

## ARE HABITATS WITH *Campanula romanica* Săvul. PREFERRED BY THE SOIL FAUNA?

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### Abstract

In 2021, from the first time in Romania, soil fauna taxa were investigated from two types of habitats: with and without *Campanula romanica*, from Măcin Mountains National Park. Some population parameters were analysed as: taxa diversity, numerical abundance, dominance, evenness, equitability, Shannon-Wiener index of diversity. In total, 24 soil fauna groups were identified, with 399 individuals, recording characteristic structure and taxa for each type of plots. Even if the number of identified taxa is almost similar between plots, the numerical abundance was higher in areas without *Campanula romanica*. In the same time sixteen environmental variables were quantified, as: the thickness of three soil layers, air temperature and humidity, soil temperature and moisture, soil pH, soil penetration resistance, amount of organic carbon, total nitrogen, C/Nt ratio, humus content, potassium content, phosphorous content and the vegetation cover. They had a significant influence on structure composition of the edaphic taxonomic groups from the two types of habitats.

**Key words:** *Campanula romanica*, environmental parameters, soil taxa.

### INTRODUCTION

Due to the ecological requirements, soil fauna could constitute a valuable bioindicators of the ecosystems, many times being possible to evaluate their conservation status through these invertebrates. Some methods for this evaluation are: to identify the taxonomical spectrum of soil fauna, to characterize the environment biotic and abiotic parameters of ecosystems where these edaphic invertebrates live and to demonstrate how these factors influence the structure and dynamics of soil fauna (Koehler & Melecis, 2010; Coleman & Wall, 2015). Many studies in all over the world demonstrated that the vegetation type influence the structure of soil fauna or vice-versa (Zhao et al., 2011; Ulrich et al., 2020; Chiriac & Murariu, 2021). The loss of biodiversity and indirectly of soil fauna would deteriorate the ecosystems functions (Iordache

& Neagoe, 2023). The invertebrate decline may contribute to relations between plants and animals, with potential negative consequences for ecosystem services like food provision and soil production (Koehler & Melecis, 2010; Zhao et al., 2011; Ulrich, 2020).

*Campanula romanica* Săvul. (*C. rotundifolia* L. subsp. *romanica* (Săvul.) Hayek) is a perennial species, hemicyptophyte, with heights between 18 and 30 cm and blooms from June to August. It is a symbol species for Măcin Mountains National Park, endemics for Dobrogea, being identified in Ponto-sarmatic steppes (62C0\*) Natura 2000 habitat, having conservative value, according to European Directive Habitats 92/43/EEC (Mihăilescu et al., 2015). It is xerophilous, saxicolous species, which grows in the cracks of the calcareous or granitic rocks, in dry and sunny habitats (Ciocârlan, 2009; Dihoru & Negrean, 2009). In order to establish its actual

conservation status, in 2021, a monitoring programme was developed in its habitats from Măcin Mountains National Park. Taking this aspect into account, we consider that the study of soil fauna taxa in monitoring plots with or without *Campanula romanica* will constitute a new and innovative approach of soil invertebrates' ecology. It is for the first time in Romania when such study was accomplished. The main objectives of the present study were: I) to highlight the structural differences of the soil fauna taxa from plots with and without *Campanula romanica*, II) to characterize the environmental parameters from investigated plots, and III) the show the different influence of the abiotic parameters on soil fauna taxa.

## MATERIALS AND METHODS

### Study area

The research was conducted in July 2021, in Măcin Mountains National Park. It is located in the South-East of Romania, in Dobrogea region, Tulcea county (45°8'49" N and 28°19'51"E). It has an area of 11151.82 hectares, being the most arid mountains from Romania. The climate is continental, with sub-Mediterranean influences in higher areas and with steppic characteristics in the south. Average annual temperatures are 10-11°C and average precipitation is 500 mm, which are extreme values within Romania (Manu et al., 2016). The study was developed in Ponto-sarmatic steppes (62C0\*), a favourable Natura 2000 habitat for *Campanula romanica* (CR) (Figure 1). A detailed description of investigated plots is presented in Table 1.

