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Exploring the homogeneity of terrestrial subterranean communities at a local spatial 1 2 scale 3 Stefano Mammola^{1,*}, Nicolò Chiappetta², Pier Mauro Giachino³, Dragan Ž. Antić⁴, Marzio 4 Zapparoli⁵, Marco Isaia^{2,**} 5 6 7 1. Molecular Ecology Group (MEG), Water Research Institute, National Research Council of Italy (CNR-8 IRSA), Verbania Pallanza, Italy 9 2. Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy 10 3. Word Biodiversity Association Onlus, Verona, Italy 11 4. University of Belgrade - Faculty of Biology, Institute of Zoology, Belgrade, Serbia 12 5. Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Tuscia University, 13 Viterbo, Italy 14 15 * corresponding author: stefano.mammola@cnr.it ** corresponding author: marco.isaia@unito.it 16 17 18 19 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.

- 33
- 34
- 35 ABSTRACT

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.

2. We explored the extent to which subterranean arthropod communities are homogenous at a local
spatial scale of less than 1 km², and which cave-specific environmental features result in a departure
from the expected homogeneous pattern. We approached this question by studying richness and
turnover in terrestrial arthropod communities of 27 caves in a small karst massif in the Western Italian
Alps.

3. Obligate subterranean species were homogeneously distributed among caves and were not
influenced by seasonality. The only factor driving their presence and abundance was the distance
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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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 within a matrix of unsuitable habitats. It is understood that island-like properties of caves emerge 68 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 (β-diversity) than those typically observed in surface habitats (Zagmajster et al., 74 2014; Mammola *et al.*, 2019). Yet, it is understood that β -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 β-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 approximately 1 km². These caves encompass a wide range of environmental conditions and host a 104 105 rich and diversified fauna, with several specialized subterranean species. Therefore, this system is 106 well-suited for exploring the ecological determinants of α - and β -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 α -109 diversity in all caves, and β -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

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

For this study, we selected 27 subterranean sites (Fig. 1) within the area of Comba dell'Infernotto, with a maximum distance of 1 km between sites. Other caves in this system were not investigated due to access difficulties. We sampled both caves and old abandoned mines in the same area, ranging from 4 to 861 m in length, from 0.5 to 35 m² in the size of the cave entrance, and from 1000 to 1200 m in the elevation of cave entrances (Table 1). All sites entrances were in a dense beech forest (*Fagus sylvatica* L.).

127

128 Sampling design

We sampled invertebrates in caves using pitfall traps (diameter 9 cm, volume 40 ml). To minimize 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.

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

141 We measured the linear distance from the cave entrance using an extendable tape. Moreover, we estimated the subjacency of each trap (i.e., the vertical distance from the surface; Mammola et al., 142 2017) using GIS, marking the position of the trap on available geological surveys of Chesta and Elia 143 144 (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.

We measured temperature at each pitfall trap with EL-USB-2+ dataloggers (Lascar Electronics, Salisbury, United Kingdom), programmed to record a temperature measure (accuracy of $\pm 0.5^{\circ}$ 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 (see author contribution for details). For the purpose of the analyses, we assigned each species to one 156 157 of the two major ecological groups commonly used to explore subterranean diversity patterns (e.g., Novak et al., 2012; Niemiller & Zigler, 2013; Trontelj et al., 2019): obligate subterranean species 158 159 ('troglobionts'; Sket, 2008; Trajano & de Carvalho, 2017), and non-obligates ('non-troglobionts'; Novak et al., 2012) (Supplementary material Appendix S1). We based this classification mainly on 160 161 the expert opinion of different taxonomists involved in this work, but also using data and morphological traits associated with subterranean life (loss of eyes and body pigment) from the 162 163 literature.

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 (α) 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:
- i) performed data exploration on the initial dataset, aiming to verify the presence of outliers, remove
 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
- and our explanatory predictors. We included potential interactions among predictors only if these
 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
- obtain a Minimum Adequate Model (MAM) for each of the dependent variables, minimizing the
 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 β -diversity among caves and between the two seasons using the framework proposed by 185 Carvalho et al. (2012) and Cardoso et al. (2014), whereby β is expressed as:

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

187 βrichness is the turnover in community composition explained by species loss/gain alone, and βreplacement 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. β-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 β -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 B_{replacement} 194 and Brichness values were significantly different between the two seasons for both obligate and non-195 obligate subterranean species.

197 **RESULTS**

198 α-diversity and abundance of species

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

204 Due to malfunctioning, half of the dataloggers did not measured temperature correctly throughout the sampling period Since the removal of these missing data would have halved the sample 205 206 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 α-diversity models were not over-dispersed [Obligates: Dispersion Ratio (DR)= 0.78; Pearson's χ^2 = 36.57; p= 0.86; Non-obligates: DR= 1.21; χ^2 = 55.56; p= 0.16]. 214 Conversely, abundance Poisson GLMs were significantly over-dispersed (Obligates: DR=12.04; χ^2 = 215 565.73; p< 0.001; Non-obligates: DR= 35.74; χ^2 = 1643.84; p< 0.001) and thus, we opted for a 216 217 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. α -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). α-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 α -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 α-diversity and abundance of non-226 obligate species, with higher values at lower elevations (Table 2).

