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## Exploring the homogeneity of terrestrial subterranean communities at a local spatial scale

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1 **Exploring the homogeneity of terrestrial subterranean communities at a local spatial**  
2 **scale**

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20 **Author contribution**

21 SM and MI conceived the study. SM, NC, and MI performed fieldwork. NC sorted specimens. MI  
22 identified spiders and harvestmen. DŽA identified millipedes. MZ identified centipedes. PMG  
23 identified coleopterans and other insect orders. MI and NC performed GIS analyses. SM performed  
24 statistical analysis and wrote the first draft of the paper. All authors contributed to the writing and  
25 approved the final submission.

26

27 **Running title**

28 Turnover in subterranean communities

29

30 **Statements**

31 Authors disclose any actual or potential conflict of interest.

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34

35 **ABSTRACT**

36

37 1. While caves are generally perceived as isolated habitats, at the local scale they are often  
38 interconnected via a network of fissures in the bedrock. Accordingly, caves in close proximity are  
39 expected to host the same, or very similar, animal communities.

40 2. We explored the extent to which subterranean arthropod communities are homogenous at a local  
41 spatial scale of less than 1 km<sup>2</sup>, and which cave-specific environmental features result in a departure  
42 from the expected homogeneous pattern. We approached this question by studying richness and  
43 turnover in terrestrial arthropod communities of 27 caves in a small karst massif in the Western Italian  
44 Alps.

45 3. Obligate subterranean species were homogeneously distributed among caves and were not  
46 influenced by seasonality. The only factor driving their presence and abundance was the distance  
47 from the cave entrance, with deeper caves yielding a greater diversity and abundance of species.

48 4. We observed significant spatial and temporal turnover in non-obligate subterranean species. In  
49 summer, there was a significant homogenization of the community and a more even distribution of  
50 non-obligate species among sites; in winter, these species were missing or found exclusively at greater  
51 depths, where environmental conditions were more stable. Furthermore, caves at lower elevations  
52 yielded, on average, a greater diversity and abundance of non-obligate species.

53 5. We demonstrated that the theoretical expectation of no turnover in community composition in caves  
54 in close proximity is not always met. Given that turnover can be mostly attributed to seasonal patterns  
55 and sampling depth, our findings have implications for planning sampling and monitoring activities  
56 in caves.

57

58 **Keywords:** Beta diversity; Community ecology; Pitfall traps; Spatial turnover; Subterranean biology;  
59 Temporal turnover

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63

## 64 INTRODUCTION

65 Ecologists and evolutionary biologists have frequently compared caves to islands (Culver, 1970;  
66 Snowman *et al.*, 2010; Esposito *et al.*, 2015; Fattorini *et al.*, 2016; Itescu, 2019; Mammola, 2019). In  
67 fact, from the perspective of a specialized subterranean organism, a cave is a patch of suitable habitat  
68 within a matrix of unsuitable habitats. It is understood that island-like properties of caves emerge  
69 mostly at broad geographical scales (Stoch & Galassi, 2010), wherever the extent of the study area is  
70 large enough to encompass geological discontinuities or other natural barriers limiting subterranean  
71 connectivity (see Fig. 2 in Mammola, 2019). For example, studies exploring the extent to which  
72 subterranean communities vary in their composition at continental scales have documented higher  
73 rates of turnover ( $\beta$ -diversity) than those typically observed in surface habitats (Zagmajster *et al.*,  
74 2014; Mammola *et al.*, 2019). Yet, it is understood that  $\beta$ -diversity patterns are strongly scale  
75 dependent (Jarzyna & Jetz, 2018), meaning that the “caves as islands” view rarely holds true at the  
76 local scale (Mammola, 2019).

77         According to a modern understanding of the subterranean realm, a cave is nothing but a  
78 human-accessible void in the ground. Subterranean organisms not only inhabit caves, but can thrive  
79 in a large breadth of lightless habitats including networks of fissures in the bedrock and shallow  
80 habitat pores not directly accessible to humans (Culver & Pipan, 2010; Giachino & Vailati, 2010;  
81 Mammola *et al.*, 2016; Pipan & Culver, 2017; Ficetola *et al.*, 2019). Indeed, we are starting to  
82 perceive caves as small “windows” opening to a vaster world, the subterranean one, allowing us to  
83 take a glimpse of what happens below our feet (Howarth, 1983; Uéno, 1987). If microcavernous  
84 habitats ensure subterranean habitat connectivity at the local scale, low values of  $\beta$ -diversity should  
85 be observed when studying biological communities of caves in close proximity. This pattern was  
86 recently confirmed by research exploring the spatial distribution of invertebrate species over the  
87 cross-section of the Trnovski Gozd massif in Slovenia, demonstrating a limited distinction between  
88 shallow (0–50 m) and deep (up to 800 m) cave communities within this karst massif (Trontelj *et al.*,  
89 2019).

90         The expectation of no community turnover among caves at restricted spatial scales is not,  
91 however, always met. Even when sampling caves in close proximity that theoretically should harbour  
92 the same fauna, slight or even conspicuous species turnover is observed. These may be the result of  
93 daily and seasonal changes in community composition (Lunghi *et al.*, 2017; Mammola *et al.*, 2017),  
94 of local habitat unsuitability due to a combination of specific environmental conditions (Jiménez-  
95 Valverde *et al.*, 2017), of competition dynamics that may lead to the exclusion of certain species from  
96 a given community (Mammola & Isaia, 2014), or even simply the consequence of imperfect detection  
97 (Ficetola *et al.*, 2018) when sampling such structurally-complex environments (Wynne *et al.*, 2018,

98 2019).

