CHARACTERIZATION OF ENVIRONMENTAL FACTORS INFLUENCING THE FUNCTIONAL GROUPS OF SOIL INVERTEBRATES FROM SOME GRASSLANDS IN SOUTH-WEST FĂGĂRAȘ MASSIF

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Abstract. Temperature and humidity are the main factors that control the biological activity in ecological systems. The great diversity of functional groups in the soil is due to the multitude of ecological niches created especially by environmental factors. The aim of this paper was to characterize the environmental factors and their influence on the functional groups of invertebrates in the soil in some grasslands in the southwest of the Făgăraș massif. The environmental variables used in this study were: air and soil temperature and humidity, amount and thickness of humus and litter-fermentation layer, organic carbon content, pH and resistance to soil penetration. The statistical analysis showed that the invertebrate species are distributed on the grassland according to the characteristics of the environmental parameters and the optimal ecological requirements of each. Physical and chemical factors determine the variability in time and space of the structural and functional parameters of invertebrate populations and therefore their ecological role in the functioning of ecosystems.

Keywords: environmental factors, invertebrates, grasslands, Făgăraș Massif.

Rezumat. Caracterizarea factorilor de mediu care influențează grupele funcționale de nevertebrate din sol în unele pajiști din sud-vestul masivului Făgăraş. Temperatura și umiditatea sunt principalii factori care controlează activitatea biologică din sistemele ecologice. Marea diversitate a grupelor funcționale din sol se datorează multitudinii de nișe ecologice create în special de factorii de mediu. Scopul acestei lucrări a fost de a caracteriza factorii de mediu și influența lor asupra grupelor funcționale de nevertebrate din sol în unele pajiști din sud-vestul masivului Făgăraș. Variabilele de mediu utilizate în acest studiu au fost: temperatura și umiditatea aerului și solului, cantitatea și grosimea stratului de humus și de litieră-fermentație, conținutul de carbon organic, pH-ul și rezistența la penetrare a solului. Analiza statistică a demonstrat faptul că speciile de nevertebrate se distribuie pe teritoriul pajiștilor în raport de caracteristicile parametrilor de mediu și de cerințele optime ecologice ale fiecăreia. Factorii fizici și chimici determină variabilitatea în timp şi spațiu a parametrilor structurali şi funcționali ai populațiilor de nevertebrate şi deci rolul ecologic al acestora în funcționarea ecosistemelor.

Cuvinte cheie: factori de mediu, nevertebrate, pajiști, Masivul Făgăraș.

INTRODUCTION

The Făgăraş Massif (also called Transylvanian Alps) is located in the central area of the Carpathian Mountains and belongs to the Southern Carpathians (NEDELEA & COMĂNESCU, 2011). Its slopes have mainly northern and southern exposure (BARLOY & PRUNAR, 2010). In the massif, the diversity of habitats (biological, speleological, paleontological, and mixed protected natural areas) is great, including grasslands from the mountain's feet to the peaks (NICOARĂ et al., 2020; ONETE et al., 2020; 2021) comprising high value biodiversity (rare, endemic, relict or new species for science).

Natural grasslands are very important because they support a great diversity of species that through their functions provide a variety of ecosystem services (quality and quantity of food production, climate change improvement, water protection, quality and cultural heritage, feed production, plant and animal diversity, protection against soil erosion, water storage, maintaining groundwater quality, habitat quality assurance, carbon storage, soil nitrogen supply). They cover most of the world's agricultural area, occupying over 40% and storing about 30% of the world's terrestrial biomass stock (MORAIS et al., 2021).

Soil invertebrates are excellent candidates for studying the quality of various types of ecosystems and for quantifying the impact of human activity on the environment, especially because they are in direct contact with soil pores and pore water (AESCHT & FOISSNER, 1996; SANTORUFO et al., 2012; KUTOVAYA et al., 2021; GEDOZ et al., 2021; DAHIYA et al., 2022). They are considered fundamental for their role in almost all processes that take place underground. They are used as bioindicators because the indices associated with these organisms are often representative of the ecological dynamics and characteristics of the habitat (BORGES et al., 2021). In addition, invertebrates are abundant, relatively easy to pick, and can respond quickly to soil disturbance (KARPIŃSKI et al., 2021; MANU et al., 2017; 2022).

The species used as a bioindicator can provide data on the appearance and amount of a certain pollutant or a wide range of pollutants and the intensity of exposure (ȘTEFĂNUȚ et al., 2018). The short life cycle and high densities of invertebrate species, as well as their high morphological and ecological diversity, allow a high capacity to colonize new habitats (MANU & ONETE, 2016). In addition, these species are associated with important ecosystem services, such as pollination, biological control, organic matter degradation, seed dispersal, and nutrient cycle (BORGES et al., 2021). The loss of their biodiversity as a result of changes in environmental variables would lead to a reduction in the resilience of ecological systems, making them more vulnerable to various types of impact and thus less able to provide certain ecosystem services. For example, in the soil ecosystem, earthworms play a crucial role.

