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1 **Spatial and functional structure of an entire ant assemblage in a lowland Panamanian**
2 **rainforest**

3

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47 **Declaration of competing interest**

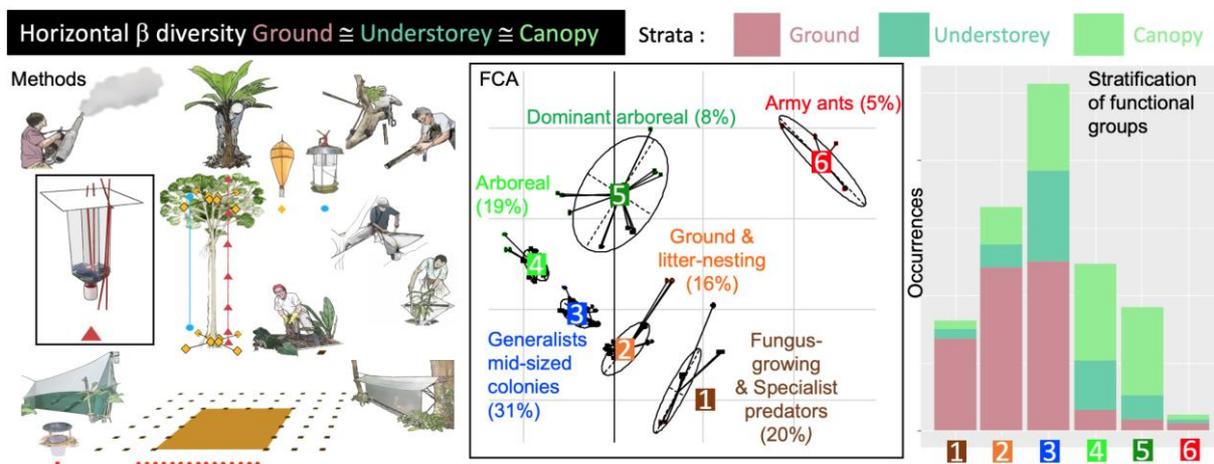
48 No conflict of interest.

49

50 **Graphical abstract**

51 Eleven sampling methods were used to study an entire rainforest ant assemblage at ground,
52 canopy and understorey levels. Ant species composition differed between strata. Horizontal
53 species turnover (β diversity) within forest strata was similar between sites. A functional
54 traits approach revealed that the ant assemblage was mainly composed of generalist species
55 with mid-sized colonies (group #3, 31% of the 405 species). However, ground- or litter-
56 dwelling species were also species-rich (#1 & 2), followed by arboreal ants (#4) including
57 ecologically important territorially dominant arboreal ants (#5), and typical army ants (#6).
58 Of the 11 sampling methods used, aerial flight interception traps placed alongside tree trunks
59 (red triangles in the figure below) collected half of the ant species and reflected the vertical
60 stratification of the ant species, demonstrating an interesting possibility for ant monitoring
61 programs.

62



63

64

65 ABSTRACT

66 Ants are a major ecological group in tropical rainforests. Few studies in the Neotropics have
67 documented the distribution of ants from the ground to the canopy, and none have included
68 the understory. A previous analysis of an intensive arthropod study in Panama, involving 11
69 sampling methods, showed that the factors influencing ant beta diversity (i.e., changes in
70 assemblage composition) were, in decreasing order of importance, the vertical (height),
71 temporal (season), and horizontal (geographic distance) dimensions. In the present study, we
72 went one step further and aimed (1) to identify the best sampling methods to study the entire
73 ant assemblage across the three strata, (2) to test if all strata show a similar horizontal beta
74 diversity and (3) to analyze the functional structure of the entire ant assemblage. We
75 identified 405 ant species from 11 subfamilies and 68 genera. Slightly more species were
76 sampled in the canopy than on the ground; they belonged to distinct sub-assemblages. The
77 understory fauna was mainly a mixture of species found in the other two strata. The
78 horizontal beta diversity between sites was similar for the three strata. About half of the ant
79 species foraged in two (29%) or three (25%) strata. A single method, aerial flight interception
80 traps placed alongside tree trunks, acting as arboreal pitfall traps, collected half of the species
81 and reflected the vertical stratification. Using the functional traits approach, we observed that
82 generalist species with mid-sized colonies were by far the most numerous (31%), followed by
83 ground- or litter-dwelling species, either specialists (20%), or generalists (16%), and arboreal
84 species, either generalists (19%) or territorially dominant (8%), and finally army ants (5%).
85 Our results reinforce the idea that a proper understanding of the functioning of ant
86 assemblages requires the inclusion of arboreal ants in survey programs.

87

88 *Keywords:* horizontal beta diversity, vertical stratification, ant diversity, canopy, ant sampling
89 methods, colony size, feeding habits, functional groups, nesting mode.

90

91 **Introduction**

92 The hot and humid climate of most intertropical areas promotes ecosystem productivity
93 and so the formation of species-rich tropical rainforests characterized by large trees (30-50 m
94 in height) and structured into three main strata (i.e., the canopy, understory and ground)
95 fostering a high plant diversity, including lianas and epiphytes (Morley, 2002; Nieder et al.,
96 2001; Tymen et al., 2017; Wright, 2002). The canopy is exposed to high insolation and large
97 differences in temperature and humidity between day and night, whereas at ground level
98 climatic conditions are more stable, cooler and wetter (Parker 1995). This environmental
99 heterogeneity induces a vertically stratified distribution of organisms adapted to local
100 environmental conditions (Shaw 2004). Stratification in rainforests has been observed in a
101 wide range of organisms, including vertebrates and invertebrates, and is responsible for
102 spatial change in species composition (i.e., vertical beta diversity) (Basham et al. 2019,
103 Basset et al. 2003). Horizontal beta diversity is also generalized and, for arthropods at least, is
104 lower than vertical beta diversity in continuous vegetation types such as lowland tropical
105 forests (Novotny et al. 2007, Basset et al. 2015). However, what is less well demonstrated is
106 whether horizontal beta diversity is similar in the three forest strata. Differences could be
107 generated by differences in habitat continuity, as the ground is continuous while the strata
108 above the ground are discontinuous, which may limit species dispersal (Shaw 2004). This
109 hypothesis is likely for amphibian assemblages, where a classic distance-decay (decrease in
110 compositional similarity with geographic distance) was observed in the canopy and
111 understory, but not at ground level, indicating different rates of beta diversity between strata
112 (Basham et al. 2019). Alternatively, tolerance to microclimatic changes might also differ,
113 with ground-dwelling species living in a more stable environment than species above ground
114 (Madigosky, 2004). In contrast, when studying ants, Antoniazzi et al. 2021, found only a
115 distance-decay relationship at ground but not at canopy level. This result may have been

116 affected by the sampling method, baits and visual searching, which only capture a fraction of
117 the ant assemblage, especially dominant species with large colonies which differ in their
118 biology and ecology between the ground and the canopy (Dejean, Corbara, Orivel, &
119 Leponce, 2007).

