

1 **Ant functional structure and diversity changes along a post-grazing succession in**
2 **Mediterranean oak woodlands**

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11

12 **Abstract**

13 Grazing exclusion may be used to promote the recovery of disturbed ecosystems. A promising way
14 for the evaluation of its effectiveness is through the monitoring of key biological groups, particularly
15 those more responsive to disturbance and playing key roles in ecosystem functioning. Ants have been
16 used as ecological indicators as they are abundant, diverse and sensitive to environmental changes.
17 Here, we aimed to evaluate changes in ant taxonomic and functional structure and diversity, using
18 functional groups, along a post-grazing succession in a Mediterranean oak woodland and to
19 understand which environmental variables drive them. The post-grazing succession comprised a
20 chronosequence of grazing excluded sites for 8, 12 and 18 years and a grazed control site. We found
21 that ant species richness, functional structure and diversity increased with years since grazing
22 exclusion: Generalist/Opportunist and the Hot Climate specialists increased in the 18 years grazing
23 excluded site, while the Cryptic Species group increased in the 12 years grazing excluded site. Yet,
24 their responses were not linear over time. Time since grazing exclusion and vegetation structure
25 explained differences in ant taxonomic and functional structure and diversity. The Invasive/Exotic
26 group dominated in all sites, except in the longest excluded site, where it occurred in the lowest

27 proportion. The invasive Argentine ant dominated the grazed site, where it may have led to ant
28 taxonomic and functional homogenization. Our results suggest that the time and changes in habitat
29 structure may favour the recovery of ant biodiversity, although the presence of the invasive Argentine
30 ant species may have slowed it down.

31 **Keywords:** chronosequence of grazing exclusion; ant biodiversity; trait-based indices; Argentine ant;
32 Mediterranean woodlands; ant functional groups

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36 **Conflicts of interest/Competing interests** The authors declare no conflicting interests

37 **Ethics approval** Not applicable

38 **Consent to participate** Not applicable

39 **Consent for publication** All authors gave their consent for publication

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41 **Code availability** Not applicable

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47

48

49 **Introduction**

50 Livestock grazing has complex interactions with the environment and it has a significant
51 impact on terrestrial arthropods (van Klink et al. 2015). For arthropod communities, the severity of
52 grazing impacts depends on several variables including scale, grazing intensity and ecosystem type
53 (e.g. drylands vs wetlands; van Klink et al. 2015; Eldridge et al. 2020). Livestock grazing may have
54 direct and indirect impacts on key insect diversity and community composition (Schmidt et al. 2012;
55 van Klink et al. 2015). Firstly, livestock grazing may directly decrease insect abundance, richness
56 and biomass, through trampling and accidental ingestion, although both events may be overlooked.
57 Secondly, the lower plant diversity and less complex vegetation structure observed in areas subjected
58 to livestock grazing (Listopad et al. 2018) may present fewer potential niches and a much lower
59 spectrum of resource availability. In this way, livestock grazing indirectly shapes insect community
60 assembly through changes in vegetation structure, as many insects move between the soil-vegetation
61 interface (Andersen 2018).

62 The cessation of the disturbance, e.g. through grazing exclusion, may be a valid strategy to
63 promote the recovery of the ecosystem and its functions (Pulsford et al. 2016). In Mediterranean
64 drylands, grazing excluded sites show an increase of vegetation complexity and of the regeneration
65 of e.g. economically important trees (Vallejo et al. 2005; Listopad et al. 2018). In particular, herb and
66 shrub cover, shrub height and shrub species richness, as well as vegetation vertical structural
67 complexity increase with time since grazing exclusion and sites with more years since livestock
68 absence (Listopad et al. 2018). Along a post-disturbance succession, time since grazing exclusion is
69 a key factor to promote shrub and tree growth, although different times may be needed to recover
70 taxa and different communities (e.g. Jing et al. 2013). For example, in a long-term grazing excluded
71 site in a Mediterranean ecosystem (more than 60 years), ant species and trait diversity increased
72 (Azcarate and Peco 2012), whereas after 11 years since deer exclusion, plant species richness and
73 diversity in a forest declined (Nishizawa et al. 2016). Monitoring post-disturbance succession is a

74 way to understand changes at the community and species level, using key groups, and infer on
75 ecosystem functioning recovery (Pulsford et al. 2016).

