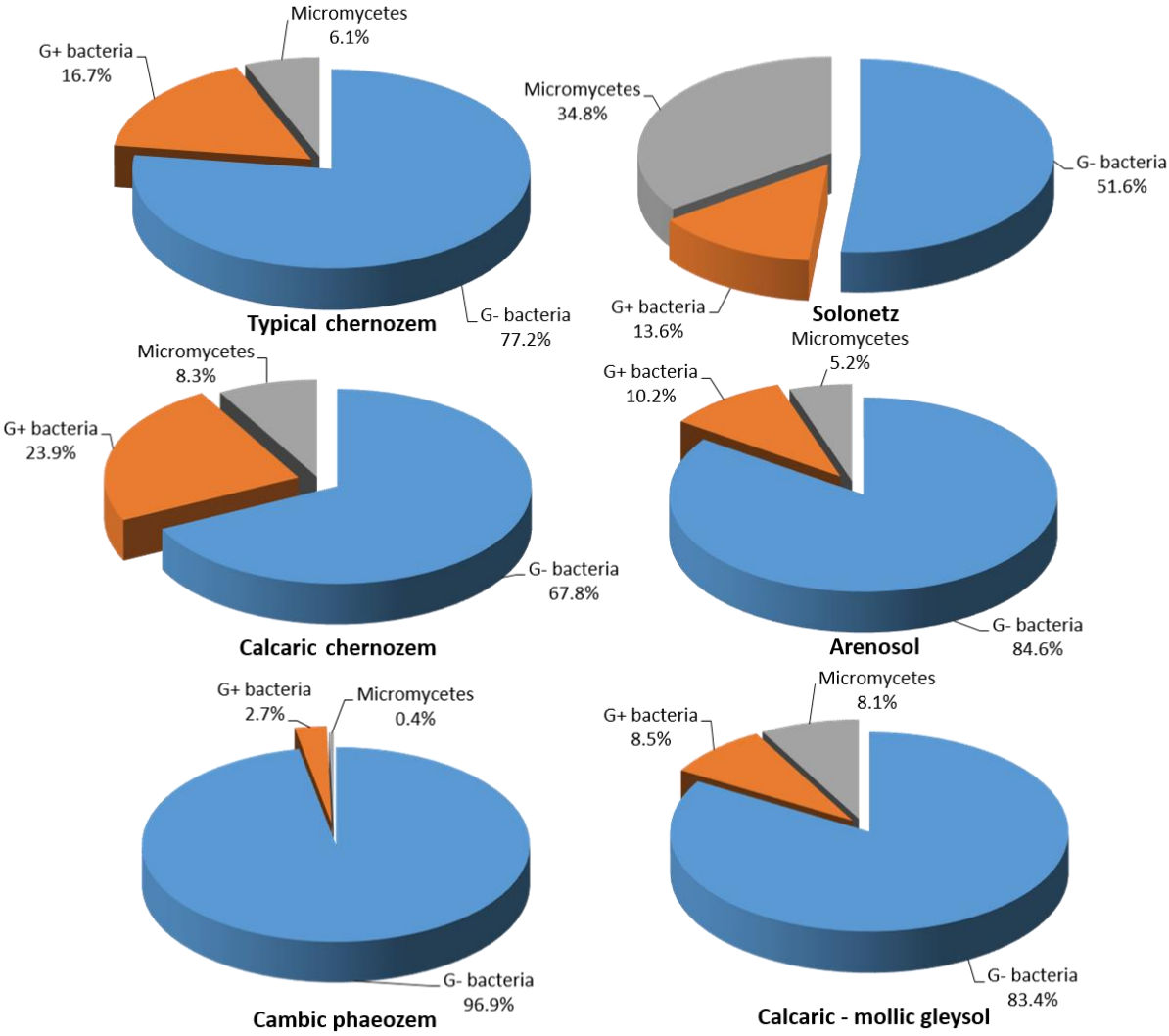

Figure 1 **Frequency of isolated microbiota on different soil types**

These results showed that the environmental factors and physicochemical properties of soil from each location could have a specific impact on soil microbial communities and ecological functions (Bissett *et al*, 2011).

