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Conference paper

Comparison of earthworm fauna in oak and spruce forests on the Western slope of Kopaonik Mountain in Serbia

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Summary. The aim of the present study was to investigate the diversity and presence of ecological categories of earthworm fauna in oak and spruce forests. We sampled a total of 96 samples (48 in oak and 48 in spruce forests) during two years of fieldwork. In total, 13 earthworm species belonging to six genera were found in the studied forests. The earthworm abundance and species richness were higher in oak forests (113 individuals/9 species) than spruce forests (82 individuals/5 species). The differences in ecological categories between the studied forests were clearly evident and expected. Namely, in spruce forests our results indicate a complete absence of anecic and endogeic species, whereas epigeic species were the most dominant. However, it seems that these patterns depend primarily on the soil type. A combination of alpha diversity index (Shannon-Weaver, Evenness, and Berger-Parker) and beta diversity (Jaccard's coefficient of similarity) were used for determining the impact of the studied forests on the earthworm community structure. Shannon's diversity and Shannon's evenness indices were higher for oak forests, while the Berger-Parker index of dominance was lower in oak forests than spruce forests. As expected, Jaccard's index of similarity showed that the earthworm community structure was clearly separated between oak and spruce forests. Overall, our results based on these indices indicate that vegetation cover and altitude strongly influence the differences in earthworm community structure in the studied forests.

Keywords: earthworm, ecological categories, forests, Kopaonik Mountain.

INTRODUCTION

In temperate regions, earthworms are among the most widespread invertebrate animals and are found mainly in the soil of forests and meadows (Lee 1985). Many factors determine earthworm population distributions in any habitat, such as available food and soil conditions (i.e. moisture, pH concentration, temperature, organic matter). Actually, Lavelle and Spain (2001) established that earthworm species richness depends on the relative significance of different ecological categories of a region, climatic factors, phylogenetic and biogeographic history, and regional parameters such as vegetation type or soil characteristics. The activity of earthworms in forest habitats improves mineralization (Wardle and Lavelle 1997), bioturbation (Scheu 1995) and soil structure (Kretzschmar 1998), resulting in a deeper organo-mineral top soil with a crumbly structure (Bouché and Aladdan 1997). Accordingly, the relationships between earthworms and factors and components of their habitat have been the topic of many studies (Omodeo and Martinucci 1987; Pop 1997; Rożen et al. 2013; Schelfhout et al. 2017).

Mt. Kopaonik is the largest mountain in Serbia. This mountain is distinguished by complex paleo-geographical changes in the past, refugial character during the Ice Age,

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very complex floristic composition, and a combination of various environmental conditions changing over a small area (Krstić et al. 2014).

To date there have been 27 earthworm species recorded in Kopaonik Mountain (Popović et al. 2020), that represent 35% out of 77 earthworm species in 13 genera within one family Lumbricidae, which have been recorded in Serbia (Stojanović-Petrović et al. 2020).

In the present study, we compare the earthworm fauna of oak and spruce forests with different environments on the Western slopes of Kopaonik Mt. Our objectives were: a) to determine the species richness and abundance of earthworm fauna in oak and spruce forests b) calculate alpha and beta diversity indices, and to examine the impact of vegetation cover and altitude on the abundance, species richness and ecological categories of earthworm fauna in the studied forests.

MATERIALS AND METHODS

Study area

Earthworm communities were sampled along the Western slopes of Kopaonik Mt. (Fig. 1) in secondary oak and primary spruce forests, within a period of two months in both 2018 and 2020 (from May and June, coinciding with the rainy season).

Kopaonik Mt. (43°16'N, 20°49'E) is situated between the central and southern part of Serbia and belongs to the Dinaric Mountain range (Vardar zone). The Dinaric mountain range belongs to the Meso-Cenozoic Alpine-Himalaya collisional belt, formed during the late Mesozoic or earliest Cenozoic closure of the Neotethian Ocean and the subsequent continental collision of Europe and Adria (Schmid et al. 2008). The eastern slopes of the Kopaonik Mt. lie in the zone of Eastern Vardar, while its western boundary along the ophiolite belt of the Dinaric Mts. indicates a narrow belt of ophiolite mélange (Dimitrijević MN and Dimitrijević MD 1987). According to some authors, this structure, located between the eastern and western Vardar zones, was a microcontinent that divided the Neotethian Ocean into two oceans (Robertson et al. 2009). In contrast, others consider it to be only a composite nappe, consisting of continental units of the more distant Adriatic border and bearing the obducted Western Vardar ophiolites (Pamić et al. 2000). It is characterized by heterogeneous geologic material conditioned by intensive geological activity, especially during the Upper Cretaceous and Tertiary periods, which resulted in the formation of a powerful complex of magmatic-eruptive rocks, displaced or poured over the Paleozoic metamorphic series (Zelić et al. 2010).

