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Don't forget the vertical dimension: assessment of distributional dynamics of cave-dwelling invertebrates in both ground and parietal microhabitats

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Abstract

Biological studies on factors shaping underground communities are poor, especially those considering simultaneously organisms with different degrees of adaptation to cave life. In this study, we assessed the annual dynamics and use of both horizontal and vertical microhabitats of a whole community with the aim of understanding whether cave-dwelling organisms have a similar distribution among vertical and ground-level microhabitats and to find out which microhabitat features influence such distribution. We monthly assessed from 2017 to 2018, by direct observation combined with quadrat sampling method on the ground and transects on the walls, richness and abundance of 62 cave-dwelling species in a cave of Northern Italy. Environmental factors such as light intensity, temperature, relative humidity and mineralogical composition of the substrates were measured during each monitoring session, influencing the dynamics of the whole community and revealing significant differences between ground and wall microhabitats. A gradient of variation of the species assemblages occurred from the entrance toward inner areas, however, evidence that the dynamics of the walls are very different from those occurring at the ground

independent from the distance from the surface are shown. Biodiversity indices highlighted sampling area diversity and a discrete total cave fauna biodiversity with the highest values found near the entrance and the lowest in the inner part of the cave.

Keywords
cave biodiversity, cave community, environmental drivers, subterranean biology, subterranean environment

Introduction

Subterranean habitats, intended as all the natural and artificial voids suitable for the occurrence of life, are intriguing scientists since the beginning of scientific disciplines (Vandel 1964) and are gaining the attention of zoologists and ecologists in recent times because of their great potential to solve broad biological questions (Mammola et al. 2020). This potential is linked to the peculiar ecological features that characterize the subterranean realm; one of the more obvious is the absence of light, with solar radiation not able to penetrate beyond the more or less short ecotonal area that connects subterranean habitats to the surface, thus preventing plant growth. Consequently, without these important primary producers, organic matter is in short supply within subterranean habitats, which are mostly depending from external inputs (Schneider et al. 2010; Iskali and Zhang 2015; Barzaghi et al. 2017). These inputs are generally vegetal and animal remains, bacteria, spores and seeds that are transported into caves by water, air, gravity or animals; animals can also deposit materials such as eggs, faeces, food and other organic material (Schneider et al. 2011). Only a small amount of primary production is carried out by autotrophic bacteria (Schneider et al. 2011). Apart from darkness, also other environmental parameters and climatic conditions have a direct effect on hypogean ecosystems and a profound influence on subterranean fauna, including relative humidity, temperature, cave morphology, lithology, water and air circulation (Culver and Pipan 2010). Lithology is important when comparing cave systems in similar areas (climate) carved in different rocks (Souza-Silva et al. 2020), but usually is less important when studying a single cave system. The climate within caves is usually very stable (Bourges et al. 2014) with the exception of the areas near the entrance, more affected by the external environment (Badino 2010; Lunghi et al. 2015). Relative humidity is probably one of the most important limiting factors for subterranean fauna, acting on the metabolism, respiration and on the absorption of water through the cuticle of different hypogean species. In the subterranean environment relative humidity is normally between 95–100 RH%, rarely less than 80 RH%, even if RH mostly depends on the geographic area and on the local subterranean settings (i.e. distance from water bodies or dripping points, local air flow) (Culver and Pipan 2014).

Absence of light and the other environmental features of the subterranean environment induce a number of physiological, metabolic, morphological and behavioural adaptations in hypogean fauna (Howarth and Moldovan 2018). Usually, subterranean-dwelling organisms are classified in biospeleological categories, based on both the oc-

currence of specific adaptations and on their necessity to complete their life cycle underground (Mammola 2019; Romero 2020). However, these categories can be poorly representative; evident connections occur through the fissures of the bedrock (Milieu Souterrain Superficiel - MSS) (Juberthie et al. 1980a, b, 1981), and the limits of the subterranean environments are less defined than expected (Giachino and Vailati 2005, 2008, 2010, 2017). The most adapted organisms are named troglobionts: they often show blindness, depigmentation and elongation of appendages and only reproduce themselves in subterranean habitats (Culver and Pipan 2019; Mammola 2019). Organisms able to breed in both subterranean and surface habitats, generally showing some adaptations to cave life are named troglophiles, while organisms occurring only accidentally in caves are called trogloxenes (Romero 2009; Mammola 2019). However, this classification is considered a ploy and a simplification which hardly mirrors the relationships among organisms and their spatial distribution underground (Romero 2020).

How these three ecological groups occur and interact within subterranean habitats is a consequence of multiple environmental conditions including not only the specific features of subterranean ecosystems (Mammola and Isaia 2016) but also seasonal changes in the regional and local climate (Novak et al. 2004; Kozel et al. 2019) and the effects of the connection with the external surface (Manenti and Barzaghi 2021).

The ensemble of subterranean-dwelling organisms is thus likely to form a gradient from the entrance to the deepest sectors of the cave that, although complex, should lead to rather simple (or more understandable) ecological dynamics compared to surface communities; however, biological studies on underground communities are poor, despite the great potential to solve broad ecological questions (Mammola et al. 2020), especially because of the habitat impediments (Mammola et al. 2021).