120 Ants are one of the dominant animal groups in rainforests because of their colony size.
121 Some species have very large colonies (i.e., several thousands to several million individuals)
122 such as, at ground level, leaf-cutting ants which are major defoliators, army ants which are
123 major predators, and at canopy level, territorially dominant arboreal ants (territories
124 distributed in a mosaic pattern) that protect their host trees from defoliators (Brady, Fisher,
125 Schultz, & Ward, 2014; Dejean et al., 2007; Hölldobler & Wilson, 1994, 2011; Majer, 1993).
126 The development of large populations is made possible by the availability of energy-rich
127 resources derived from plants. Most ant species are partly herbivorous (primary consumers)
128 feeding on extrafloral nectar, food bodies, pollen, sap and leaves through fungiculture; they
129 are also ‘cryptic herbivores’ feeding on hemipteran honeydew (Davidson et al., 2003; Rico-
130 Gray & Oliveira, 2007; Tobin, 1994). Most of these ants are generalists as they also scavenge
131 dead animals and feces and capture different kinds of prey. Finally, some species are
132 predators, either generalists or specialists (Cerdá & Dejean, 2011; Hölldobler & Wilson,
133 1994).

134 Few studies in the Neotropics have documented ant distribution from the ground to the
135 canopy. Using a single method, baiting 20 trees from the base to the canopy, Yanoviak and
136 Kaspari (2000) recorded 48 ant species. Ryder Wilkie, Mertl, and Traniello (2010) obtained
137 489 ant species in Ecuador with six methods (i.e., subterranean probes, pitfall traps, hand
138 collecting, Winkler devices, baiting and canopy fogging). Longino and Colwell (2020) noted
139 539 species with seven methods (i.e., Malaise, light traps, Berlese, Winkler, baiting, hand
140 collecting and fogging). Finally, Antoniazzi et al. (2021) using baits and hand collection in

141 the canopy and at ground level found 43 species on 10 trees. Unfortunately, none of the
142 studies included the understorey stratum, often dominated by palms in the Neotropics
143 (Popma, Bongers, & Meave, 1988).

144 A previous analysis of an intensive arthropod study in Panama, involving 11 sampling
145 methods, showed that species turnover for arthropods, including ants, was driven primarily
146 by vertical and seasonal variation, and less by horizontal variation (Basset et al., 2015). We
147 analyze here this dataset in further detail to determine three approaches related to studying
148 ants. (1) The complementarity and the pertinence of the methods for studying the entire ant
149 assemblage, including the understorey species. (2) The validity of the null hypothesis that all
150 three strata show similar horizontal species turnover based on the best method for studying
151 the ant assemblages in multiple strata. We predicted the following. (a) The turnover will be
152 higher in the arboreal strata (canopy and understorey), representing islands of habitats
153 (Adams et al. 2017), than at ground level, a continuous habitat (Theunis et al., 2005). (b) The
154 local site conditions, whether abiotic or biotic, likely influence local ant species composition
155 (i.e., the non-random distribution of species diversity within-sites will be lower than between
156 sites, or distance-decay). (3) The functional structure of the entire forest ant assemblage
157 based on our survey and on a thorough review of the literature on the biology and ecology of
158 each species. Our prediction is that key ant groups, with large populations, will differ
159 according to the strata considered.

160

161 **Material and methods**

162

163 *Study sites*

164 This study was conducted in Panama during the IBISCA-Panama project in the 6,000
165 ha evergreen seasonal mixed rainforest in the San Lorenzo Protected Area which has been

166 free of major disturbance for the past 200 years (9°16'N, 79°58'W; 130 m a.s.l.). The climate
167 is moist tropical, with a mean annual rainfall of 3,139 mm, and the daily mean temperature is
168 26°C (see STRI, 2017 for details). Ants were collected from 11 study sites that were
169 representative of the forest environment of the area, each measuring 40 x 40 m and centered
170 around a 20 x 20 m botanical plot (Basset et al., 2012, 2015). The present study was
171 conducted over a total surface area of ≈1.76 ha and the sites were distributed from 24 to 1941
172 m apart (median: 700m). Field permits were granted by the *Autoridad Nacional del Ambiente*
173 of Panama.

174

175 *Ant sampling methods*

176 During the IBISCA-Panama project four surveys were conducted spanning the dry and
177 the wet seasons (September 2003 - November 2004) (Basset et al., 2012, 2015). Because the
178 San Lorenzo rainforest remains humid and experiences almost no loss of canopy cover during
179 the January-April dry season, the ground-level fauna is not thought to suffer much from
180 drought stress (see Roisin et al., 2006 for termites).

181 A combination of 11 sampling methods, resulting in an exceptionally large dataset, was
182 used (Table 1; Fig.S1). (1) Berlese funnel. Three trees per site were sampled. For each tree,
183 16 soil cores of 15 cm³ were gathered at ground level (n=8) and at the bases of main branches
184 (n=8) and placed in Berlese funnels for 2 days. (2) Winkler. This sampling was conducted on
185 51 quadrats of 1 m² distributed at intervals of 5 m around each 20 x 20 m plot. The leaf-litter
186 present within each quadrat was sifted and extracted during 48 hours by a Winkler extractor.
187 (3) Pitfall traps. At each study site 15 pitfall traps (424 ml) were buried in a line at 1.3 m
188 intervals. They contained a solution of ethanol, salt and detergent and were examined after 3
189 days. (4) Aerial composite flight interception traps (aFIT). Each trap consisted of two vertical
190 perspex sheets (60 x 23 cm) above a collecting funnel; a preserving jar was suspended from