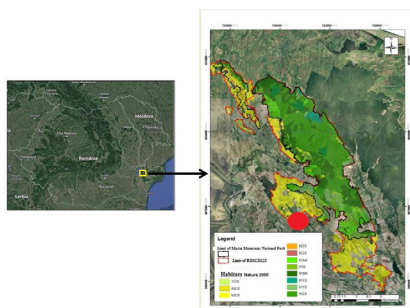


Figure 1. Geographical characterisation of the investigated areas in Măcin Mountains National Park-Romania, in 2021 (red bullet = geographical position of investigated plots with and without *Campanula romanica*)

### Soil fauna

In July 2021, twenty two soil samples were collected, using a MacFadyen soil core (5 cm diameter) to 10 cm depth. Due to the fact that the study was made in a Natura 2000 protected area, the number of soil samples was limited. The samples were collected taking into account the presence or absence of *Campanula romanica* (CR) species (eleven samples in plots with *Campanula romanica* and eleven samples in plots without this plant species). The soil fauna groups were extracted using the Tullgren-Berlese method (by natural drying for 20 days) (Macfadyen 1953, 1961; Koehler & Melecis, 2010). Identification of soil fauna groups was performed on the Carl Zeiss stereomicroscope, and their preservation was made in ethylic alcohol of 90<sup>0</sup>. The identification of taxa was made using the published identification keys (Brussaard et al., 1997; Ceuca, 2010; Coleman & Wall, 2015; Dindal, 1990; Gindei & Popescu, 2009; Krantz & Walter, 2009; Orgiazzi et al., 2016; Platnick, 2018).

### Environmental variables

In total, sixteen environmental variables were quantified. Within the soil the following parameters were quantified: the thickness of the litter fermentation layer- TOLF (cm); the thickness of humus layer- TOH (cm); the thickness of soil layer- TOS (cm); the temperature -T<sub>soil</sub> (°C); acidity-pH; moisture content - H<sub>soil</sub>(%); penetration resistance- RP (PSI), humus (%), organic carbon - C<sub>org</sub> (%), total nitrogen - Nt (%), carbon: nitrogen ratio - C/N; the content of phosphorus - P<sub>AL</sub>(mg/kg); content of potassium - K<sub>AL</sub>(mg/kg). On 5 cm above the soil level the temperature - T<sub>air</sub> and air moisture content - H<sub>air</sub> were measured. In total, 20 soil samples were analysed in order to measure these abiotic factors. The thickness of the litter-fermentation layer (OLF), of the humus layer (OH) and of the soil layer (OS) was measured with a graduated ruler (in centimetres), taking into account the morphological properties of the soil sample (colour, texture, consistency) (Chiriță, 1974). A digital thermo-hygrometer PCE-310 was used to measure air and soil moisture and temperature. Penetration resistance was determined with a soil penetrometer, Step System GmbH, 41010. The pH was measured with a C532 Jasco Consort pH-meter. Quantified chemical

analyses were: the amount of organic carbon (humus: wet oxidation; STAS 7184/21-82; PTL 12); total N (Kjeldahl method; STAS 7184/2-85; PTL 09); P<sub>AL</sub> (extractable phosphorus) was also analysed in ammonium acetate-lactate; STAS 7184/19-82; PTL 19P); K<sub>AL</sub> (potassium extractable in ammonium acetate-lactate; STAS 7184/18-80; PTL 22). The vegetation cover was determined using pratological method, which

take into consideration the percentage participation in biomass of botanical components by economic groups (as grasses, legumes, mosses and lichens, wood species). It is one of the most recommended fast method for determining grassland vegetation coverage (Ivan & Donița, 1975; Onete et al., 2021). The average values of environmental variables are presented in Table 2.

Table 1. A detailed description of investigated plots, from Măcin Mountains National Park, 2021