76 Ants are ecosystem engineers with key roles in ecosystem functioning, i.e. they mediate soil
77 turnover, seed dispersal, nutrient cycling and pest control among others (Offenberg 2015). Changes
78 in ant community composition and functional traits have been linked with different land use practices
79 and because their sensitivity towards environmental changes is high, and their response to disturbance
80 and restoration efforts is quick and measurable, they are often used as ecological indicators to monitor
81 post-disturbance changes (Ottonetti et al. 2006; Dahms et al. 2010; Ellison 2012; Tiede et al. 2017;
82 Jiménez-Carmona et al., 2020). Disturbances, such as fire and deforestation and changes in land-use,
83 such as plantations, may have severe consequences for the soil-plant interface, where most ground-
84 dwelling ants live and forage (Vasconcelos et al. 2017; Martello et al. 2018; de Queiroz et al. 2020).
85 For example, *Eucalyptus* deforestation has been linked to the loss in ant taxonomic and functional
86 diversity (Martello et al. 2018), due to the simplification in vegetation vertical structure, loss in leaf
87 litter and changes in the microclimate.

88 Analysing the response of this key insect group to grazing exclusion over time, both in terms
89 of ant species and traits, may provide valuable information on the role of ant communities on
90 ecosystem functioning recovery along a post-grazing succession. To do so, we selected a
91 chronosequence of grazing excluded sites in a typical Mediterranean oak woodland ecosystem,
92 known as *Montado*. The *Montado* is characterized by a heterogeneous landscape of open woodlands
93 with low-density of cork oak (*Quercus suber*) and/or holm oak trees (*Quercus ilex*), and an understory
94 devoted to livestock grazing and/or crop cultivation (Pinto-Correia et al. 2011). Grazing by cattle,
95 sheep, goats and Iberian pigs is one of the main activities in the *Montado* and it represents a
96 fundamental practice for the existence of this ecosystem (Plieninger 2007). At the European level,
97 the *Montado* ecosystem is recognized as a High Nature Value Farmland (HNVF) due to the utilization
98 of sustainable agro-silvopastoral practices, which foster high levels of biodiversity (Paracchini et al.
99 2008). However, climate change, invasive species, land abandonment as well as high-intensity human

100 practices may represent a threat to the functioning of this ecosystem (e.g. Fernández-Manjarrés et al.,
101 2018). For example, sites under intensive livestock grazing may be characterized by a decline in *Q.*
102 *suber* regeneration, which is considered an economically important tree species in the Montado
103 ecosystem (e.g. Köbel et al. 2021). Thus, allowing *Q. suber* regeneration via grazing exclusion
104 promotes shrub and tree vegetation regrowth without any further human interventions in delimited
105 areas (Vallejo et al. 2005; Listopad et al. 2018; Köbel et al. 2021).

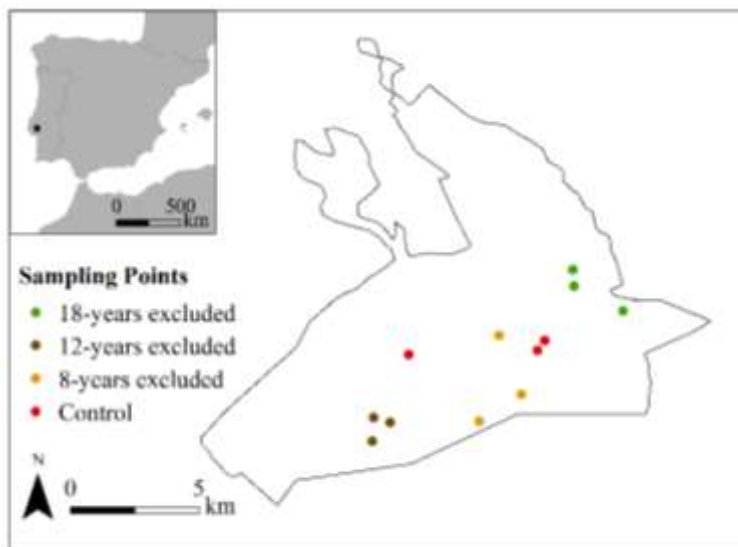
106 We formulated the following research questions: i) How do ant species richness, functional structure
107 and single- and multi-trait diversity change along a post-grazing succession? and ii) Which abiotic
108 factors drive these changes? We expect that ant species richness and functional diversity increase
109 along the post-grazing succession, coupled with changes in ant functional structure. Also, given the
110 strong link between plant and ant communities (Frenette-Dussault et al. 2013) we expect changes in
111 ant communities to be influenced by grazing exclusion duration (i.e. number of years after exclusion)
112 together with vegetation characteristics and heterogeneity.