191 canopy branches by sturdy nylon ropes. The funnel functioned as a large arboreal pitfall
192 collecting many ant workers and a few winged sexuals. Six to seven traps were placed in
193 each of five sites at different heights (0, 1.3, 7, 14, 21, 28 m and in some cases 35 m).
194 Because baiting experiments showed that canopy ants are generally present from 7 m above
195 the ground (Leponce et al., 2019), we considered that the traps installed above this height
196 intercepted ants from the canopy, whereas those placed 1.3 m above the ground rather
197 intercepted ants from the understorey and those placed at 0 m rather permitted ground-
198 dwelling ants to be captured. Left in place for 1 year, these traps were sampled every 10 days
199 yielding 1659 samples from the three forest strata. (5) Understorey Flight Interception Traps
200 (uFIT). Each uFIT consisted of a fine mesh screen (3 x 1 m that was left at each study site
201 during three consecutive 2-day-long periods). (6) Beating. Ants were dislodged by beating
202 the foliage (three strong strokes using a stick) and collected on a square beating sheet
203 measuring 0.4 m². Beating was conducted in the canopy and the understorey at seven sites
204 during the first survey and two sites during the second, third and fourth surveys (10 canopy
205 and 10 understorey samples per site). A total of 560 beating samples were collected. (7) Palm
206 tree inspection. Small *Geonoma congesta* H. Wendl. ex Spruce (Arecaceae) dominated the
207 understorey. The clustering of their leaf petioles gathers litter, favoring the installation of ant
208 nests (crown base at ≈1.5 m in height). During the first survey, 391 palm trees were
209 monitored at three sites and the ants sheltered therein were collected using aspirators. (8)
210 Light traps. At each study site, three traps were installed in the understorey 2 m above the
211 ground and three others in the canopy at a height of 25-35 m. Replicated one night during
212 four seasonal periods they resulted in a total of 96 samples. (9) Malaise traps. A Malaise trap
213 (2 x 1 m) was operated during 10 days at eight sites during the first survey and occasionally
214 during the three other surveys yielding a total of 63 samples. (10) Climbers (branch-clipping).
215 During the first survey, climbers cut off two to four branches (diameter >10 cm) from the

216 crown of 174 canopy trees situated in the vicinity of seven plots. Just after the branches had
217 fallen to the ground, the ants crawling on them or hiding in parts of nests were collected
218 using aspirators and forceps. Note that this method is particularly useful for capturing
219 territorially dominant arboreal ants (Dejean et al., 2018, 2019). (11) Fogging. A knockdown
220 insecticide (natural pyrethrum quickly broken down by UV rays) was sprayed up into the tree
221 crowns. Stunned arthropods fell onto six collecting sheets (5 x 4 m). This method was used
222 during the three surveys on 120 of the 174 previously mentioned trees.

223

224 *Ant identification and voucher conservation*

225 The ants were identified by comparing them with the collection kept at the
226 Myrmecology Laboratory, Cocoa Research Center, in Ilhéus, Bahia, Brazil and by consulting
227 specialized literature. Bolton's catalogue nomenclature was used (Bolton, 2019).

228 Representative samples ("vouchers") of each species were deposited in this collection and at
229 the Royal Belgian Institute of Natural Sciences (RBINS). This study focused on ant workers,
230 but winged sexuals were identified when possible to increase taxonomic coverage but were
231 excluded from the analyses on the stratification of ant assemblages.

232

233 *Complementarity of each sampling method and its pertinence for studying the entire* 234 *ant assemblage*

235 To estimate the rate of species accumulation by each method (all strata pooled) or by
236 strata (all methods pooled), species rarefaction curves were plotted on the species
237 occurrences data matrices using the Mao Tau algorithm in EstimateS 9.1.0 software (Colwell,
238 2016) with 100 randomizations of the sampling order without replacement. Methods were
239 grouped based on their compositional similarity according to the UPGMA algorithm with the
240 Bray-Curtis similarity index available in the PAST 3.26 software.

241

242 *Vertical stratification of the local ant assemblage*

243 To show differences and overlaps in species composition and richness between the
244 three strata, we used proportional Venn diagrams in which the area of each shape is
245 proportional to the number of species it includes.

246

247 *Horizontal beta diversity within strata*

248 To measure how species composition changed horizontally, we performed an additive
249 partition of the species richness of each horizontal stratum (Barton et al., 2013, Veech et al.,
250 2002): $\gamma = \alpha + \beta$ where γ is the total species richness, α the average species richness within
251 sites and β the average difference in species richness between sites. This analysis was based
252 on datasets from the three most efficient multi-strata sampling methods: aFITs (three strata),
253 Berlese (two strata) and beating (two strata). We used the software PARTITION3 (Veech, &
254 Crist, 2009) to compare the observed diversity value to a corresponding null distribution
255 (individual-based randomization method, 1000 randomizations). The mean observed richness
256 is equal to the expected richness if the distribution of richness is spatially homogeneous. This
257 allows us to test the significance of the observed value as either a significantly high or low
258 value. In addition, we followed the approach developed by Baselga (2010) to separate beta
259 diversity into its turnover (species change) and nestedness (species gain/loss) components.
260 The total compositional variation between assemblages was calculated using the Jaccard
261 pairwise dissimilarity index. For each combination of strata and method, pairwise beta
262 diversity measures were calculated using the “betapart” package (Baselga, & Orme, 2012) in
263 R software (R core Team 2015).

264

265 *Functional traits of the ant species distributed across strata*

266 Based on previous studies of the nesting and feeding preferences of Neotropical rainforest
267 ant species and the results obtained here on the distribution of morphospecies throughout the
268 three forest strata (Appendix A1), we defined nominal categories for three functional traits
269 (*sensu largo*, see Wong, Guénard, & Lewis, 2019): (1) size of the colony (five categories:
270 <300 individuals; 300-1,000; 1,000-10,000; 10,000-100,000; and >100,000); (2) nesting
271 habits (seven categories: bivouac; subterranean; leaf-litter; plant-ants associated with
272 myrmecophytes; arboreal nesting in hollow twigs; arboreal nesting in tree cavities; and
273 arboreal building carton or silk nests); and (3) feeding habits (six categories: consumers of
274 plant nectar or food bodies; honeydew feeders; fungus-growing leaf-cutters and debris
275 collectors; scavengers; generalist predators; specialized predators), the whole resulting in 18
276 categories of functional traits.

