

ASSESSING CHARACTERISTICS OF MICROBIAL COMMUNITIES IN SOILS FROM HIGH NATURAL VALUE AGRICULTURAL SYSTEMS FROM CARAS-SEVERIN COUNTY

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Abstract

Romania owns important areas with High Natural Value (HNV) agriculture systems. The aim of the paper was to assess the biodiversity aspects of microbial communities in two soils from the area selected for HNV payments, that belongs to the Pilot Zone II in Lupac, Caras-Severin County: P3-cultivated in conventional system with maize for forage and P4-under HNV agriculture system with forage plants, mainly leguminous, between abandoned orchard trees. Microbial counts and taxonomy of bacteria and fungi have been determined (by dilution plate method). Diversity index of Shannon and similarity index between the habitats have been calculated, as well as soil respiration potential (estimated by substrate-induced respiration method). Microbial diversity varied from 3 to 9 species. Dominant species included *Pseudomonas fluorescens*, *Bacillus* spp. and cosmopolitan genera *Penicillium* and *Trichoderma*, with role in improving the control of phytopathogens in rhizosphere and nutrients uptake by plants, aggregation of soil, cellulose decomposition and carbon sequestration. Circular paper chromatograms provided information on biological quality of studied soils.

Key words: HNV agriculture system, biodiversity, bacteria, fungi, paper chromatograms.

INTRODUCTION

Various agricultural practices target the reduction of CO₂ and greenhouse gases accumulation in atmosphere by increasing the sequestration of carbon in soil, thus mitigating the effects of climate change and preventing the global change (Alidoust et al., 2018).

Land-management practices including organic matter amendments proved their beneficial effect on carbon and nitrogen dynamics in grassland soils (Ryals et al., 2014), resulting in improvement of carbon sequestration, plant nutrition and preventing soil degradation. Recent studies underlined the importance of land use and agronomic practices on carbon sequestration mediated by soil microbiota and the influence of edaphic microbial diversity on preventing soil loss caused by various types of degradation (Guo et al., 2014; Lal et al., 2015; Raducu et al., 2017; Lalichetti & Sultan, 2020), as well as the beneficial role of microflora in sustaining soil fertility (Valensa et al., 2017).

Houghton & Nassikas (2017) reported that global and regional fluxes of carbon were affected by land use and land cover change.

Research reports from China evidenced the beneficial effects of long-term application of manure and other fertilizers on the fractions of soil organic matter and microorganisms under a cropping system with wheat-maize rotation (Gong et al., 2009).

Results from a long-term field experiment in Sweden evidenced quantitative changes in organic carbon and the structure and taxonomy of microbial communities caused to repeated treatments with organic and N fertilizers (Katterer et al., 2014).

Soil microorganisms play an important role in organic matter cycling, regulation of gas exchange, carbon sequestration, improving plant growth by increasing nutrients availability and uptake, suppression of pathogens or by eliciting of plants immune response, all these activities contributing to soil services in ecosystem (Aislabie & Deslippe, 2013; Matei et al., 2020; Yan et al., 2021).

Studies on soils from areas with high natural value (HNV) agriculture systems underlined their importance in preservation of vegetal and microbial biodiversity, because they are the source of valuable species which intervene in

the main processes influencing ecosystem dynamics (Matei et al., 2018; Dumitrascu et al., 2020).

This paper presents the results of research in order to evaluate the biodiversity aspects of microbial communities in two soils from a selected areal, eligible for HNV payments, that belongs to Pilot Zone II, Caras-Severin County from South-West region of Romania.

MATERIALS AND METHODS

Soils have been collected from a selected areal belonging to Pilot Zone II, in Caras-Severin County, eligible for HNV payments.

P3-Lupac (Clocotici) (arable in conventional system)

Profile no. 3 is situated nearby village Clocotici and the road towards locality Lupac, (Longitude: 21.823421°E, Latitude: 45.244880°N), at 232 m altitude, slope 10%, on an arable terrain, cultivated in conventional system.

Culture sowed is maize for forage. It belongs to a landscape of mosaic type, characteristic for the type 2 of HNV terrain. The terrain was fertilized with manure (Figure 1).

Soil Type: Endostagnic Luvisol (WRB, 2014). Soil presented medium texture; loose in surface layer; medium permeability; acid reaction in surface (pH - 4.95), oligomesobasic; medium humus content (4.59%), medium organic matter reserve in the first 50 cm (132 t/ha); medium nitrogen content (Nt - 0.238%), low and very low available phosphorus and potassium contents P_{AL} - 5 mg x kg⁻¹ and K_{AL} - 180 mg x kg⁻¹.