In the soil sample prevailed from the unmanaged permanent grassland soil (solonetz) was recorded the filamentous fungi maximum count (5.8 x 10^5 CFU g^{-1}), while the minimum (3,2 x 10^5 CFU g^{-1}) was found in case of sunflower (arenosol) under conventional agricultural condition (C) (data not shown). The high number of microbial population from the unmanaged permanent grassland soil is attributable to litter inputs, root exudates and dead roots that provide a great source of organic carbon favoring microbial growth (Zhang *et al*, 2011).

Chen *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. Ulea *et al* (2017) showed that in the same soil type the bacterial community depends on land use and management ($p<0.05$). Similarly, Francioli *et al* (2014) reported that different land usages and seasons strongly influenced the dynamics and the composition of the bacterial community. The statistical analysis of our results demonstrated that only soil types have no significant impact on bacterial community richness.

In our study, the microbial population as total count ranged from 0.6 x 10^6 CFU g^{-1} (typical chernozem) to 12,1 x 10^6 CFU g^{-1} (cambic

phaeozem) in case of chernisols class, Other three soil classes, each containing one soil type, were present in this research: protisols class with soil type arenosol 0.65 x 10^6 CFU g^{-1}), salsodisols class with soil type solonetz (0.2 x 10^6 CFU g^{-1}) and hydrisols class with soil type calcaric-mollic gleiosol (0.55 x 10^6 CFU g^{-1}).

Six land use types and their associated management inputs were investigated in order to establish the relationship between management factors and vegetation type with soil communities abundance. Our findings about different land use and soil management practices (conventional, organic, and unmanaged) showed that the bacterial richness, assessed through the plate counting method, was ranked hay crop > arable land under agricultural rotation > permanent grassland. Similar observations have been made in other studies (Francioli *et al*, 2014). 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 *et al*, 2008). The intensity of human intervention, the environmental and edaphic factors from each location could have a specific impact on the results. We found that bacterial abundance and habitat disturbance are not correlated.

We noticed that the number of isolated fungus genera in the all observed soil types under different usage and land management variants were not identical. The isolated species belonging to eight micromycetes genera: *Penicillium*, *Aspergillus*, *Fusarium Rhizopus*, *Trichoderma*, *Thielaviopsis*, *Chaetomium* and *Mucor*. Among the determined micromycetes in all the studied variants, we pointed out *Penicillium* genus, which was isolated at a rate comprised between 30.5 (solonetz) and 87.0% (cambic phaeozem) of the total identified genera (*figure 2*).

