Temporal and spatial dynamics of arthropod groups in terrestrial subsurface habitats in central Portugal

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Abstract

The mesovoid shallow substratum (MSS) can act as a climatic refuge for invertebrates, as a biogeographic corridor to deeper substrates or as a permanent habitat for some species. This study characterizes the seasonal invertebrate diversity and abundance of MSS ecosystems in central Portugal focusing on Diplopoda, Diplura, Orthoptera and Coleoptera during one year.

Sampling was performed with standard MSS pitfalls in scree slopes (colluvial MSS) of karst areas and environmental parameters (temperature, pH, conductivity, water content, organic carbon, nitrate, phosphate and ammonium) were quantified. Our results show that winter was the season with the highest arthropod abundance and that the MSS acts as a permanent habitat for chordeumatidan millipedes and as a climatic refuge for orthopterans and most beetles. All Diplura collected belong to a single species known previously from surface habitats in the Iberian Peninsula, which does not seem to use the Portuguese MSS as a refuge. MSS habitats in central Portugal, classified as western Mediterranean and thermophile deposits protected by the Natura 2000 network based on plant communities and geology, revealed an abundant and diverse invertebrate community that urges characterization and protection.

Key-words: mesovoid shallow substratum; Diplopoda; Diplura; Orthoptera; Coleoptera; Portugal.

1. Introduction

The world´s terrestrial biodiversity is dominated by insects and other arthropods (Grimaldi and Engel, 2005; Stork, 2018), and these are distributed between surface and subterranean ecosystems. Among subterranean ecosystems, two main types can be classified in terms of depth: the deep subterranean habitat, traditionally represented by caves, and the shallow subterranean habitats, including the mesovoid shallow substratum and the epikarst, which are typically more affected by fluctuations of temperature, humidity and organic matter from the surface (Culver and Pipan, 2009b, 2014; Ortuño et al., 2014). Out of the two, the most common are the shallow subterranean habitats, which can be found all over the globe and have been very poorly studied when compared with caves (Mammola et al., 2016).

The Mesovoid Shallow Substratum, originally discovered and described as “Milieu Souterrain Superficiel” (MSS) by French biologists (Juberthie et al., 1980), is a common type of shallow subterranean ecosystem that can be found worldwide (Mammola et al., 2016). This habitat consists of a network of cracks and small air-filled voids found in the bedrock horizon (Gers, 1992; Mammola et al., 2016) and can be categorized into four different types, based on its genesis and type of rock: i) colluvial (formed by the erosion of exposed bedrock and accumulation of the fragments on sloping grounds. When exposed is called a scree slope) (Juberthie et al., 1980, 1981; Jiménez-Valverde et al., 2015); ii) bedrock (formed by weathering of the bedrock at the interface between soil and parent rock) (Mammola et al., 2016); iii) volcanic (accumulation of volcanic materials on the substrate) (Oromí et al., 1986; Mammola et al., 2016) or iv) alluvial (accumulation of rocky fragments, pebbles and gravel in streambeds of temporary watercourses) (Ortuño et al., 2013).

The MSS constitutes a unique and very important ecotone (Prous et al., 2004). Due to its contact with both the surface and the deep subterranean habitats (Gers, 1998) it can act as a climatic refuge for invertebrates (Nitzu et al., 2010, 2014) or as a biogeographic corridor for animals inhabiting deeper substrates (Culver, 1982; Ortuño et al., 2013; Jiménez-Valverde et al., 2015; Mammola et al., 2016). However, the MSS is more than just a transitional habitat as several species live there permanently and some display troglomorphisms, i.e. traits similar to those found in subterranean animals that evolved due to the convergent selective pressures in the deep subterranean environment (Culver and Pipan, 2009a, 2015; Pipan et al., 2011; Ortuño et al., 2013). Troglomorphisms include depigmentation, loss of eyes and wings, elongation of appendages, and cuticle thinning, among others (Christiansen, 2012).

Most of the subterranean arthropods are rare and endemic species, endangered by global changes, simply by being unknown (Reboleira et al., 2011; Mammola et al., 2019; Castaño-Sánchez et al., 2020a, b). This lack of knowledge is most likely due to the technical difficulties involved in sampling subterranean environments, recently described as the “Racovitzan Impediment” (Ficetola et al., 2019; Mammola et al., 2019).