The biological studies trying to consider the ensemble of subterranean organisms, generally focus only on caves used by troglophile and more or less accidental species (Di Russo et al. 1997; Fenolio et al. 2005; Novak et al. 2010; Manenti et al. 2013; Lunghi et al. 2018), while ecological studies dealing with troglobionts often focus only on single species and rarely consider the whole community (Kozel et al. 2019). External seasonality and organic matter or prey abundance in the less deep sectors of caves seem to affect occurrence, abundance and interactions of troglophile species (Mammola et al. 2017; Ficetola et al. 2018). On the contrary, for communities composed mainly of troglobionts, local subterranean features seem to have a stronger importance; in particular, in a Slovenian cave, Kozel et al. (2019) recently observed that the dynamics of troglobiont communities across a year are strongly affected by microclimatic conditions of the substrate, cave morphology and even pH of substrate. In a French cave, relations between the distribution of five species, cave wall morphology and climatic conditions have been observed, although no general rule could be formulated (Bourne 1976). However, studies dealing with the dynamics of the whole community, considering the environmental drivers of both troglobionts and troglophiles/trogloxenes at the same time remain scarce (Lunghi and Manenti 2020). Moreover, an important aspect to consider is that subterranean habitats are three-dimensional, with the substrates, walls and ceilings of the subterranean spaces accessed by humans (caves), in turn con-

nected with an intricate adjacent fissure network not directly accessible for direct exploration (Giachino and Vailati 2010; Mammola 2019).

The study of environmental drivers of subterranean-dwelling organisms may allow to achieve a better understanding of how substrate and wall microhabitats affect the occurrence and interactions of organisms with different degrees of adaptations to subterranean life. In the present research, we studied the annual dynamics and use of both horizontal and vertical microhabitats of a whole community in a cave in northern Italy, with the aim of understanding whether subterranean-dwelling organisms have a similar distribution among vertical and ground-level microhabitats and to find out which microhabitat features influence their distribution. We hypothesise that *i*) underground environmental conditions affect the whole community of a cave irrespective to the single species with different degrees of adaptation to subterranean life and that *ii*) the mineralogical composition of the substrate plays a major role in shaping subterranean communities; moreover we hypothesise that, if there is a main gradient of adaptation of the cave communities from the entrance toward the inner areas (Mammola 2019), *iii*) cave sectors, wall and ground microhabitats, should show different fauna composition according to their distance from the entrance.

Material and methods

Study area

Surveys were performed in the Baraccone Cave (309 Pi/CN) (44°16.5192'N, 8°5.0674'E UTM WGS84 32T, 1040 m a.s.l., Bagnasco, CN, Piedmont, Northern Italy) which is hosted in a Special Area of Conservation (IT1160020 "Bosco di Bagnasco"), on the right bank of the Tanaro River (Fig. 1A). The area is characterized by a well-preserved beech and maple-lime-ash forest, on the border between the Alpine and Apennine-Mediterranean environment (25 km from the sea) (Regione Piemonte 2017). During the observation period, from March 2017 to March 2018, rainfall occurred in March and May 2017, and in January and March 2018, whereas the most extreme events happened in November and December 2017. External monthly average temperature at the meteorological station of Perlo (3.6 km from the cave) ranged between 22 °C in August 2017 to slightly below 0 °C in February 2018.

From a geomorphological point of view, the area is formed by a uniform mountain slope composed of an alternation of limestones and dolomites, with W-NW exposure, drained by the Gambulogni torrent. Baraccone cave has a length of about 40 m and a vertical drop of – 8 m (Fig. 1B). It is rich in decaying vegetal matter, feces and fungi and hosts different microhabitats. The entrance, narrow and with a steep slope, favors the fall of vegetal debris which is then transported throughout the cave. The small size of the cave allows it to be investigated in detail, covering different ecological niches.

