

## STRUCTURAL CHARACTERISTICS OF THE SOIL INVERTEBRATE COMMUNITIES FROM TWO FRAGMENTED NATURA 2000 SITES FROM ROMANIA

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### **Abstract**

*Wetland ecosystems are dependent of groundwater. They provide goods and ecosystem services. Any anthropic activity will affect their structure. This ecological damage could be revealed by using the biological indicators, as soil invertebrate communities. In 2018, two fragmented Natura 2000 sites were studied: Forest and Eutrophic Marshes from Prejmer (ROSCI0170) and Lempeș Fortress Hill-Hărman Marsh (ROSCI0055). 80 soil samples were investigated, from four fragments in each sites. Two structural parameters were analysed: numerical abundance and constancy. In total, 19 taxa were identified, with 1108 individuals. The highest values of numerical abundances were obtained by the Oribatida mites and Collembola. In Prejmer, 23.52% from the total number of taxa were euconstant, 17.64% constant, 41.17% accessory and 17.64% accidental. The soil fauna from Hărman was represented only by accessory (53.84%) and accidental taxa (46.15%). The dominance of the accidental and accessory taxa demonstrating that the two protected area were not characterized by stable communities. The canonical analysis revealed that the type of habitat influenced the spatial distribution of soil invertebrate communities, defining distinct groups for marsh ecosystems, alluvial forests and deciduous forests.*

**Key words:** fragment, invertebrate, soil, structure.

### **INTRODUCTION**

Groundwater dependent ecosystems are natural ecosystems that integrate different components dependent of groundwater (cave and aquifer ecosystems, springs, streams, lakes, rivers, swamps, estuaries and coastal ecosystems, wetlands-swamps, riparian systems, alluvial systems and other terrestrial systems - wetlands, meadows) (Eamus et al., 2006; Kløve et al., 2014). Wetlands come in many different forms. They can be tidal zones, marshes, bogs or swamps among many other types. These types of ecosystems offer to human societies a wide range of essential goods and services (Daily et al., 1997). Wetlands provide several ecosystem services such as reducing erosion, recharging aquifers, flood control, pollution filter, storm and wind buffer, carbon sink and providing habitat for several wildlife species (Eamus et al., 2005). Groundwater dependent ecosystems are often hydricly and ecologically connected to terrestrial ecosystems through transition zones (Tomlinson &

Boulton, 2010). Therefore, we consider that an important component of the biodiversity of terrestrial ecosystems dependent on groundwater, but also on the surface water (as wetlands-swamps), is represented by the soil (edaphic) fauna. In Europe, several biological indicators were used, which were based on groups of organisms (simple indicators) or on whole community of soil fauna (compound indicators). Over the decades, different groups of edaphic invertebrates have been used as bioindicators of natural or anthropogenic ecosystems (Collembola, Nematoda, Acari, Chilopoda, Diplopoda, Protura, Isopoda, Diplura, Coleoptera, Mollusca, etc.). Any anthropic impact (as ecosystem fragmentation) will reflect into modification on structure and functions of soil invertebrates communities (Lavelle & Bignell, 1997; Ruf, 1998; Ponge et al., 2003; Sanchez-Moreno & Navas, 2007; Bedano et al., 2011; Santamaria et al., 2012; Skubała & Zaleski, 2012; Manu et al., 2019). In this context, the present paper aims to highlight the structural characteristics of the

soil invertebrate communities from two main hypotheses: the investigated ecosystems were characterized by stable edaphic fauna and how the type of vegetation habitats influenced the structure of these communities?

## MATERIALS AND METHODS

### The study area

The present study was made in November 2018, in two fragmented Natura 2000 sites from Braşov County, Romania: Forest and Eutrophic Marshes from Prejmer (ROSCI0170) and Lemeş Fortress Hill-Hărman Marsh (ROSCI0055). The nature reserve Forest and Eutrophic Marshes from Prejmer, with an area of 345 hectares, was declared as protected area by law no. 5 of March 6, 2000, published in the Official Monitor of Romania, no. 152 of April 12/2000. In this area there are terrestrial ecosystems (forests, shrubs, meadows) and freshwater aquatic ecosystems (swamps). Lemeş Fortress Hill-Hărman Marsh has an area of 374 hectares, and was declared as protected area in 2000, by the same law as above.