113 **Materials and Methods**

114 *Study Area*

115 This study was conducted in the state-run property *Companhia das Lezírias* (38°50' N, 8°49' W),
116 located northeast of Lisbon, Portugal (Fig 1). This is the largest continuous area of *Montado* in the
117 country and a Long-Term Socio-Ecological Research (LTSER) site, where several studies on
118 different taxa, including lichens, biocrusts and plants have been carried out (Listopad et al. 2018;
119 Köbel et al. 2021). The study area is an 11000 ha property characterized by high land-use
120 heterogeneity resulting from diverse farming activities. The property is economically managed using
121 a multifunctional approach of different activities, such as cork extraction, cattle grazing, cereal and
122 rice cultivation and timber production from pine and eucalyptus forests. In the grazed plots, stocking
123 rate is between 0.5 and 1.1 livestock units/ha. The climate is dry sub-humid with a mean annual
124 rainfall and mean annual temperature of 662 mm and 16.3°C, respectively (www.ltsermontado.pt).
125 In a first instance, we identified all the areas from the property that were under grazing exclusion.

126 Then, in order to have a chronosequence of grazing exclusion, we selected three layers of polygons,
127 where grazing was excluded for different years, namely 8, 12 and 18 years since grazing has ceased.
128 For each polygon, 3 sampling plots (replicates) were chosen randomly after ensuring they were
129 homogeneous in terms of confounding factors such as altitude, dominant tree species, and tree
130 density. Additionally, we identified a control site (0 years-excluded site) currently grazed by cows at
131 low-density and randomly selected 3 sampling plots following the same requirements. Overall, 12
132 sampling plots were selected for this study (Fig 1). Distance between sampling plots (replicates)
133 within each sampling site varied between 450 and 5600 m.



134
135 **Figure 1.** Spatial distribution of the 12 sampling plots in Companhia das Lezírias, Portugal. Sampling plots with the
136 same colour are included in the same site and the four sites represent a chronosequence since grazing exclusion. The
137 studied post-grazing succession comprise a control site (0 years-excluded) and sites where grazing was excluded for 8,
138 12 and 18 years.

139
140 **Ant Sampling**

141 In each sampling plot, 10 pitfall traps were placed 5 m apart, along two parallel lines (5 m
142 apart). Pitfall traps consisted of plastic cups (100 ml, $\varnothing = 60$ mm), suitable to capture ground-dwelling
143 insects (Bestelmeyer et al. 2000). Traps were filled with liquid car antifreeze (ethylene glycol 5%)
144 and a few drops of liquid detergent to reduce surface tension and were protected by a plastic roof to
145 prevent flooding. Sampling lasted 5 days and was done once per site in September 2016. Ants are

146 usually active from spring to autumn, when temperatures drop, thus sampling in late
147 summer/beginning of autumn allowed us to gain representative data on the ground-dwelling ant
148 diversity occurring in the sampled area. After sampling, the specimens were transferred to 70%
149 ethanol and brought to the laboratory, where they were sorted and the ants identified to species level
150 following Collingwood and Prince (1998).

151 ***Biodiversity Metrics***

152 We used incidence data, meaning the number of pitfall traps in each sampling plot that had a
153 given species. We used incidence rather than abundance, as the latter is strongly influenced by the
154 proximity of the traps to an ant nest entrance (Gotelli et al. 2011).