Figure 1. Soil profile P3 in arable (conventional system) - Lupac (Clocotici)

P 4 - Lupac (Clocotici) (abandoned orchard, in HNV system) Soil profile no. 4 is located at 50 m distance from Profile no. 3 nearby village

Clocotici and on the left side of road towards locality Lupac (Longitude: 21.824058°E, Latitude: 45.244671°N), at 234 m altitude.

The terrain is covered with natural and semi-natural vegetation, in mosaic, characteristic to the type 2 of HNV terrain. The vegetal cover between species of trees from abandoned orchard (plum and cherry) consists in forage plants, the majority belonging to leguminous plants (Figure 2). Fibrous roots of plants are present in surface soil layer (0–20 cm).

Soil Type: Endostagnic Luvisol (WRB, 2014). Soil presented medium texture; loose in surface layer; medium permeability; moderate acid reaction (pH - 5.61), mesobasic; medium humus content (3.63%), medium organic matter reserve in the first 50 cm (160 t/ha); medium nitrogen content (Nt - 0.343%), very low and low available phosphorus and potassium contents P_{AL} - 7 mg x kg⁻¹ and K_{AL} - 262 mg x kg⁻¹.



Figure 2. Soil profile P4 in abandoned orchard (HNV system) - Lupac (Clocotici)

Surface samples (0-20 cm) from soils under conventional (P3) and HNV (P4) agriculture systems have been analysed using microbial indicators of biodiversity and microbiological activity of bacterial and fungal communities, as well as image forming methods for evidencing the specific aspects concerning the biological quality.

Microbiological analysis was performed according to soil serial dilution method. Specific culture media have been utilised: Nutrient Agar (NA) purchased by Difco, USA for bacteria and potato - dextrose agar (PDA) purchased by Merck, Germany. Colonies developed after 7 days incubation were counted and results expressed as Total Number of Bacteria - TNB and Total Number of Fungi reported to 1gram of dry soil (Dumitru & Manea, 2011). Taxonomy study utilised the morphologic criteria by optical microscope

(MC 5. A) examination and measurements according to determinative manuals: Bergey & Holt (1994) for bacteria and Domsch & Gams (1970) and Watanabe (2002) for fungi. In order to evaluate microbial diversity of bacterial and fungal communities, total number of species (S), Relative abundance (A%), Species richness (SR₂ index) and Diversity index of Shannon (H') have been utilised for soils under conventional and HNV agriculture systems (Mohan & Ardelean, 1993). According to Morris et al. (2014), a high value of H' index is registered when the number of species is high and have a high homogeneity. The homogeneity (evenness) or equitability (ε) was calculated according to Stugren (1982) and Similarity index (SI%) between the lists of species from the two soils (according to Tiwari et al., 1994).

The global physiological activities of edaphic communities were determined by substrate induced respiration method (SIR) and expressed as mg CO₂ x 100 g⁻¹ soil (Matei, 2011).

Soil biological quality was assessed by interpreting the characteristics of images of circular chromatograms obtained as a result of migration of soil extracts on filter paper previously impregnated with developing substance, accordingly to procedure recommended by Pfeiffer (1984).

All data are the mean of three replicates. The results were interpreted by one-way analysis of variance (ANOVA) and the value p<0.05 was considered statistically significant (Student test).

RESULTS AND DISCUSSIONS

Analysis of results revealed significant differences between the microbial characteristics of soils from areas under arable (conventional) and forage plants in abandoned orchard (HNV) agriculture systems concerning both total counts of bacteria, fungi and global physiological activities (Table 1).

P3- Soil from P3 Lupac, in conventional system of agriculture presented moderate to low total counts of heterotrophic bacteria (11.892 x 10⁶ viable cells x g⁻¹ dry soil), a high number of fungi (148.914 x 10³ cfu x g⁻¹ dry soil) and, as a consequence, a more intense

global activity of microbial community (39.436 mg CO₂ x 100 g⁻¹ soil, value considered to characterize a moderate potential of soil respiration) as compared to microflora from P4.