The ecological investigations were made in four fragments, in each protected area. These were codified as following: PF1 (the first fragment from Prejmer); PF2 (the second fragment from Prejmer), PF3 (the third fragment from Prejmer); PF4 (the fourth fragment from Prejmer); HF1 (the first fragment from Hărman); HF2 (the second fragment from Hărman), HF3 (the third fragment from Hărman); HF4 (the fourth fragment from Hărman) (Figure 1).



Figure 1. Geographical location of fragments from Forest and Eutrophic Marshes from Prejmer (ROSCI0170) (yellow color) and Lemeş Fortress Hill-Hărman Marsh (ROSCI0055) (green color) ecosystems, from Romania, 2018

fragmented Natura 2000 sites, proposing two In the Forest and Eutrophic Marshes from Prejmer, the samples from PF1 were located at 45°43'46.04"N and 25°44'09.03"E; at 514 m altitude. PF2 was located at 45°44'52.22"N and 25°43'48.66"E; at 503 m altitude. In PF3, the soil samples were taken from 45°44'56.90"N and 25°42'08.94"E, 508 m altitude. PF4 was situated at 45°44'55.46"N and 25°41'15.64"E and 501 m altitude. The PF1 fragment was characterized by the habitat 7210 \* calcareous fens with *Cladium mariscus*. The rest of fragments were characterized by the following type of habitat: 91 EO alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*).

The fragments from Lemeş Fortress Hill-Hărman Marsh were located and described as following: HF1 at 45°43'03.63"N and 25°40'03.61"E, 514 m altitude, habitat type: 7210 \* calcareous fens with *Cladium mariscus*; HF2 at 45°43'07.30"N and 25°40'00.08"E, 498 m altitude, habitat type: 9170 Galio-Carpinetum oak-hornbeam forests; HF3 at 45°44'12.63"N and 25°40'27.07"E, 511 m altitude, habitat type: 9170 Galio-Carpinetum oak-hornbeam forests; HF4 at 45°43'07.30"N and 25°40'00.08"E, 498 m altitude, habitat type: 9130 Asperulo-Fagetum beech forests.

### Soil samples

The investigated area in each swamp was by 500 square meters. In total 80 cores (40 samples in each protected area) were sampled for soil fauna, to a depth of 10 cm with a MacFadyen corer, by 5 cm diameter. The samples were taken randomly. The fauna were extracted with a modified Berlese-Tullgren funnel, in ethyl alcohol. The published identification keys were used (Dindal, 1990; Orgiazzi et al., 2016; Krantz, 2009).

### Data analysis

The constancy was obtained using the formula:  $C = 100 \cdot pA/P$ , where: pA - number of samples with taxa A; P - total number of samples. The taxons were classified in four constancy classes: euconstant taxa having constancy of 75.1-100% (C4), constant taxa having constancy of 50.1-75% (C3), accessory taxa having constancy of 25.1-50% (C2) and

accidental taxa having constancy of 1-25% (C1) (Selvin & Vacca, 2004).

The correspondence analysis (CA) between identified taxonomical groups and analysed fragments from the two protected areas; the individual rarefaction were calculated using the BioDiversity Pro 2.0 software, PAST (Hammer et al., 2001).

## RESULTS AND DISCUSSIONS

Taking into consideration the taxonomical spectrum of two investigated Natura 2000 sites, we identified in total 19 taxa, with 1108 individuals. These were grouped in seven taxonomic classes: Clitellata, Diplopoda, Chilopoda, Entognatha, Insecta, Arachnida and Gastropoda (Table 1). The highest values of numerical abundances were obtained by the taxa from Oribatida suborder (mites), with 411 individuals and Collembola order (springtails), with 308 individuals. On the opposite there are taxa from Chilopoda, with a total of 4 individuals. Making a comparison between the two investigated fragmented protected areas, we observed that in Forest and Eutrophic Marshes from Prejmer, the both structural parameters recorded higher values (17 taxa and 655 individuals), in comparison with Lempeş Fortress Hill-Hărman Marsh (13 taxa with 453 individuals). In each area, the Oribatida mites and Collembola taxa were numerical dominant (411 individuals and respectively 308 individuals), instead Chilopoda was less represented (4 individuals). From all 17 identified taxa, 58.82% were common for both protected areas, 41.17% were characteristics for Prejmer and only 17.64% from Hărman. In the scientific world is well known that soil invertebrates constitute a valuable bioindicator tool (Gardi et al., 2009; Manu et al., 2019). The obtained data are comparable with other studies from all over the world, which revealed that Collembola, Enchtreidae, Oribatida and Mesostigmata were the most abundant taxa in wetlands (Plum, 2005; Reynolds et al., 2007; Huhta et al., 2011). If we compare the obtained results from Romanian marshes with other