The majority of studies regarding MSS invertebrate fauna have been conducted in Europe, more specifically in France, Spain, Romania and Slovakia, and deal mainly with arthropods and gastropods (Mammola et al., 2016). Phenology and biodiversity in the MSS has been studied for groups like Araneae, Pseudoscorpiones, Opiliones, Acari, Chilopoda, Diplopoda, Isopoda, Collembola, Hymenoptera and Coleoptera in the Czech Republic, Slovakia and Spain (Růžička et al., 2010; Rendoš et al., 2012, 2016; Rudy et al., 2018).

In Portugal the MSS habitat was first studied in the islands of Madeira (Oromí and Borges, 1991) and Azores (Borges, 1993), while in continental Portugal only a millipede and an isopod species have been reported from the MSS (Reboleira and Enghoff, 2014; Reboleira et al., 2015). Portugal is one of the Mediterranean biodiversity hotspot regions for subterranean organisms (Reboleira et al., 2011, 2013b) and the MSS habitats found in Portuguese karst areas are classified as western Mediterranean and thermophile deposits protected by the Natura 2000 network, under the 92/43/CEE directive (EU, 1992; ICNB, 2000). This classification is based on the peculiar geology and plant communities, while invertebrate communities remain unstudied.

The aim of this work is to study the annual seasonal invertebrate diversity and richness in colluvial MSS of central Portugal, and to evaluate if environmental variables such as temperature, vegetation coverage and chemical properties of the sediment have any effect on the biodiversity. In order to accomplish these goals we selected three scree slope habitats in two karst areas of central Portugal and the invertebrate groups Diplopoda, Diplura, Orthoptera and Coleoptera (selecting the most abundant families Carabidae, Leiodidae and Staphylinidae).

2. Material and methods

2.1 Sampling

The sampling localities are situated in colluvial MSS areas (scree slopes) in central Portugal: Serro Ventoso and Fórnea in the Estremenho karst massif, and Columbeira valley in the Cesaredas karst plateau (Figure 1, Table 1). The sampling localities were chosen according to the following criteria: 1) located in Portuguese karst scree slopes; 2) located in a natural protected area.

In Serro Ventoso there was a high moss coverage in the majority of the sites selected, and all were located circa 2 meters from the forest edge (composed mainly of *Quercus coccifera* shrubs). The sediment collected was dark and not aggregated, and it was present in low quantities.

In Fórnea there was no moss coverage but some of the sites had *Quercus coccifera* leaf litter. All the sites were located circa 1 meter from the forest edge (composed mainly of *Quercus coccifera* and *Rubus ulmifolius* shrubs, and *Olea europea var. sylvestris* trees). The sediment collected was dark and not aggregated, and it was present in low quantities.

In Cesaredas only one of the sites was fully covered by mosses, while the other four were exposed. Three of the sites were located circa 1 meter from the forest edge (composed mainly of ferns, *Quercus coccifera* and *Rubus ulmifolius* shrubs. The other two sites were located under treetops and circa 6 meters above a stream (Ribeira da Zambujeira). The sediment collected was mildly aggregated with clay properties, and it was present in high quantities. The specimens were collected using pitfall traps modified from the model described by López and Oromí (2010): 50 cm long PVC tube with 8 mm perforations and three levels of baited traps. Propylene glycol was used as preserving liquid and pork liver as bait. The traps were installed in the scree slopes by digging a narrow vertical hole, 60 cm deep, and placing the PVC tube 10 cm below the surface. Five traps were installed per site at 10 m distance from each other and 0.5–1 m away from the surrounding trees/shrubs. Specimens were collected every season for a year (January – December 2019) only at the deepest level of each trap. The seasons were defined based on sampling dates as follows: winter from January to beginning of April, spring from April to beginning of July, summer from July to beginning of October and fall from October until the end of December.

Sediment samples (250 g) were collected during each pitfall trap installation in the hole dug to place the trap (along the 50 cm depth gradient), corresponding to five replicates per locality, and kept refrigerated until laboratory processing.