Table 1. Total counts and global physiological activity of soil bacterial and fungal communities in HNV and conventional agriculture systems

Soil profile location and land use	TNB (x 10 ⁶ viable cells x g ⁻¹ d. s.)	TNF (x 10 ³ cfu x g ⁻¹ d. s.)	Soil respiration (mg CO ₂ x 100 g ⁻¹ soil)
LUPAC P3 (Conventional)	11.892a ¹	148.914a	39.436a
LUPAC P4 (HNV)	5.905b	46.316b	24.785b

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

Fungal microflora (Figure 3) presented the highest diversity (9 species), characterized by the dominance of antagonists *Trichoderma viride* and *Penicillium chrysogenum*, able to control, in conditions from this soil, the development of some potential phytopathogens represented by species of genera *Fusarium* and *Pythium*.

These species were accompanied by accessory or accidental species (less abundant in community from genera *Penicillium*, *Paecilomyces*, *Mucor*) well-known for their diverse enzyme equipment (cellulase, pectinase, chitinase), very efficient in degradation of various materials of vegetal origin.

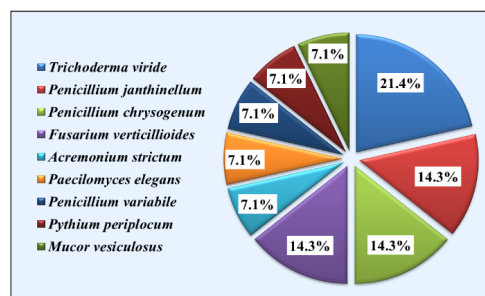


Figure 3. Relative abundance of fungal species in soil from P3 under arable (in conventional system)

The population of heterotrophic bacteria was dominated by fluorescent and non-fluorescent representatives of genus *Pseudomonas* and various bacillaceae, both with role in suppressing pathogens and carbon sequestration in stable forms (humus) (Figure 4).

P4 - Soil from P4 Lupac (HNV system) situated in abandoned orchard was populated with low numbers of heterotrophic bacteria (5.905×10^6 viable cells \times g^{-1} dry soil) and fungi (46.316×10^3 cfu \times g^{-1} dry soil), low values being also registered for the level of global physiological activities of microbiota (24.785 mg $CO_2 \times 100$ g^{-1} soil).

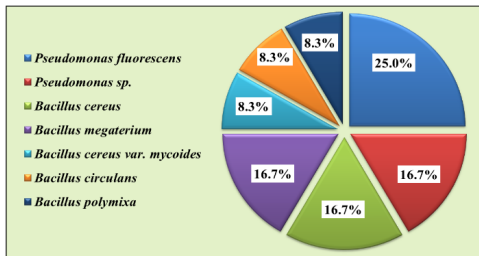


Figure 4. Relative abundance of bacterial species in soil from P3 under arable (in conventional system)

Species diversity of fungal microflora from this soil was low (only 3 species identified). Dominant species was potential phytopathogen from genus *Fusarium* (*F. culmorum* var. *roseum*), its development being stimulated by supplementary nitrogen fixed from atmosphere by leguminous plants between the rows of trees, accompanied by representatives of two cosmopolitan, cellulolytic genera (*Penicillium* and *Aspergillus*) (Figure 5).

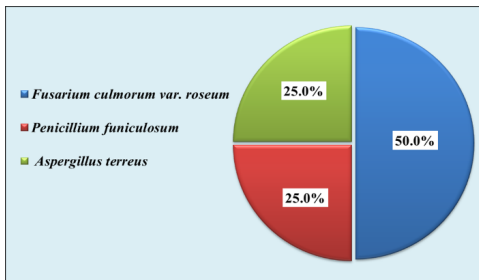


Figure 5. Relative abundance of fungal species in soil from P4 under arable (in conventional system)

The community of heterotrophic bacteria from P4 (Figure 6) consisted of 8 species as compared with 7 species in the case of nearby soil from P3, representatives of genus *Arthrobacter* accompanying the bacilli and pseudomonads (fluorescent, non-fluorescent). Other results in literature (Attitalla et al., 2001; Weller et al., 2002) attribute the soil

suppressiveness to the presence of a population of fluorescent bacteria from genus *Pseudomonas*, able to control the development of phytopathogens (species from genus *Fusarium*). According to results of Ahmed & Holstrom (2014), the suppressiveness is also influenced by the siderophores, exometabolites released by various microbial species in the soil (e. g. pseudomonads, bacilli, fungi from genera *Trichoderma*, *Penicillium*, *Aspergillus*, *Paecilomyces*), that inhibit the proliferation of phytopathogenic bacteria and fungi, species also found in both Endostagnic Luvisols from Lupac (Clotocici).