In the hilly belt up to 1000 m a.s.l., three plots of the oak forest were sampled. The climatogenic community of this part of the oak belt is the community of *Carpino orientalis-Quercetum frainetto cerris* (Knapp 1944; Jovanović 1953). These forests are located mainly where sierozem on serpentines and humus silicate soil on serpentines occurs. Also, three plots of the spruce forest were sampled from the subalpine and alpine belt from above 1500 m a.s.l. Spruce, from 1550 m up to 1800 m above sea level, forms an altitude belt, in which deciduous tree species do not appear. Spruce forest (*Oxali acetosellae-Piceetum abietis* Mišić and Popović 1960 syn. *Piceetum mabietis serbicum* Mišić and Popović 1960) is

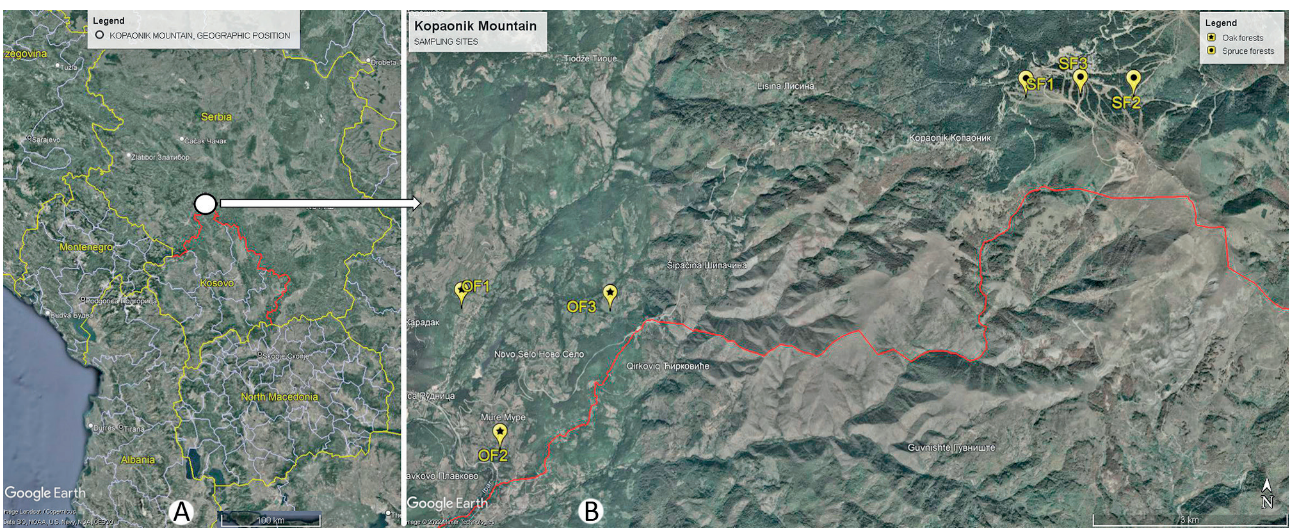

Fig. 1. A, Kopaonik Mountain, geographic position; **B**, Kopaonik Mountain, sampling sites (OF1-OF3 oak forests, SF1-SF3 spruce forests). Google Earth Image ©2022.

the highest quality and most productive on a granite base located at Ravni Kopaonik.

Spruce forest still thrives on Kopaonik Mt., and is found located at the very top of the mountain. It is represented by a special altitude breed (*Picea abies ecosubspec. subalpina*), which is characterized by a number of morphological, phenological, physio-ecological and other characteristics of typical spruce, as shown by detailed studies of group variability of spruce on "Kopaonik", especially the characteristics of this ecological form (Mišić et al. 1973). The community of subalpine spruce, juniper and blueberry (*Piceo subalpinae-Juniperetum sibiricae*, Mišić and Popović (1954; 1986) syn. *Piceo-subalpinae-Vacinio-Juniperetum* Mišić and Popović (1954; 1986)) is widespread in the belt from 1800 to 2000 m a.s.l. Soil types are brown podzolic and humus silicate soils (Ostojić et al. 2018).