99 Here, we seek to understand the extent to which habitat connectivity determines a  
100 homogenization of subterranean biological communities at local scales, and which cave-specific  
101 environmental conditions may determine a departure from this expected pattern. We used a  
102 geographically restricted area, the cave system of Comba dell’Infernotto (Chesta & Elia, 2004), as a  
103 uniquely suited study site for this purpose, in that it hosts a great diversity of caves in an area of  
104 approximately 1 km<sup>2</sup>. These caves encompass a wide range of environmental conditions and host a  
105 rich and diversified fauna, with several specialized subterranean species. Therefore, this system is  
106 well-suited for exploring the ecological determinants of  $\alpha$ - and  $\beta$ -diversity patterns at the local scale.

107 Our null hypothesis was that community composition is the same in all caves within the  
108 considered subterranean system. In other words, we expected to observe comparable levels of  $\alpha$ -  
109 diversity in all caves, and  $\beta$ -diversity values close to zero in pairwise comparisons among caves. We  
110 hypothesize that departures from such theoretical expectations should be linked to seasonal patterns  
111 in community composition and/or due to a combination of unsuitable local environmental conditions.

112

## 113 **MATERIAL AND METHODS**

### 114 **Study area**

115 The study was carried out in the cave system of Comba dell’Infernotto (or Infernetto) (7.4° E, 44.2°  
116 N), in the municipality of Valdieri, Maritime Alps, Province of Cuneo, Piedmont, Italy (Figure 1).  
117 With more than 40 caves and mines currently documented, this small karst area yields the greatest  
118 number of subterranean sites in the province of Cuneo (Chesta & Elia, 2004). This area is included  
119 within the boundaries of the Site of Community Importance IT1160056 “Alpi Marittime” (European  
120 Habitat Directive 43/92).

121 For this study, we selected 27 subterranean sites (Fig. 1) within the area of Comba  
122 dell’Infernotto, with a maximum distance of 1 km between sites. Other caves in this system were not  
123 investigated due to access difficulties. We sampled both caves and old abandoned mines in the same  
124 area, ranging from 4 to 861 m in length, from 0.5 to 35 m<sup>2</sup> in the size of the cave entrance, and from  
125 1000 to 1200 m in the elevation of cave entrances (Table 1). All sites entrances were in a dense beech  
126 forest (*Fagus sylvatica* L.).

127

### 128 **Sampling design**

129 We sampled invertebrates in caves using pitfall traps (diameter 9 cm, volume 40 ml). To minimize  
130 impacts on invertebrate populations, only one pitfall trap per site was used. We installed traps at the

131 ground level away from accumulation of organic material. We covered each trap with a flat stone to  
132 shelter it from percolating water (Růžička, 1982). We filled traps with brine (supersaturated  
133 preserving solution of water and NaCl; Giachino & Vailati, 2010) and baited them with cheese. We  
134 acknowledge that the specific use of a bait, by exerting different attraction on different species, may  
135 bias the composition of the sampled arthropod assemblages. Nevertheless, this represented a  
136 homogeneous bias across the sampling, thus did not compromise our specific aims.

137 To evaluate seasonal patterns in community composition, we trapped invertebrates once in  
138 summer, from June to September 2017, and once in winter, from November 2017 to April 2018. We  
139 were forced to overextend the winter sampling period (6 vs 4 months in summer) due to a late snow  
140 melt.

141 We measured the linear distance from the cave entrance using an extendable tape. Moreover,  
142 we estimated the subjacency of each trap (i.e., the vertical distance from the surface; Mammola et al.,  
143 2017) using GIS, marking the position of the trap on available geological surveys of Chesta and Elia  
144 (2004) and plotting the latter in plan view on a georeferenced topographical map. We derived  
145 subjacency for traps in caves below 10 m in length by direct measurements. We derived total cave  
146 length and maximal subjacency of each cave from Chesta and Elia (2004), or measured these  
147 parameters directly in the field whenever this information was not available in speleological literature.  
148 We approximated the area of each cave entrance by multiplying its base by its height. For each cave,  
149 we also recorded by eye the presence of percolating water in the two seasons.

150 We measured temperature at each pitfall trap with EL-USB-2+ dataloggers (Lascar  
151 Electronics, Salisbury, United Kingdom), programmed to record a temperature measure (accuracy of  
152  $\pm 0.5^{\circ}\text{C}$ ) every six hours (0:00, 6:00, 12:00, 18:00) throughout the two sampling periods.

153

#### 154 **Species identification and ecological classification**

155 Specimens were identified to the lowest possible taxonomic rank by experts of the different groups  
156 (see author contribution for details). For the purpose of the analyses, we assigned each species to one  
157 of the two major ecological groups commonly used to explore subterranean diversity patterns (e.g.,  
158 Novak et al., 2012; Niemiller & Zigler, 2013; Trontelj et al., 2019): obligate subterranean species  
159 ('troglobionts'; Sket, 2008; Trajano & de Carvalho, 2017), and non-obligates ('non-troglobionts';  
160 Novak et al., 2012) (Supplementary material Appendix S1). We based this classification mainly on  
161 the expert opinion of different taxonomists involved in this work, but also using data and  
162 morphological traits associated with subterranean life (loss of eyes and body pigment) from the  
163 literature.