types of ecosystems, at international level, we observed that the number of taxon is higher than that from forest ecosystems (9-14 taxa), shrubs (9-11 taxa), arable land (6-12 taxa) or grasslands (16 taxa) (Parisi et al., 2005; Yan et al., 2012). According to these studies, species numbers and abundances of Lumbricidae, Isopoda, Chilopoda and Diplopoda tended to be lower in frequently and/or extensively flooded sites. In bogs, even when they are waterlogged the entire year, species numbers are distinctly higher than the most frequently flooded sites (Plum, 2005; Sterzyńska et al., 2015). At national level these types of studies, which take into consideration the functional groups of invertebrates, are few (Manu et al., 2020). Analysing this literature, we observed that the number of taxa and the numerical abundance of the taxons from the two fragmented marshes recorded lower values, in comparison with those obtained in a protected area "Springs Complex of Corbii Ciungi", characterized by the meadows and riverine scrub habitats (34 functional groups, with 4180 individuals) (Manu et al., 2020).

If we put into discussion the constancy index, quantified for each invertebrate communities from the two investigated protected areas, the study revealed that in Prejmer, 23.52% from the total number of taxa were euconstant, 17.64% constant, 41.17% accessory and 17.64% accidental. On the other hand, the soil fauna from Hărman was represented only by accessory (53.84%) and accidental taxa (46.15%) (Table 1). The dominance of accessory and accidental species (with few exceptions (Collembola, Oribatida, Opiidae and Mesostigmata) revealed the fact that the rest of invertebrates communities are only occasional present in investigated fragments of the two areas. We could suppose that the fragmentation of the investigated ecosystems impact the soil invertebrate communities, being known that the taxons as Chilopoda, Isopoda, Coleoptera, etc., were identified in optimal conditions, in alluvial forest (Herlitzius, 1987; Manu et al., 2013; Kolesnikova et al., 2016).

Table 1. The structural parameters (numerical abundance and constancy) of identified taxa from Forest and Eutrophic Marshes from Prejmer (ROSCI0170) and Lempeş Fortress Hill-Hărman Marsh (ROSCI0055), Romania, 2018

Taxa	PF1	PF2	PF3	PF4	Total PF	HF1	HF2	HF3	HF4	Total HF
Phylum Annelida										
Class Clitellata										
Subclass Oligochaeta										
Order Haplotaxida										
Family Lumbricidae - Lum	2/ac	4/ac		3/ac	9/ct	2/ac				2/ac
Family Enchytreidae - Enc	13/as	6/as	2/as	1/as	22/eu	5/as	2/ac			7/ac
Phylum Arthropoda										
<i>Subphylum Myriapoda</i>										
Class Diplopoda - Dip	3/ac	1/ac			4/as					
Class Chilopoda - Chi	2/ac		1/ac		3/as		1/ac			1/ac
<i>Subphylum Crustacea</i>										
<i>Subphylum Hexapoda</i>										
Class Entognatha										
Order Collembola- Collem	57/ct	83/eu	37/as	29/as	206/eu	48/eu	34/as	10/ac	10/ac	102/as
Order Diplura - Dip	6/ac				6/ac					
Order Protura - Pro	1/ac				1/ac					
Class Insecta										
Order Coleoptera - Col	1/ac				1/ac					
Order Psocoptera - Pso				1/ac	1/ac					
Order Hymenoptera										
Superfamily Formicoidea- For							20/ac			20/ac
Insect larvae- Ins.larv	20/ct	3/ac			23/ac		1/ac	1/ac	1/ac	3/as
<i>Subphylum Chelicerata</i>										
Class Arachnida										
Order Opiliones-Opi		1/ac			1/ac					
Supraorder Acariformes										
Order Trombidiformes										
Suborder Prostigmata										
Family Trombidiidae- Tro	1/ac			2/ac	3/ac					
Family Bdellidae- Bde	2/ac	4/as		2/ac	8/ct		1/ac	12/as	1/ac	14/as
Order Sarcoptiformes										
Suborder Oribatida-Ori	140/eu	54/eu	15/ct	17/as	226/eu	113/eu	22/ct	47/eu	3/eu	185/as
Family Opiidae- Opi	32/as	33/ct	2/ac	2/ac	69/eu	8/ac	3/ac	29/as	2/ac	42/as
Suborder Astigmata- Ast								6/ac	2/ac	8/ac
Family Acaridae- Aca	6/as	8/as			14/as	29/eu	1/ac	13/as	4/ac	47/as
Order Mesostigmata- Mes	27/ct	15/as		16/ac	58/ct	14/ct	1/ac	4/as	2/ac	21/as
Phylum Mollusca										
Class Gastropoda- Gas						1/ac				1/ac
Total no of taxa	15	11	5	9	17	8	10	8	8	13
Total no of individuals	313	212	57	73	655	220	86	122	25	453
Total no of euconstant species (eu)	1	2			4	3		1	1	
Total no of constant species (ct)	3	1	1		3	1	1			
Total no of accessory species (as)	3	4	2	3	7	1	8	3		7
Total no of accidental species (ac)	8	4	2	6	3	3	1	4	7	6