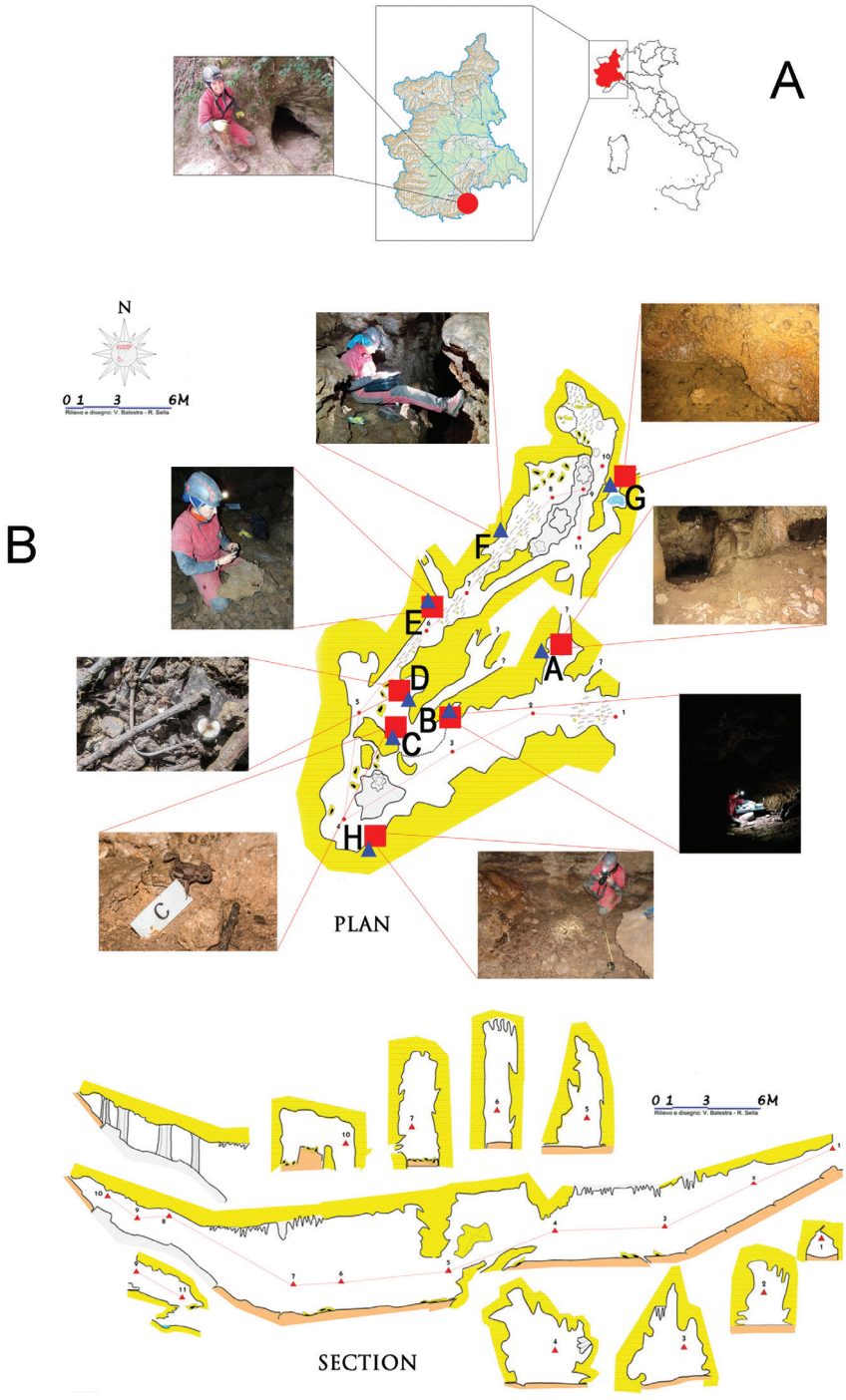

Figure 1. A Location of Baraccone Cave, Piedmont, Italy, and the entrance of the cave (photo by E. L.) **B** baraccone Cave map with monitoring areas. Red quadrats for ground fauna monitoring and blue triangles for parietal fauna monitoring (map by V. B. and R. Sella, photos by E. L. and V. B.).

Many fauna samplings have been carried out in Baraccone Cave before this study (Suppl. material 1). From 1928 to 2013, 17 species of invertebrates have been reported. Only two of the reported species can be considered troglobitic (obligate specialized species) while eight are troglophile (facultative cave-dwelling organisms) and the others are accidentals.

Investigation method

Different methods to collect data on subterranean fauna exist (e.g. Bichuette et al. 2015; Wynne et al. 2019; Mammola et al. 2021), however, there are few studies comparing these sampling methods (e.g. Weinstein and Slaney 1995). Several authors agree that a combination of different methods is essential to investigate subterranean communities (Bichuette et al. 2015; Kozel et al. 2017) and some hypogean species have preferential microhabitats (Kozel et al. 2017; Pacheco et al. 2020), therefore, sampling simultaneously different habitats in more locations allows to increase the range of detected taxa respect to a single-set-alone sampling (Kozel 2018). For these reasons, a combination of sampling and monitoring methods in characterizing cave fauna and assessing cave fauna assemblages according to seasonality and microhabitat differentiation have been used in this work.

Pre-evaluations on site allowed us to establish eight sampling areas (Fig. 1B) to monitor invertebrates, ranging from cave ground to walls and representative of the diverse microhabitats in the cave. Seven areas include one sampling quadrat for ground fauna monitoring and one transect for parietal fauna monitoring, while one area only has a transect for parietal fauna monitoring (F area), being the space on the ground less than 1 m^2. To select these areas, in order to evidence the different microhabitats of the cave, the diverse conditions of temperature (T), relative humidity (RH%), light intensity (LI), type of substrate, presence and abundance of macrofungi, faeces and decomposing organic matter, presence, position and shape of stones, rocks on the ground and minerals, presence of water and fractures (Table1) have been taken into account. Being the cave entrance of small dimension and characterized by considerable slope, it was not possible to establish a monitoring area there; therefore, visual investigations were carried out during the transit of researchers to point out any additional species useful only for the fauna characterization.

Surveys were carried out monthly, from March 2017 to March 2018. Only in February 2018 it was not possible to reach the Baraccone Cave due to the presence of large amounts of ice and snow. In the eight areas, we performed visual encountered surveys, supported by the acquisition of macrophotographs of the observed species. Macrophotography has several advantages, including to highlight details not visible at naked eye and to review behaviour and characteristics of the observed individuals on a computer at home. However, sometimes photographing animals in some areas of the cave, as fissures or on the ceiling, can be difficult, and bringing photographic equipment in caves can be complex due to habitat impediments (see Mammola et al. 2021). Visual encountered surveys required a priori biological knowledge of the taxa observed for

Table 1. Sampling areas representative factors. Mean values of temperatures and relative humidity are considered.

	AREA	GROUND	WALL
A	Low humidity (< 90 RH%), very high temperature (> 11.0 °C), light availability, high climatic external influence	Big amount of decomposing organic vegetal matter	Wet and smooth wall
B	Low humidity (< 90 RH%), high temperature (10.5–11.0 °C), light availability	Decomposing organic vegetal matter	Fractures, aragonite minerals
C	Medium humidity (90–94 RH%), high temperature (10.5–11.0 °C), light availability	Decomposing organic vegetal matter, step of speleothem, stones	Wet wall, minerals, speleothems
D	Medium humidity (90–94 RH%), medium temperature (10.0–10.4 °C), light availability	Decomposing organic vegetal matter, fungi, stones	Fractures, speleothems
E	Medium humidity (90–94 RH%), low temperature (< 10.0 °C), no light	Decomposing organic vegetal matter, stones, mud	Wet wall
F	Medium humidity (90–94 RH%) medium temperature (10.0–10.4 °C), no light	-	Aragonite minerals, fungi
G	High humidity (> 94 RH%), medium temperature (10–10.4 °C), no light	Water, mud, sediments, speleothems	Wet wall, mud, sediments, speleothems
H	Medium humidity (90–94 RH%), medium temperature (10.0–10.4 °C), light availability	Decomposing organic vegetable matters, faeces, fungi, mud, stones	Wet wall, speleothems