277 We added the nesting strata (i.e., ground and litter, understory and canopy) as three more
278 traits obtaining a matrix of [405 ant species x 21 categories of traits]. The scores
279 corresponding to the functional traits ranged from “0”, indicating “no affinity” for a given
280 trait category, to “3”, indicating “high affinity”. Information on the traits was structured using
281 a Fuzzy-Coding technique (Chevenet, Dolédec, & Chessel, 1994). A Fuzzy Correspondence
282 Analysis (FCA) was conducted on this [ant species x traits] matrix. We determined the
283 optimal number of clusters (low variance within clusters and high variance between clusters)
284 based on the majority rule after computing 23 clustering indices (Euclidean distance; K-
285 means clustering method). To ease interpretation, bar plots were used to show the distribution
286 of the samples from the different forest strata within clusters. These analyses were conducted
287 with the ADE4, NbClust and ggplot2 packages in R software (R Development Core Team,
288 2015).

289

290 **Results**

291 *Complementarity of each sampling method and its pertinence for studying the entire*
292 *ant assemblage*

293 The combination of methods allowed us to record 405 ant species (11 subfamilies and 68
294 genera) in the ≈ 1.76 ha of rainforest surveyed (Table 1, Appendix A1). The rarefaction curve
295 for the 11 sampling methods used indicates a near asymptote (Fig. 1A) as $\approx 81\%$ of the
296 estimated local species richness was recorded (Chao2 = 498, CI95% 459-564). The curves
297 corresponding to the different methods used accumulated species at different rates, those for
298 aerial and understorey FITs being similar, whereas palm trees accumulated the fewest species
299 and light traps attracted only a few winged sexuals (Fig. 1B).

300 A cluster analysis showed that the methods fell into four groups according to the ant
301 species collected (Fig. 2). The first group consists of soil and leaf-litter samples from the
302 ground or the canopy collected with Berlese funnels and Winkler devices. The second group
303 consists of understorey or canopy samples collected with Malaise traps, beating, fogging and
304 by climbers. The third group consists of samples from all three strata obtained with aerial and
305 understorey flight interception traps (aFITs and uFITs), pitfall traps and through palm tree
306 inspection. Finally, the fourth group consists of light traps, less effective than the other
307 methods.

308 The 10 most species-rich genera make up 50.9% of the occurrences of the 405 species
309 recorded, whereas 11 ant species out of 405 account for more than one quarter of the
310 occurrences (exactly 25.3% of 7,274 occurrences; Appendix A1). We collected few winged
311 sexuals (100 occurrences) for 48 species of which eight were the sole sample of their species
312 (Appendix A1).

313

314 *Vertical stratification of the local ant assemblage*

315 Winged sexuals were excluded from this analysis resulting in 397 ant species studied
316 here. Ant species composition showed a moderate vertical stratification. Globally, a total of
317 253 species out of 397 were recorded on the ground, 199 in the understorey and 261 in the
318 canopy. The slightly higher presence of ants in the canopy compared to the ground and
319 understorey was confirmed by rarefaction (standardized richness for 1000 occurrences;
320 ground: 176 ± 13 species; understorey: 166 ± 13 ; and canopy: 198 ± 14) (Fig. 1C). However, 87
321 species (22%) were sampled exclusively on the ground, 23 in the understorey (6%), and 72
322 (18%) in the canopy (Fig. 3A). Overall, this indicates that certain species can patrol in two or
323 three strata, as confirmed by individual multi-strata collection methods (Fig. 3B-D). Aerial
324 FITs alone captured 53% of the species present and reflected species stratification (Fig. 3B).
325 The ground stratum was dominated by the Myrmicinae, Ponerinae and Ectatomminae, while
326 the canopy had a higher proportion of Formicinae, Dolichoderinae and Pseudomyrmicinae
327 (Fig.4). An intermediate situation was found in the understorey.

328

329 *Differences in horizontal beta diversity between strata*

330 Whatever the method considered, there was no significant difference between strata in
331 horizontal beta diversity between sites, (aFIT: $F_{(2,12)}=0.39$, $P=0.69$; Berlese: $t=-0.84$, $P=0.41$;
332 beating: $t=0.29$, $P=0.78$) (Fig. 5). Furthermore, the observed β diversity between sites was
333 significantly higher than expected with randomized datasets, except in the understorey for
334 aFITs (Fig. 5), suggesting some site effect on horizontal species distribution. Species
335 turnover was the main contributor to beta diversity ($88 \pm 6\%$, $n=7$) while nestedness
336 contributed to $12 \pm 6\%$.

337

338 *Ant functional traits*

339 The FCA analysis based on the functional traits of the 405 ant species clearly delimited
340 six clusters (Fig. 6A). Cluster 1 groups together 83 ground- and litter-nesting species with
341 specialized feeding habits (e.g., specialized predators and fungus-growers) and small colonies
342 except for some fungus-growing species that have large to very large colonies. The 66
343 species in Cluster 2 are mostly ground and litter nesters with small to slightly larger colonies
344 (i.e., up to 1,000 individuals) and generalist feeding habits (e.g., scavenging, predatory and
345 feeding on sugary substances). Cluster 3 is comprised of 127 species most of which are
346 ground and litter nesters (e.g., *Pheidole* spp.) but some are arboreal that nest in preformed
347 cavities (e.g. *Procryptocerus* spp.; *Pseudomyrmex* spp.). Compared to the previous clusters
348 they are generalist feeders that most frequently feed on sugary substances, whereas the size of
349 the colonies can be relatively large (i.e., up to 10,000 individuals).

350 Comparatively, Cluster 4 groups together 77 arboreal species with mid-sized colonies
351 that nest in preformed cavities in the trees. Generalist feeders, they mostly exploit sugary
352 substances, particularly honeydew from the hemipterans they tend. By comparison, most of
353 the 31 species in Cluster 5 are characterized by their ability to build carton or silk nests, many
354 of them being territorially dominant arboreal ants with very large colonies. Exceptionally,
355 *Wasmannia iheringi* has small colonies. Finally, all 21 species in Cluster 6 are highly
356 predatory army ants with very large colonies that nest in bivouacs.

357 The ant species in these six clusters were noted in all forest strata, but to different
358 degrees as clusters 1, 2 and 3 dominated at ground level, cluster 3 in the understory and
359 clusters 3, 4 and 5 in the canopy (Fig. 6B).

360

361

362 **Discussion**

363 In this Panamanian lowland rainforest, the estimated species richness was between 459
364 and 564 species (CI 95%). This figure is lower than in Costa Rica and Amazonian Ecuador
365 with 584-636 and 647-736 ant species, respectively (Longino & Colwell, 2020; Ryder Wilkie
366 et al., 2010). We sampled slightly more ant species in the canopy than on the ground (261 vs.
367 253 species) and their species assemblages were distinct with 87 species observed only on the
368 ground and 72 in the canopy. The understory ant fauna was mostly a mixture of species
369 from the other two strata, but 23 species were specific to this stratum.