Making an analysis in spatial dynamics of soil invertebrate communities, in each studied area, we observed differences for each studied fragments, especially in Prejmer. The highest numbers of identified taxa and of numerical abundances, in the Prejmer protected area, were obtained in PF1 and PF2, in comparison with PF3 and PF4. This fact was highlighted by the individual rarefaction analysis (Figure 2). Common taxa for each fragment were the following taxa: Enchytreidae, Collembola, Oribatida and Oppiidae, with represent 23.52% from the total identified taxons. In the same time, the first fragments were the only ones, which were characterized by few euconstant (Oribatida and Collembola) and constant taxa (Opiidae, Mesostigmata, insect larvae). The second fragments PF3 and PF4, were dominated by the accessory and accidental species (Table 1).

In Lemeș Fortress Hill-Hărman Marsh, the differences between the four transects are not so evident. In HF1, HF3, HF4 the number of taxa was the same, only in HF2 this parameter recorded the highest value (Table 1; Figure 3). If we put into discussion the numerical abundance, in the HF1 and HF3 were recorded the highest values, in comparison with the other two fragments HF2 and HF4. 38.46% were common taxa for the four fragments from Hărman ecosystems, as following: Collembola, Oribatida, Oppiidae, Acarida and Mesostigmata. Considering the constancy index, the fragments were better represented by accessory and accidental taxa, than euconstant-constant taxons (as Collembola, Oribatida and Acarida) (Table 1).

In order to demonstrate if the types of habitat influence the spatial distribution of soil invertebrate communities, the correspondence analysis (CA) between identified taxonomical groups and investigated fragments was analysed. In Prejmer protected area, four groups were defined: soil invertebrates communities characteristics for PF1: Coleoptera and insect larvae; for PF2 and PF3: Collembola, Opiidae; for PF4: as Psocoptera, Trombidiiidae, Bdellidae, Mesostigmata and Lumbricidae (Figure 4).

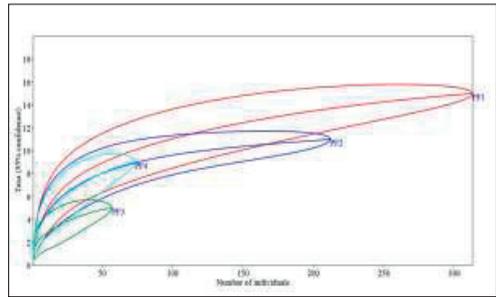


Figure 2. The individual rarefaction of the soil invertebrate communities from Forest and Eutrophic Marshes from Prejmer (ROSCI0170), Romania, 2018

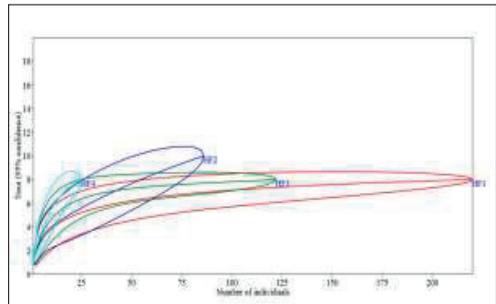


Figure 3. The individual rarefaction of the soil invertebrate communities from Lemeș Fortress Hill-Hărman Marsh (ROSCI0055), Romania, 2018

Even if the PF1 was defined by the calcareous marsh habitat, it is possible that due to the environmental conditions (dryness period), there weren't favorable conditions for development of soil invertebrates communities. On the other hand, the alluvial forest with *Alnus glutinosa* and *Fraxinus excelsior* constituted a proper habitat for the majority of the soil functional groups.