155 First, we measured ant species richness for each sampling site, while for the functional analysis, we
156 selected three continuous traits (which directly relate to habitat complexity) and one categorical trait
157 (which describes ant response to disturbances). Continuous traits were measured from ant specimens
158 using a binocular Olympus SZX7 furnished with a micrometer, and included: Weber's length (the
159 length of the alitrunk), relative leg length (ratio between the hind leg length and head length; RLL)
160 and relative eye length (ratio between eye and head length; REL), as these traits provide valuable
161 biological information and are often used in ant ecological research (Frenette-Dussault et al. 2013;
162 Frasconi Wendt et al. 2020). Weber's length is often used as a surrogate for ant body size and is
163 related to habitat heterogeneity (Yanoviak and Kaspari 2000). The RLL relates to habitat structure
164 (Kaspari and Weiser 1999), while REL relates to feeding preferences and strategies (Weiser and
165 Kaspari, 2006). These continuous traits change according to the habitat structure, with ants with large
166 body sizes and leg length being advantaged in a more planar environment ("size-grain hypothesis";
167 Kaspari and Weiser 1999). The disturbance-response trait follows the group classification system
168 proposed by Roig and Espadaler (2010). This system was constructed specifically to the Iberian
169 myrmecofauna, considering the biogeographic particularities of the Iberian Peninsula (high diversity
170 and endemism) and includes eight different functional groups (Roig and Espadaler 2010). The

171 presence or absence of a certain functional groups is indicative of a disturbed/undisturbed ecosystem,
172 e.g. the presence of the Invasive/Exotic group is indicative of disturbed ecosystem.

173 To examine the functional structure of the ant community we used the Community Weighted Mean
174 (CWM), which corresponds to the average trait value weighted by the relative abundance (here we
175 used species incidence) of the species carrying that trait (Garnier et al. 2004). In the case of the
176 disturbance-response trait, each group is weighted by the number of individuals belonging to the
177 group, meaning that they are relative to each other, so if some increase (their relative proportion in
178 the community) it is at the expense of the decrease in others. To assess functional diversity, we
179 measured single-and multi-trait Rao quadratic entropy (RaoQ), which is calculated considering
180 species relative abundance (Botta-Dukát 2005). We used Gower dissimilarity distance between ant
181 species, as it takes into account mixed variable types (continuous and categorical traits; Laliberté and
182 Legendre 2010).

183 *Statistical Analysis*

184 Prior to analysis, we applied a $\log_{10}(x + 1)$ transformation to the ant incidence matrix, as we
185 recorded a high incidence of the invasive Argentine ant in most sampling points.

186 We performed a spatial auto-correlation analysis (Mantel test) between community composition
187 based on Bray-Curtis distance and geographic distance between sampling sites to evaluate if ant
188 community composition were spatially autocorrelated.

189 To understand if grazing exclusion was the main environmental driver of ant community
190 composition in the four sampling sites, we performed a non-metric multidimensional scaling (NMDS)
191 using the site per species incidence matrix (McCune et al. 2002). NMDS uses non-normally
192 distributed data and ranked distances, overcoming the “zero-truncation problem” which characterizes
193 other ordination techniques (McCune et al. 2002). The NMDS was based on the Bray-Curtis distance
194 and data underwent 500 iterations per run, each one beginning at a random start. We calculated the
195 percentage of data variability represented by each NMDS axis, using the coefficient of determination
196 (r^2). Afterwards, we overlaid vectors of the CWMs and RaoQ of the ant traits on the NMDS ordination

197 using the “envfit” function (Oksanen et al., 2017), and correlated them (Spearman, $P < 0.05$) with the
198 ordination.