| Average values of parameters  | With CR                                                                                                                                                                                                                                                                 | Without CR                                                                                                                                                                                                                             |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GIS coordinates               | From N 45° 03'28.9";<br>E 28°10'46.7"<br>To: N 45° 15'19.8";<br>E 28°21'21.1"                                                                                                                                                                                           | From N 45° 02'48.7";<br>E 28°10'43.4"<br>To: N 45° 15'28.1";<br>E 28°21'17.9"                                                                                                                                                          |
| Altitude                      | 155 m                                                                                                                                                                                                                                                                   | 176 m                                                                                                                                                                                                                                  |
| Exposure                      | 50% West; 20% South, 20% North, 10% East                                                                                                                                                                                                                                | 20% West; 40% South, 40% East                                                                                                                                                                                                          |
| Slope                         | 21.10                                                                                                                                                                                                                                                                   | 13.50                                                                                                                                                                                                                                  |
| Habitat                       | 80% rocky habitat; 20% pastures                                                                                                                                                                                                                                         | 20% rocky habitat; 80% pastures                                                                                                                                                                                                        |
| Type of soil                  | Sandy                                                                                                                                                                                                                                                                   | Sandy                                                                                                                                                                                                                                  |
| Total vegetation cover        | 52.3%                                                                                                                                                                                                                                                                   | 59.55%                                                                                                                                                                                                                                 |
| Herbaceous layer cover        | 47.8%                                                                                                                                                                                                                                                                   | 65.91%                                                                                                                                                                                                                                 |
| Shrub layer cover             | 2.64%                                                                                                                                                                                                                                                                   | 0.36%                                                                                                                                                                                                                                  |
| Tree layer cover              | 0.91%                                                                                                                                                                                                                                                                   | 0                                                                                                                                                                                                                                      |
| Plant species dominant        | <i>Trifolium pratense</i> L., <i>Campanula romanica</i> Săvul., <i>Festuca</i> sp., <i>Rumex</i> sp., <i>Achillea quartata</i> L., moss, <i>Ailanthus altissima</i> (Mill.) Swingle, <i>Moeringia</i> sp., <i>Dianthus</i> sp., <i>Thymus</i> sp., <i>Centaurea</i> sp. | <i>Thymus zigioides</i> Griseb., <i>Teucrium polium</i> L., <i>Bromus</i> sp., <i>Trifolium pratense</i> L., <i>Achillea quartata</i> L., <i>Scleranthus</i> sp., moss, <i>Trifolium fragiverum</i> L., <i>Achillea millefolium</i> L. |
| Type of anthropic impact      | Grazing, invasive species, tourism                                                                                                                                                                                                                                      | Grazing                                                                                                                                                                                                                                |
| Intensity of anthropic impact | Medium                                                                                                                                                                                                                                                                  | Medium                                                                                                                                                                                                                                 |

Table 2. The mean values of investigated environmental variables in plots with and without *Campanula romanica* (CR), from Măcin Mountains National Park, 2021 ( $\pm$  standard error)

| Environmental parameters | TOLF              | TOH               | TOS               | Tair                |
|--------------------------|-------------------|-------------------|-------------------|---------------------|
| With CR                  | 0.89 $\pm$ 0.192  | 0.32 $\pm$ 0.139  | 3.55 $\pm$ 0.207  | 35.77 $\pm$ 0.706   |
| Without CR               | 0.62 $\pm$ 0.202  | 0.2 $\pm$ 0.2     | 4.4 $\pm$ 0.266   | 36.22 $\pm$ 0.921   |
| p                        | 0.387             | 0.873             | 0.046             | 0.948               |
| Environmental parameters | H air             | T soil            | H soil            | RP                  |
| With CR                  | 56.97 $\pm$ 2.856 | 26.32 $\pm$ 0.699 | 48.55 $\pm$ 3.490 | 126.37 $\pm$ 10.640 |
| Without CR               | 59.72 $\pm$ 2.539 | 27.25 $\pm$ 0.846 | 58.6 $\pm$ 4.023  | 164 $\pm$ 13.182    |
| p                        | 0.325             | 0.601             | 0.041             | 0.022               |
| Environmental parameters | VegCov            | pH                | Humus             | Corg                |
| With CR                  | 65.45 $\pm$ 8.981 | 4.81 $\pm$ 0.157  | 12.74 $\pm$ 1.423 | 7.39 $\pm$ 0.826    |
| Without CR               | 58 $\pm$ 11.333   | 5.54 $\pm$ 0.152  | 8.88 $\pm$ 1.145  | 5.15 $\pm$ 0.664    |
| p                        | 0.696             | 0.004             | 0.044             | 0.044               |
| Environmental parameters | Nt                | C/N               | PAL               | KAL                 |
| With CR                  | 0.67 $\pm$ 0.077  | 12.91 $\pm$ 0.414 | 26.11 $\pm$ 5.439 | 156.91 $\pm$ 26.348 |
| Without CR               | 0.46 $\pm$ 0.053  | 12.8 $\pm$ 0.359  | 7.24 $\pm$ 1.303  | 187.2 $\pm$ 14.369  |
| p                        | 0.043             | 0.735             | 0.002             | 0.384               |

## Data analysis

The population parameters used in the statistical analysis were: the number of taxa, the numerical abundance (number of individuals), dominance (D%), species diversity (Shannon-Wiener index), evenness ( $e^H/S$ ) and equitability (J index). For the environmental parameters, the mean values were evaluated, including the standard error ( $\pm$  SE).