Methods

We sampled eight samples, 50 × 50 cm, from each of the sampling units (plots) (Zicsi 1958). Sampling efforts were conducted over two consecutive years of sampling from the oak and spruce forests. In total, 96 samples (48 in oak and 48 in spruce forests) were collected over two years of fieldwork (Table 1). Earthworms were collected by the diluted formaldehyde method (Raw 1959), complemented with digging and hand-sorting. The specimens were killed and fixed in 75% ethanol or 4% formaldehyde. Some specimens of taxonomic importance were placed in 96% ethanol for further molecular studies. Specimen identification was carried out based on the works of Mršić (1991) and Csuzdi and Zicsi (2003) using a stereo microscope. Earthworms were identified to the species level and only mature individuals were counted.

Species richness was represented by the number of species on the forests, and total abundance by the total number of individuals of each species on the forests. The categorization of earthworm taxa was based on their zoogeographic distribution, as proposed by Csuzdi and Zicsi (2003), Pop et al. (2010) and Csuzdi et al. (2011). In addition, earthworm fauna was classified into five ecological categories (epigeic, anecic and endogeic, coprophagic and hydrophilic), based on their ecological behaviors (Bouché 1972; Lee 1985; Paoletti et al. 2013). Materials from the present study have been deposited in the Earthworm Collection of the University of Kragujevac, Serbia (CEKUS). All of the specimens included in this study are archived in the institutions stated above and are publicly accessible.

Table 1. List of studied sites and their plots in the western slopes of Kopaonik Mt.

Sites/altitude	Latitude	Longitude	Plots/ abbreviations
Rudnica 411 m	43.25°	20.69°	Oak forest (OF1)
Mure 670 m	43.23°	20.70°	Oak forest (OF2)
Novo Selo 800 m	43.25°	20.72°	Oak forest (OF3)
Malo jezero 1750 m	43.28°	20.80°	Spruce forest (SF1)
Jaram 1830 m	43.28°	20.82°	Spruce forest (SF2)
Marine vode 1950 m	43.28°	20.81°	Spruce forest (SF3)

Statistical analysis

For diversity of communities within habitats, we used alpha diversity, while for diversity between habitats, we used beta diversity. Alpha diversity was determined using the Shannon-Weaver (Shannon and Weaver 1963), Evenness (Pielou 1966), and Berger-Parker (Berger and Parker 1970) indices.

Shannon-Weaver (H') index:

H'= - Σ pi lnpi;

pi - the total number of individuals of one species

In ecological analyzes, Shannon-Weaver index values are usually between 1.5 and 3.5; and rarely 4.5. In essence, higher values of this index indicate greater diversity (Southwood 1978).

Evenness (Shannon-Weaver J') index:

J'= H' / lnS,

H'- Shannon-Weaver Index

S - wealth of species

The values of this index are between 0 and 1, and when the value of this index is close to zero, the uniformity decreases. This means that the relative number of certain species in a certain locality is smaller than in other localities, as a result, the fauna has less uniformity (Heip 1974).

Berger-Parker (D_{BP}) index:

D_{BP} = Nmax / N,

Nmax - total number of individuals of the most common species,

N - total number of individuals in the community.

The values of this index are between 0 and 1, and when the value of this index is close to 1, the dominance is high (Southwood 1978).

Beta diversity between the two communities can be described by applying one of the many coefficients of diversity. We used cluster analysis (UPGMA) based on Jaccard's coefficient of similarity (Krebs 1998).

Paleontological statistics software (PAST) was utilized for calculating the alpha diversity index (H', J' and D_{BP}) and beta diversity (cluster analysis UPGMA based on Jaccard's coefficient of similarity) in the studied forests (Hammer et al. 2001).

RESULTS

A total of 195 earthworm individuals were recorded in the studied forests (113 individuals from the oak forest/82 individuals from the spruce forest). Also, the present research resulted in reporting altogether 13 earthworm species belonging to six genera of family Lumbricidae, of which 9 species *Allolobophora chlorotica* (Savigny 1826), *A. leoni* Michaelsen 1891, *Apporectodea rosea* (Savigny 1826), *Cernosvitovia strumicae* (Šapkarev 1973), *Dendrobaena vejdovskyi* (Černosvitov 1935), *D. octaedra* (Savigny 1826), *Eisenia fetida* (Savigny 1826), *Lumbricus rubellus* Hoffmeister 1843 and *Proctodrilus antipai* (Michaelsen 1891) were recorded in the oak forest; while subspecies *Dendrobaena alpina alpina* (Rosa 1884), *Dendrobaena attemsi* (Michaelsen 1902), *D. byblica* (Rosa 1893), *D. illyrica* (Cognetti 1906) and *D. octaedra* were recorded in the spruce forest. The registered species belong to the following zoogeographical distribution and ecological categories, as shown in Table 2. The number of peregrine earthworms is high in secondary oak forests, which could be explained by the fact that peregrine is mainly distributed with tree seedlings during afforestation.