164

## 165 **Statistical analyses**

166 Data supporting the study are deposited in figshare (doi: 10.6084/m9.figshare.12005934 **Note that**  
167 **the hyperlink will become available upon acceptance**). All analysis were conducted in R (R Core  
168 Team, 2018). We used Poisson generalized linear models (GLMs) to explore factors driving alpha  
169 diversity ( $\alpha$ ) and the abundance of obligate and non-obligate subterranean species in our study sites.  
170 We followed the general protocol by Zuur & Ieno (2016) for conducting regression-type analyses,  
171 whereby we:

- 172 i) performed data exploration on the initial dataset, aiming to verify the presence of outliers, remove  
173 collinear predictors, and detect potential interactions among predictors (Zuur *et al.*, 2009);
- 174 ii) constructed Poisson GLMs to test for significant relationships between the dependent variables  
175 and our explanatory predictors. We included potential interactions among predictors only if these  
176 were detected during data exploration (see point i);
- 177 iii) performed a step-wise model selection on each model with the ‘*step1*’ R function, in order to  
178 obtain a Minimum Adequate Model (MAM) for each of the dependent variables, minimizing the  
179 Aikaike Information Criterion (AIC) value; and
- 180 iv) validated each model with the aid of the R package ‘*performance*’ (Lüdecke *et al.*, 2020). In this  
181 phase, we tested each model for over-dispersion, and switched to a negative binomial distribution if  
182 models were over-dispersed (Gelman & Hill, 2007).

183 To investigate spatial and temporal turnover in community composition, we estimated  
184 pairwise  $\beta$ -diversity among caves and between the two seasons using the framework proposed by  
185 Carvalho *et al.* (2012) and Cardoso *et al.* (2014), whereby  $\beta$  is expressed as:

$$186 \quad \beta_{\text{total}} = \beta_{\text{replacement}} + \beta_{\text{richness}}$$

187  $\beta_{\text{richness}}$  is the turnover in community composition explained by species loss/gain alone, and  $\beta_{\text{replacement}}$   
188 is turnover in community composition explained by replacement of species alone. Thus, this  
189 framework allowed us to distinguish between these two processes (loss/gain vs replacement)  
190 underlying changes in community composition.  $\beta$ -diversity was calculated with the function ‘*beta*’  
191 in the R package ‘*BAT*’ (Cardoso *et al.*, 2015, 2020), using species abundances as input data. We  
192 graphically explored changes in  $\beta$ -diversity values among caves in the two sampling seasons using  
193 density plots. We used a Wilcoxon rank sum test with continuity correction to test if median  $\beta_{\text{replacement}}$   
194 and  $\beta_{\text{richness}}$  values were significantly different between the two seasons for both obligate and non-  
195 obligate subterranean species.

196

## 197 **RESULTS**

### 198 **$\alpha$ -diversity and abundance of species**

199 In total, we trapped 3,026 specimens belonging to 11 arthropod orders, 12 molluscs, and 1  
200 nematode (Supplementary material Appendix S1). Dipterans, both adults and larvae, were the most  
201 abundant organisms in our traps (1,673 specimens), followed by coleopterans (616), diplopods (308),  
202 and acari (208). The pitfall trap placed in cave 11 (a small cave near Barôn Litrôn) was lost in summer,  
203 and therefore this site was excluded from the analysis.

204 Due to malfunctioning, half of the dataloggers did not measure temperature correctly  
205 throughout the sampling period. Since the removal of these missing data would have halved the sample  
206 size of the dataset, and considering that cave temperature was significantly associated with sampling  
207 season, we did not include any temperature-derived variable in the regression analysis. Furthermore,  
208 we found that the categorical variable “presence of percolating water” was significantly associated  
209 with cave length. Cave length, cave maximum subjacency, and pitfall trap subjacency were also  
210 collinear with the distance of the pitfall from the entrance (all Pearson  $r > 0.7$ ). Therefore, we  
211 included only the distance of the pitfall from the entrance, cave elevation, cave entrance size, and  
212 sampling season in the initial regression models. We successfully fitted and validated models for all  
213 four dependent variables. Poisson  $\alpha$ -diversity models were not over-dispersed [Obligates: Dispersion  
214 Ratio (DR)= 0.78; Pearson’s  $\chi^2= 36.57$ ;  $p= 0.86$ ; Non-obligates: DR= 1.21;  $\chi^2= 55.56$ ;  $p= 0.16$ ].  
215 Conversely, abundance Poisson GLMs were significantly over-dispersed (Obligates: DR= 12.04;  $\chi^2=$   
216 565.73;  $p< 0.001$ ; Non-obligates: DR= 35.74;  $\chi^2= 1643.84$ ;  $p< 0.001$ ) and thus, we opted for a  
217 negative binomial distribution to model abundance. Results of model selection are presented in Table  
218 2, while estimated regression coefficients and p-values are given in Table 3.  $\alpha$ -diversity (Figure 2a)  
219 and abundance of obligate subterranean species (Figure 2c) were best explained by the distance of  
220 the pitfall trap from the entrance; other variables had no significant effects and were dropped during  
221 model selection (Table 2).  $\alpha$ -diversity (Figure 2b) and abundance (Figure 2d) of non-obligate species  
222 were best explained by the distance of the pitfall trap from the entrance in interaction with the  
223 sampling season. Specifically, we observed higher  $\alpha$ -diversity and abundance at lower depth in  
224 summer, and at greater depth in winter. Moreover, abundance values were significantly lower in  
225 winter (Figure 2e, 2f). Finally, there was an effect of elevation on  $\alpha$ -diversity and abundance of non-  
226 obligate species, with higher values at lower elevations (Table 2).