the identification and it does not allow a correct determination at a specific level of all taxa observed (e.g., for some it was possible to carry out an analysis of the demographic dynamics at the genus level only). However, it is a non-invasive method and samples of the taxa can be collected manually. The samples taken for the determination of the uncertain species were placed directly in 96% ethanol in sampling tubes and have been determined by reference experts (see acknowledgements). We decided not to install pitfall traps and to use a less invasive method. We applied the stratified quadrat sampling method, using seven 1×1 m squares fixed with nails and strings, to count superficial ground invertebrates. The observed individuals were collected in a box to avoid multiple counts and released at the end of the evaluation. Parietal fauna was monitored along eight wall-roof transects of 2 m. Death individuals were not counted. For each plot and wall surface sampling area, 15 minutes counting sessions were carried out by the same two researchers at the same time (h 10:00–16:00).

Temperature and relative humidity were measured in each sampling area before the visual encountered survey with a HD 2101.1 Delta Ohm Thermo-hygrometer equipped with a HP 472AC RH% and T probe Pt100 (operating range –20±80 °C, 0–100 RH%, accuracy ±2% (5–95 RH%), ±3% (95–99 RH%), ±0.3 °C (-20±80 °C)). Thanks to a 2.5 m long extension for the thermo-hygrometer probe all parameters were recorded without a close human presence. An HD 2302.0 Delta Ohm Luxmeter with LP471PHOT photometric probe was used for light quantity measurements (measuring range 0.0–200 000 Lux).

The photographs of the specimens in the cave were made by VB using a Canon EOS 70D reflex camera equipped with EF 100 mm 1:2.8 USM Macro lens and integrated flash.

Mineralogical samples, collecting only broken speleothems for the conservation of the cave, were characterized by X-ray powder diffraction (XRD). XRD analyses

were performed on a Philips PW3710 diffractometer (current: 20 mA, voltage: 40 kV, range: 2θ 5–80°, step size: 0.02° 2θ, time per step: 2 sec.) equipped with a Co-anode and interfaced with Philips High Score software package for data acquisition and processing, at DISTAV (University of Genova).

Statistical analysis

The software PAST, Version 4.02 (Hammer et al. 2001) was used to perform the following statistical analyses:

Canonical Correspondence Analysis, in order to assess the relationships between environmental factors, mineral substratum and detected taxa at the class level. A more in depth analysis was made considering classes with a number of specimens exceeding 5% and the orders with a number of specimens exceeding 5% of the total of each class considered.

ANOSIM test and SIMPER analysis to highlight differences between the faunal assemblages of different sampling sites and, where present, the contribution of each taxon to such differences. Jaccard similarity index and Bonferroni correction were adopted. A UPGMA clustering based on Jaccard similarity index (1000 bootstrap replicates) on the cumulate data of each sampling point was made for a graphical representation of the similarity/distance relationships among their assemblages.

Equitability (Pielou's evenness) (J), Dominance (1-Simpson index) (D) and Shannon diversity (H) indices were calculated for each sampling point and for each month in order to compare the biodiversity of the assemblages of different parts of the cave and to outline the monthly trend of the faunal diversity.

A rarefaction analysis was performed to verify the completeness of the species richness observed.

Results

In the Baraccone Cave, from March 2017 to March 2018, 62 different species of invertebrates were observed thanks to our standardized monitoring (Suppl. material 4: Table S1). The total number of counted invertebrate specimens was 3630: 992 in sampling area A, 343 in B, 441 in C, 404 in D, 273 in E, 126 in F, 122 in G and 929 in H. At least 20 species are troglophile and 5 are troglobionts (Suppl. material 4: Table S1, Suppl. material 2: Fig. S1).

Environmental drivers of the whole community

The seasonal T and RH% variations (Suppl. material 3: Figure S2) followed the same trends for all the sampling areas in the cave, except for points A and B, more influenced by the external climate and therefore warmer and less humid than the others. Light was absent in the areas E, F and G; except for A (mean of 0.06 Lux), in the other sampling areas only very low values were measured (mean of 0.02 Lux) (Suppl. material 3: Fig. S2).

XRD results performed on mineralogical samples evidenced that minerals are mostly characterized by calcite and aragonite and minor amounts of goethite, dolomite, quartz and clay minerals. The average of the mineral values found in the samples for each sampling area are listed in Suppl. material 5: Table S2. Minerals on the walls were mainly calcite, except for B and F where aragonite was observed. Aragonite was found also in C and H but only in the inner part of the sampled speleothems, covered by calcite.

Most of the minerals found in the samples were calcite, consequently only a weak but equally significant correlation between substratum and fauna was pointed out. Thanks to the field monitoring and the mineralogical analysis, it was possible to observe that most of the fauna on the cave wall was found on calcite. *Amilenus aurantiacus* (Simon, 1881), *Dolichopoda azami* Saulcy, 1893, *Limonia nubeculosa* Meigen, 1804, Diptera Culicidae indet. and Diptera Limoniidae indet. were observed occasionally on aragonite. Instead, a species of fungus (still unidentified) was observed exclusively on aragonite walls.

Canonical Correspondence Analysis (CCA) on ground fauna highlighted that Diplopoda, Arachnida and Malacostraca were positively related to temperature and calcite while light and clay positively influenced Gastropoda, Chilopoda and Insecta. The other taxa seem to prefer cooler and more humid microhabitats (Fig. 2A). Considering only dominant orders of the prevailing classes of Arachnida, Entognatha and Insecta (see methods) CCA showed that temperature, light and goethite positively influence Mites and Diptera, while calcite positively influenced Opiliones, Pseudoscorpions, Aranea and Coleoptera. Collembola seemed to prefer more humid microhabitats (Fig. 2B). CCA analysis on the classes of parietal fauna showed that light, goethite and dolomite positively influenced Clitellata, while humidity, calcite and clay positively influenced Arachnida and Diplopoda (Fig. 2C). Considering dominant orders of the prevailing classes of Arachnida and Insecta, CCA analysis showed a positive relationship between light, calcite, goethite, dolomite and Spiders, while humidity and clay positively influenced Opiliones (Fig. 2D).

