MICROBIAL DIVERSITY IN SOILS FROM HIGH NATURAL VALUE AGRICULTURAL SYSTEMS WITH PASTURES AND NATURAL MEADOW IN SUCEAVA COUNTY

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Abstract

Agriculture with High Natural Value (HNV) is a new concept, develped in the last two decades to describe those agricultural systems in Europa owing the widest biodiversity. Its main characteristics are the low intensity and the presence of semi-natural vegetation. Romania has one of the most important resource of areas classified as HNV due to the great variety of species associated to agricultural land utilised as permanent meadows, by traditional mowing and grazing activities. The aim of this paper is to present the results of research carried out to evaluate the biodiversity of microbial communities in three soils under traditional management of HNV pastures (Vicovu de Jos, Comănești) and natural meadow (Valea Moldovei), Suceava county. The total counts and species of bacteria and fungi (estimated by dilution plate), soil respiration (by substrate-induced respiration method), diversity index of Shannon (H) and similarity index between habitats were calculated and presented as Venn diagram. Biodiversity of microbial communities consists in 6 to 9 species with important roles in main processes in plant rhizosphere, soil organic matter recycling, cellulose decomposition, soil aggregation, biocontrol.

Key words: HNV agricultural systems, biodiversity, microbial communities, pastures, natural meadow.

INTRODUCTION

The concentration of CO₂ and other greenhouse gases (GHGs) in the atmosphere is increasing as a result of fossil-fuel combustion, industrial activities and land-use change, leading to climate change and global warming.

The process of soil C sequestration or flux of C into the soil is part of the global carbon balance (Blujdea et al., 2014). The factors affecting the flow of C into and out of the soil are affected by land-management practices (Oancea, 2003). Kirschbaum, (1995) evidenced the temperature dependence of soil organic matter decomposition, and predicted that soil organic C stocks will decline as an effect of global warming. Land-management options that improve C sequestration, increasing plant productivity and simultaneously preventing soil erosion are of major interest in regions with desertification risk. Recent studies reported the impact of agronomic practices on soil organic carbon dynamics by their influence on various labile/non-labile fractions equilibrium (Abril & Bucher, 2001; Jackson et al., 2002; Guo et al., 2014; Lalichetti & Sultan, 2020).

Studies on the ecology of grassroots underlined that complex plant-microbe-soil interactions proved to be important drivers of plant and microorganisms, community structure and dynamics (Reynolds et al., 2003; Prommer et al., 2020).

Organic amendments and land management affect soil microbial community composition, diversity and biomass (Gomez et al., 2006; Lejon et al., 2007; Bonilla et al., 2012; Eftene et al., 2014; Moreno et al., 2019; Bonanomi et al., 2020).

Soil microbiota has an important contribution to soil services (Aislabie & Deslippe, 2013; Dobrovol'skaya et al., 2015; Matei et al., 2020).

An important source of biodiversity for both plants and soil microorganisms is represented by HNV areas.

This paper presents the results of research carried out to evaluate the biodiversity of microbial communities in three soils under traditional management of high natural value (HNV) pastures (Vicovu de Jos, Comănești) and natural meadow (Valea Moldovei), Suceava county, North-East Romania.

MATERIALS AND METHODS

The period analysed in this study was autumn 2020-spring 2021.

Surface samples (0-20 cm) from soils under traditional management of HNV pastures (Vicovu de Jos, Comănești) and natural meadow (Valea Moldovei), Suceava county were analysed by using microbial indicators (microbiological activity from soil, number and micro-organisms types) to characterize the biodiversity of microbial communities.

Microbiological analyses were performed by soil dilution method on specific culture media with agar-agar: Nutrient agar (NA) for aerobic heterotrophic bacteria and potato-dextrose agar (PDA) for fungi. After 7 days incubation at dark, colonies were counted and microbial density (Total Number of Bacteria-TNB and Total Number of Fungi-TNF) was reported to gram of dry soil.

Taxonomic identification was done using morphologic criteria, according to the manual (Bergey & Holt, 1994) for heterotrophic bacteria and to Domsch & Gams (1970) and Watanabe (2002) determinative manuals for fungi. Morphological characteristics were measured and photographed under a MC 5.A optic microscope.