2.2 Environmental variables

All localities have a temperate warm climate and are classified as Csb ("cool dry-summer") by the Köppen and Geiger scale (Andrade and Contente, 2020).

The following environmental variables were used to characterize each sampling locality during the trap installation: 1) elevation; 2) average temperature; 3) vegetation coverage; 4) pH; 5) conductivity; 6) water content; 7) organic carbon (%); 8) nitrate (NO_3) (µg/g sediment); 9) phosphate (PO_4) and 10) ammonium (NH_4).

Average temperature was recorded every 2 hours with data loggers installed at the bottom of each trap (TidbiT v2 Temp UTBI-001, Onset, MA, US).

The vegetation coverage parameter was quantified according to the type of vegetation found in a two-meter area from the trap: 1) high, tall shrubs and trees; 2) medium, tall shrubs; and 3) low, small shrubs and creeping herbaceous plants (Table 1).

Conductivity and pH were measured following the standard procedures (Patriquin et al., 1993). Water content was measured by weight loss in percentage. Soil organic matter (SOM) was measured through loss on ignition (2 g of dried sediment placed in a crucible and ignited at 550°C for 6 hours) and organic carbon as half of SOM value for each sampling site (Dean, 1974). Available nitrate, phosphate and ammonium content was measured using a FIAstar 5000 analyser unit (FOSS, Hillerød, Denmark) with sediment samples being suspended in purified water.

2.3 Specimen sorting and identification

Specimens were sorted and identified to species level where possible using a Leica Wild M10 stereomicroscope and are deposited in the Natural History Museum of Denmark, University of Copenhagen. The selected four groups (Diplopoda, Diplura, Orthoptera and Coleoptera) were identified to species level and are representative of different trophic levels. Diplura specimens were prepared in a hydro soluble medium and mounted in slides for identification. The data was organized by phylogenetic order following the life trees from the "Tree of Life: Web project" (Maddison et al., 2007).

2.4 Statistical analysis

All analyses have been performed in R software version 3.5.0 (R Team, 2013). In order to test significant differences between localities, for each environmental parameter we used a one-way ANOVA analysis alongside a Tukey's test. The five replicates of each locality were tested against the five replicates of each of the other localities, for every single parameter.

To assess the richest locality per season, we used the Shannon-Wiener diversity index for all groups (except Diplura and Orthoptera, with only one species each), using the following the formula (pi is the proportion of individuals that belong to the species I and R is the number of species in the sample):

$$H' = -\sum_{i=1}^{R} pi \ln{(pi)}$$

For each season we used the abundance of each species, while for each locality we summed the abundance values of all seasons for each species. We used this index to verify which locality and season within locality were the richest.

To test the correlation between environmental variables and total abundance, we used the Shapiro-Wilk test to verify if the data was normally distributed, and then either a Pearson correlation test for normally distributed samples or a Kendall correlation test for not normally distributed samples. A canonical-correlation analysis (CCA) was performed using the “vegan” package (Oksanen et al., 2007) using total abundance data per replicate and environmental variables.

3. Results

3.1 Environmental variables

Water content ranged from 37 % in Fórnea to 45 % in Serro Ventoso and organic carbon ranged from 6.92 % in Cesaredas to 9.54 % in Serro Ventoso. Phosphate values were very similar in Serro Ventoso and Fórnea, 0.53 and 0.56 µg/g sediment respectively and higher in Cesaredas 0.97µg/g sediment. Ammonium ranged from 0.11 µg/g sediment in Fórnea to 0.23 µg/g sediment in Serro Ventoso (Table 2).

The highest temperature was 22.7 ºC registered in Fórnea during summer and the lowest was 8.0 ºC in Serro Ventoso during winter. In the transition from winter to spring there was an increase of 6.8 ºC in Serro Ventoso, 5.1 ºC in Cesaredas and 4.3 ºC in Fórnea. The average spring temperature was 17.6 ºC and the average summer temperature was 18.8 ºC. The average annual temperature range per season was 6.6 ºC for winter and spring, 6 ºC for summer and 4.6 ºC for fall. As for the localities, the range was 14.1 ºC for Serro Ventoso, 11.8 ºC for Fórnea and 12.2 ºC for Cesaredas (Figure 2, **Suppl. material 1**).