227

### 228 **$\beta$ -diversity**



229 Results of  $\beta$ -diversity analysis are illustrated in Figure 3. In obligate subterranean species, the density  
230 of  $\beta_{\text{Total}}$  values across sites showed a bimodal concentration around 0 and 1, indicating the existence  
231 of two types of communities;  $\beta_{\text{Total}}$  values close to zero corresponded to the comparisons between  
232 pitfall traps with similar depth and environmental conditions, and values close to 1 to the comparisons  
233 between pitfall traps installed close to versus far from the entrance. This bimodal distribution was  
234 more pronounced in summer than in winter (Figure 3a). Nevertheless,  $\beta_{\text{Replacement}}$  values were mostly  
235 concentrated towards zero (Figure 3b), indicating that community turnover in obligate-species was  
236 not due to a difference in species composition. There was a significant difference between  $\beta_{\text{Replacement}}$   
237 values for summer and winter ( $W= 235950$ ,  $p< 0.001$ ), whereas no significant differences between  
238  $\beta_{\text{Richness}}$  values in the two seasons were observed ( $W = 9418.5$ ;  $p= 0.22$ ; Figure 3c).

239 We observed pronounced seasonal variations in the distribution of non-obligate  
240 arthropod communities across sites (Figure 3d). In summer, there was a homogenization of values for  
241 non-obligate species, indicating a more even distribution of species among sites (Figure 3f, 3g). The  
242 richness component of  $\beta$ -diversity was found to fluctuate more across sites. Summer values were  
243 mostly concentrated either between 0.25 or between 0.75, whereas in winter the highest density of  
244 values was between 0.75 and 1. Observed differences between the two seasons were highly significant  
245 for  $\beta_{\text{Richness}}$  ( $W= 156080$ ;  $p<0.001$ ) and approached significant for  $\beta_{\text{Replacement}}$  ( $W= 207350$ ,  $p= 0.05$ )

246

## 247 **Discussion**

248 Our expectation that community composition should be equal across the caves of the cave system of  
249 Comba dell’Infernotto was not entirely met. While a pool of nine obligate subterranean species was  
250 consistently found in most caves (Appendix S1), we observed significant turnovers in species  
251 composition between external and internal sectors of caves. When considering non-obligate  
252 subterranean species, there was also substantial turnover in richness and community composition,  
253 which was mostly attributable to seasonality.

254 The main driver of  $\alpha$ -diversity and abundance was the distance of the pitfall trap from the  
255 entrance. Pitfalls at greater distance from the cave entrance yielded greater diversity and abundance  
256 of obligate subterranean species, independently from the sampling season. In our study site, a higher  
257 distance from the entrance (corresponding to greater values of subjacency) ensured the maintenance  
258 of more stable environmental conditions, including higher relative humidity and constant temperature  
259 (Figure 4). These are the optimal microclimatic conditions for the specialized subterranean fauna, and  
260 indeed subterranean habitats with greater environmental stability often support a more diverse and  
261 abundant subterranean arthropod community (Tobin *et al.*, 2013; Bento *et al.*, 2016; Mammola *et al.*,  
262 2017).

263 In a few cases, obligate subterranean species also occurred in close proximity to the surface,  
264 although in low numbers. This was not an unexpected pattern, as it has been shown that obligate  
265 subterranean species within different cave systems may present a bimodal distribution, with peaks in  
266 richness and abundance in the nearby of the surface and deeper inside caves (Novak *et al.*, 2012;  
267 Kozel *et al.*, 2019). Yet, in the system of caves system of Comba dell'Infernotto, the predicted and  
268 observed abundance in the vicinity of the surface was close to zero (Figure 2d), which explains the  
269 non-significance of this pattern.

270 While obligate subterranean species were substantially unaffected by seasonal dynamics, the  
271 occurrence of non-obligate species varied significantly between winter and summer in relation to the  
272 distance of the sampling plot from the cave entrance. In winter, we mostly collected non-obligate  
273 species far from the cave entrance, where environmental conditions are relatively stable through the  
274 year and where temperatures never drop below the freezing point (Novak *et al.*, 2014) (Figure 4).  
275 Winter abundance of accidental species was in general low, following the general reduction of activity  
276 in surface habitats in winter and the typical winter dormancy pattern in most arthropods. In winter,  
277 we collected the few non-obligate species in pitfall traps installed far from the entrance; these were  
278 mostly species overwintering in caves – a seasonal behaviour documented in several taxa (Chelini *et*  
279 *al.*, 2011; Lipovšek *et al.*, 2016, 2019; Balogová *et al.*, 2017; Mammola & Isaia, 2018).

280 On the other hand, in summer we observed a massive colonization of the shallow sectors of  
281 caves by external species. The importance of these species for the ecology of subterranean ecosystems  
282 is noteworthy, as they provide significant inputs of carbon into the system (Novak *et al.*, 2013).  
283 Concomitantly, there was also a migration of non-obligate species from deep toward shallow cave  
284 sectors. This pattern is often observed when studying spatial and temporal dynamics of temperate  
285 cave invertebrate communities (Tobin *et al.*, 2013; Lunghi *et al.*, 2017; Mammola & Isaia, 2018). In  
286 our case, the most abundant species involved in this migration was the predatory beetle *Sphodropsis*  
287 *ghiliani* (Schaum). In summer, this species seemingly moves into warmer, entrance sectors where the  
288 availability of prey is usually higher (e.g., Novak *et al.*, 2013; Mammola & Isaia, 2018). The same  
289 migration pattern has been also observed for the same species in a cave of the North-Western Italian  
290 Alps (Mammola *et al.*, 2015).