Differences between sampling points, walls and ground

The faunal comparison of the various sampling areas and between the ground-level and parietal fauna, carried out by means of the One-Way ANOSIM analysis (Fig. 3A), evidenced that the differences between the sampling areas were significant; moreover, at the same sampling point the parietal fauna was very distinct from that on the ground. Fauna detected on the diverse ground areas were also significantly different from each other, while the only points on the wall that differ from the others (except for B) were A and C. UPGMA clustering showed two clusters: the former gathering ground assemblages, the latter those of the wall transects (Fig. 3B).

The overall average dissimilarity, obtained by SIMPER analysis, indicated that fauna in the sampling areas differ on average by 90.89%. Taxa responsible for the observed differences evidenced by One-Way ANOSIM are listed in Fig. 3C and Suppl. material 6: Table S3, of which 2 are troglobiont and 14 troglophile. The greatest contributions

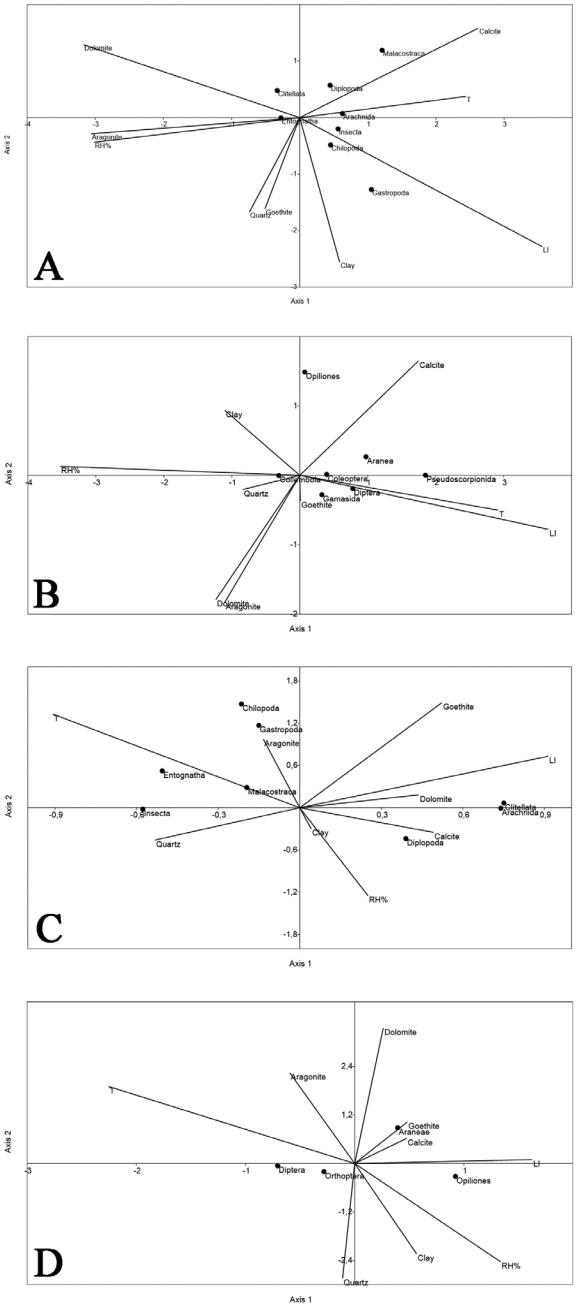

Figure 2. Canonical Correspondence Analysis. Hypogean fauna related to environmental factors and mineral substratum **A** classes of ground fauna **B** orders of ground fauna (Arachnida, Entognatha and Insecta) with a number of specimens exceeding 5% of each considered class total **C** classes of parietal fauna **D** orders of parietal fauna (Arachnida and Insecta) with a number of specimens exceeding 5% of each considered class total.