The most abundant taxon in the oak forests was *Apporectodea rosea* (N = 27; 23.89% of all observed individuals in oak forests), while in spruce forests (N = 36; 43.9% of all observed individuals in spruce forests), as well in the overall study *Dendrobaena octaedra* (N = 56; 28.71% of all observed individuals) was most abundant. Regarding ecological categories, epigeic earthworm species were dominant in spruce forests, while in oak forests endogeic earthworm species were most represented. Large-bodied, deep burrowing species are completely absent in the studied forests. However, *Cernosvitovia strumicae* belongs to a deep-burrowing-endogeic species (Fig. 2): this species displays remarkable adaptation to life in deep soil and strong development of soil displacement capacities. Also, the finding of Vardar endemic species *C. strumicae* in Kopaonik Mt., expands the geographical range of this endemic lumbricid to the Balkan Peninsula (Popović et al. 2022).

Moreover, oak forests were richer in earthworm species (average 6 species). Upper montane spruce forests contained fewer earthworm species (average 4 species). Shannon-Weaver and Evenness indices indicate that oak forests have greater species diversity than spruce forests. In contrast, the Berger-Parker index showed higher species dominance in spruce forests (Table 3).

Cluster analysis showed a clear separation of earthworm community structure in the studied forests (Fig. 3). Namely, only *Dendrobaena octaedra* was consistently present in all six of the studied forests.

Table 2. List of earthworm species with zoogeographic distribution and ecological categories as well as their number of individuals observed in studied oak and spruce forests on the Western slopes of Kopaonik Mt. Numbers refer to the total percentage contribution of each species to the total sample.

Species	Distribution	Ecological categories	Studied forests						
			OF1	OF2	OF3	SF1	SF2	SF3	Σ
All. chlorotica	Peregrine	Endogeic	3.58	–	–	–	–	–	3.58
All. leoni	Trans-Aegean	Endogeic	2	–	–	–	–	–	2
Ap. rosea	Peregrine	Endogeic	6.66	4.1	3	–	–	–	13.76
C. strumicae	Vardar Endemic	Endogeic	–	3	1	–	–	–	4
D. alpina alpina	Balkanic-Alpine	Epigeic	–	–	–	1	1.53	2	4.53
D. attemsi	Balkanic-Alpine	Epigeic	–	–	–	4.1	3	4.1	11.2
D. byblica	Circum-Mediterranean	Hydrophilic	–	–	–	–	2	–	2
D. illyrica	Illyric	Epigeic	–	–	–	–	3	3	6
D. octaedra	Peregrine	Epigeic	2.56	3.58	4.1	6.66	5.6	5.1	27.66
D. vejdovskyi	Balkanic-Alpine	Epigeic	–	–	2	–	–	–	2
E. fetida	Peregrine	Coprophagic	3.58	2	–	–	–	–	5.58
L. rubellus	Peregrine	Epigeic	3.58	4.1	2	–	–	–	9.68
P. antipai	Central European	Endogeic	–	3.58	3	–	–	–	6.58

All. = Allolobophora; C. = Cerrnosvitovia; Ap. = Apporectodea; D. = Dendrobaena; E. = Eisenia; L. = Lumbricus; P. = Proctodrilus.

Fig. 2. Living specimen of species *Cernosvitovia strumicae* in an oak forest on the Western slopes of Kopaonik Mt.

DISCUSSION

In the studied forests, the earthworm fauna was found to be different: oak forests are richer in earthworm taxa relative to spruce forests. Shannon's diversity and Shannon's evenness index values were lower values in spruce forests, while values for the Berger-Parker index of dominance were higher in spruce forests than in oak forests. This is because of the optimum environmental factors in oak forests, which can also provide suitable habitats for earthworms (i.e., more humidity and food). Not surprisingly, the species *Apporectodea rosea* is most represented in oak forests. According to Stojanović et al. (2018), the peregrine species *Ap. rosea* is one of the most represented earthworms in Serbia. Tóth et al. (2020) considered that peregrine species are restricted to