There were no significant differences between the three localities for any of the environmental parameters measured, except for ammonium, which in Serro Ventoso was significantly higher than in Fórnea ($p = 0.0212$), and for average temperature, which was significantly different between all localities ($p < 0.0001$) (**Suppl. material 2**).

3.2 Annual diversity variation

In the MSS arthropod abundance reached its highest value during the winter in Serro Ventoso and Fórnea, while in Cesaredas it occurs during fall (Figure 3A, Table 3). Coleoptera were consistently the most diverse of the studied groups ($H' = 2.23$) (**Suppl. material 3**).

3.2.1 Diplopoda

A total of 429 specimens belonging to three species of the orders Chordeumatida and Polydesmida were collected. The chordeumatidan *Haplobainosoma lusitanum* Verhoeff, 1900 represented 98% of the Diplopoda diversity (Table 3).

Cesaredas was the locality with the highest Diplopoda abundance. In Serro Ventoso and Fórnea abundance increased from fall to winter and decreased until spring, whereas in Cesaredas it decreased from fall to summer. During summer no specimens were found in Serro Ventoso or Fórnea and only one was collected in Cesaredas. The season with the highest Diplopoda abundance was winter for Serro Ventoso and Fórnea, and fall for Cesaredas (Figure 3B, Table 3).

Serro Ventoso was the most diverse locality ($H' = 0.23$) and its most diverse season was spring ($H' = 1.04$). In Fórnea the most diverse season was also spring ($H' = 0.41$) (**Suppl. material 3**).

Serro Ventoso was the only locality where a correlation between temperature and Diplopoda abundance was found ($p = 0.036$), meaning that the Diplopoda abundance increased with lower temperatures (Figure 4, **Suppl. material 4**).

3.2.2 Diplura

Diplura were entirely represented by the species *Campodea arrabidae* Wygodzinski, 1944 in a total of 193 specimens collected in the three localities: 78 in Serro Ventoso; 109 in Fórnea; and 6 in Cesaredas (Table 3, **Suppl. material 4**).

Diplura's highest abundance was found in Fórnea, where the abundance increased slightly from fall to spring and then decreased until summer. In Serro Ventoso no specimens were collected during fall, and abundance increased from winter to summer, while in Cesaredas abundance remained constant during fall, winter and summer, increasing slightly during spring (Figure 3C, Table 3).

Diplura abundance did not correlate to the studied environmental variables in any locality (**Suppl. material 4**).

3.2.3 Orthoptera

A total of 11 specimens belonging to the genus *Petaloptila (Petaloptila)* Pantel, 1890 were collected (Table 3). Orthoptera specimens were only collected in two of the localities in very small numbers. The season with the highest abundance was summer (Figure 3D, Table 3).

3.2.4 Coleoptera

A total of 454 Coleoptera specimens were collected and identified as 20 morphospecies. In terms of abundance the Coleoptera community was composed of 42% Leiodidae, 33% Staphylinidae and 25% Carabidae (Table 3).

Specimens of the most abundant family, Leiodidae, were only collected during winter and spring, with abundance increasing towards spring. Serro Ventoso was the locality with the highest Leiodidae abundance (Figure 3F, Table 3, **Suppl. material 4**). Among them, the species *Catops coracinus* Kellner, 1846 and *Sciodrepoides watsoni watsoni* (Spence, 1813) were dominant in Serro Ventoso and Cesaredas, while *Ptomaphagus (Ptomaphagus) tenuicornis tenuicornis* (Rossenhauer, 1856) dominated the diversity in Fórnea. *C. coracinus* represented 40% of all collected leiodids, followed by 36% of *P. tenuicornis* and 21% of *S. watsoni.* In Serro Ventoso and Cesaredas *C. coracinus* abundance increased towards spring, while in Fórnea only one specimen was collected in winter. *P. tenuicornis* was only collected during spring in Serro Ventoso and in Fórnea abundance increased considerably towards spring. *S. watsoni* abundance also increased considerably towards spring in Serro Ventoso while in in Fórnea and Cesaredas this species was only collected during spring (Table 3).

