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Original article

Earthworm community structure in grassland habitats differentiated by climate type during two consecutive seasons

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ABSTRACT

Earthworms are increasingly used as soil indicator species in soil quality assessment. Apart from the abundance and biomass, the important parameter that is accounted for is their community composition. The latter is regulated by various abiotic and biotic factors. The aim of this study was to qualitatively and quantitatively analyze the characteristics of earthworm communities in grassland habitat differentiated by climate type and elevation during two consecutive seasons. The results obtained indicated an influence of climate and biogeographic history on species composition, species richness and population dynamics at the highest observed scale. The locations situated on the maritime (Mediterranean) slope differed significantly from the locations on the continental slope, according to species composition, the ratio of ecological categories and juvenile to adult ratio. On the lower scale, interspecific interactions were observable. Namely, a pairwise comparison of species pairs showed a low co-occurrence of most species. An understanding of earthworm assemblages and population dynamics in the Mediterranean region is important for biological soil quality assessment, evaluation of climate change impact and similar studies, which present a current challenge in the applied soil ecology research, particularly as research on earthworms has been underrepresented in this region.

1. Introduction

The body of knowledge about earthworm biology and ecology has grown considerably during the past few decades. Comprehension of this topic is important since the species composition of earthworm communities has been proposed as a potential endpoint for an assessment of biological soil quality [1]. The number of species that constitute an earthworm community depends on spatio-temporal relationships, ecological strategies, species niches and resource partitioning within the community. The number of earthworm species within a given community may vary from one to fifteen species, with three to six species in most communities regardless of habitat type or geographical position [2]. The earthworm community diversity at a specific location depends on soil characteristics, climate and quality and quantity of organic matter, together with current and previous site management and the degree of site disturbance. Additionally, interspecific interactions are considered to be an important driving factor for earthworm communities [3,4]. The competition in earthworm communities influences the spatial structuring of species assemblages [5–7] and a non-linear relationship over a scale from regional to local species richness [3,4].

Apart from the difference in species number, earthworm communities differ in the ratio of ecological categories. This ratio changes successively with the change of vegetation cover [8,9] at a small and medium scale, but also on the large scale of latitudinal–vegetational sequence [10]. Studies conducted at a regional scale with the aim of describing how environmental factors and biotic interactions relate mutually on different spatio-temporal scales to drive earthworm community patterns [9,11–16] concluded that the spatial structure of the earthworm populations on a regional scale exists.

Biogeographic history or more specifically the border of ice sheet during the Quaternary glaciation had a great influence on the earthworm species composition in the territory of Europe [17,18,2]. The region of Southern Europe, which has avoided the detrimental effects of the Ice Age, consequently, has a much higher earthworm species richness with a high proportion of endemic species [19–22].

A mountainous region in Croatia encompasses two earthworm speciation centers; the Dinara Mt. on the East and the European Southern Alps on the West [23]. In this area we have chosen a transect across Velika Kapela Mt. that consists of two slopes, each belonging to a different climate (continental and maritime) and consequently to different vegetational zones. As the transect is

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vertically stratified, the locations were differentiated not only by climate but also by elevation. All sampling locations were grasslands with sub-locations arranged in a regular grid to investigate a co-occurrence of species on a small scale. To account for a temporal variation in earthworm community, assembly sampling was conducted in two consecutive seasons. In this research emphasis was given on differences in earthworm species composition and population dynamics between seasons and between continental and maritime, i.e. Mediterranean, an area which generally has a lack of such studies [24].

2. Materials and methods

2.1. Site description

Sampling was carried out along a vertically stratified transect across Velika Kapela Mt. in Croatia at seven locations. The mountain slopes belong to two different climate zones; the North East slope belongs to the continental climate zone and the South West slope belongs to the maritime climate zone, i.e. Cfb and Cfa climate according to Köppen climate classification, respectively. Five locations (L1–L5) were situated on the continental slope and two locations (L6–L7) on the maritime slope. All coordinates are given in Table 1. This imbalance is a consequence of both the geographic profile (Fig. 1) and the characteristics of slopes. Namely, the maritime slope is much steeper and with less grassland areas available, which is typical of Sub Mediterranean/Mediterranean habitats. The altitudes of sampling locations ranged from 327 m a.s.l. on the continental slope to 183 m a.s.l. on the maritime slope, with highest elevation that of 700 m a.s.l. (location L4). The average annual temperature along the transect ranges from 9 °C–10 °C at the continental slope foothill, to 5 °C–6 °C at the highest elevation and 12 °C–13 °C at the foothill of the maritime slope. The maximum soil temperatures (at 5 and 20 cm) occur in July and the minimum in January. The average annual precipitation ranges from 1500 mm to 1750 mm at the continental slope foothill, 2500 mm–3000 mm at the highest point to 1300 mm–1400 mm at the maritime slope foothill. The average duration of snow cover of at least 30 cm is 30 days on the continental slope. According to the Croatian National Habitat Classification, locations on the continental slope mostly belong to the mesophilous meadows of Middle Europe and locations on the maritime slope to the sub-Mediterranean and epi-Mediterranean dry grasslands [25]. The vegetation at all seven locations belongs to secondary grasslands under anthropogenic influence, with slight differences in land use.