The correspondence analysis (CA) was used as an ordination method between biological components. The relationship between the environmental parameters and the number of species was established using canonical correspondence analysis (CCA). The used software also includes standard statistical tests for univariate data, such as the ANOVA test. This analysis of variance is a statistical procedure for testing the null hypothesis, for several univariate samples that are taken from within mite communities that have the same average. The samples are assumed to have a normal distribution and a similar variance ( $p =$  is the probability of obtaining a result at least as extreme as the one actually observed, given that the null hypothesis is true). The statistical software package PAST was used (Hammer et al., 2001).

## RESULTS AND DISCUSSIONS

Considering the environmental parameters, the plots with *Campanula romanica* were characterized by the highest values of the thickness of litter-fermentation and humus layers, of air humidity, the highest percent of vegetation cover, the highest content of humus, organic carbon, total nitrogen and phosphorous in soil, as well as the C/N ratio (Table 2). Plots without *Campanula romanica*, were characterized by the highest values of the soil thickness, air and soil temperature, soil moisture content, soil resistance at penetration, pH and content of potassium from soil. Making a comparison between the two types of investigated plots, significant differences were obtained between thickness soil layer, soil

moisture content, soil resistance at penetration, pH, content of humus, organic carbon, total nitrogen and phosphorous from soil ( $p < 0.005$ ) (Table 2). A precious indicator of soil quality is the C/Nt ratio, which indicates of the mineralization capacity of nitrogen. According to soil specialists, a ratio lower than 15 (C/Nt < 15) indicates a high rate of decomposition of organic matter and organic nitrogen, (Chiriță, 1974; Klarner et al., 2013). In both type of plots, the C/Nt ratio is lower than 15, which indicate a proper rate of organic matter decomposition and a favorable habitat for soil taxa. The presence of organic matter (as higher content of  $C_{org}$  or humus) is a favorable factor for development of edaphic invertebrate taxa (as Nematoda, Collembola, Enchytraeidae, Oribatida, etc.), which in turn represent the food source for predator groups (such as Gamasina, Trombididae) (Krantz & Walter, 2009; Klarner et al., 2013).

Analyzing the taxonomical spectrum of identified faun groups, we obtained twenty four soil taxa, belonging to two phyla Annelida and Arthropoda (Table 3). Considering the number of soil taxa, the differences between two types of plots is insignificant, the same tendency being observed at characteristic taxa for each investigated areas, even if the Shannon diversity index is slightly increased in plots with *Campanula romanica* (Table 3). If we put into discussion the number of individuals, in plots without *Campanula romanica* (204 individuals), this parameter recorded higher value than that from plots with *Campanula romanica* (195 individuals). The total numerical abundance was by 399 individuals. The dominant soil taxa in both types of plots were Collembola (with a total numerical abundance by 172 individuals), Oribatida (63 individuals) and Chamobatidae (64 individuals). Higher dominance index was obtained in plots without *Campanula romanica*, which demonstrated the increased values of numerical abundance of few taxa, phenomenon highlighted by the decreased values of evenness and equitability indices (Table 3).

Table 3. The number of individuals of each identified taxa in studied plots, with and without *Campanula romanica* (CR), from Măcin Mountains National Park, 2021