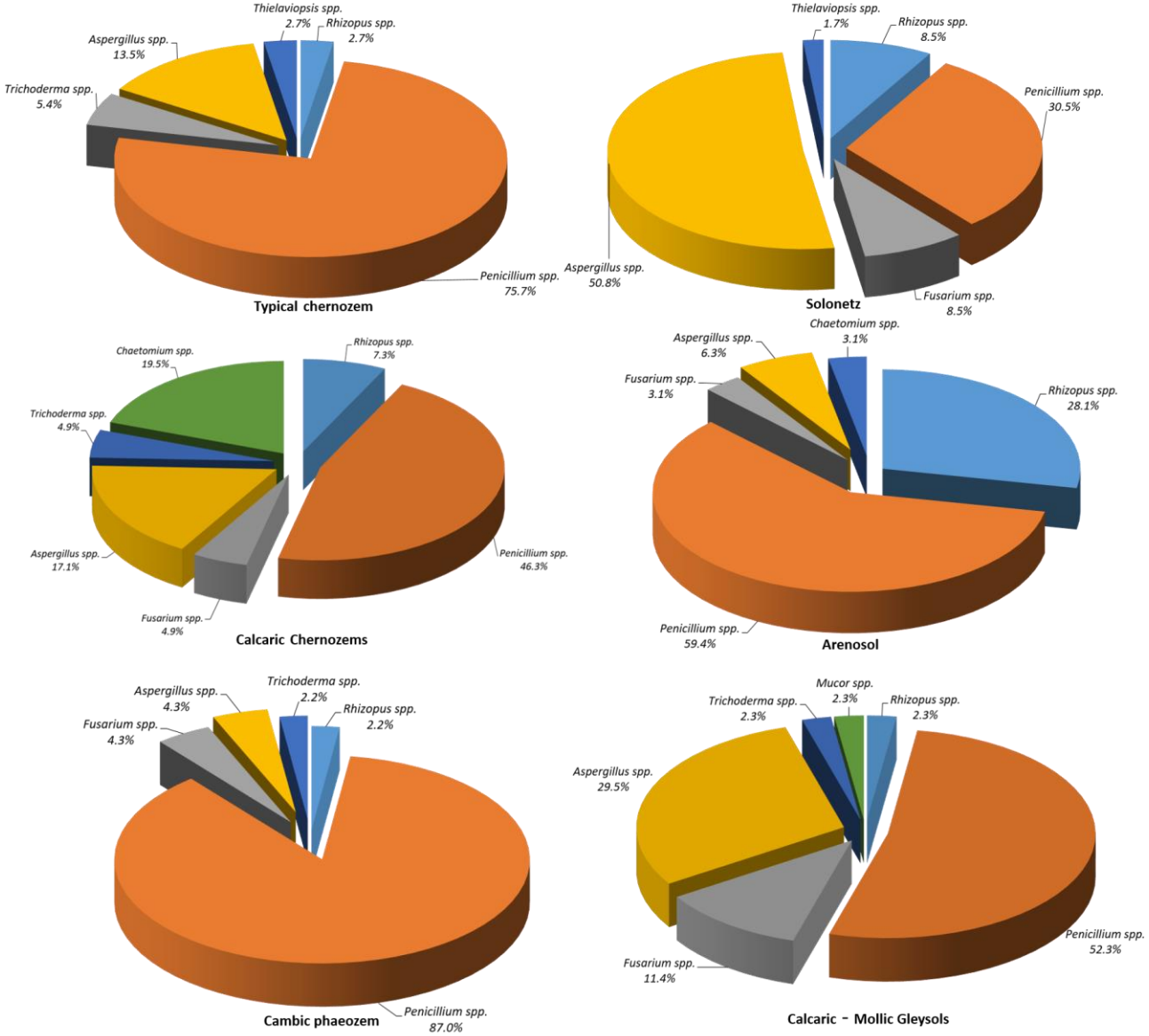

Figure 2 **Taxonomical compositions of filamentous fungi from different soil types**

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*, *Aspergillus*, *Fusarium* and *Rhizopus* as dominant genera in cases of typical chernozem (A, C), calcaric chernozem (A, C), cambic phaeozem (A, O) and arenosol (A, C) with more that 82.0% from all fungi (*figure 2*).

In case of the permanent grassland (P) placed under unmanaged agricultural conditions (solonetz) was noticed that the number of isolated fungus genera was almost the same with the other soil types and the isolated species belong to five micromycetes genera (*Aspergillus*, *Penicillium*, *Fusarium Rhizopus* and *Thielaviopsis)* Among the determined filamentous fungi in all the studied soil types, we pointed out *Aspergillus* and *Penicillium* genera, which were isolated at a rate of 50.8% and 30.5%, respectively (*figure 2*). It is the single variant in which *Aspergillus* spp. is the dominant genus. In very small ratio was present *Thielaviopsis* spp. with 1.7%.

For calcaric-mollic gleiosol soil type (P, O) the most isolated species belonging to six micromycetes genera (*Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Rhizopus* and *Mucor*).

Our findings about different land use and soil management practices (conventional, organic, and unmanaged) showed that the filamentous fungi richness was ranked permanent grassland > hay crop > arable land under agricultural rotation.

The results of Laudicina *et al* (2010) showed that intensive tillage caused a higher soil aeration and organic substrates accessibility than reduced tillage and this speed up the mineralization of organic matter from soil. The best practices for improving soil fertility are represented by reduced land use systems coupled with high input of compost.

CONCLUSIONS

Our observation on microbiological activity showed different reactions on soil types, land use and management. Between the analyzed variants the microbial activity was the highest in the cambic phaeozem sample soil from the arable land under agricultural rotation system when organic fertilizers were applied. In case of the soil type under unmanaged agricultural condition (solonetz) was noticed the greatest number of isolated filamentous fungi in compares with the other variants of soil type, land use and management.

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.

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