Staphylinidae was the most diverse family, and its abundance increased from fall to winter (highest abundance of all seasons) and decreased towards summer. Cesaredas was the locality with the highest Staphylinidae abundance (Figure 3G, Table 3). *Proteinus* Latreille, 1796 (54%), Pselaphinae (17%) and Aleocharinae (14%) were dominant in all localities, representing 85% of the Staphylinidae total abundance (Table 3). The subfamily Aleocharinae was only collected during spring and summer with abundance decreasing towards the latter season. In Serro Ventoso and Cesaredas *Proteinus* sp. was only collected during winter and spring with abundance decreasing towards spring, and in Fórnea this species was only collected during winter (Table 3). Specimens of the subfamily Pselaphinae were collected during the whole year in Serro Ventoso and Fórnea with abundance reaching its peak during spring. In Cesaredas these specimens were only collected during fall (Table 3).

Abundance of the family Carabidae reached its peak during fall in Serro Ventoso and Cesaredas. In Fórnea winter and spring Carabidae abundances were very similar (Figure 3E, Table 3). In all localities more than half of the carabid beetle diversity belongs to the tribe Pterostichini (68% in Serro Ventoso, 53% in Fórnea and 96% in Cesaredas). The genus *Platyderus* Stephens, 1828 occurred only in Fórnea and contributed to 57% of the total winter abundance (Table 3).

Serro Ventoso was the most diverse locality for all beetles (H' = 2.06) and winter was the most diverse season (H' = 1.72). The same pattern was observed in Fórnea (winter: H' = 2.21), while the most diverse season in Cesaredas was spring (H' = 1.53). Between the three beetle families, Staphylinidae was the most diverse (H' = 1.47). Spring was the most diverse season for Carabidae in Serro Ventoso and Fórnea and for Leiodidae and Staphylinidae in Serro Ventoso. Winter was the most diverse season for Leiodidae and Staphylinidae in Fórnea and for Leiodidae in Cesaredas (**Suppl. material 3**).

No correlations were found between the environmental variables and the total abundance of any of the Coleoptera families in any of the localities (**Suppl. material 4**).

4. Discussion

The temporal and spatial dynamics of invertebrate communities of the terrestrial subsurface habitats remain largely unstudied, and whether these habitats constitute “a gateway to colonize deep zones” is currently one of the fundamental questions in subterranean biology (Mammola et al*.,* 2020). This study presents the first data on invertebrate (Diplopoda, Diplura, Orthoptera and Coleoptera) seasonal abundance variation for the MSS in continental Portugal, and reveals a large seasonal variation for these groups, but little influence of sediment properties on abundance.

The chemical and physical properties of the three sites were very similar, and also vegetation did not appear as sufficiently distinct to lead to differences between sites, which differ not much in elevation. In contrast, season had a strong impact on arthropod abundance, across sites. Both at surface and in caves there is usually a decrease in arthropod abundance during the winter season (Reddy and Venkataiah, 1990; Moldovan et al., 2018). In the MSS this seasonal decrease has also been reported in several localities in Romania and Slovakia (Nitzu et al., 2011, 2014; Rendoš et al., 2012). In two of our localities however, we observed the opposite trend, since the highest abundance was recorded during winter. This can most likely be explained by the fact that in the MSS temperature and humidity ranges are smaller than at surface and, therefore, winters are less harsh in the MSS (Gilgado et al., 2015; Jiménez-Valverde et al., 2015; Ledesma et al., 2020), possibly providing a seasonal climatic refuge for some species during the colder season. The big exception were millipedes, in two of the localities. Diplopoda annual variation in the MSS was dominated by *Haplobainosoma lusitanum* (Diplopoda: Chordeumatida), found in great numbers when compared with previous occurrence data for the species. Prior to this study *H. lusitanum* was considered rare at surface and in caves (Reboleira and Enghoff, 2014). In temperate climates chordeumatidan millipedes mate and lay eggs in early spring and die before the heat of the summer (Meyer, 1990; Spelda, 2015). Juveniles hatch in late summer and adults appear in fall with a normal activity period from late fall to early spring (Spelda, 2015). Each locality showed a different *H. lusitanum* annual abundance pattern. In Cesaredas abundance generally followed the pattern described by Spelda (2015), suggesting that this chordeumatidan may be well established in the MSS habitat. In Serro Ventoso, *H. lusitanum* annual abundance pattern can be explained by the annual temperature variation which rose 6.8 ºC from winter to spring (the highest increment for the same period among the three localities). This sharp thermal increment most likely promoted the oviposition in deeper parts of the MSS where the first phase of postembryonic development might have occurred also over summer, i.e., at deeper levels than our sampling traps, since no specimens were collected during spring and summer. On the other hand, in Fórnea *H. lusitanum* was only found during winter and spring with abundance decreasing drastically towards spring, suggesting that it used the MSS as a climatic refuge habitat during winter (Gilgado et al., 2015; Jiménez-Valverde et al., 2015; Ledesma et al., 2020). Among the three localities, Cesaredas had the highest *H. lusitanum* abundance, which can be explained by the "Rain shadow effect", that states that on the windward side of the mountain the warm moist air favours higher biodiversity while on the leeward side the dry air promotes lower biodiversity (Antonelli et al., 2018). The scree slope in Cesaredas faces west (windward side of the mountain), while in Serro Ventoso and Fórnea the slopes face east and southeast (leeward side).