They are called "ecological engineers" because of their ability to decompose, mineralize, recycle and accumulate heavy metals in their bodies. Earthworms contribute to soil fertility and moisture (SINGH et al., 2022). Also, mites are considered bioindicators of soil quality, especially in forest ecosystems, where they find adequate environmental conditions for their development (MANU & ONETE, 2013; 2014). The specific composition of invertebrate groups is closely related to the environmental variables' characteristic of the micro-habitat in which they are found (DAVIS et al., 2006).

Soil temperature is one of the important factors that influence the physical, chemical and biological processes of the soil. It also influences the gas exchange processes between the atmosphere and the soil. The amount of radiation received by the soil affects soil temperature, biological processes such as: seed germination, plant root growth and nutrient availability. Soil temperature changes the decomposition rate of organic matter and the mineralization of various organic materials in the soil (ONWUKA, 2016).

Soil moisture is influenced by pore size, osmotic potential (presence of dissolved substances in the soil) and gravity, these factors determining the movement of water on the soil profile and therefore its availability for plant and invertebrate species (VILLANI & WRIGHT, 1990).

Soil pH is the concentration of hydrogen ions. It determines the alkalinity or acidity of the soil. The pH is influenced both by biological and chemical processes carried out in the soil, by the plant layer, fertilizers, carbon dioxide resulting from the respiration of plant species or invertebrates, and by the decomposition of organic substances by microorganisms. Invertebrates can be used as "early warning" organisms to detect possible acidification of the micro-habitat (LARSEN et al., 1996). PH has been shown to play a key role in determining the composition and abundance of invertebrate taxa (FELDMAN & CONNOR, 1992).

Soil penetration resistance (compaction) can be defined as the process by which soil particles are rearranged, thus reducing the empty space, they being in contact with each other, thus increasing the density of the soil. This involves changes in the physical properties of the soil (density and porosity), thus changing its chemical properties and thus the soil fauna and plant growth (NAWAZ et al., 2013).

Carbon and nitrogen mineralization processes performed by microorganisms are directly and indirectly affected by invertebrates. The processes of denitrification and nitrogen fixation performed by microorganisms are regulated by the redox conditions in the soil and the availability of nitrogen for plants, which is strongly influenced by fungi and bacteria. Denitrification rates, for example, are highest in waterlogged soils, where anaerobic conditions predominate (ANDERSON, 1988). Organic soil carbon is a material of plant, animal and microbial origin that is in different stages of decomposition and is associated with the mineral fraction with different degrees of privacy (KAY, 2018). In addition to its importance in the global cycle, soil organic carbon contributes positively to a number of important biological, physical and chemical properties in defining a soil's productivity (BALDOCK, 2007).

The purpose of this article was to measure environmental variables (air and soil temperature and humidity, amount and thickness of humus and litter-fermentation layer, organic carbon content, pH, resistance to soil penetration) and their correlation with the diversity of functional groups of invertebrates.

MATERIALS AND METHODS

We realized a screening of the literature for information about the region, localities, habitats and species. Our research focused on two grasslands from the Făgăraș Massif (Galbena and Vemeșoaia), with different grazing impact intensities (higher in Vemeșoaia, lower in Galbena) and previous knowledge about the plant and soil invertebrates' species diversity (***. CONTRACT 131/2018). Following the field trips performed in 2021, we set 10 soil sampling plots along one transect. We established 10 such transects based on altitudinal gradient (Line 1 to Line 10) in each grassland (totally 200 sampling plots). Soil sampling was performed using a MacFadyen probe with a diameter of 5 cm to a depth of 10 cm (MACFADYEN 1953; 1961). The soil was collected on the three horizons: the litter-fermentation layer (OLF) which consists of partially decomposed animal and plant debris, the humus horizon (OH) containing well-fermented material and a large amount of organic matter and the soil horizon. (OS) which is the area where plant material and animal residues are decomposed (NEHER & BARBERCHECK, 1998). The soil was separated both for the extraction, sorting and identification of functional groups of soil fauna and for performing chemical analyses in the laboratory.

In situ, we also performed environmental factors measurements: air (T air) and soil (T soil) temperature, air (U air) and soil (U soil) humidity, degree of penetrability of the soil (coil compaction) (RP). The air temperature and humidity were measured with a digital thermo-hygrometer at a distance of 10 cm from the ground level. Soil temperature and humidity were measured with a PCE-310 digital thermo-hygrometer. Penetration resistance was determined with a soil penetrometer, Step System GmbH, 41010. It allows the measurement and monitoring of soil density at all stages of vegetation development. The thickness of the litter-fermentation layer (OLF), the humus layer (OH) and the soil layer (OS) was measured using a graduated ruler (in centimeters), taking into account the morphological properties of the soil sample (color, texture, consistency) (CHIRIȚĂ, 1974). The soil was collected in plastic bags, labelled and stored in the refrigerator, then sent for chemical analysis to the laboratory of the National

Research-Development Institute for Pedology, Agro-chemistry and Environmental Protection - ICPA Bucharest who provided analysis bulletins that we integrated in the database.