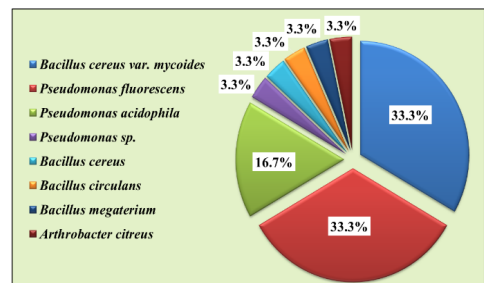


Figure 6. Relative abundance of bacterial microflora in soil from P4 under abandoned orchard (HNV system)

Present data evidencing the beneficial microorganisms from both bacterial and fungal populations with role in biocontrol of pathogens, organic matter recycling and humification are in concordance with previous results on edaphic microflora from HNV agriculture systems in Suceava county, North-East Romania (Matei et al., 2022) and South-Eastern Transylvania (Dumitrascu et al., 2019). Comparative analysis of lists with taxonomic composition of bacterial communities showed that 80% of species were common (present in both soils) and two species were unique, *Bacillus polymixa* only for P3 (conventional) and *Arthrobacter citreus* only for P4 (HNV). Each soil owns a specific taxonomic composition and structure of fungal community and there are no common species (SI = 0). Authors of a study in grassland soils reported that composition and structure of microbial communities from rhizosphere were influenced by soil type and grass plant species (Singh et al., 2007). On the other hand, the interactions between plants, microorganisms and soil were

considered by Reynolds et al. (2003) as drivers of the structure and dynamics of grass plants community.

The values of all diversity indices calculated for bacterial and fungal communities from Endostagnic Luvisols from Lupac (Clocotici) under arable and abandoned orchard are presented in Table 2.

According to the value of biodiversity index (S), microbial communities in studied soils consisted in minimum 3 and maximum 9 species. An increased value of diversity index of Shannon was found for bacterial community in soil from P3, under maize for forage cultivated in conventional system and fertilized

with manure ($H' = 1.864$ bits and evenness $\epsilon = 0.737$), higher than those from P4 (with $H' = 1.598$ bits and lower evenness value $\epsilon = 0.558$).

The highest diversity of fungi was also found in soil from P3 conventional system ($H' = 2.107$ bits and the highest evenness $\epsilon = 0.756$).

Accordingly, other research presented the use of manure and compost as a practice for restoring the functions of most soils, particularly those with low levels of organic matter and poor structure by improving the soil microbial activity, nutritional status and physical characteristics (Tavali, 2021).

Table 2. Taxonomic composition and biodiversity indices of microbial communities in soils under conventional and HNV agriculture systems from Lupac (Clocotici)

Soil profile location and land use	Fungal species	Bacterial species
Lupac (Clocotici) P3 Arable (Conventional)	<i>Trichoderma viride</i> <i>Penicillium janthinellum</i> <i>Penicillium chrysogenum</i> <i>Fusarium verticillioides</i> <i>Acremonium strictum</i> <i>Paecilomyces elegans</i> <i>Penicillium variable</i> <i>Pythium periplocum</i> <i>Mucor vesiculosus</i>	<i>Pseudomonas fluorescens</i> <i>Pseudomonas</i> sp. <i>Bacillus cereus</i> <i>Bacillus megaterium</i> <i>Bacillus cereus</i> var. <i>mycoides</i> <i>Bacillus circulans</i> <i>Bacillus polymixa</i>
	S = 9; SR ₂ = 0.642 Shannon H' = 2.107; $\epsilon = 0.756$ Hmax = 2.197; H1' = 1.662 var(H') = 0.069	S = 7; SR ₂ = 0.583 Shannon H' = 1.864; $\epsilon = 0.737$ Hmax = 1.946; H1' = 1.435 var(H') = 0.084
Lupac (Clocotici) P4 Abandoned orchard (HNV)	<i>Fusarium culmorum</i> var. <i>roseum</i> <i>Penicillium funiculosum</i> <i>Aspergillus terreus</i>	<i>Bacillus cereus</i> var. <i>mycoides</i> <i>Pseudomonas fluorescens</i> <i>Pseudomonas acidophila</i> <i>Pseudomonas</i> sp. <i>Bacillus cereus</i> <i>Bacillus circulans</i> <i>Bacillus megaterium</i> <i>Arthrobacter citreus</i>
	S = 3; SR ₂ = 0.750 Shannon H' = 1.040; $\epsilon = 0.643$ Hmax = 1.099; H1' = 0.706 var(H') = 0.737	S = 8; SR ₂ = 1.333 Shannon H' = 1.598; $\epsilon = 0.558$ Hmax = 2.079; H1' = 1.160 var(H') = 0.144
	Similarity index SI = 0	Similarity index SI = 80%