The total number of species (S) was recorded for each microbial community, as well as relative abundance and dominance of each species. The ratio between the number of species and microbial counts in communities expressed species richness (SR₂ index).

The Shannon index (H') that takes into account both the richness and evenness (ε) of a given ecosystem was used to evaluate the microbial biodiversity in soil samples (Mohan & Ardelean, 1993). Its value increases with the increased number of species and is also higher when the species are evenly distributed (Morris et al., 2014).

The index of Brillouin (1956) and Simpson (D) index (Stugren, 1982) were also used to calculate the diversity and "equitability" component of microbial species diversity (distribution of "individuals" on species). Similarity indices (SI) between habitats were calculated (Tiwari et al., 1994) and the results of comparative analysis of the microbiomes

composition were presented as Venn diagrams (Gentlemen & Ihaka, 1994).

The global physiological activities of soil active microflora were determined by substrate induced respiration method (SIR) and results were expressed as mg CO₂x100 g⁻¹ soil (Matei, 2011). Circular paper chromatograms were made by migration of soil extracts to obtain information on soil biological quality (Papacostea, 1976).

All assays were carried out in triplicate. Results were interpreted by one-way analysis of variance (ANOVA). The value p<0.05 was considered statistically significant (Student test).

RESULTS AND DISCUSSIONS

Data analysis revealed statistically significant differences between the three HNV areas concerning both total counts of bacteria, fungi and global physiological activities (Table 1).

Table 1. Total microbial counts and global physiological activity of soil bacterial and fungal microflora in HNV pastures and natural meadow

No crt.	Soil profile location (land use)	TNB (x 10 ⁶ viable cells x g ⁻¹ d.s.)	TNF (x 10 ³ cfus x g ⁻¹ d.s.)	Soil respiration (mg CO ₂ x100 ^{g-1} soil)
1	Vicovu de Jos P1 (pasture)	7.669b ¹	125.798a	41.137a
2	Comănești P3 (pasture)	6.513c	101.394b	34.201b
3	Valea Moldovei P5 (natural meadow)	9.425a	125.613a	32.168c

 1 The values in a column followed by the same letter are not significantly different for P< 0.05 (Student test)

P1 - Soil from Vicovu de Jos P1 (pasture) presented low density of aerobic heterotrophic bacteria, high values for the representatives of fungal community and moderate values of soil respiration potențialului (41.137 mg CO₂ x 100 g⁻¹ soil).

6 fungal species have been identified (Figure 1), two belonging to the group of Zygomycetes, developed on cattle excrements on the pasture, and actively involved in their decomposition, as well as in humification processes (Zygorrhynchus moelleri, Mucor circinelloides) and associated with species belonging to other genera with strong cellulolytic, pectinolytic or chitinolytic capacities (Cladosporium, Penicillium, Paecilomyces, Mortierella).

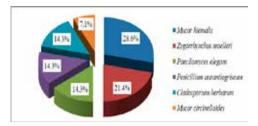


Figure 1. Percent mean relative abundance of fungal microflora composition in soil under pasture from Vicovu de Jos

Bacterial microflora, formed by representatives of 9 species (Figure 2), was dominated by fluorescent pseudomonads (well-known as microbial antagonists and implicated, along with fungal species identiffied, in the processes of humus formation), associated with bacillaceae, *Arthrobacter* and accompanied by actinomycetes with role in aggregation of soil particles. The role of pseudomonads in biological control of fusarium wilt was confirmed by *in vivo* experiments on tomato, carried out by Attitalla et al. (2001).

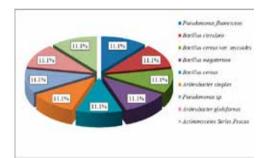


Figure 2. Percent mean relative abundance of bacterial microflora composition in soil under pasture from Vicoyu de Jos

P3 – Soil from **Comănești P3 (pasture)**, presented a low density of bacteria (6.513 x10⁶ viable cells x g⁻¹ dry soil) and a relative high level of total counts of fungi (101.394 x10³ cfu x g⁻¹ dry soil).