In the soil invertebrates' laboratory from the Institute of Biology Bucharest, we extracted the soil invertebrates using Berlese – Tullgren method (BERLESE, 1905; TULLGREN, 1917; KRANTZ & WALTER, 2009). This method is based on the reaction capacity of soil invertebrates to leave the soil sample in a certain direction under the action of external factors, especially drought (BALOGH, 1958); the invertebrates in the soil will leave the soil and migrate to the bottom of the sample, thus reaching alcohol. For taxonomic identification and counting we used Zeiss stereomicroscope and published identification keys (BRUSSAARD et al., 1997; GÎDEI & POPESCU, 2009; KRANTZ & WALTER, 2009; CEUCA, 2010; COLEMAN & WALL, 2015; PLATNICK, 2018). Statistical analyses were performed using PAST software (HARPER, 1999; HAMMER et al., 2001).

RESULTS AND DISCUSSION

The grazing impact is high in Vemeșoaia grassland and low in Galbena grassland, with the dominance of some plant species in different micro-habitats highlighting the fact that the plant species finds the optimal survival conditions. These conditions are determined by plant itself (CRAWLEY, 2009) through both amending the underground architecture and growth of their own roots and physical-chemical parameters of the soil present in their rhizospheres.

The environmental factors acting on soil invertebrates' groups might be biotic (plant communities, other invertebrates, etc.) and abiotic (environmental variables). Plant species and environmental factors' diversity generate a great variety of ecological niches inside the soil, thus, providing different resources and significant functional differentiation of the organisms from the soil.

Canonical Correspondence Analysis shows the fact that the plant species spread on grasslands territory with respect to environmental parameters characteristics and optimal requirements of every plant species. Spatial diversity and abundance-dominance of plant species is high, determining significant differences among established transects (Line 1 to Line 10) in both studied grasslands (Fig. 1), leading to high diversity of soil invertebrates' groups.

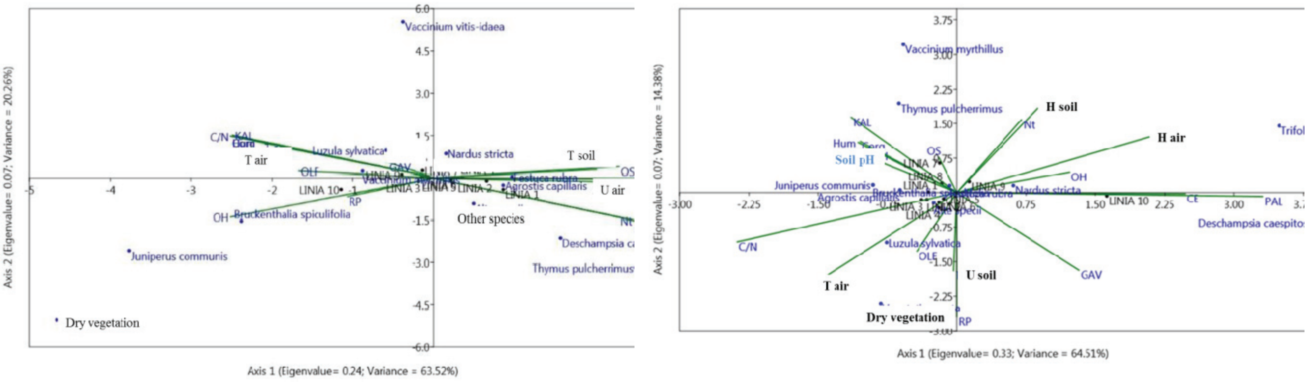

Figure 1. Canonical Correspondence Analysis between plant species and environmental parameters from Galbena (left) and Vemeșoaia (right).

Physical and chemical parameters of the soil generate variability in time and space of the structural and functional parameters of plant species and by default of invertebrates' communities (from above- and below-ground) and their ecological role in ecosystems' functioning. In the studied grasslands, the air temperature measurements made in the field throughout the day reveal the hourly variation (Fig. 2), but also the local variation at the micro-habitat level.