291 We also found higher richness and abundance of non-obligate subterranean species at lower  
292 elevations (Table 3). In the study area, the elevation difference determines a change in the average  
293 temperature of about 1 °C between lower (1000 m) and higher (1200 m) caves, which may in turn  
294 drive the observed biological pattern. Alternatively, the explanation for this relationship may be  
295 linked to gravitation-assisted patterns in the distribution of species. Trontelj *et al.* (2019) documented  
296 how the distribution of animals within karst massif can be influenced by gravity. They argued that

297 “[...] *animals from a wider area of shallow subterranean spaces can be funneled towards deeper*  
298 *parts of vertical caves by flowing water or gravity.*” This may explain why, including our case, caves  
299 at lower elevations yielded a greater diversity and abundance of species.

300

### 301 **Conclusions**

302 Our results indicate that at a spatial scale of less than 1 km<sup>2</sup>, there is a single pool of obligate species  
303 that permanently occurs in the subterranean domain, and a pool of non-obligate species whose  
304 presence in the subterranean domain is more influenced by seasonality. This finding corroborates the  
305 idea that caves in close proximity are often interconnected by networks of subterranean habitat spaces  
306 (Giachino & Vailati, 2010; Trontelj *et al.*, 2019; Mammola, 2020). Yet, the theoretical expectation of  
307 no turnover in the community composition of these caves is not always met. Turnover is primarily  
308 attributable to active migrations of fauna from deep to superficial habitats, and vice versa. From a  
309 methodological perspective, our results imply that sampling a few caves across different seasons  
310 should provide a general indication of the resident subterranean fauna of a certain area, having in  
311 mind that a large depth is essential for sampling specialized subterranean taxa and that a greater  
312 diversity is generally expected at lower elevations within a karst massif.

313

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319

### 320 **Supplementary material**

321 **Appendix S1.** List of species recorded in the system of caves of Maissa.

322 **Literature cited**

323

- 324 Balogová, M., Jelić, D., Kyselová, M. & Uhrin, M. (2017) Subterranean systems provide a suitable  
325 overwintering habitat for *Salamandra salamandra*. *International Journal of Speleology*, **46**, 321–  
326 329.
- 327 Bento, D. de M., Ferreira, R.L., Prous, X., Souza-Silva, M., Bellini, B.C. & Vasconcellos, A. (2016)  
328 Seasonal variations in cave invertebrate communities in the semiarid Caatinga, Brazil. *Journal of*  
329 *Cave and Karst Studies*, **78**, 61–71.
- 330 Cardoso, P., Mammola, S., Rigal, F. & Carvalho, J.C. (2020) BAT: Biodiversity Assessment Tools.
- 331 Cardoso, P., Rigal, F. & Carvalho, J.C. (2015) BAT – Biodiversity Assessment Tools, an R package  
332 for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity.  
333 *Methods in Ecology and Evolution*, **6**, 232–236.
- 334 Cardoso, P., Rigal, F., Carvalho, J.C., Fortelius, M., Borges, P.A. V., Podani, J., *et al.* (2014)  
335 Partitioning taxon, phylogenetic and functional beta diversity into replacement and richness  
336 difference components. *Journal of Biogeography*, **41**, 749–761.
- 337 Carvalho, J.C., Cardoso, P. & Gomes, P. (2012) Determining the relative roles of species  
338 replacement and species richness differences in generating beta-diversity patterns. *Global Ecology*  
339 *and Biogeography*, **21**, 760–771.
- 340 Chelini, M.C., Willemart, R.H. & Gnaspini, P. (2011) Caves as a Winter Refuge by a Neotropical  
341 Harvestman (Arachnida, Opiliones). *Journal of Insect Behavior*, **24**, 393–398.
- 342 Chesta, M. & Elia, E. (2004) L’Infernotto e le grotte miniere della Maissa. *Mondo Ipogeo*,  
343 *Bollettino del Gruppo Speleologico Alpi Marittime*, **16**, 62–77.
- 344 Culver, D.C. (1970) Analysis of Simple Cave Communities I. Caves as Islands. *Evolution*, **24**, 463–  
345 474.
- 346 Culver, D.C. & Pipan, T. (2010) Superficial subterranean habitats – gateway to the subterranean  
347 realm? *Cave and Karst Science*, **35**.
- 348 Esposito, L.A., Bloom, T., Caicedo-Quiroga, L., Alicea-Serrano, A.M., Sánchez-Ruiz, J.A., May-  
349 Collado, L.J., *et al.* (2015) Islands within islands: Diversification of tailless whip spiders  
350 (*Amblypygi*, *Phrynus*) in Caribbean caves. *Molecular Phylogenetics and Evolution*, **93**, 107–117.
- 351 Fattorini, S., Borges, P.A.V., Fiasca, B. & Galassi, D.M.P. (2016) Trapped in the web of water:  
352 Groundwater-fed springs are island-like ecosystems for the meiofauna. *Ecology and Evolution*, **6**,  
353 8389–8401.
- 354 Ficetola, G.F., Barzaghi, B., Melotto, A., Muraro, M., Lunghi, E., Canedoli, C., *et al.* (2018) N-  
355 mixture models reliably estimate the abundance of small vertebrates. *Scientific Reports*, **8**, 10357.
- 356 Ficetola, G.F., Canedoli, C. & Stoch, F. (2019) The Racovitza impediment and the hidden  
357 biodiversity of unexplored environments. *Conservation Biology*, **33**, 214–216.
- 358 Gelman, A. & Hill, J. (2007) *Data analysis using regression and multilevel/hierarchical models*.