In Lemeș Fortress Hill-Hărman Marsh protected areas, we discovered that the spatial distribution of the soil invertebrate communities was influenced by the type of habitat, describing four groups. The first group HF1 (alkaline marsh) was the characterized by the following taxa: Lumbricidae, Mesostigmata, Acaridae and Oribatidae; HF2 (oak-hornbeam forest) another distinct group, contains the following taxa as: Chilopoda, Formicoidea, insect larvae; HF3 and HF4 offered proper habitats (oak-hornbeam forest and beech forest) for mites' taxa, Opiidae, Bdellidae and Astigmata.

We observed that in the two protected areas, the soil invertebrate communities formed a distinct group in marsh ecosystems, possible due to the specifically environmental conditions.

International ecological researches concerning the influence of the type of habitat on the structure of soil invertebrates' communities were well developed in Europe. These studies revealed that there are specifically environmental conditions (taking into account the abiotic and biotic factors) for each investigated habitats, which influenced directly or indirectly the soil invertebrate fauna (Plum, 2005; Manu, 2013; Skubala & Zaleski, 2012; Sterzynska et al., 2015; Manu et al., 2020). The results of our study is in concordance with those from Europe.

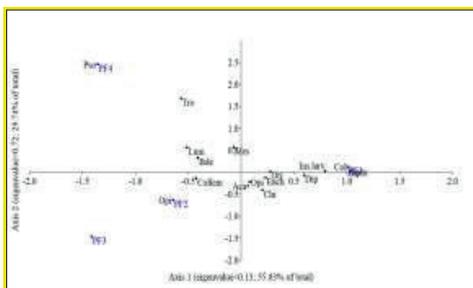


Figure 4. Correspondence analysis (CA) between identified taxonomical groups and analysed fragments from Forest and Eutrophic Marshes from Prejmer (ROSCI0170), Romania, 2018 (the short names of the taxa are mentioned in Table 1)

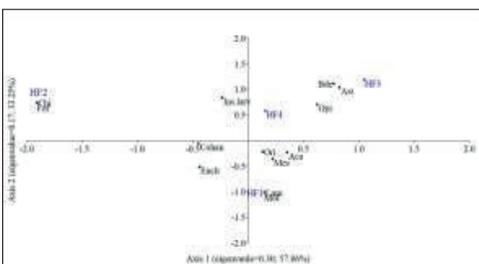


Figure 5. Correspondence analysis (CA) between identified taxonomical groups and analysed fragments from Lempeş Fortress Hill-Hărman Marsh (ROSCI0055), Romania, 2018 (the short names of the taxa are mentioned in Table 1)

## CONCLUSIONS

In order to highlight the structure characteristic of the soil invertebrate fauna from two

fragmented Natura 2000 sites (Forest and Eutrophic Marshes from Prejmer - ROSCI0170 and Lempeş Fortress Hill-Hărman Marsh - ROSCI0055), 80 soil samples were analysed, from four fragments/each protected areas, in 2018. Two main structural parameters analysed were numerical abundance and constancy. In total, 19 taxa were identified, with 1108 individuals. The highest values of numerical abundances were obtained by the Oribatida mites and Collembola. On the opposite there are taxa from Chilopoda. In both protected areas the dominant taxa were accessory and accidental ones, only in Prejmer were identified euconstant and constant functional groups, but there are poorly represented. These data revealed that the analysed fragmented areas are not characterized by stable soil invertebrate communities. Using the correspondence analysis, we demonstrated that the type of habitat influenced the spatial distribution of soil invertebrate communities, defining distinct groups for marsh ecosystems, alluvial forests and deciduous forests.

## ACKNOWLEDGMENTS

This study was funded through project PED number 453/2020 code PN-III-CERC-CO-PED-2-2019, supported by Executive Unit for Financing Higher Education, Research, Development and Innovation, for Romania (UEFISCDI). The theme of the article is according to the general framework of the project number RO1567-IBB01/2021 of the Institute of Biology Bucharest of the Romanian Academy.

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