In Portugal a total of 16 Diplura species have been reported (Sendra, pers. obs.), two of which are known to inhabit subterranean habitats (Reboleira et al., 2011). The only dipluran species found in the Portuguese MSS, *Campodea arrabidae,* had previously been collected in upper soil layers in Portugal and Spanish Galicia (Sendra and Moreno, 2004; Sendra and Reboleira, 2020). This species showed a strong seasonal abundance variation, same as other *Campodea* species studied in forest and meadow soils, and in MSS habitats with reduction of abundance during colder seasons (Blesic, 1987; Gunn, 1992; Sendra et al., 2017). This abundance pattern is opposite to what was previously observed in subterranean members of the same family which did not show any apparent seasonal abundance variation (Sendra, 2015; Sendra et al., 2020). Such high seasonal variability in soil and MSS species can be partly explained by the interruption of the breeding period during colder seasons (Bareth, 1968). In Cesaredas the Diplura specimens seem to be accidental dwellers, as only 6 were collected in one year compared to 78 and 109 specimens in the other two localities.

A very common group of MSS inhabitants in the Iberian Peninsula are the orthopterans (Olmo-Vidal and Hernando, 2000; Barranco, 2012; Barranco et al., 2013; Jiménez-Valverde et al., 2015). They were collected in two of the sampling localities, all belonging to the genus *Petaloptila (Petaloptila)*, already known from surface ecosystems in Portugal (Ferreira and Grosso-Silva, 2008). Based on the abundance patterns, *Petaloptila* seems to use this MSS habitat as a climatic refuge to escape the heat of the summer at surface or to look for food, as both adults and nymphs have been captured, including one in first instar (Nitzu et al., 2010, 2014; Jiménez-Valverde et al., 2015; Mammola et al., 2016; Ledesma et al., 2020).

The most diverse and abundant among the studied groups in the MSS in central Portugal was found to be Coleoptera, as already reported by Rendoš et al. (2012) for the MSS in central Europe. The most diverse beetle family was Staphylinidae, which matches the pattern for world's beetle diversity (Newton, 2015) and for the MSS in Slovakia (Rendoš et al., 2012). However, in two of the localities the most abundant family was actually Leiodidae, in accordance to the results of studies on the MSS in the Czech Republic and Canada (Dolný, 2000; Zeran et al., 2007). The six Leiodidae species collected in our sampling localities were already known from other Portuguese habitats (Barros, 1907, 1913, 1924; Jeannel, 1936, 1941;

Blas, 1979; Giachino and Vailati, 1993; Faria e Silva et al., 2013), and *C. coracinus* had already been found in the subterranean environment in caves of southern Portugal (Reboleira, pers. obs.). The three dominant leiodids (*C. coracinus, P. tenuicornis* and *S. watsoni*) exhibited seasonal abundance patterns in the MSS similar to those already known at surface (Salgado, 1996; Salgado and Fernández, 1998; Faria e Silva et al., 2013).