370 The stratification shown in the San Lorenzo rainforest for various groups of arthropods
371 (Bourguignon, Leponce, & Roisin, 2009; Roisin et al., 2006; Ribeiro, & Basset, 2007; Basset
372 et al., 2015) was also noted for ants for which some species were sampled only in one stratum
373 (Fig. 1C and Fig. 3), in accordance with Ryder Wilkie et al. (2010) and Longino and Colwell
374 (2020).

375 This stratification results from the following distinct environmental conditions in the
376 three strata. At ground level, fallen leaves, wood and different debris are decomposed by
377 detritivores (Stahl et al., 2013). Their abundance spurs ant diversification through predation
378 involving omnivorous and strictly predatory species (Cerda & Dejean, 2011; Hölldobler &
379 Wilson, 1994; Appendix A1). The ground layer also included leaf-cutting, fungus-growing
380 ants which are preyed upon by specialized army ants (i.e., *Nomamyrmex*; Appendix A1) and
381 detritivorous fungus-growing species preyed upon by other ants (i.e., *Megalomyrmex* as a
382 social parasite of *Attina*; Appendix A1). Also, certain ant species feed on the honeydew of
383 hemipterans attended on the roots of trees (*Acropyga*; Appendix A1).

384 The understory was previously neglected in studies dealing with tropical rainforest ants.
385 This gap is filled by this study showing that 23 species were specific to this stratum, whereas
386 the species richness is intermediate between that for the ground and that for the canopy (176

387 *versus* 166 and 198 ant species, respectively, Fig. 1C; Fig. 3). Small palm trees allow several
388 ant species to nest in the litter between the clusters of their leaf petioles (Gibernau, Orivel,
389 Delabie, Barabé, & Dejean, 2007), whereas the workers of several generalist ground- and
390 canopy-nesting species also foraged on understory plants (Table 1 and Appendix A1).

391 In the canopy, the presence of populous territorially dominant arboreal ant colonies is
392 possible *via* the presence of large numbers of attended honeydew-producing, sap-sucking
393 hemipterans, whereas extrafloral nectar, rather produced by liana, is mostly exploited by non-
394 dominant ants, the latter forming the core of the species richness in this stratum (Blüthgen et
395 al., 2000; Blüthgen & Stork, 2007; Dejean et al., 2007). Food bodies in the canopy are mostly
396 produced by myrmecophytic *Cecropia* and are the main, if not the only, food source for
397 mutualistic plant-ants specifically associated with these trees such as *Azteca constructor*
398 (Appendix A1).

399 Contrary to our predictions, which were based on the rationale that ground, understory
400 and canopy level assemblages were not subject to the same environmental variations because
401 of differences in habitat continuity, we did not find any difference in horizontal beta diversity
402 between strata. This result contrasts with the findings of Antoniazzi et al. (2021) who
403 observed that horizontal beta diversity was different between the canopy and the ground in a
404 Mexican lowland tropical rainforest. These authors collected ants on a limited number of
405 trees during one-day sessions of baiting and hand collection. Thus, we cannot exclude that
406 our results differ due to a different sampling approach (i.e., different spatiotemporal scale and
407 sampling coverage) (Barton et al. 2013). Furthermore, we observed that the beta diversity
408 was greater than expected for a random spatial distribution of species (Veech, 2005). This
409 suggests that general conditions at the local scale (40 x 40 m site) had a prevailing effect on
410 horizontal species distribution, increasing differences in species composition between sites
411 (Soininen et al., 2007). This could be due to local biotic or abiotic conditions affecting the

412 overall ant species distribution across strata (Hortal et al., 2013, Kaspari et al., 2003, Klimes
413 et al., 2012). Another possibility is that the geographical scale of the study is too small (i.e.,
414 sites too close to each other) to show difference in beta diversity between strata. The beta
415 diversity was mainly driven by species turnover and much less by nestedness, indicating that
416 assemblages at species poor sites were not subsets of species rich sites.

417 The six clusters corresponding to the ants' functional traits were composed of ant species
418 collected from all three forest strata, but with some notable differences due to the interplay
419 between their nesting and foraging activities, and their colony size (Fig. 6). Cluster 1 is
420 characterized by specialist feeders including all fungus-growing ants of the New World
421 subtribe *Attina* (subfamily Myrmicinae; tribe Attini) plus specialized predators (e.g.,
422 *Acanthognathus* and *Strumigenys* prey on collembollans; *Stegomyrmex*, *Discothyrea* and
423 *Proceratium* on arthropod eggs; *Leptogenys* on diplopods; and *Thaumatomyrmex* on
424 myriapoda of the order Polyxenida) that are ground or litter nesters with small colonies. Yet,
425 some fungus-growing species have large to very large colonies (e.g., *Cyphomyrmex*
426 *transversus*, *Trachymyrmex cornetzi*, *T. isthmicus* and the leaf-cutting species of the genera
427 *Acromyrmex* and *Atta*) (see details in Appendix A1). Note that leaf-cutting ants are ecological
428 engineers and the main Neotropical defoliator (Hölldobler & Wilson, 2011). Cluster 2 groups
429 ground- and litter-nesting species not belonging to Cluster 1. The exceptions noted in the
430 canopy are colonies nesting in suspended soil (e.g., *Hypoponera* sp.08) or *Solenopsis* from
431 the subgenus *Diplorhoptrum* that are parasites of other ant species from which they steal
432 brood (see Appendix A1), some of them nesting in the forest canopy.

433 Cluster 3 is species rich because it groups together all generalist ant species with mid-
434 sized colonies, some of which are arboreal (Fig. 6). Cluster 4 corresponds to arboreal ants
435 with medium-sized colonies (Fig. 6). The colonies of *Odontomachus hastatus* noted in this
436 cluster nest in the litter accumulated in understory palm trees or in association with hemi-

437 epiphytes (Gibernau et al., 2007); other species nest in suspended soils in the canopy (e.g.,
438 *Pheidole* spp.), something facilitated by the presence of epiphytes (DaRocha et al., 2015,
439 2016, Longino, & Nadkarni, 1990).