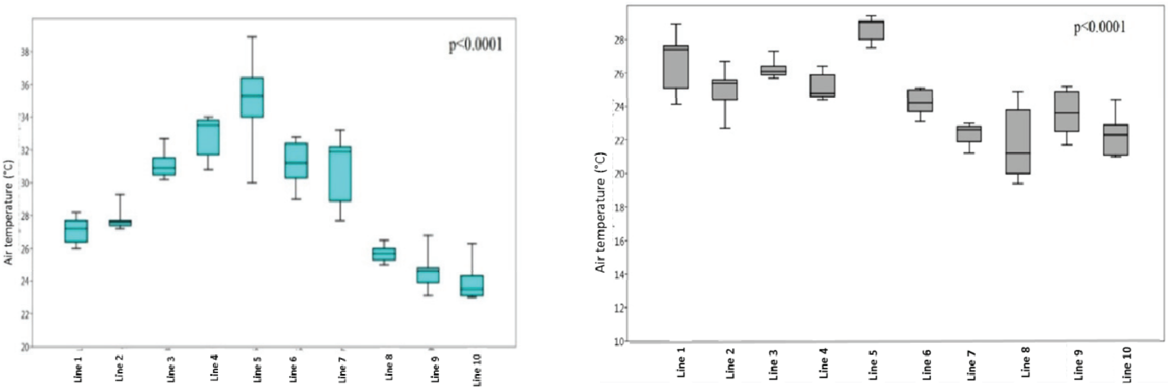

Figure 2. Air temperature in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

The temperature of the soil (Fig. 3) is not as variable as that of the air and highlights the fact that it depends on the specific structure and composition of the plant coverage that protects the soil.

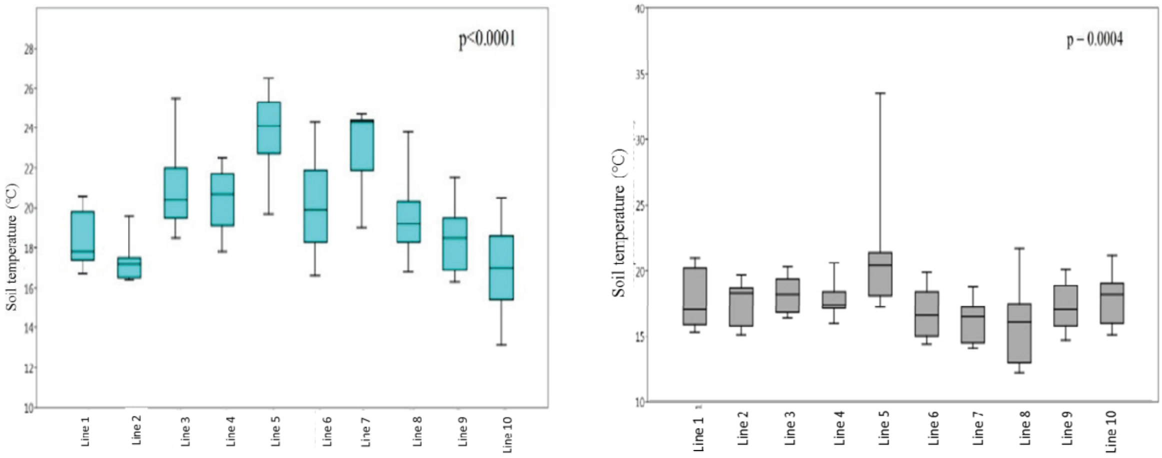

Figure 3. Soil temperature in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

The temperature of the soil has the role of modelling the rate at which the physical processes of the soil take place, the exchanges of matter and energy with the atmosphere, the chemical reactions that take place in it and also the biological processes in the soil. It is influenced by several factors such as: soil color, sun position, slope, soil moisture, porosity, mineral composition, percentage of organic matter and specific composition of vegetation (VILLANI & WRIGHT, 1990).

The average global temperature has increased by 0.8°C since 1880 and is estimated to increase by at least 1.5°C in the next century (ROBINSON et al., 2018; WARNER et al., 2021). Rising temperatures and early melting of snow lead to an increase in the richness of herbivorous insect species, causing changes in plant species diversity and distribution. Such vegetation changes could alter habitat characteristics and therefore the associated invertebrate communities (ROBINSON et al., 2018). Species in the invertebrate community have different types of response to these temperature changes. Species-level responses to warming in the last century may be of the type of change in geographical boundaries within which certain species meet, phenology, seasonal calendar of life cycle events (WARNER et al., 2021).

Conversely, when the average temperature decreases, some species of nematodes and arthropods show a seasonal pattern of vertical movement associated with soil temperature. Migration to deeper horizons has the role of protecting invertebrates from very low soil surface temperatures (VILLANI & WRIGHT, 1990). Temperature plays a key role in structuring invertebrate communities, especially areas with strong seasonal weather dynamics. Higher temperatures in early spring can lead to faster community development, faster growth rates and / or higher activity levels. Thus, global warming can change the dynamics of soil invertebrate communities, with potential cascading effects at all levels of biological organization (ROBINSON et al., 2021).

Air humidity is inversely proportional to the increase in air temperature (Fig. 4). Soil moisture (Fig. 5) follows the same trend of variation as air humidity but depends on the dominant plant species and the degree of vegetation cover of the investigated quadrats and the composition of invertebrate communities.