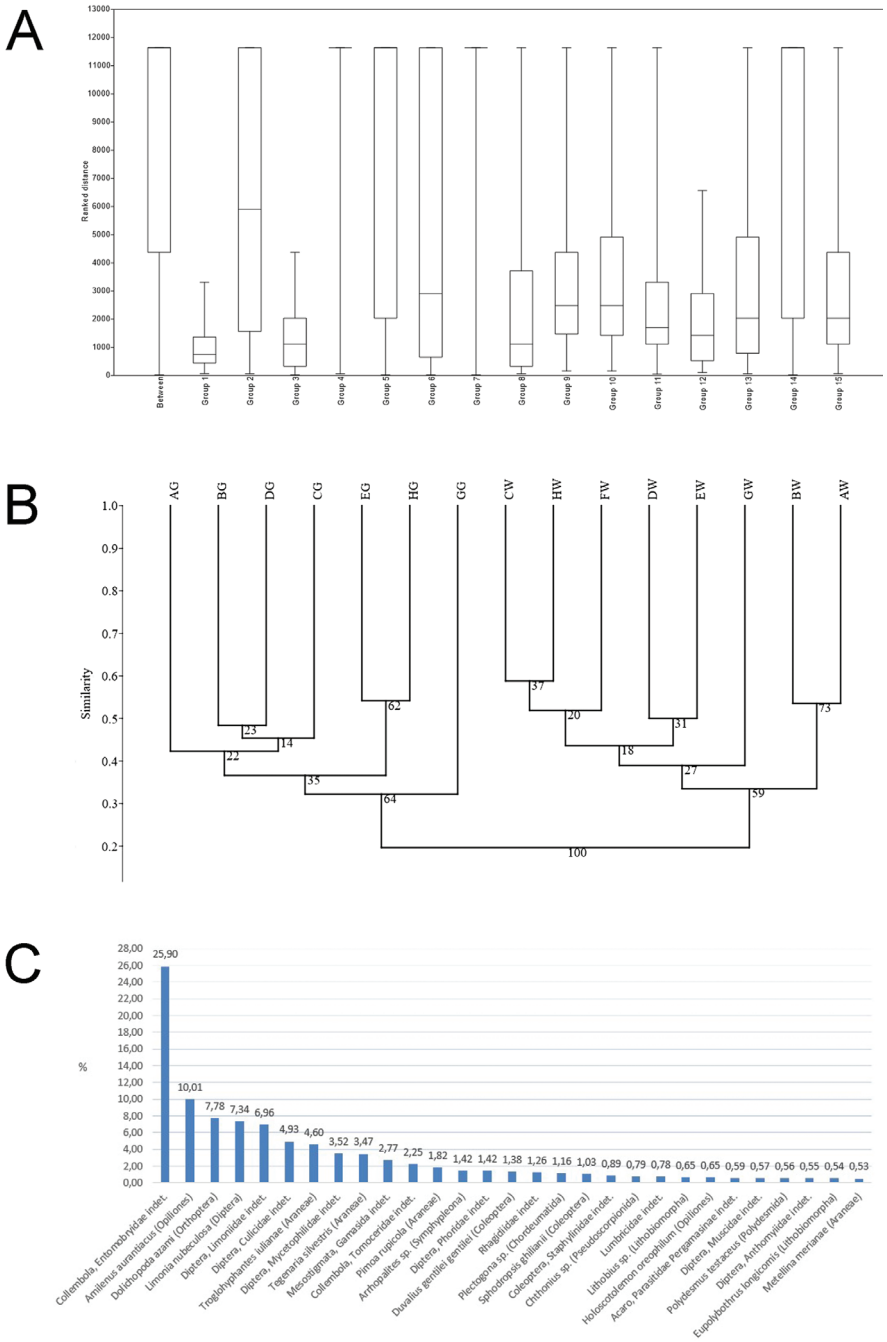

Figure 3. A one-Way ANOSIM test. Wall in eight sites (A-H, Group 1–8), Ground in seven sites (A-E and G-H, Group 9–15) **B** similarity between ground (from AG to HG) and wall (from AW to HW) faunal samples (UPGMA clustering based on Jaccard similarity index - bootstrap values are shown under each node) **C** SIMPER Analysis. Taxa responsible for the observed differences between faunal assemblages in different sampling areas in percentage.

to the average dissimilarity were given by Collembola, Entomobridae indet., *Amilenus aurantiacus*, *Dolichopoda azami*, *Limonia nubeculosa*, Diptera, Limoniidae indet., Diptera, Culicidae indet. (4.93%), *Troglohyphantes iulianae* Brignoli, 1971, Diptera, Mycetophilidae indet., *Tegenaria silvestris* L. Koch, 1872 and Mesostigmata, Gamasida indet. Among these, in ground sampling area Collembola were almost exclusive with only few individuals found on the walls and Mesostigmata, Gamasida indet. was exclusive. Along the transects *Amilenus aurantiacus* and *Dolichopoda azami* were preponderant while Diptera, Limoniidea and Culicidae were almost exclusive, except one or two individuals observed on the ground. Diptera, Mycetophylidae were dominant on the ground but a lot of individuals were also found on the walls. The spiders *Troglohyphantes iulianae* and *Tegenaria silvestris* were observed both on the ground and on the walls. The greatest part of these animals was found on the walls in A, B and H, while on the ground they were observed in sampling areas A, C and D. The innermost sampling areas E, F and G were the less influential. The greatest contributions to the average dissimilarity were given by the species found in A and H, followed by B, C and D.

Biodiversity indices (Fig. 4, Table 2) highlighted a maximum diversity, corresponding to the highest equitability and to the lowest dominance, for ground area A (closest to the entrance). On the contrary, the lowest diversity, related to a maximum dominance value and a minimum equitability, was recorded in ground area H (deepest into the cave). Moreover, biodiversity indices highlighted a discrete total cave fauna biodiversity.

Monthly trend of Equitability (Pielou's evenness), Dominance (1-Simpson index) and Shannon diversity indices for cave invertebrates are shown in Fig. 5A. A peak of diversity was recorded during April-May 2017, while lower values were detected in March and November 2017.

The rarefaction curve (Fig. 5B) shows that an asymptotic value of species richness was reached in December 2017, after 10 monitoring sessions.

Discussion

Increasing sampling sites considering different habitats and a combined use of different methods allowed us to increment the range of detected taxa, as suggested in different research (Bichuette et al. 2015; Kozel et al. 2017; Kozel 2018; Wynne et al. 2018). Being the cave size limited (ca. 40 m), one sampling area for each encountered microhabitat can be representative enough, but in more developed and complex caves it would be necessary to increase the number of sampling areas for each observed microhabitat. Our cave entrance morphology did not allow us to insert a sampling area in this zone. Since this area is the connection point with the outside, where possible, it should be taken into account, even if the invertebrates observed here are often occasional and not subterranean-adapted animals (Lunghi et al. 2017; Galli et al. 2021).

The behaviour and size of the animals influence the effectiveness of each sampling method (Bichuette et al. 2015) and direct observation does not allow a correct determination at a specific level of all the animals observed. However, since the trend of

Table 2. Biodiversity indices calculated for each sampling area. Maximum and minimum values for each index are evidenced in bold and italics, respectively.