440 Cluster 5 is composed of canopy-dwelling generalist feeders that build carton or silk
441 nests (only *W. iheringi* has small colonies). Most are territorially dominant arboreal ants (e.g.,
442 genera *Azteca*, *Cephalotes* and *Crematogaster*) that protect their host trees from defoliators
443 (Majer, 1993). Some workers were collected from the understory and the ground as they
444 were hunting or forming columns to move from one tree in their territory to another whose
445 crowns are not in contact (Dejean et al., 2007, 2019).

446 Cluster 6 is composed of typical, nomadic army ants (Dorylinae) that organize raids from
447 the soil to understory plants, and certain of them (e.g., *Eciton hamatum*) even climb trees to
448 hunt in their crowns, preying on the brood of social wasps and arboreal *Dolichoderus* and
449 *Camponotus* colonies (Brady et al., 2014; Hölldobler & Wilson, 1994, 2011). Although less
450 species rich than the other clusters, with their large to very large colonies, army ants have a
451 major impact on the rainforest ecosystem as they regulate other arthropods, including other
452 ant taxa (McGlynn & Poirson, 2012). Note that among the subfamily Dorylinae,
453 *Cylindromyrmex meinerti* and *Syscia augustae*, which prey on termites, have small colonies
454 (de Andrade, 1998; Mackay and Mackay, 2002; Appendix 1), so that they belong to Cluster
455 2.

456 In conclusion, this study allowed us to link for the first time the diversity and distribution
457 of ants and the functional ecology of the entire above-ground ant community in a lowland
458 Neotropical forest. Generalist species with mid-sized colonies were the most frequent.
459 Ground- or litter-dwelling species with a specialized diet were also species rich, whereas this
460 was not the case for the ecologically important territorial dominant arboreal ants, leaf-cutting
461 ants and typical army ants. Half of the species foraged in more than one stratum. Slightly

462 more species were present in the canopy than at ground level and an intermediate number of
463 species was found in the understorey. At the scale of a site, local conditions appear to
464 influence the horizontal distribution of species, but there does not seem to be a different
465 horizontal species turnover between strata. Finer scale studies are needed to determine in
466 more detail which factors may be responsible for this pattern (Klimes et al., 2012). When
467 used for a prolonged period of time, aerial flight interception traps, acting as pitfall traps,
468 collected half of the species in the three forest layers and may be useful for programs
469 monitoring the entire ant assemblage in future studies. By complementing them with
470 subterranean pitfall traps, the hypogeous ant fauna might be even better documented (Wong
471 & Guénard, 2017). Overall, this study emphasizes the importance of comprehensive, multi-
472 strata surveys and thorough reviews of methods to better understand the functioning of ant
473 assemblages in tropical rainforest environments.

474

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502

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687

688 **Table and figure legends**

689

690 **Table 1**

691 Sampling effort used to collect ants in the three strata of the San Lorenzo rainforest with the number
692 of samples for each method, the number of samples containing ants (also expressed as a percentage),
693 the number ant species collected, the number of sites where these methods were used, the number of
694 seasonal replicates, and the total number of trapping days. Abbreviations: aFIT: aerial composite
695 Flight Interception Trap installed every 7 meters along a tree trunk (plus one at 1.3 m above ground);
696 uFIT: understorey Flight Intercept Trap.

697

698 **Fig. 1.** Sample-based rarefaction curves for the 11 sampling methods used in the San Lorenzo
699 protected area (405 ant species and 4,547 samples). A) Global view, all strata combined; B) Close-up;
700 C) Individual-based rarefaction (all methods pooled, winged sexuals excluded; 397 species) showing
701 the number of species found in each strata.

702

703 **Fig. 2.** Faunal similarity between the ant species captured using the different sampling methods for
704 the three forest levels.

705

706 **Fig. 3.** Proportional Venn diagram of the number of species collected from the ground, understorey
707 and canopy levels showing both differences and overlaps between the three strata (based on workers;
708 winged sexuals excluded). A) The combined 11 sampling methods resulted in 397 ant species being
709 captured. B) Aerial FITs, acting as pitfall traps, captured 209 species combined from the ground (traps
710 at 0 m, with buried collector funnel), the understorey (traps suspended 1.3 m above the ground) and
711 the canopy (traps at 7, 14, 21, 28, 35 m). C) Beating enabled 67 species to be sampled. D) Berlese
712 funnels resulted in 97 species being captured. Values indicate the number of ant species in each
713 category.

714

715 **Fig. 4.** Proportion of ant subfamilies observed in the three strata and by stratum-specific methods
716 (Winkler, palm inspection, fogging, respectively). Subfamily abbreviations: DOLI: Dolichoderinae,
717 DORY: Dorylinae, ECTA: Ectatomminae, FORM: Formicinae, HETE: Heteroponerinae, MYRM:
718 Myrmicinae, PARA: Paraponerinae; PONE: Ponerinae; PROC: Proceratiinae; PSEU:
719 Pseudomyrmecinae.

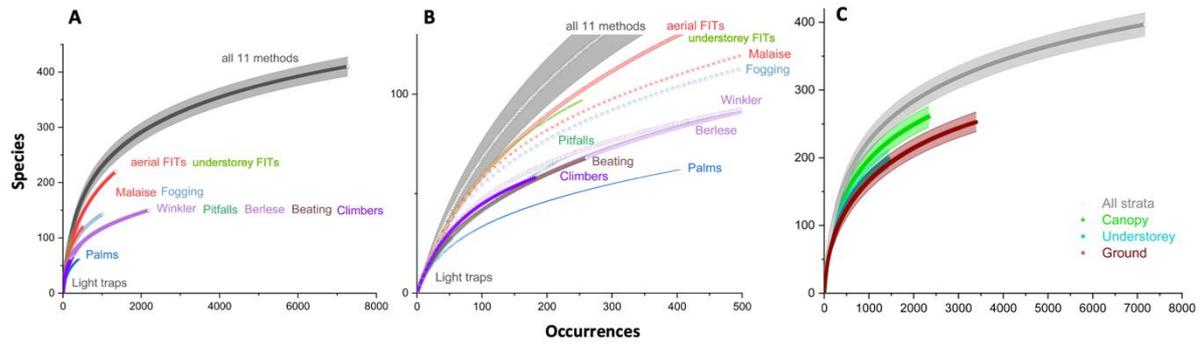
720

721 **Fig. 5.** Comparison of the horizontal species turnover within each stratum for species collected either
722 with aFITs, Berlese or beating. Total (gamma) diversity for each stratum was decomposed into
723 average alpha (local) diversity of 40x40m sites and the beta diversity between sites. The observed and
724 expected contribution of alpha and beta diversity are provided. An asterisk (*) refers to significant
725 differences between observed and expected values based on 1000 randomizations of the dataset.