| Taxonomical classification   | Investigated taxa | With CR    | Without CR   |
|------------------------------|-------------------|------------|--------------|
| <b>Phylum Annelida</b>       |                   |            |              |
| Class Clitellata             |                   |            |              |
| Subclass Oligochaeta         |                   |            |              |
| Order Haplotaxida            |                   |            |              |
| Family Lumbricidae           | Lumbricidae       | 1 ± 0.301  | 1 ± 0.301    |
| Family Enchytraeidae         | Enchytraeidae     | 6 ± 1.35   | 2 ± 0.603    |
| <b>Phylum Arthropoda</b>     |                   |            |              |
| <i>Subphylum Myriapoda</i>   |                   |            |              |
| Class Diplopoda              | Diplopoda         | 2 ± 0.603  |              |
| Class Chilopoda              | Chilopoda         | 4 ± 0.674  | 3 ± 0.646    |
| <i>Subphylum Hexapoda</i>    |                   |            |              |
| Class Entognatha             |                   |            |              |
| Order Collembola             | Collembola        | 56 ± 5.430 | 116 ± 13.094 |
| Order Diplura                | Diplura           | 1 ± 0.404  |              |
| Order Protura                | Protura           | 2 ± 0.301  |              |
| Class Insecta                |                   |            |              |
| Order Coleoptera             |                   |            |              |
| Family Curculionidae         | Curculionidae     | 1 ± 0.301  |              |
| Order Psocoptera             | Psocoptera        | 4 ± 0.674  | 3 ± 0.646    |
| Insect larva                 | Insect larva      | 7 ± 0.809  | 8 ± 0.786    |
| <i>Subphylum Chelicerata</i> |                   |            |              |
| Class Arachnida              |                   |            |              |
| Order Araneae                | Araneae           |            | 1 ± 0.301    |
| Supraorder Acariformes       |                   |            |              |
| Order Trombidiformes         |                   |            |              |
| Suborder Prostigmata         |                   |            |              |
| Family Trombidiidae          | Trombidiidae      | 1 ± 0.301  |              |
| Family Cunaxidae             | Cunaxidae         |            | 2 ± 0.603    |
| Order Ixodida                |                   |            |              |
| Superfamily Ixodoidea        |                   |            |              |
| Family Ixodidae              | Ixodidae          |            | 1 ± 0.301    |
| Order Sarcoptiformes         |                   |            |              |
| Suborder Oribatida           | Oribatida         | 41 ± 5.728 | 22 ± 2.756   |
| Family Galumnidae            | Galumnidae        |            | 1 ± 0.301    |
| Family Camissidae            |                   |            |              |
| Family Ceratozetiidae        | Ceratozetiidae    |            | 1 ± 0.301    |
| Family Chamobatidae          | Chamobatidae      | 37 ± 7.579 | 27 ± 6.846   |
| Family Oribatellidae         | Oribatellidae     | 7 ± 1.120  | 5 ± 0.687    |
| Suborder Astigmata           |                   |            |              |
| Family Acaridae              | Acaridae          | 1 ± 0.301  |              |
| Family Glycyphagidae         | Glycyphagidae     |            | 1 ± 0.301    |
| Order Mesostigmata           |                   |            |              |

|                               |                 |              |              |
|-------------------------------|-----------------|--------------|--------------|
| Suborder Gamasina             | Gamasina        | 6 ± 0.934    | 7 ± 0.924    |
| Suborder Uropodina            | Uropodina       | 1 ± 0.301    |              |
| Acari immatures               | Acari-immatures | 17 ± 4.803   | 3 ± 0.904    |
| Total number of taxa          |                 | 18           | 17           |
| Total number of individuals   |                 | 195 ± 14.900 | 204 ± 23.870 |
| Number of characteristic taxa |                 | 7            | 6            |
| Dominance_D                   |                 | 0.18         | 0.36         |
| Shannon_H                     |                 | 2.08         | 1.60         |
| Evenness_e^H/S                |                 | 0.45         | 0.29         |
| Equitability_J                |                 | 0.72         | 0.56         |

In plots with *Campanula romanica* some characteristic soil fauna taxa were identified, as: Diplura, Protura, Diplopoda, Trombidiidae, Acaridae, Curculionidae (Figure 2). Diplura is considered the sister group to insects and thus the closest group among the three basal hexapods that include Collembola and Protura. All inhabit soil and subsurface of terrestrial habitats. These invertebrates, due to their soft body and thin cuticle, depend on high humidity and moderate temperatures (Sendra et al., 2021). In soils, diplurans occupy all soil horizons. Larger species occupy the upper layers represented by O and A-horizons, smaller species live in the narrower pores of the B-horizon, where only diplurans with a tiny body (less than 2 mm body length and short appendages) can move (Sendra et al., 2021). Many Acaridae species are associated with bark beetles, even with Curculionidae, which was identified in plots with *Campanula romanica* (Klimov & Khaustov, 2018).