Contrary to what was observed for seasonal Staphylinidae abundance at surface (Irmler and Lipkow, 2018), in the MSS the abundance peak was observed during winter. *Proteinus* sp. was the biggest contributor to this pattern, suggesting that it might use the habitat as a climatic refuge during winter. Aleocharinae and Pselaphinae are the other two most abundant staphylinids in our localities, which are frequent inhabitants of subterranean habitats (Assing, 2018) and extremely difficult to identify to species level (Ferreira, 2014; Jałoszyński et al., 2013). Aleocharinae were only collected during the warmer seasons, mainly in spring, when the average temperatures were within the range of the optimal breeding temperatures described by Zagaja et al. (2017) for myrmecophilous Aleocharinae. Therefore, in these localities Aleocharinae might descend into the MSS for optimal breeding conditions, migrating back to the surface as adults to complete the rest of their life cycles. In Serro Ventoso and Fórnea Pselaphinae abundance peaks occurred during spring suggesting that the communities in these localities might be using the MSS during reproductive season to lay eggs just like the Aleocharinae.

The third most abundant beetle family were the ground beetles (Carabidae). Their life cycles are extremely variable depending on the species and habitat (Butterfield, 1996; Sota, 1996; Traugott, 1998; Reboleira and Ortuño, 2010; Rusdea, 2013; Ortuño et al., 2019). In continental Portugal, carabids are the most abundant subterranean beetles (Reboleira et al., 2013a). More than half of the carabid abundance was represented by the tribe Pterostichini. *Platyderus* was found to be most abundant genus during winter and spring in Portuguese surface habitats (Oliveira, 2016), while in the MSS we only found a species of this genus during winter.

This study revealed a surprisingly high diversity and abundance of invertebrates in the MSS in central Portugal showing that it acts either as a climatic refuge for surface invertebrates or as a permanent habitat for several species in the studied areas (Culver and Pipan, 2009a; Nitzu et al., 2010, 2014; Ortuño et al., 2013; Jiménez-Valverde et al., 2015; Mammola et al., 2016). In Portugal this habitat has been neglected regarding its importance for invertebrate fauna, therefore its conservation is critical. The main threats this habitat faces are anthropic destabilization of the scree slopes and destruction of habitat (ICNB, 2000). Although the MSS habitat is protected by legislation under the Natura 2000 Network (EU, 1992) its biodiversity only started to be revealed in the last few years. The data provided by this study can be used as a contribution to increase knowledge on the living communities of the MSS and to stimulate the protection of this habitat and its biodiversity.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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Figure Captions

Figure 1. Subterranean traps for invertebrate collection. (A) Map of the three sampling localities in central Portugal, implemented over national protected areas: 1 – Serro Ventoso; 2 – Fórnea; 3 – Cesaredas; (B) Sampling trap installed in the MSS; (C) Three level collecting system; (D) Installation of the collecting system in the trap.

Figure 2. Annual temperature variation per month for the three localities (air temperature inside the trap).

Figure 3. Invertebrate abundance per locality and season. (A) Total invertebrate abundance; (B) Diplopoda; (C) Diplura; (D) Orthoptera; (E) Carabidae; (F) Leiodidae; (G) Staphylinidae.

Figure 4. Correlation scatterplot between Diplopoda abundance and average temperature in Serro Ventoso (Pearson correlation).

Table 1. Sampling localities in central Portugal.

Locality	Karst area	Latitude	Longitude	Elevation (m a.s.l.)	Slope facing direction	Vegetation coverage
Serro Ventoso	Estremenho	39.56028	-8.836083	298	East	Medium
Fórnea	Estremenho	39.55943	-8.804452	327	Southeast	Medium
Cesaredas	Cesaredas	39.29988	-9.199631	72	West	High

Table 2. Average pH, conductivity (mS/cm), organic carbon (%), water content (%), available nitrate (NO_3) (µg/g sediment), available phosphate (PO_4) (µg/g sediment) and available ammonium (NH_4) (µg/g sediment) values and standard error for the three localities.