227

228 β-diversity

229 Results of β -diversity analysis are illustrated in Figure 3. In obligate subterranean species, the density 230 of B_{Total} values across sites showed a bimodal concentration around 0 and 1, indicating the existence of two types of communities; B_{Total} values close to zero corresponded to the comparisons between 231 232 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 β_{Richness} values in the two seasons were observed (W = 9418.5; p= 0.22; Figure 3c). 238

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

246

247 Discussion

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

254 The main driver of α -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 grater 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).

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

We also found higher richness and abundance of non-obligate subterranean species at lower elevations (Table 3). In the study area, the elevation difference determines a change in the average temperature of about 1 °C between lower (1000 m) and higher (1200 m) caves, which may in turn drive the observed biological pattern. Alternatively, the explanation for this relationship may be linked to gravitation-assisted patterns in the distribution of species. Trontelj et al. (2019) documented how the distribution of animals within karst massif can be influenced by gravity. They argued that "[...] animals from a wider area of shallow subterranean spaces can be funneled towards deeper
parts of vertical caves by flowing water or gravity." This may explain why, including our case, caves
at lower elevations yielded a greater diversity and abundance of species.

300

301 Conclusions

Our results indicate that at a spatial scale of less than 1 km², there is a single pool of obligate species 302 303 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 should provide a general indication of the resident subterranean fauna of a certain area, having in 310 311 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.

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323

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447 TABLES & FIGURES CAPTIONS

Table 1. Main features of the investigated caves.

Code (Fig.1)	Cave name	Cadaster code	Туре	Elevation [m]	Pitfall distance from entrance [m]	Pitfall subjacency [m]	Total length [m]	Depth [m]	Entrance size [m ²]	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 Grotta dell'Ola –	1221 Pi	Natural	1169	4	7	12	6	4	3	7.4054	44.2615
4	Maissa 23 Grotta Diana – Maissa	1228 Pi	Natural	1105	4.5	2	6	-2	2.8	2	7.4060	44.2627
5	22 Small cave near Grotta	1227 Pi	Natural	1048	19.1	14	39	-11	5	18	7.4061	44.2627
6	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	Barôn Litrôn – Maissa 6	1214 Pi	Natural/M ine	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 Small cave near	1219 Pi	Natural	1090	13.3	12	92	-20	2.6	14	7.4106	44.2613
18	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 Mineshaft near Grotta	1054 Pi	Natural	1023	19	15	84	-16	10	15	7.4131	44.2611
22	Infernotto Superiore	n.c.	Mine	1041	5.5	4	6	-1	4.2	4	7.4113	22.2605
23	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 Natural cave	n.c.	Natural	1177	8.2	3	10	4	2	3	7.4148	44.2580
26	"Salamandre" Natural cave "Salamandre	n.c.	Natural	1100	3.6	4	7	-2	2.5	4	7.4144	44.2580
27	Superiore"	n.c.	Natural	1110	2	5	10	2	1.5	5	7.4144	44.2580

450 n.c. = mines or caves not included in the speleological cadaster; subjacency = vertical distance from

451 the surface; Mammola et al. 2017)

Table 2. Results of model selection. For each dependent variable, models are listed from the least to

456 t	he most supported.	AIC = Aikaike	Information	Criterion.
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Dependent variable	Model structure	AIC
	Pitfall depth + Season + Elevation + Entrance size	129.24
Richness of obligate subterranean species (α)	Pitfall depth + Elevation + Entrance size	127.46
	Pitfall depth + Elevation	126.17
	Pitfall depth	125.18
	Pitfall depth + Season + Elevation + Entrance size	264.60
Abundance of obligate subterranean species	Pitfall depth + Elevation + Entrance size	262.62
	Pitfall depth + Elevation	261.17
	Pitfall depth	259.75
Richness of non-obligate	Pitfall depth * Season + Elevation + Entrance size	227.16
species (a)	Pitfall depth * Season + Elevation	225.15
Abundance of non-obligate	Pitfall depth * Season + Elevation + Entrance size	437.80
species	Pitfall depth * Season + Elevation	435.82

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- **Table 3.** Estimated regression parameters in the Minimum Adequate Models. $R^2 =$ Nagelkerke's
- 471 pseudo-R² (Nagelkerke, 1991).

Dependent variable	Variable	Estimated $\beta \pm S.E.$	<i>p</i> -value	R ²	
Dishuass of Tradahiants (a)	Intercept	-1.98 ± 0.50	-	0.50	
Richness of Troglobionis (α)	Distance from the entrance	0.85 ± 0.18	< 0.001	0.30	
Abundance of Tradahianta	Intercept	-3.10 ± 0.69	-	0.70	
Abundance of froglobionis	Distance from the entrance	1.98 ± 0.28	< 0.001		
	Intercept	8.66 ± 1.54	-		
	Distance from the entrance	-0.44 ± 0.12	< 0.001	0.87	
Richness of Troglophiles (a)	Season (Winter)	-3.12 ± 0.51	< 0.001		
	Distance from the entrance * Season	istance from the entrance * Season 0.97 ± 0.21			
	Elevation	-0.01 ± 0.01	< 0.001		
	Intercept	11.93 ± 2.82	-		
Abundance of Troglophiles	Pitfall depth	-0.38 ± 0.24	0.110		
rioundance of fregrephiles	Season (Winter)	-6.34 ± 0.88	< 0.001	0.001 0.91	
	Distance from the entrance * Season	1.54 ± 0.34	< 0.001		
	Elevation	-0.01 ± 0.01	0.015		

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

477

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.

486

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

492

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