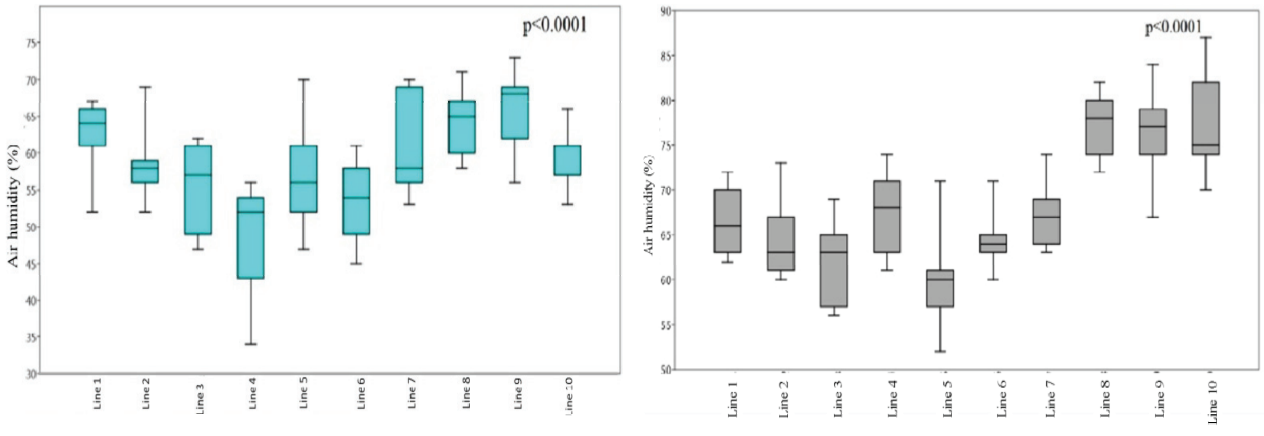

Figure 4. Air humidity in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

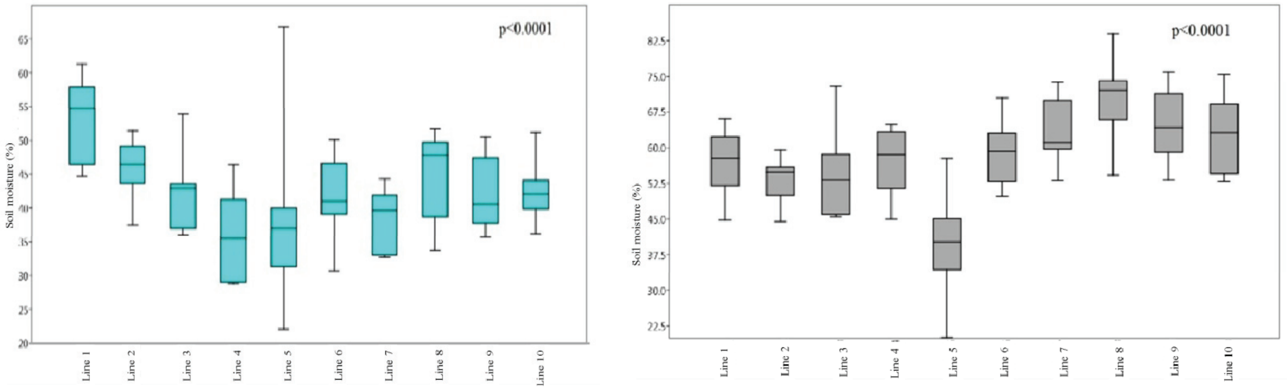

Figure 5. Soil moisture in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

Although the soil moisture at the surface (litter-fermentation layer - OLF) in a certain area can be relatively constant, it can have strong variations in the substrate (well-decomposed and well-fermented layer). This can influence the movement and retention of water (VILLANI & WRIGHT, 1990). When temperatures or precipitation oscillate close to long-term seasonal environments, organisms respond physiologically and/or behaviorally to ameliorate possible negative effects of change. When extreme seasonal fluctuations occur, the limits of the physiological or behavioral response are exceeded. At first, this can cause reductions in growth and ability to multiply, but if the stress becomes severe, the organisms will die (SOUSA, 1984).

Soil moisture conditions play an essential role in the distribution and abundance of soil organisms, which have sensitive reactions to this environmental parameter. For example, arthropods are sensitive to changes in humidity because they have a high surface-to-volume ratio. When rainfall is low, most arthropods migrate, sometimes building shelters. Structurally, soft-bodied arthropods (isopods and myriapods) do not have that wax cuticle found in arachnids and insects, which prevents or reduces evaporation. This, in combination with differences in excretion-related water losses, suggests that soft-bodied arthropods will be more vulnerable to reduced water availability. Thus, changes in precipitation can be expected to affect hard and soft invertebrates differently, leading to changes in community structure (BARNETT & FACEY, 2016).