	Taxa S	Individuals N	Dominance D	Shannon H	Equitability J
A Wall	22	**791**	0.2090	1.928	0.6237
B Wall	21	132	0.1481	2.356	0.7739
C Wall	17	105	0.2813	1.803	0.6364
D Wall	10	61	0.1954	1.854	0.8051
E Wall	11	53	0.2339	1.778	0.7417
F Wall	10	126	0.2164	1.785	0.7754
G Wall	*6*	*45*	0.2820	1.454	0.8116
H Wall	10	484	0.2341	1.617	0.7023
A Ground	**37**	201	*0.06869*	**3.019**	**0.8362**
B Ground	21	211	0.4448	1.548	0.5083
C Ground	25	336	0.4497	1.439	0.4471
D Ground	25	343	0.3366	1.819	0.5650
E Ground	17	220	0.4503	1.432	0.5056
G Ground	15	77	0.2903	1.675	0.6184
H Ground	20	445	**0.6721**	*0.9479*	*0.3164*
Cave invertebrates (total)	61	3630	0.1352	2.643	0.6430

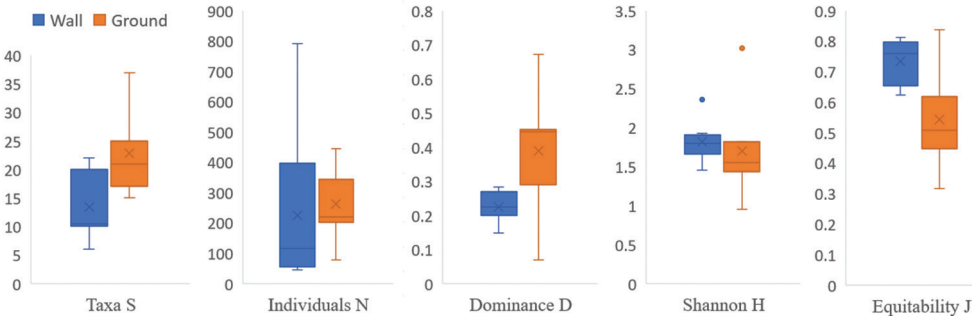

Figure 4. Biodiversity indices for wall and ground cave fauna.

the rarefaction curve was asymptotic, the recorded number of species was considered very close (almost identical) to the total richness of the cave detectable based on the applied methodology. This confirms the reliability of the sampling scheme in terms of frequency and number of inspections relatively to the methods adopted in this study.

Many of the collected species were new for the investigated area, such as *Plectogona* sp. and *Campodea* sp. (Suppl. material 2 Fig. S1). Other species were previously considered endemic to other caves like *Eukoenenia strinatii* Condé, 1977 (Suppl. material 2: Fig. S1), known from Bossea Cave (Frabosa Soprana), 20 km from Baraccone Cave. To date *Eukoenenia strinatii* has been observed also in other nearby cavities (Balestra et al. 2019).

The subterranean-dwelling organisms had a different distribution along the cave and among vertical and ground-level microhabitats, due to the different environmental conditions, including the mineralogical composition of the substrate. Moreover, the crystal habit of the minerals could have an important role in the subterranean fauna distribution. In Baraccone cave, acicular aragonite was found exclusively on some walls,