In plots without *Campanula romanica*, were identified another characteristic taxa: Cunaxidae, Ixodidae, Glycyphagidae and Araneae (Figure 2). Mites from Cunaxidae family are present in various types of ecosystems (forest, grasslands, agricultural fields). They are opportunistic predators, feeding with nematodes, collembolans, phytophagous mites or thrips (Skvarla et al., 2014). Glycyphagidae mites generally are associated with the nests of rodents and insectivores (Krantz & Walter, 2009). Araneae taxon was identified in plots where 80% from investigated habitats were grasslands, with higher soil and air temperature.

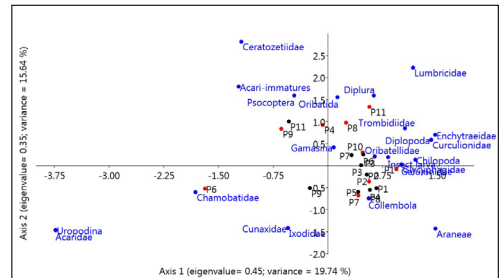


Figure 2. Correspondence analysis (CA) between identified soil fauna taxa and analysed plots (red dots = plots with *Campanula romanica*; black dots = plots without *Campanula romanica*) from Măcin Mountains National Park, 2021

Considering the influence of environmental parameters on the soil fauna taxa, we observed that in plots with *Campanula romanica*, that Gamasina, Chamobatidae were influenced by the thickness of humus layer (TOH), Diplura by the soil content of humus, Oribatida and Oribatellidae by the soil moisture content (Figure 3). In plots without the *Campanula romanica*, taxa as Chamobatidae, Uropodina and Acari-immatures were influence by the Nt and Corg, two abiotic factors which characterize the presence of organic matter in soil. Lumbricidae, Galumnidae and Gamasina were influenced by the soil temperature; Oribatellidae by the soil pH, Araneae by the thickness of soil layer, insect larva by the air temperature and Collembola by the content of potassium from soil (Figure 4).

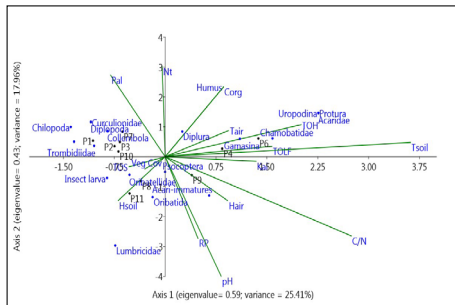


Figure 3. Canonical correspondence analysis (CCA) between identified soil fauna taxa and environmental parameters, in plots with *Campanula romanica*, from Măcin Mountains National Park, 2021

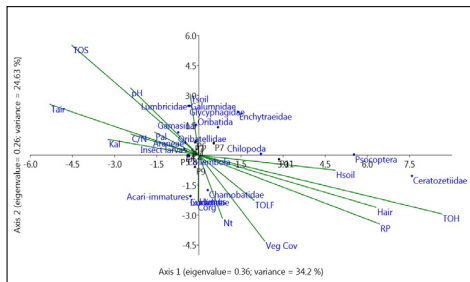


Figure 4. Canonical correspondence analysis (CCA) between identified soil fauna taxa and environmental parameters, in plots without *Campanula romanica*, from Măcin Mountains National Park, 2021

## CONCLUSIONS

In 2021, a study focused on soil fauna taxa was made, in plots with and without *Campanula romanica* from Măcin Mountains National Park. *Campanula romanica* is an endemic species for Dobrogea. It is xerophilous, saxicolous species, which grows in the cracks of the calcareous or granitic rocks, in dry and sunny habitats. These ecological requirements had influenced the composition of soil fauna taxa. We identified 24 soil fauna groups, with 399 individuals, recording characteristic structure and taxa for each type of plots. Even if the number of identified taxa is almost similar between plots, the numerical abundance was higher in areas without *Campanula romanica*. In the same time sixteen environmental parameters were analyzed. They recorded specifically values, characteristic for each type of plots, influencing in various ways the structure of soil fauna. There is a significant interdependence between these environmental variables, influencing the

abundance and distribution of edaphic taxonomic groups in studied plots.

As a general conclusion, the microhabitats with *Campanula romanica* are proper for certain soil fauna taxa (as Enchytraeidae, Diplura, Protura, Acari immatures), and for the most abundant groups as Collembola and Acari, but on the other hand the habitats without *Campanula romanica* are preferred by the same dominant soil invertebrates, and by the Cunaxidae and Insect larva, as well.

The present study demonstrated that soil invertebrate groups recorded different structural patterns, in correlation with the type of vegetation and environmental parameters.

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