A moderate microbial activity (34.201 mg CO₂ x 100 g⁻¹ soil) was registered for this soil biota. Fungal microflora (7 species) was dominated by species from the group of Zygomycetes (*Mucor*, *Actinomucor*) accompanied by less abundant ubiquitary, cellulolytic species from genera *Aspergillus*, *Penicillium*, *Acremonium*, *Cladosporium* and *Fusarium* (Figure 3).

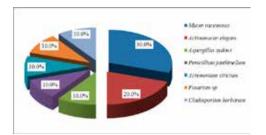


Figure 3. Percent mean relative abundance of fungal microflora composition in soil under pasture from Comănesti

The group of heterotrophic bacteria consisted of 7 species belonging to bacillaceae, pseudomonadaceae (fluorescent, non-fluorescent) and arthrobacteriaceae (Figure 4).

The presence of species *Bacillus subtilis* indicate an environment higher humidity conditions and an easily degradable organic material (possibly excrements from animals grazing on the pasture).

Pseudomonas aeruginosa is known as saprophyte and parasite of animal and vegetal organisms (Papacostea, 1976).

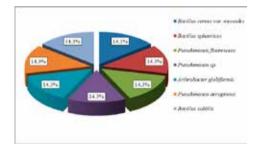


Figure 4. Percent mean relative abundance of bacterial microflora composition in soil under pasture from Comănești

P5 - Soil from Valea Moldovei P5 (natural meadow) presented a low to moderate level of total counts of bacteria (9.425 x 10⁶ viable cells x g⁻¹ dry soil) and a high level of total counts of fungi (125.613 x 10³ cfu x g⁻¹ dry soil). The global physiological activities of soil active microflora were moderate as intensity (32.168 mg CO₂ x 100 g⁻¹ soil) but significantly lower than in soils under pastures. Fungal microflora consists of 8 species. It was identified the species *Zygorrhynchus moelleri*, (Figure 5), dominant în community, frequently isolated from rhizosphere and especially from

acid debasified soils, with important role in degradation of organic matter.



Figure 5. Zygorrhynchus moelleri dominant in soil under natural meadow from Valea Moldovei (x200)

Termophilic species (Fusarium pallidoroseum, two species of the genus Aspergillus) were accompanied by other strong cellulolytic species of genera Penicillium, Verticillium and Paecilomyces (Figure 6).

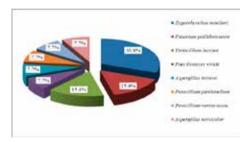


Figure 6. Percent mean relative abundance of fungal microflora composition in soil under natural meadow from Valea Moldovei

Bacterial microflora, consisting of 6 species was characterized by the co-dominance of bacillaceae with pseudomonads, each represented by 3 species (Figure 7).

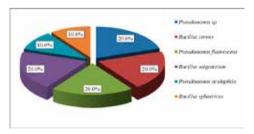


Figure 7. Percent mean relative abundance of bacterial microflora composition in soil under natural meadow from Valea Moldovei

Bacillus species are considered beneficial in soil communities and pseudomonads contribute

to the biocontrol of plant pathogens, as well as to C sequestration in stable forms (humus), as reported in literature (Kucuc & Kivanc, 2003; Matei et al., 2018; Dumitrascu et al., 2019).

Ecologic spectra of fungal and bacterial groups in each soil profile evidenced the percent relative abundance of a species in the structure of microbial cenosis and revealed species status (e.g., as dominant species).

Analysis of bacterial species composition lists and the logic diagram (Venn) from Figure 8a showed that two species representing fluorescent pseudomonads and non-fluorescent pseudomonads were shared between the three lists. Our results are in concordance with other studies (Weller et al., 2002), that attribute the suppressiveness character of the soil to the pseudomonads, able to control plant pathogens. In fungal communities, no species was shared by all the three lists (Figure 8b).