Low soil moisture has a strong impact on feeding, survival and egg laying behavior. In response to drought conditions, several species of herbivorous larvae feed from the bottom in the soil profile. In addition, the laying behavior of invertebrate eggs in the soil is often altered by low soil moisture, which can lead to a delay in oviposition, as the eggs are laid at a greater depth of the soil or fewer eggs are produced.

The development of other stages may also be delayed in dry conditions. Thus, both the behavior and the size of the underground arthropod population are likely to be affected by changes in the amount of water in the soil (STALEY et al., 2007). Litter is that layer of leaves, needles or other various plant and animal debris that, through decomposition, contributes to the formation of soil features (CHIRIȚĂ, 1953).

The soil horizon with organic matter (O) is represented by the area where plant material (high C / N ratio) and animal residues accumulate. The variation of the thickness (Fig. 6) of the humus layer in the two grasslands highlights the increased intensity of grazing in Vemeșoaia compared to Galbena.

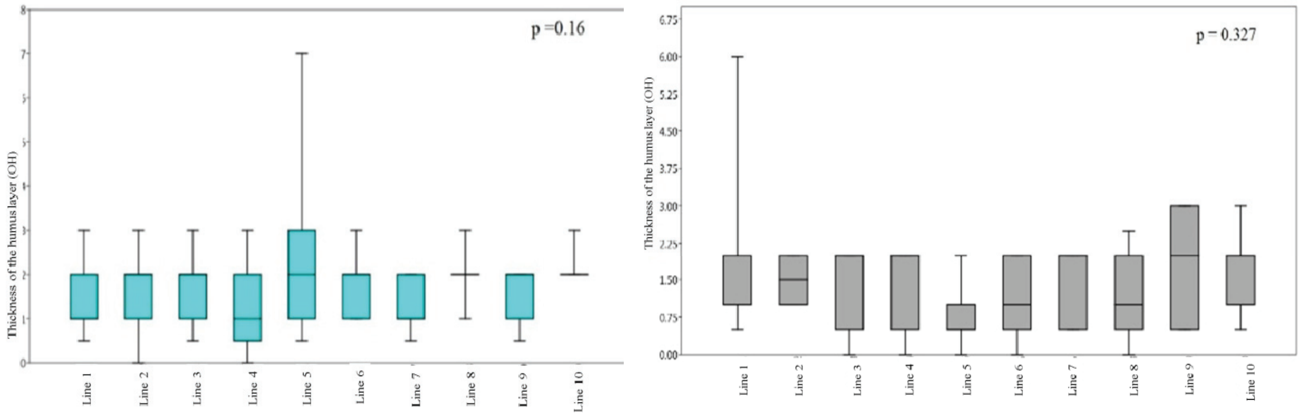

Figure 6. Thickness of the humus layer (OH) in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

This impact leads to the destruction of the vegetation mat by grazing and its trampling by sheep, so it degrades and dries excessively on large areas. Partially decomposed animal and plant residues are found in the litter-fermentation layer (OLF) (Fig. 7), and well-decomposed and well-fermented ones as well as a larger amount of organic material are found in the humus layer. The thickness of the soil layer (Fig. 8) depends on the geological structure of the studied grasslands and on the vegetation cover. The skeletal soils are small and distributed in fragments.

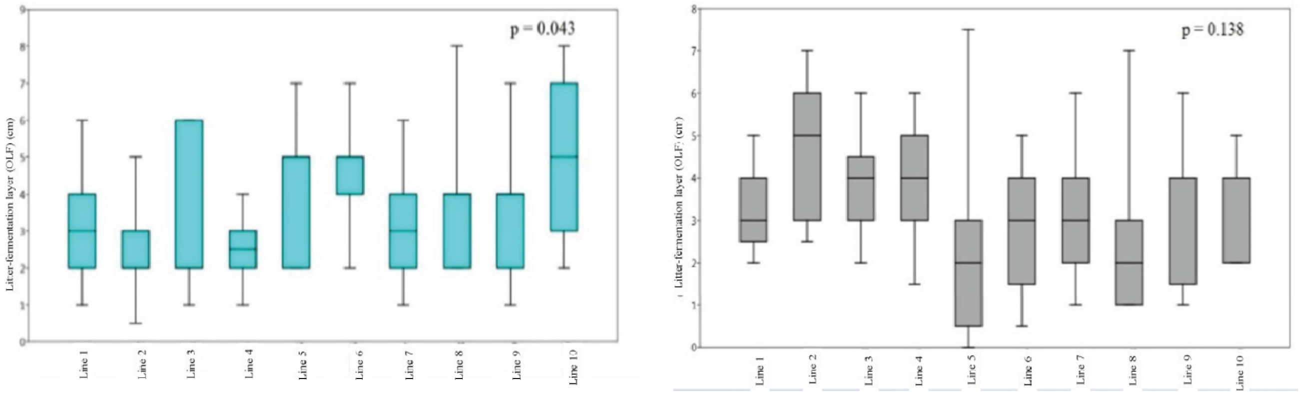

Figure 7. Litter-fermentation layer (OLF) thickness in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