2.2. Experimental design

At each location 49 samples were taken, arranged in a regular grid (7 × 7) with 5 m inter-sample distance. Sampling was performed twice; the first sampling was conducted in spring (mid April) and the second sampling in summer (early July) in 2008. To avoid sampling at the same points, for the second sampling the grid was shifted spirally, counterclockwise. The difference in location was considered negligible as compared to inter-sample distance and the sample position was therefore treated as identical for both samplings.

2.3. Earthworm sampling

The earthworms were sampled using the standard hand sorting-formalin expulsion method [26] with a 0.25 m^2 sampling area. According to this method, the upper 10 cm of soil with above vegetation and roots were removed and hand sorted, while on the denuded area 5 L of 0.2% formalin were poured twice with an interval of 15 min. All emerged earthworms were briefly rinsed with water to remove formalin and excessive soil, placed in 10% ethanol to prevent excessive curling and finally placed in 75% ethanol. Preserved earthworms were taken to the laboratory where the adult specimens were identified to the species level [20,17] and classified according to the three main ecological categories. As juveniles couldn't be determined to species level, they were allocated to five categories according to the body pigmentation, shape of the prostomium and setal ratio. All individuals were counted and weighted.

2.4. Soil sampling

At each location three soil samples were taken and pooled for a basic soil analysis. Soil analyses included soil texture determination, soil water holding capacity, pH (H_2O, $CaCl_2$), soil organic matter by loss on ignition and soil porosity [27]. Results are given in Table 1.

2.5. Data analysis

The diversity of earthworms at each location and sampling date was evaluated through the average number of species, the cumulative number of species, average biomass, and the Shannon diversity index (H'), as well as its evenness (J'). Density data did not conform to a normal distribution with the Wilk–Shapiro test ($P = 0.05$). Statistical significance was tested with the Kruskal–Wallis test and Gao's non-parametric multiple test procedure for all-pairs comparisons was performed as a post-hoc test with $P = 0.05$ [28]. Indicator species for both climate types and location land management were determined using the Indicator Value (IndVal) method [29]. The statistical significance of observed IndVal values was assessed by means of a permutation test (1000 randomization). The threshold level of 25% for the index was adopted as suggested by Dufrêne and Legendre [29]. Earthworm sampling completeness was tested by calculating non-parametric species richness estimators [30]. The estimators were calculated separately for each season and cumulatively for both seasons. Detailed descriptions of the estimators can be found in Colwell and Coddington [31] and Colwell [30]. Species pair-wise relationships

Table 1
Coordinates, elevation and soil characteristics of sampled locations.

	Coordinates		Elevation (m a.s.l.)	Water holding capacity (%)	Organic matter (%)	pH		Soil texture				Slope
						dH_2O	$CaCl_2$	Clay (%)	Silt (%)	Sand (%)		
L1	45.27778	15.17741	327	35.10	4.80	7.43	7.25	3.50	24.98	71.52	Sandy loam	Continental
L2	45.2492	15.17996	559	29.58	3.59	6.82	6.65	39.20	20.91	39.89	Clay loam	Continental
L3	45.24694	15.12626	629	32.28	4.12	6.44	6.10	8.25	20.95	70.80	Sandy loam	Continental
L4	45.23382	15.12519	700	35.67	4.72	6.95	6.70	18.75	13.23	68.02	Sandy loam	Continental
L5	45.23789	15.05434	630	34.16	7.44	6.43	6.21	9.48	5.32	85.20	Loamy sand	Continental
L6	45.16747	14.8571	652	34.23	10.76	5.71	5.31	9.83	5.15	85.02	Loamy sand	Maritime
L7	45.13217	14.84246	183	26.58	7.33	6.17	5.84	26.42	15.85	57.73	Sandy clay loam	Maritime