- 359 Cambridge University Press, Cambridge.
- 360 Giachino, P.M. & Vailati, D. (2010) *The subterranean environment. Hypogean life, concepts and*  
361 *collecting techniques*. WBA Handbo. Verona.
- 362 Howarth, F.G. (1983) Ecology of Cave Arthropods. *Annual Review of Entomology*, **28**, 365–389.
- 363 Itescu, Y. (2019) Are island-like systems biologically similar to islands? A review of the evidence.  
364 *Ecography*, **42**, 1298–1314.
- 365 Jarzyna, M.A. & Jetz, W. (2018) Taxonomic and functional diversity change is scale dependent.  
366 *Nature Communications*, **9**, 2565.
- 367 Jiménez-Valverde, A., Sendra, A., Garay, P. & Reboleira, A.S.P.S. (2017) Energy and speleogenesis:  
368 Key determinants of terrestrial species richness in caves. *Ecology and Evolution*, **7**, 10207–10215.
- 369 Kozel, P., Pipan, T., Mammola, S., Culver, D.C. & Novak, T. (2019) Distributional dynamics of a  
370 specialized subterranean community oppose the classical understanding of the preferred  
371 subterranean habitats. *Invertebrate Biology*, **138**, e12254.
- 372 Lipovšek, S., Leitinger, G., Janžekovič, F., Kozel, P., Dariš, B., Perc, M., *et al.* (2019) Towards  
373 understanding partial adaptation to the subterranean habitat in the European cave spider, *Meta*  
374 *menardi*: An ecocytological approach. *Scientific Reports*, **9**, 9121.
- 375 Lipovšek, S., Novak, T., Janžekovič, F., Weiland, N. & Leitinger, G. (2016) Malpighian tubule cells  
376 in overwintering cave crickets *Troglophilus cavicola* (Kollar, 1833) and *T. neglectus* Krauss, 1879  
377 (Rhaphidophoridae, Ensifera). *PLoS ONE*, **11**, e0158598.
- 378 Lüdecke, D., Makowski, D. & Waggoner, P. (2020) performance: Assessment of Regression Models  
379 Performance.
- 380 Lunghi, E., Manenti, R. & Ficetola, G.F. (2017) Cave features, seasonality and subterranean  
381 distribution of non-obligate cave dwellers. *PeerJ*, **5**, e3169.
- 382 Mammola, S. (2019) Finding answers in the dark: caves as models in ecology fifty years after  
383 Poulson and White. *Ecography*, **42**, 1331–1351.
- 384 Mammola, S. (2020) On deepest caves, extreme habitats, and ecological superlatives. *Trends in*  
385 *Ecology & Evolution*.
- 386 Mammola, S., Cardoso, P., Angyal, D., Balázs, G., Blick, T., Brustel, H., *et al.* (2019) Local- versus  
387 broad-scale environmental drivers of continental  $\beta$ -diversity patterns in subterranean spider  
388 communities across Europe. *Proceedings of the Royal Society B: Biological Sciences*, **286**,  
389 20191579.
- 390 Mammola, S., Giachino, P.M., Piano, E., Jones, A., Barberis, M., Badino, G., *et al.* (2016) Ecology  
391 and sampling techniques of an understudied subterranean habitat: the Milieu Souterrain Superficiel  
392 (MSS). *The Science of Nature*.
- 393 Mammola, S. & Isaia, M. (2014) Niche differentiation in *Meta bourneti* and *M. menard* (Araneae,  
394 Tetragnathidae) with notes on the life history. *International Journal of Speleology*, **43**, 343–353.
- 395 Mammola, S. & Isaia, M. (2018) Day-night and seasonal variations of a subterranean invertebrate

- 396 community in the twilight zone. *Subterranean Biology*, **27**, 31–51.
- 397 Mammola, S., Piano, E., Giachino, P.M. & Isaia, M. (2015) Seasonal dynamics and micro-climatic  
398 preference of two alpine endemic hypogean beetles. *International Journal of Speleology*, **44**, 239–  
399 249.
- 400 Mammola, S., Piano, E., Giachino, P.M. & Isaia, M. (2017) An ecological survey of the invertebrate  
401 community at the epigeal/hypogean interface. *Subterranean Biology*, **24**, 27–52.
- 402 Nagelkerke, N.J.D. (1991) A note on a general definition of the coefficient of determination.  
403 *Biometrika*, **78**, 691–692.
- 404 Niemiller, M.L. & Zigler, K.S. (2013) Patterns of cave biodiversity and endemism in the  
405 Appalachians and Interior Plateau of Tennessee, USA. *PLOS ONE*, **8**, e64177.
- 406 Novak, T., Janžekovič, F. & Lipovšek, S. (2013) Contribution of non-troglobiotic terrestrial  
407 invertebrates to carbon input in hypogean habitats. *Acta Carsologica*, **42**, 301–309.
- 408 Novak, T., Perc, M., Lipovšek, S. & Janžekovič, F. (2012) Duality of terrestrial subterranean fauna.  
409 *International Journal of Speleology*, **41**, 181–188.
- 410 Novak, T., Šajna, N., Antolinc, E., Lipovšek, S., Devetak, D. & Janžekovič, F. (2014) Cold  
411 tolerance in terrestrial invertebrates inhabiting subterranean habitats. *International Journal of*  
412 *Speleology*, **43**, 265–272.
- 413 Pipan, T. & Culver, D.C. (2017) The unity and diversity of the subterranean realm with respect to  
414 invertebrate body size. *Journal of Cave and Karst Studies*, **79**, 1–9.
- 415 R Core Team. (2018) R: A Language and Environment for Statistical Computing.
- 416 Růžička, V. (1982) Modifications to improve the efficiency of pitfall traps. *Newsletter of the British*  
417 *Arachnological Society*, **34**, 2–4.
- 418 Sket, B. (2008) Can we agree on an ecological classification of subterranean animals? *Journal of*  
419 *Natural History*, **42**, 1549–1563.
- 420 Snowman, C. V., Zigler, K.S. & Hedin, M. (2010) Caves as islands: mitochondrial phylogeography  
421 of the cave-obligate spider species *Nesticus barri* (Araneae: Nesticidae). *Journal of Arachnology*,  
422 **38**, 49–56.
- 423 Stoch, F. & Galassi, D.M.P. (2010) Stygobiotic crustacean species richness: a question of numbers,  
424 a matter of scale. *Hydrobiologia*, **653**, 217–234.
- 425 Tobin, B.W., Hutchins, B.T. & Schwartz, B.F. (2013) Spatial and temporal changes in invertebrate  
426 assemblage structure from the entrance to deep-cave zone of a temperate marble cave. *International*  
427 *Journal of Speleology*, **42**, 203–214.
- 428 Trajano, E. & Carvalho, M.R. de. (2017) Towards a biologically meaningful classification of  
429 subterranean organisms: A critical analysis of the schiner-racovitza system from a historical  
430 perspective, difficulties of its application and implications for conservation. *Subterranean Biology*,  
431 **22**, 1–26.
- 432 Trontelj, P., Borko, Š. & Delić, T. (2019) Testing the uniqueness of deep terrestrial life. *Scientific*