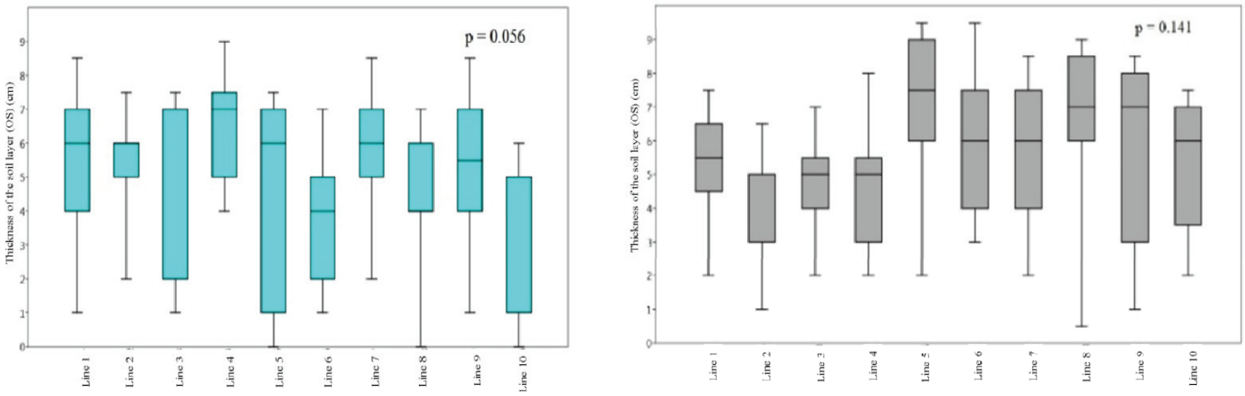

Figure 8. Thickness of the soil layer (OS) in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

The plant species present on the territory of the two studied grasslands, are adapted to the reaction of the soil solution (pH), from plants that grow on moderately acid soils to plants that grow on acid soils (Fig. 9).

Nutrient mineralization is the result of soil microflora activity, while soil fauna populations increase nutrient release by litter fragmentation thus improving soil structure (CHIRIAC et al., 2020). Humus is the main organic component of the soil; it is formed by humidifying litter, plant debris and dead animal organisms. This process takes place due to the action of fauna and microflora (fungi and bacteria) and as a result is the accumulation inside the soil of a mixture of organic substances that are in different stages of transformation.

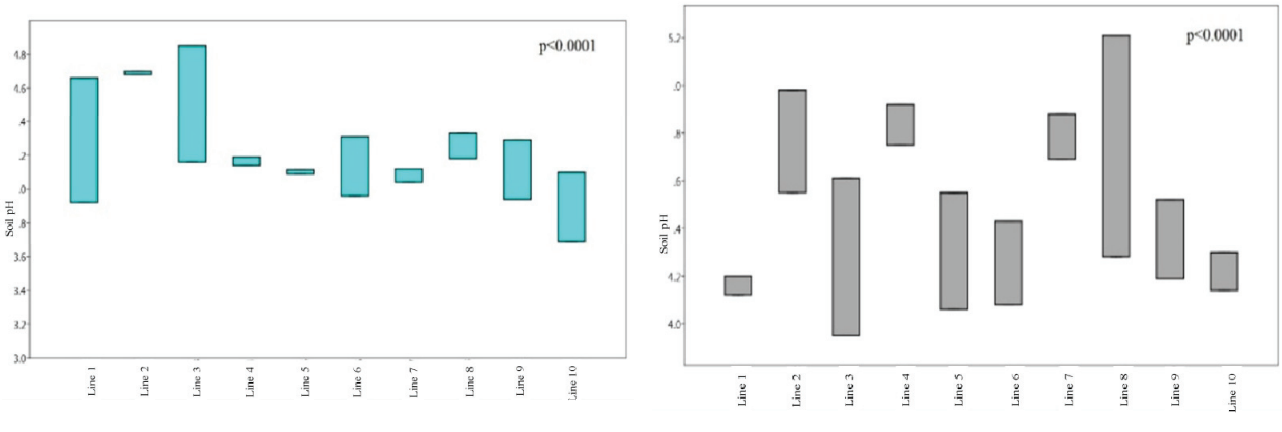

Figure 9. Soil pH in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

In summer, when the temperature is high, the activity of microorganisms is more intense, with the accumulation of more mineral and organic salts, the pH becoming lower. During the rainier and colder seasons, the pH is higher (NEAGOE & IORDACHE, 2021). The two grasslands display different expositions and slopes, compact vegetation (dense shoots and roots) and soils, being characteristic of high mountains (Fig. 10). Soil penetration resistance is variable in relation to micro-habitats and plant species diversity and density.