726

727 **Fig. 6** Fuzzy Correspondence Analysis (FCA) of functional traits composition corresponding to the
728 405 ant species recorded in this study. **A.** Ordination of the ant species on the two first axes of the
729 FCA. The percentage of species in each cluster is indicated in brackets. **B.** Ordination of the
730 functional trait modalities on the two first axes of the FCA. **C.** Distribution of the ants from the six
731 clusters defined in Fig. 6A among the rainforest strata based on their occurrence in samples.

732

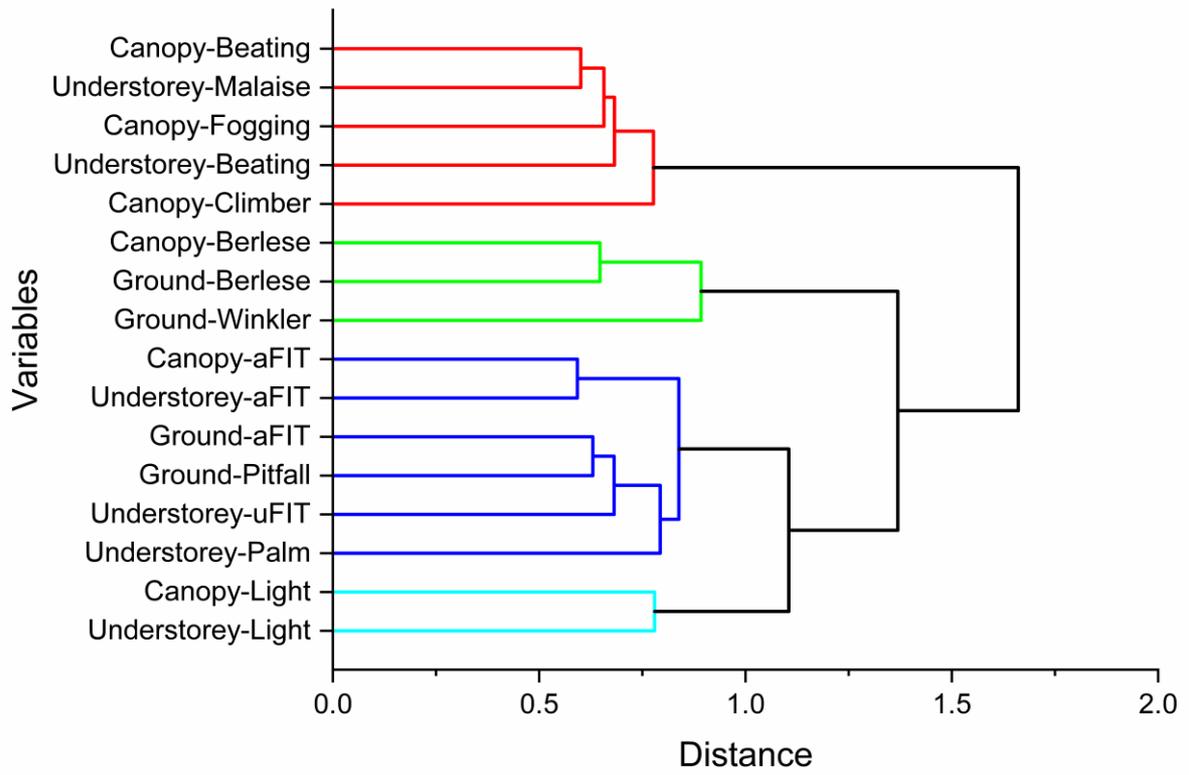


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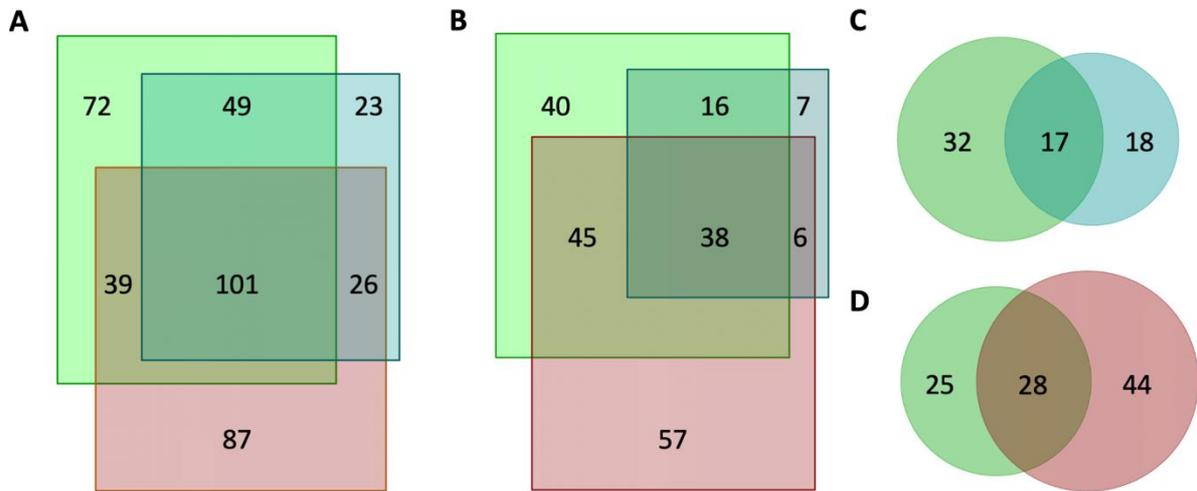
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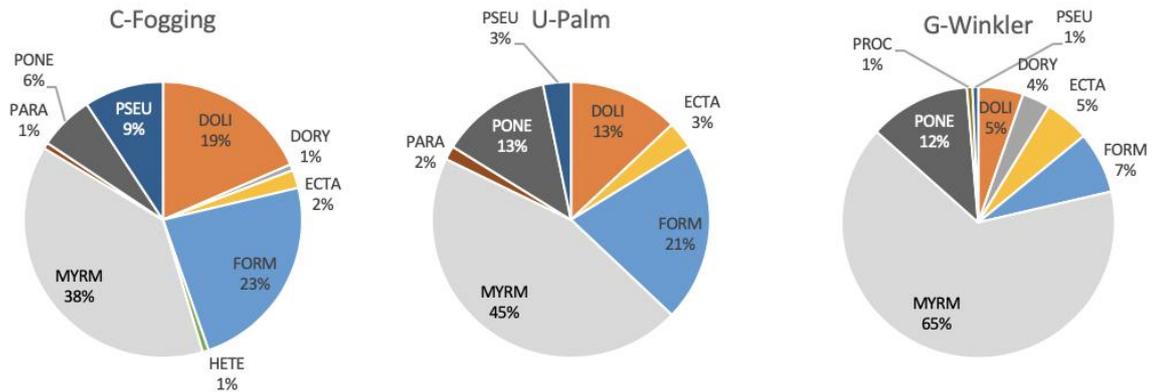
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753