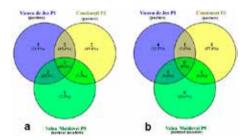


Figure 8. The Venn diagram denoting the unique and shared number and proportion of bacteria (a) and fungal (b) species in the communities from HNV soils

Biodiversity (S) of edaphic microbial communities consisted in 6 to 9 species. The highest value of diversity index of Shannon for bacterial community was found in soil under pasture from Vicovu de Jos (H'=2.364 bits and evenness ϵ =0.864). The communities from the two pasture soils were characterized by high homogeneity, as calculated by Brillouin formula (value E=1).

For the group of fungi, the highest diversity was found in soil under the natural meadow from Valea Moldovei (diversity index of Shannon H'=1.925 bits and evenness ε =0.796). The homogeneity, as calculated by Brillouin formula, reflected that the mycocenoses were formed by few dominant species, with higer abundance and more species with homogen distribution of less abundant individuals (see Table 2 for the values of all diversity indices).

Table 2. Taxonomic composition and biodiversity indices of bacterial and fungal microflora in HNV soils under pastures and natural meadow

Soil profile location (land use)	Fungal species	Bacterial species	
Vicovu de Jos P1	Mucor hiemalis	Pseudomonas fluorescens	
(pasture)	Zygorrhynchus moelleri	Bacillus circulans	
	Paecilomyces elegans	Bacillus cereus var. mycoides	
	Penicillium aurantiogriseum	Bacillus megaterium	
	Cladosporium herbarum	Bacillus cereus	
	Mucor circinelloides	Arthrobacter simplex	
		Pseudomonas sp.	
		Arthrobacter globiformis	
		Actinomycetes Series Fuscus	
	S=6 SR ₂ =0.476	S=9 SR ₂ =1.173	
	Shannon H'=1.710 ε=0.855	Shannon H'=2.364 ε=0.874	
	Brillouin H=0.563 B= 7.879 E=0.902	Brillouin H=0.618 B=5.560 E=1	
	Simpson Index D=0.806	Simpson Index D=0.889	
Comănești P3	Mucor racemosus	Bacillus cereus var. mycoides	
(pasture)	Actinomucor elegans	Bacillus sphaericus	
	Aspergillus sydowi	Pseudomonas fluorescens	
	Penicillium janthinellum	Pseudomonas sp.	
	Acremonium strictum	Arthrobacter globiformis	
	Fusarium sp.	Pseudomonas aeruginosa	
	Cladosporium herbarum	Bacillus subtilis	
	S=7 SR ₂ =0.690	S=7 SR ₂ =1.074	
	Shannon H'=1.834 ε=0.789	Shannon H'=1.946 ε=0.780	
	Brillouin H=0.498 B=4.481 E=0.934	Brillouin H=0.529 B=3.702 E=1	
	Simpson Index D=0.820	Simpson Index D=0.875	
Valea Moldovei P5	Zygorrhynchus moelleri	Pseudomonas sp.	
(natural meadow)	Fusarium pallidoroseum	Bacillus cereus	
	Verticillium leccani	Pseudomonas fluorescens	
	Paecilomyces viride	Bacillus megaterium	
	Aspergillus terreus	Pseudomonas acidophila	
	Penicillium janthinellum	Bacillus sphaericus	
	Penicillium verrucosum		
	Aspergillus versicolor		
	S=8 SR ₂ =0.636	S=6 SR ₂ =0.636	
	Shannon H'=1.925 ε=0.796	Shannon H'=1.748 ε=0.836	
	Brillouin H=0.601 B= 7.812 E=0.964	Brillouin H=0.536 B=5.356	
	Simpson Index D=0.828	E=0.871	
		Simpson Index D=0.820	

Circular paper chromatograms were used to obtain information on soil biological quality, by image analysis, that allow the evaluation of features such as soil vitality, fertility, the intensity of biotic activity, the degree of complexity of organic matter and the presence of stable humus (Figure 9).



Figure 9. Sections of circular chromatograms of soils under pastures from Vicovu de Jos (a), Comănești (b)and natural meadow from Valea Moldovei (c)

The sections of circular chromatograms highlight the qualitative differences between the soils under the influence of land use as pastures and natural meadow.