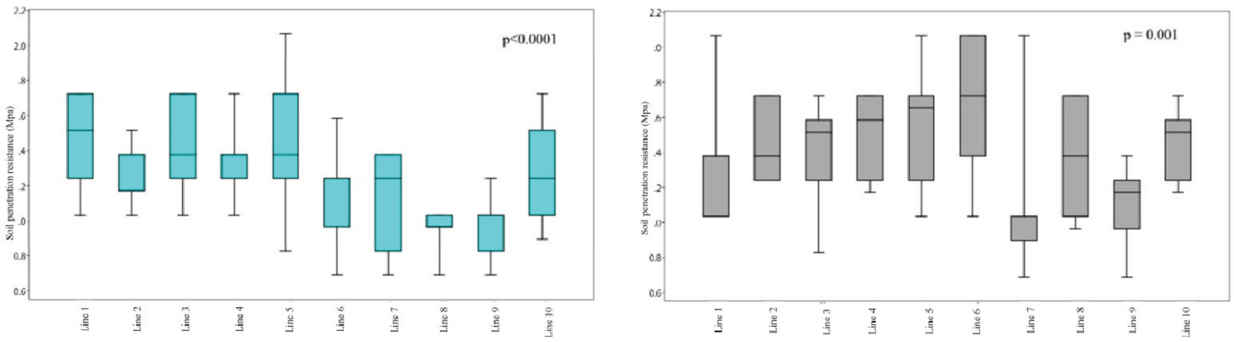

Figure 10. Soil penetration resistance in relation to the 10 lines in Galbena (left) and Vemeșoaia (right).

Soil penetration resistance (compaction) is a strong determinant of the abundance of earthworms (GILROY et al., 2008). Among the changes induced by human activities (especially using heavy machinery), soil compaction is a major problem due to its impact in terms of preventing the penetration of plant roots, shrinking spaces inside the soil filled with water and air and diminishing biological activity (BATEY, 2009; NAWAZ et al., 2013; DEVIGNE et al., 2016). The soil fauna has used the interstitial spaces as its habitat. By compaction, these spaces are reduced in size, thus preventing the access of invertebrates. Sometimes soil compaction does not lead to the disappearance of a community but most often influences its distribution (NAWAZ et al., 2013).

The microfauna tracks the temporal changes of bacterial and fungal populations in soil microhabitats, especially in the rhizosphere, which changes the dynamic balance between nitrogen mobilization and immobilization. Mesofauna feeding activities can determine the distribution, activities and composition of fungal communities. Macrofauna has major effects on fungal and bacterial activities, both directly, by feeding and intestinal passage, and indirectly, by affecting the microbial environment in the litter and soil.

CONCLUSIONS

The invertebrate communities in the Făgăraş Massif are characterized by high structural and functional variability and play an essential role in assessing the natural state of grasslands. Invertebrates in the soil are sensitive to changes in environmental variables but also to changes in the environment in which they are found and respond quickly to the natural or anthropogenic impact on the soil, so they can be considered valuable indicators of grassland status. Some invertebrate species are used as biomarkers of heavy metal pollution (i.e., nematodes) due to several factors: high density, short life cycle, low mobility and high capacity to accumulate toxic substances.

The physical and chemical factors of the soil determine the variability in time and space of environmental variables at the level of microhabitat and implicitly of invertebrate populations and therefore their ecological role in the functioning of ecosystems. It is known that temperature and humidity influence the dynamics of invertebrate communities. Both soil pH and organic matter influence the availability of essential nutrients for plants, soil functions and the diversity of functional groups of invertebrates in the soil. Environmental conditions and biotic communities shape complex ecological processes, such as decomposition. Soil invertebrates are key factors in decomposition rates in ecosystems. Also, the interactions between temperature, humidity and soil fauna are essential in the functioning of ecosystems.

The functional diversity of the invertebrate community plays an important role in the carbon and nitrogen cycle in ecosystems, with reduced soil biodiversity limiting nutrient decomposition and cycle. The study of soil invertebrates is particularly important, as they have several roles: they link primary production to secondary consumers, play a key role in the nutrient cycle by facilitating the decomposition of organic matter and decaying roots, improve soil fertility and structure, influence species composition, thus changes in vegetation and primary production. By understanding how abiotic factors interact with soil invertebrates we can anticipate the potential impacts of environmental changes on them. We can also evaluate the success of a restoration that modifies environmental variables at the microhabitat level on invertebrate species. The effects of the abiotic environment on the survival, growth and multiplication of invertebrate species, as well as the ways in which these relationships are altered in time and space, should receive considerable attention, not only from those working in these fields, but also from the part of a wider audience with interests about the ecological environment. More studies are needed on invertebrates that have a systemic and experimental approach and that correlate their behaviour with the environmental variables in their characteristic microhabitat.

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