Locality	**pH**	**Conductivity**	**Water content**	**Organic carbon**	**NO_3**	**PO_4**	**NH_4**
Serro Ventoso	7.74 ± 0.02	196.52 ± 19.5	45 ± 4	9.54 ± 1.2	36.79 ± 12.6	0.53 ± 0.08	0.23 ± 0.03
Fórnea	7.76 ± 0.003	190.86 ± 12.4	37 ± 3	7.95 ± 0.6	29.51 ± 7.7	0.56 ± 0.2	0.11 ± 0.02
Cesaredas	7.77 ± 0.004	170.18 ± 16.4	38.2 ± 5	6.92 ± 1.5	20.09 ± 6.4	0.97 ± 0.4	0.14 ± 0.03

Table 3. Species abundance and diversity per group, locality and season. Fall (F), Winter (W), Spring (Sp) and Summer (S).

	Serro Ventoso				**Fórnea**				**Cesaredas**			
	F	W	Sp	S	F	W	Sp	S	F	W	Sp	S
Diplopoda	37	75	4	0	0	42	7	0	107	76	80	1
Haplobainosoma lusitanum Verhoeff, 1899	37	71	2	0	0	42	6	0	107	76	80	1
Polydesmus coriaceus Porat, 1871	0	4	1	0	0	0	1	0	0	0	0	0
Polydesmus sp.	0	0	1	0	0	0	0	0	0	0	0	0
Diplura	0	7	27	44	11	33	37	28	1	1	3	1
Campodea arrabidae Wygodzinski, 1944	0	7	27	44	11	33	37	28	1	1	3	1
Orthoptera	0	0	2	8	0	0	0	1	0	0	0	0
Petaloptila (Petaloptila) sp.	0	0	2	8	0	0	0	1	0	0	0	0
Coleoptera	36	80	84	10	0	19	75	8	34	44	52	12
Carabidae	32	10	2	3	0	7	4	6	23	0	15	9
Laemostenus sp.	10	3	1	1	0	1	1	1	2	0	0	0
Platyderus sp.	0	0	0	0	0	4	1	0	0	0	0	0
Pterostichini spp.	22	7	1	2	0	2	2	5	21	0	15	9

Leiodidae	0	39	64	0	0	3	61	0	0	4	22	0
Catops coracinus Kellner, 1846	0	31	35	0	0	1	0	0	0	2	7	0
Catops fuliginosus Erichson, 1837	0	2	1	0	0	0	0	0	0	0	0	0
Nargus (Demochrus) wilkinii (Spence, 1813)	0	0	0	0	0	0	0	0	0	1	0	0
Ptomaphagus (Ptomaphagus) tenuicornis tenuicornis (Rossenhauer, 1856)	0	0	8	0	0	2	60	0	0	0	0	0
Sciodrepoides watsoni watsoni (Spence, 1813)	0	4	20	0	0	0	1	0	0	0	15	0
Speonemadus transversostriatus (Murray, 1856)	0	2	0	0	0	0	0	0	0	1	0	0
Staphylinidae	4	31	18	7	0	9	10	2	11	40	15	3
Aleochara sp.	0	0	1	1	0	0	0	0	0	0	0	1
Aleocharinae spp.	0	0	5	5	0	0	4	1	0	0	5	1
Anotylus sp.	0	0	0	0	0	1	0	0	0	0	0	0
Cephennium sp.	0	0	0	0	0	0	0	0	0	1	0	0
Ilyobates sp.	0	1	0	0	0	0	0	0	0	0	0	0
Ischnosoma sp.	0	0	0	0	0	0	0	0	0	0	0	1
Micropeplus latus C.Hampe, 1861	0	5	0	0	0	0	0	0	0	0	0	0
Proteinus sp.	0	23	5	0	0	4	0	0	0	39	10	0
Pselaphinae spp.	4	2	7	1	0	1	6	1	3	0	0	0
Rybaxis sp.	0	0	0	0	0	1	0	0	0	0	0	0
Tasgius globulifer (Geoffroy, 1785)	0	0	0	0	0	1	0	0	0	0	0	0
Xantholinus sp.	0	0	0	0	0	1	0	0	8	0	0	0
Species diversity (number of species)	18				17				15			
	4	13	15	7	1	13	10	6	6	7	7	6