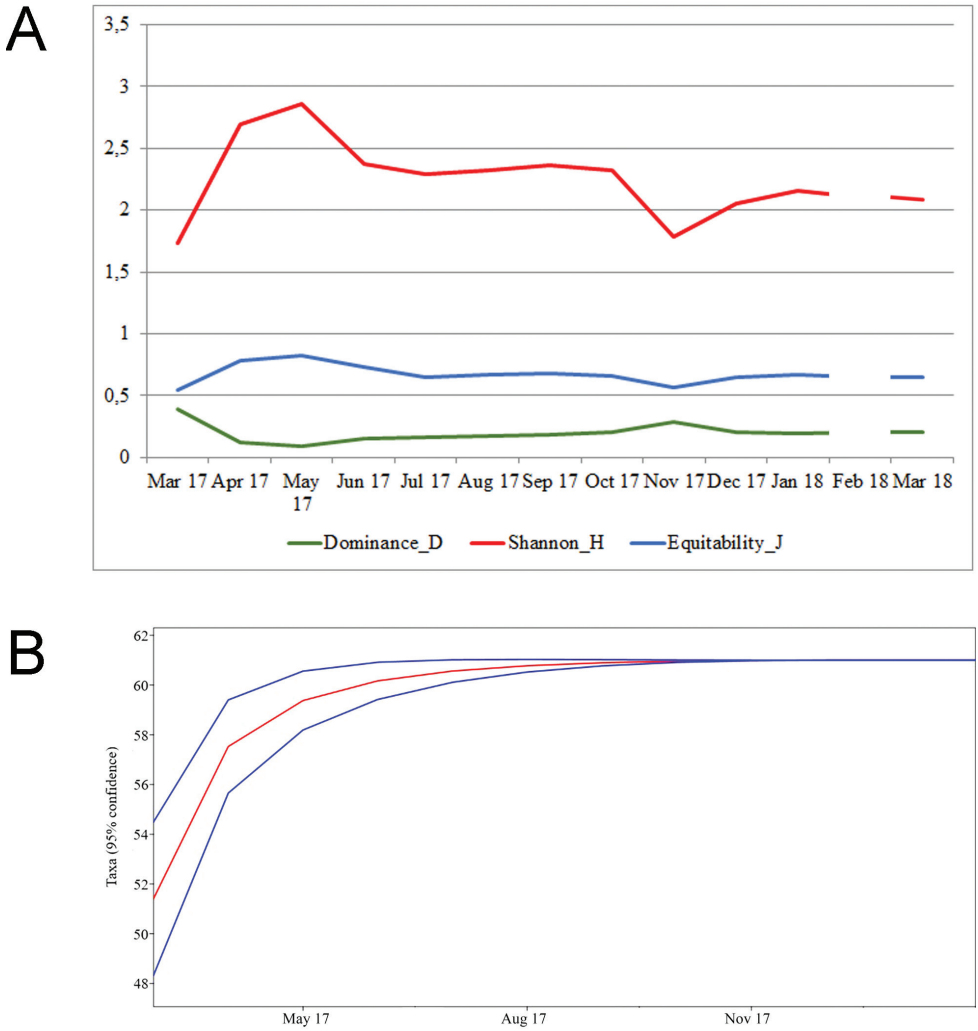

Figure 5. A trend of Equitability (Pielou's evenness), Dominance (1-Simpson index) and Shannon diversity (H) indices from March 2017 to March 2018 **B** rarefaction curve (in red). In blue the 95% confidence interval.

on which only specimens with elongated limbs and/or wings have been occasionally observed. Probably animal movements on aragonite are disadvantaged because of its needle shape, so they seem to prefer the smooth walls of calcite. Seasonality affects the subterranean climate especially close to the cave entrance while inner part climate variations are less evident. Climate and environmental variations affected the presence/absence of certain species (e.g. Carchini et al. 1982; Di Russo et al. 1997; Mammola et al. 2015; Bento et al. 2016; Lunghi et al. 2017; Mammola and Isaia 2018), therefore, sampling during different periods all year round was necessary to outline a more complete picture on species diversity and seasonal variations. An increase of fauna diversity was recorded

during spring, while lower values were detected in colder months. This is related to the climate variations and probably to the presence of organic resources brought in from the surface. However, for some species it is probably linked also to their reproductive activity or to the availability of potential preys (Kane 1975; Di Russo et al. 1997; Mammola and Isaia 2018). Fauna variations can also be related to seasonal precipitation changes (infiltrating waters also bring nutrients into the cave) (Bento et al. 2016).

Taxa responsible for the observed differences evidenced by One-Way ANOSIM were found especially in sampling areas most influenced by light and external climate variations. In fact, presence/absence of light is the environmental factor that has the greatest influence on the ground and on the wall fauna. In addition, temperature decreased and stabilized moving towards the inner areas influencing the ground fauna while relative humidity decreased moving towards the entrance influencing parietal fauna. Temperature and relative humidity in the inner part of the cave were more constant, moreover, inner sampling areas had also less trophic resources.

The high difference of Shannon diversity values between ground assemblages in A and H can be at least partly related to the influence of the cave entrance, but also to the different trophic resources and substrates. Area A was characterized only by calcite substratum and was located near the entrance, therefore more influenced by external climate changes and rich in decomposing vegetal debris. However, H was characterized by calcite, aragonite and quartz substratum and rich in vertebrate feces (rodents, bats and badger). Moreover, H was muddy, more humid and cold respect to A.

Conclusions

Research on subterranean-dwelling organisms has a long history of single-species focused or single-groups focused studies that rarely consider the subterranean realm as a three-dimensional environment. Our results confirm the first hypothesis, underlining that the environmental conditions seem to affect the occurrence and abundance of most taxa composing subterranean communities, irrespective of the fact that scientists classify them with forced categories among troglobionts or troglophiles/trogloxenes. Particularly, humidity and light levels seem to affect most organisms with some exceptions. At the same time, we cannot state that substrate mineralogical composition of both walls and ground seems to be a major determinant of subterranean communities within the same site as we detected low variability among the different microhabitats sampled; however, some organisms showed a preference for peculiar substrate typologies.

Caves are extreme and fragile environments that host unique ecosystems and fascinating creatures in a world still to be explored in detail. Conserving and preserving these habitats is increasingly important, given the amount of information that can be obtained from the studies of these environments. This study highlights the importance of fauna monitoring in caves for better understanding subterranean biodiversity and how species can be distributed in cave microhabitats. The main outcome of our results is the strong difference

that we recorded between the species assemblages occurring at the ground and wall levels and the variation occurring between ground microhabitats of different areas. On one hand, these results confirm the general idea of a gradient of variation occurring from the entrance toward inner areas, but on the other hand, they evidence that the dynamics of the walls can be very different from those occurring at the ground independent of the distance from the surface. These results can be a starting point for further researches directed to verify if variation occurring at the ground level reflects also a variation of environmental pressures that can influence adaptation of organisms towards underground habitats and for a broader study of subterranean environments as three-dimensional spaces.

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Supplementary material 1

Information on the study area

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Information on the study area.

Link: https://doi.org/10.3897/subtbiol.40.71805.suppl1

Supplementary material 2

Fauna observed in Baraccone Cave

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Fauna observed in Baraccone Cave.

Link: https://doi.org/10.3897/subtbiol.40.71805.suppl2

Supplementary material 3

Monthly temperature, relative humidity and light intensity in Baraccone Cave for each sampling area

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Monthly Temperature, Relative Humidity and Light Intensity in Baraccone Cave for each sampling area.

Link: https://doi.org/10.3897/subtbiol.40.71805.suppl3

Supplementary material 4

Richness and abundance of Baraccone Cave invertebrate fauna

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Richness and abundance of Baraccone Cave invertebrate fauna.

Link: https://doi.org/10.3897/subtbiol.40.71805.suppl4

Supplementary material 5

Percentage of minerals found in each sampling area

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Percentage of minerals found in each sampling area.

Link: https://doi.org/10.3897/subtbiol.40.71805.suppl5

Supplementary material 6

SIMPER Analysis

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Taxa responsible for the observed differences between faunal assemblages in different sampling areas.

Link: https://doi.org/10.3897/subtbiol.40.71805.suppl6