754

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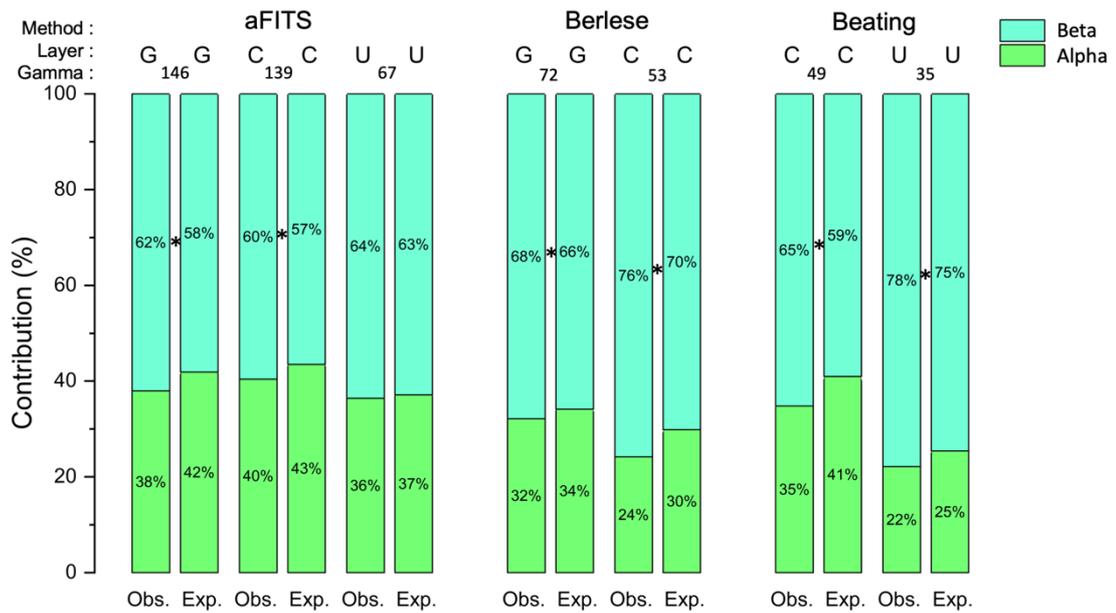
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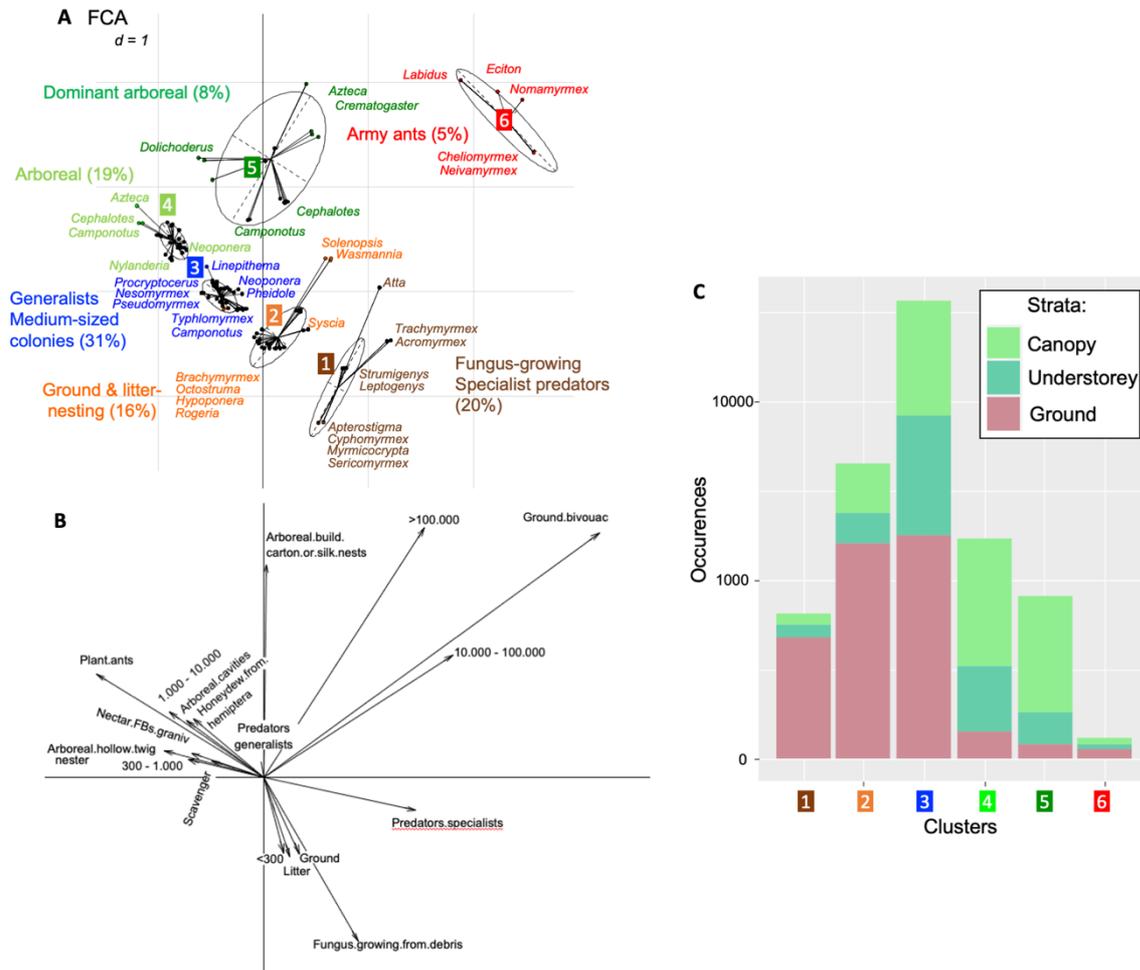
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