Analysis of the chromatograms revealed different capacities of humus formation, reflected in reduced content in labile organic carbon, increased content in nitrogen and in the dynamics of organic carbon decomposition. Processes of intermediary development with row organic matter, accumulated but also with tendency of integration in soil were evidenced. There are not evidences of unfavorable conditions in soil.

Mineral diversity was observed especially in chromathgrams from P1 (pasture) and P5 (natural meadow).

The qualitative/quantitative content in accesible minerals appeared very well evidenced at P5, with intense mineralization and reduced at P3. Protein content was high and very well structured, as evidenced by specific features, especially for chromatograms of P5 and P1, excepting P3 chromatogram, where no tendency of structuration of protein material was registered. P3 chromatogram revealed formation of acid humic substances with low stability and in P1, P5, the formation of brown colloidal humus with high stability. The most intense processes of mineralization with reduction of organic matter reservoir were evidenced in soil under pasture from Comănești.

Chromatograms revealed the presence of aggregates in solution, as well as elements for construction of soil. Microbial activity was very well evidenced on chromatogram of P1. The lowest level of microbial activity and functional diversity appeared for P3.

Functional diversity was well outlined by specific aspect of characteristic zone on chromatograms P1, and P5. Conditions relatively favorable for organic aggregation and floculation appeared mainly in P5, followed by those from P1.

Nutritional potential and carbon sources were represented by fragmented chemical compounds, enreached in nitrogen and with a relatively equal distribution (excepting P3).

Generally, the chromatograms revealed a moderate to low level of enzymatic activity.

P3 presented the lowest level of enzymatic activity and P1 the highest.

Our results on microbial bio diversity in soils from HNV areas are in concordance with other studies from literature (Grayston et al., 2001; Singh et al., 2007; Liu et al, 2012).

Zhao et al. (2018) found that relative abundance of individual bacterial taxa varied distinctly among different samples (by over several ranges of magnitude) as a result of the differences in soil properties and conditions.

Parton et al. (1995) presented the impact of climate change on grassland production and soil carbon worldwide and Reeder & Schuman (2002) underlined the influence of livestock grazing on C sequestration in semi-arid mixed-grass and short-grass rangelands.

Present research findings are in concordance with previous results (Eftene et al., 2014) on

the influence of 4 land use types (cultivated land, vineyard, *Acacia* forest and pasture) on microbiological activity of sandy soils from Băilești Plain (southwest of Romanian Plain), in climatic conditions with hot dry summers and low precipitations. The most intense global microbial activity, measured as soil respiration, was in soil under pasture and the lowest values of total counts of bacteria and fungi were recorded under vineyard, as response of edaphic microbiota to anthropic interventions. Further research results application are necessary for introduction of sustainable pasture

Further research results application are necessary for introduction of sustainable pasture management practices among farmers/ shepherds, aiming biodiversity conservation, combating of desertification/land degradation and avoidance of climate changes.

CONCLUSIONS

Natural meadow soil from Valea Moldovei presented significantly higher density of both bacteria and fungi and lower levels of CO₂ released by global physiological activities as compared with the two pastures.

Biodiversity of edaphic microbial communities consisted in 6 to 9 species.

The highest value of diversity index for bacterial community was found in soil under pasture from Vicovu de Jos (H'=2.364 bits and evenness ϵ =0.864).

For the group of fungi, the highest diversity was found in soil under the natural meadow from Valea Moldovei (H'=1.925 bits and evenness ε=0.796).

Edaphic microorganisms identified contribute to important soil services in HNV pastures and natural meadow by recycling of nutrients and formation of soil, cellulose decomposition, C sequestration and synthesis of stable organic matter (humic acids), improving soil structure by aggregation of soil particles and biological control of pathogens by production of active metabolites with inhibitory role.

Specific paper chromatograms revealed good conditions in all HNV soils but higher enzyme activity, functional diversity, higher level of nutritional reserve, more intense humification processes (with colloidal substances and mineral compounds well integrated in the organic material) for land use as natural

meadow, followed by pasture from Vicovu de Jos than for the pasture from Comănești.

Protection and conservation of microbiological diversity in soils from HNV areas contributes to sustainable use of biodiversity components and is imperative for the preservation of important functions, structures and processes of natural, as well as agro-ecosystems.

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