Circular paper chromatograms provided information on biological quality of the soils, by image analysis concerning the general aspect and harmony, color, shape and size, useful for evaluation of vitality, fertility, the complexity of organic matter, the stable humus or the intensity of biologic activity in soils (Figure 7).

There are no evidences of unfavorable soil conditions for plant growth.

Generally, the chromatograms of the two soils reveal some problems of integration of mineral compounds, low mineral diversity, abiotic conditions preponderant, relatively weak conditions for organic aggregation and flocculation.

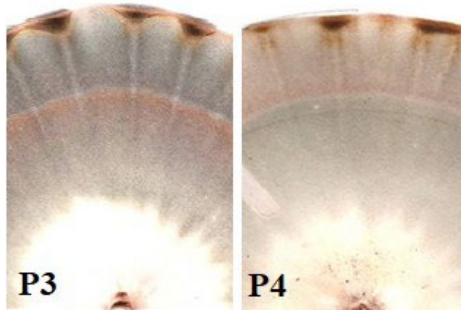


Figure 7. Sections of circular chromatograms of soils under arable (P3) and abandoned orchard (P4) from Lupac (Clucotici)

The aspect, color and radius of internal zone of both chromatograms are characteristic for increased contents in non-accessible minerals.

Low accessibility of mineral contents is more evident on chromatogram from P4 but a higher accessibility is evidenced on chromatogram from P3.

Analysis of external zone of chromatograms reveals a weak enzymatic activity. Yet, the level of enzymatic activity is higher in P3 soil than in P4.

Functional diversity is better evidenced in chromatogram from P3 as compared to P4, where a weaker microbial activity and a lower functional diversity is evidenced in image of external zone, in concordance with the values presented in Tables 1 and 2.

Studies on image forming methods sustain the use of circular chromatograms for rapid characterization of quality, applicable to various soil types and land management (More et al., 2014). Our results on microbial diversity and quality of soils from HNV areas are similar to studies from literature (Gomez et al., 200; Fritz et al., 2011) and underline the influence of land use on soil organic status that in turn, induces modifications of density and genetic structure of microbial communities (Lejon et al., 2007), as well as the role of soil heterotrophic respiration patterns in terrestrial ecosystems for global carbon dynamics (Tang et al., 2020; Matei et al., 2023).

Present research findings are in concordance with results confirming that land use shapes the resistance of soil microbiota and the carbon cycling response to global warming,

contributing to preventing the effects of climate changes (Moreno et al., 2019).

CONCLUSIONS

Soil from P3 cultivated with maize for forage in conventional system presented significantly higher values of total counts of both bacteria, fungi and CO₂ released by global physiological activities as compared with P4 (HNV) from abandoned orchard.

Biodiversity index (S) of microbial communities in studied soils consisted in minimum 3 and maximum 9 species.

Bacterial community from soil cultivated with maize for forage in conventional system presented the highest value of diversity index ($H' = 1.864$ bits and evenness $\epsilon = 0.737$).

Biodiversity of fungal community was higher in soil from conventional system P3 ($H' = 2.107$ bits and the highest evenness $\epsilon = 0.756$).

Dominance of fluorescent pseudomonads, bacilli and fungi from cosmopolitan genera *Penicillium* and *Trichoderma* presented importance in improving nutrients availability, uptake and the control of pathogens in plants rhizosphere, soil aggregation, cellulose decomposition and carbon sequestration processes.

Circular paper chromatograms provided useful evidences on biological quality of soils.

Areas with HNV agriculture practices contribute to protection and preservation of microbial diversity in soils, sustaining its important functions and services for ecosystem.

ACKNOWLEDGEMENTS

This work was supported by two grants of the Romanian Ministry of Research, Innovation and Digitization, Project number PN 23 29 03 01/2023 and Project number 44PFE/2021, Program 1 – Development of national research-developing system, Subprogramme 1.2 - Institutional performance - RDI Excellence Financing Projects.

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