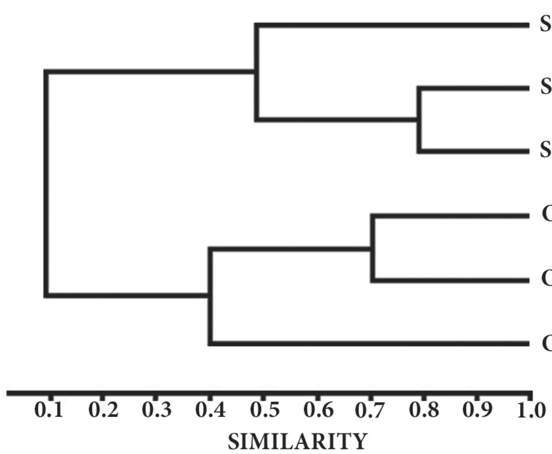

Fig. 3. Cluster analysis (UPGMA) using the Jaccard's index of similarity among the oak and spruce forests on the Western slopes of Kopaonik Mt.

Table 3. Alpha diversity index of earthworm fauna in the studied oak and spruce forests on the Western slopes of Kopaonik Mt.

Alpha diversity index	Studied forests					
	OF1	OF2	OF3	SF1	SF2	SF3
Taxa_S	6	6	6	3	5	4
Individuals	43	40	30	23	31	28
H'	1.69	1.71	1.66	0.82	0.91	0.66
J'	0.90	0.92	0.87	0.76	0.50	0.48
D_{BP}	0.30	0.28	0.28	0.70	0.74	0.82

human-modified habitats and that the natural vegetation of coniferous forests and low pH would impede the spread of these species. Anecic species (large-bodied and deep burrowing) were absent in oak forests, which was surprising given that are mainly closely associated with eutrophic deciduous forests (Zicsi et al. 2011; Jänsch et al. 2013). However, due to the xerothermic character of oak forests, which is caused by the small depth of useful soil, the skeleton presence on the surface and in profile, was well as water permeability, conditions are actually very unfavourable for anecic species.

We have shown that ecological earthworm categories are highly influenced by the forests via several leaf litter and/or soil characteristics, but not all categories and species are affected similarly. Essentially, spruce forests are represented by podzol-type soil, which is characterized by acidified topsoil and recalcitrant litter, as well as significantly lower soil moisture content, having a negative impact on the anecic earthworm category. Besides, endogeic earthworms feed on largely humified soil organic matter and dead roots: meaning that they literally eat their way through shallow soil and, thereby, ingest large quantities of soil, which is mixed with organic material, i.e., bioturbation (Curry and Schmidt 2006). Actually, most of these species are very sensitive to acidic soil (Muys and Granval 1997). Ecological studies of the earthworm fauna along altitudinal gradients indicate that epigeic species are dominant at high altitudes, respectively in spruce forests (Rożen et al. 2013; Zenkova and Rapoport 2013). Specifically, epigeic earthworms are known to be more tolerant to low pH values (Bouché 1977; Sims and Gerard 1999; Schelfhout et al. 2017). Actually, the main reasons for the presence of epigeic earthworm in spruce forests are freeze-hardiness and/or dry periods, a high degree of reproduction (parthenogenesis), and the ability to tolerate acid soils and exploit poor quality litter (Tiunov et al. 2006; Holmstrup 2007; Terhivuo and Saura 2008; Eggleton et al. 2009; Zenkova and Rapoport 2013; Meshcheryakova and Berman 2014). As a consequence of these facts, epigeic species of *Dendrobaena octaedra* were the most common in the studied forests.

In conclusion, rankings based on cluster analysis of the select forest ecosystems confirm the existence of characteristic earthworm communities and an obvious earthworm vegetation soil relationship at the ecosystem level (Pop 1997). Also, we must emphasize that climatic change is one of the major drivers of biodiversity loss in forests (Millenium Ecosystem Assessment 2005). Moreover, future climate change may come in the form of higher summer temperatures and/or increased droughts in temperate European regions (European Environment Agency 2017), which would jeopardize endogeic and epigeic earthworms that are sensitive to drought (Schelfhout et al. 2017). To prevent loss of earthworm biodiversity, foresters can mitigate these expected global changes by careful choice of tree species at lower altitudes.

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Google Earth Image. ©2022. A, Kopaonik Mountain, geographic position (Google Earth Image Landsat / Copernicus; Imagery Date: 14 December 2015); B, Kopaonik Mountain, sampling sites (Google Earth Image ©2022 Maxar Technologies; Imagery Date: 12 December 2018). Google Earth Pro, PC Version 7.3.4.8248.

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