- 433 *Reports*, **9**, 15188.
- 434 Uéno, S.-I. (1987) The derivation of terrestrial cave animals. *Zoological Science*, **4**, 593–606.
- 435 Wynne, J.J., Howarth, F.G., Sommer, S. & Dickson, B.G. (2019) Fifty years of cave arthropod  
436 sampling: Techniques and best practices. *International Journal of Speleology*, **48**, 33–48.
- 437 Wynne, J.J., Sommer, S., Howarth, F.G., Dickson, B.G. & Voyles, K.D. (2018) Capturing arthropod  
438 diversity in complex cave systems. *Diversity and Distributions*, **24**, 1478–1491.
- 439 Zigmajster, M., Eme, D., Fišer, C., Galassi, D., Marmonier, P., Stoch, F., *et al.* (2014) Geographic  
440 variation in range size and beta diversity of groundwater crustaceans: Insights from habitats with  
441 low thermal seasonality. *Global Ecology and Biogeography*, **23**, 1135–1145.
- 442 Zuur, A.F. & Ieno, E.N. (2016) A protocol for conducting and presenting results of regression-type  
443 analyses. *Methods in Ecology and Evolution*, **7**, 636–645.
- 444 Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2009) A protocol for data exploration to avoid common  
445 statistical problems. *Methods in Ecology and Evolution*, **1**, 3–14.
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## 447 TABLES &amp; FIGURES CAPTIONS

448 Table 1. Main features of the investigated caves.

Code (Fig.1)	Cave name	Cadaster code	Type	Elevation [m]	Pitfall distance from entrance [m]	Pitfall subjacency [m]	Total length [m]	Depth [m]	Entrance size [m <sup>2</sup> ]	Subjacency [m]	Longitude (°)	Latitude (°)
1	Maissa 1	1209 Pi	Natural	1186	16	16	37	-9	10	16	7.4050	44.2615
2	Topalinda – Maissa 2	1210 Pi	Natural	1179	36	30	334	-63	20	83	7.4052	44.2615
3	Maissa 16	1221 Pi	Natural	1169	4	7	12	6	4	3	7.4054	44.2615
4	Grotta dell'Ola – Maissa 23	1228 Pi	Natural	1105	4.5	2	6	-2	2.8	2	7.4060	44.2627
5	Grotta Diana – Maissa 22	1227 Pi	Natural	1048	19.1	14	39	-11	5	18	7.4061	44.2627
6	Small cave near Grotta Diana	n.c.	Natural	1111	3.1	8	4	0	2.7	8	7.4065	44.2622
7	Maissa 21	1226 Pi	Natural	1145	3	4	6	-2	2	4	7.4168	44.2614
8	Maissa 9	1217 Pi	Natural	1085	10.3	7	11	-1	2.5	7	7.4084	44.2617
9	Maissa 8	1216 Pi	Natural	1077	6.4	7	6	-2	1.2	9	7.4084	44.2618
10	Maissa 7	1215 Pi	Natural	1071	35	13	29	-11	1.3	13	7.4086	44.2618
11	Small cave near Baron Litron	n.c.	Natural	1063	5.5	3	10	-1	12	5	7.4093	44.2616
12	Baròn Litròn – Maissa 6	1214 Pi	Natural/Mine	1063	16	38	861	-59	10	75	7.4093	44.2616
13	Sweet Inny – Maissa 10	1218 Pi	Natural	1045	14.3	13	116	-30	35	35	7.4098	44.2615
14	Small cave near Maissa 12	n.c.	Natural	1027	12	6	12	-7	1.5	6	7.4099	44.2613
15	Small mine shaft near Maissa 11	n.c.	Mine	1038	2.5	1	4	-1	0.5	1	7.4102	44.2617
16	Small cave near Maissa 11	n.c.	Natural	1051	3.5	2	5	0	26	1	7.4104	44.2613
17	Maissa 12	1219 Pi	Natural	1090	13.3	12	92	-20	2.6	14	7.4106	44.2613
18	Small cave near Maissa 10	n.c.	Natural	1037	3.5	2	8	3	3	2	7.4105	44.2617
19	Maissa 11	n.c.	Natural	1054	6.8	7	37	-20	0.8	22	7.4104	44.2613
20	Maissa 13	n.c.	Natural	1023	22	5	20	-4	3	5	7.4113	44.2619
21	Grotta dei Morti	1054 Pi	Natural	1023	19	15	84	-16	10	15	7.4131	44.2611
22	Mineshaft near Grotta Infernotto Superiore	n.c.	Mine	1041	5.5	4	6	-1	4.2	4	7.4113	22.2605
23	Grotta Infernotto Superiore	1055 Pi	Natural	1041	6.1	4	19	-1	2.5	12	7.4130	44.2609
24	Maissa 31	n.c.	Natural	1181	3.4	3	7	-2	3	3	7.4148	44.2580
25	Maissa 32	n.c.	Natural	1177	8.2	3	10	4	2	3	7.4148	44.2580
26	Natural cave “Salamandre”	n.c.	Natural	1100	3.6	4	7	-2	2.5	4	7.4144	44.2580
27	Natural cave “Salamandre Superiore”	n.c.	Natural	1110	2	5	10	2	1.5	5	7.4144	44.2580

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450 n.c. = mines or caves not included in the speleological cadaster; subjacency = vertical distance from  
 451 the surface; Mammola et al. 2017)

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455 **Table 2.** Results of model selection. For each dependent variable, models are listed from the least to  
 456 the most supported. AIC = Akaike Information Criterion.

<b>Dependent variable</b>	<b>Model structure</b>	<b>AIC</b>
Richness of obligate subterranean species ( $\alpha$ )	Pitfall depth + Season + Elevation + Entrance size	129.24
	Pitfall depth + Elevation + Entrance size	127.46
	Pitfall depth + Elevation	126.17
	Pitfall depth	125.18
Abundance of obligate subterranean species	Pitfall depth + Season + Elevation + Entrance size	264.60
	Pitfall depth + Elevation + Entrance size	262.62
	Pitfall depth + Elevation	261.17
	Pitfall depth	259.75
Richness of non-obligate species ( $\alpha$ )	Pitfall depth * Season + Elevation + Entrance size	227.16
	Pitfall depth * Season + Elevation	225.15
Abundance of non-obligate species	Pitfall depth * Season + Elevation + Entrance size	437.80
	Pitfall depth * Season + Elevation	435.82

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470 **Table 3.** Estimated regression parameters in the Minimum Adequate Models.  $R^2$  = Nagelkerke's  
 471 pseudo- $R^2$  (Nagelkerke, 1991).

Dependent variable	Variable	Estimated $\beta \pm$ S.E.	<i>p</i> -value	$R^2$
Richness of Troglobionts ( $\alpha$ )	Intercept	$-1.98 \pm 0.50$	-	0.50
	Distance from the entrance	$0.85 \pm 0.18$	<0.001	
Abundance of Troglobionts	Intercept	$-3.10 \pm 0.69$	-	0.70
	Distance from the entrance	$1.98 \pm 0.28$	<0.001	
Richness of Troglophiles ( $\alpha$ )	Intercept	$8.66 \pm 1.54$	-	0.87
	Distance from the entrance	$-0.44 \pm 0.12$	<0.001	
	Season (Winter)	$-3.12 \pm 0.51$	<0.001	
	Distance from the entrance * Season	$0.97 \pm 0.21$	<0.001	
	Elevation	$-0.01 \pm 0.01$	<0.001	
Abundance of Troglophiles	Intercept	$11.93 \pm 2.82$	-	0.91
	Pitfall depth	$-0.38 \pm 0.24$	0.110	
	Season (Winter)	$-6.34 \pm 0.88$	<0.001	
	Distance from the entrance * Season	$1.54 \pm 0.34$	<0.001	
	Elevation	$-0.01 \pm 0.01$	0.015	

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474 **Figure 1. Map of the study area.** Dots show the distribution of the sampled caves on the left slope  
475 of the Comba dell’Infernotto (municipality of Valdieri, Province of Cuneo, Italy). Photographs  
476 illustrate the morphology of some examples of cave entrances.

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478 **Figure 2. Drivers of richness and abundance of species in the system of caves of Comba**  
479 **dell’Infernotto.** Predicted relationships between the richness and abundance of obligate (a, b) and  
480 non-obligate (c, d) species and the depth at which the pitfall trap was placed in each cave, based on  
481 the results of regression analysis. Note that for non-obligate species, there was a significant  
482 interaction between the depth of the pitfall trap and the sampling season; therefore, the predicted  
483 relationship is shown separately for each season. In all predictions of non-obligate species, elevation  
484 is set at the mean values. Black lines are predicted values, while grey surfaces are 95% confidence  
485 intervals.

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487 **Figure 3. Community turnover in the system of caves of Comba dell’Infernotto.** Density of  $\beta$ -  
488 diversity values for obligate (a, b, c) and non-obligate (d, e, f) terrestrial arthropod communities in  
489 winter and summer samples. Total  $\beta$ -diversity is split in two components:  $\beta_{\text{richness}}$  is the turnover in  
490 community composition explained by species loss/gain alone, and  $\beta_{\text{replacement}}$  is turnover in community  
491 composition explained by replacement of species alone.

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493 **Figure 4. Example of cave microclimate in the system of caves of Comba dell’Infernotto.** Daily  
494 temperature was registered by two dataloggers, one placed at a depth of 36 m in Maissa 2 and one at  
495 4.5 m in Maissa 23.