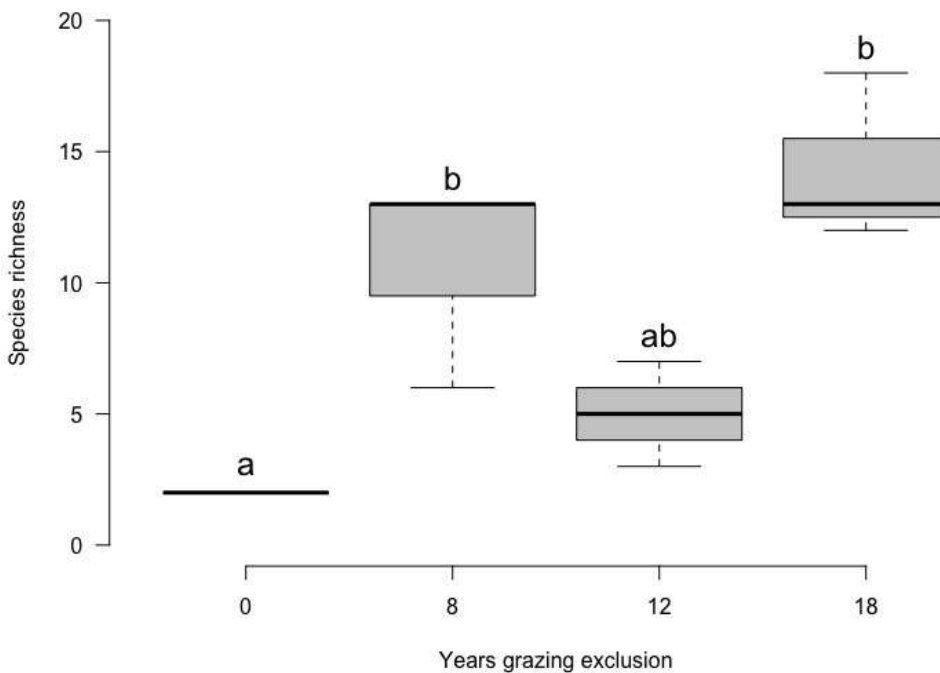
199 Differences in ant species richness, CWMs and single- and multi-traits RaoQ along the post-grazing
200 succession were assessed by Kruskal-Wallis tests ($P < 0.05$) followed by non-parametric multiple
201 comparison tests (Dunn’s test, with a Benjamini-Hochberg adjustment). In addition to the effect of
202 years after grazing exclusion on the ant community, we also considered other potential explanatory
203 variables related to vegetation vertical structure to summarize the environment in which ants move.
204 In particular, we considered shrub cover, plant species number, plant functional diversity and the
205 CWM of shrub height. These variables were retrieved from a previous study along the same post-
206 grazing succession (Köbel et al. 2021). Shrub cover and shrub height, which are indicative of the
207 habitat openness and vertical structure, were measured in the field recording respectively the length
208 of the canopy interception along a transect and the maximum height of all woody species which
209 touched the transect. Plant functional diversity was assessed through functional dispersion (FD_{is})
210 (Laliberté and Legendre 2010), based on three traits: leaf area, seed mass and shrub height (Köbel et
211 al. 2021). All potential explanatory variables were scaled and then checked for collinearity (Table
212 S1). We excluded plant species number from subsequent analysis, as it was highly correlated with
213 years of post-grazing (Spearman, $P > 0.7$; Dormann et al. 2012). Then, we built general linear models
214 using the selected predictors to explain species richness, single-trait CWMs and single- and multi-
215 traits RaoQ, after standardizing all response variables. All analyses were conducted within the R
216 statistical environment (R Core Team 2016), using packages “ade4”, “vegan” and “FD” (Dray and
217 Dufour 2007; Laliberté et al. 2014; Oksanen et al. 2017).

218 **Results**

219 A total of 25 ant species were recorded along the chronosequence of grazing exclusion (Table
220 S2). The 18 years-excluded site showed the highest species richness (22 species), followed by the 8
221 years-exclusion site (17 species), the 12 years-excluded site (7 species) and the grazed site with just
222 1 species (Fig 2). We found the Argentine ant in 10 out of 12 plots; this invasive species was not

223 detected in two sampling plots belonging to the 18 years-excluded site. The Mantel test showed no
224 autocorrelation between the ant community composition and the spatial distance between sampling
225 sites ($r = 0.14$, $P = 0.11$).

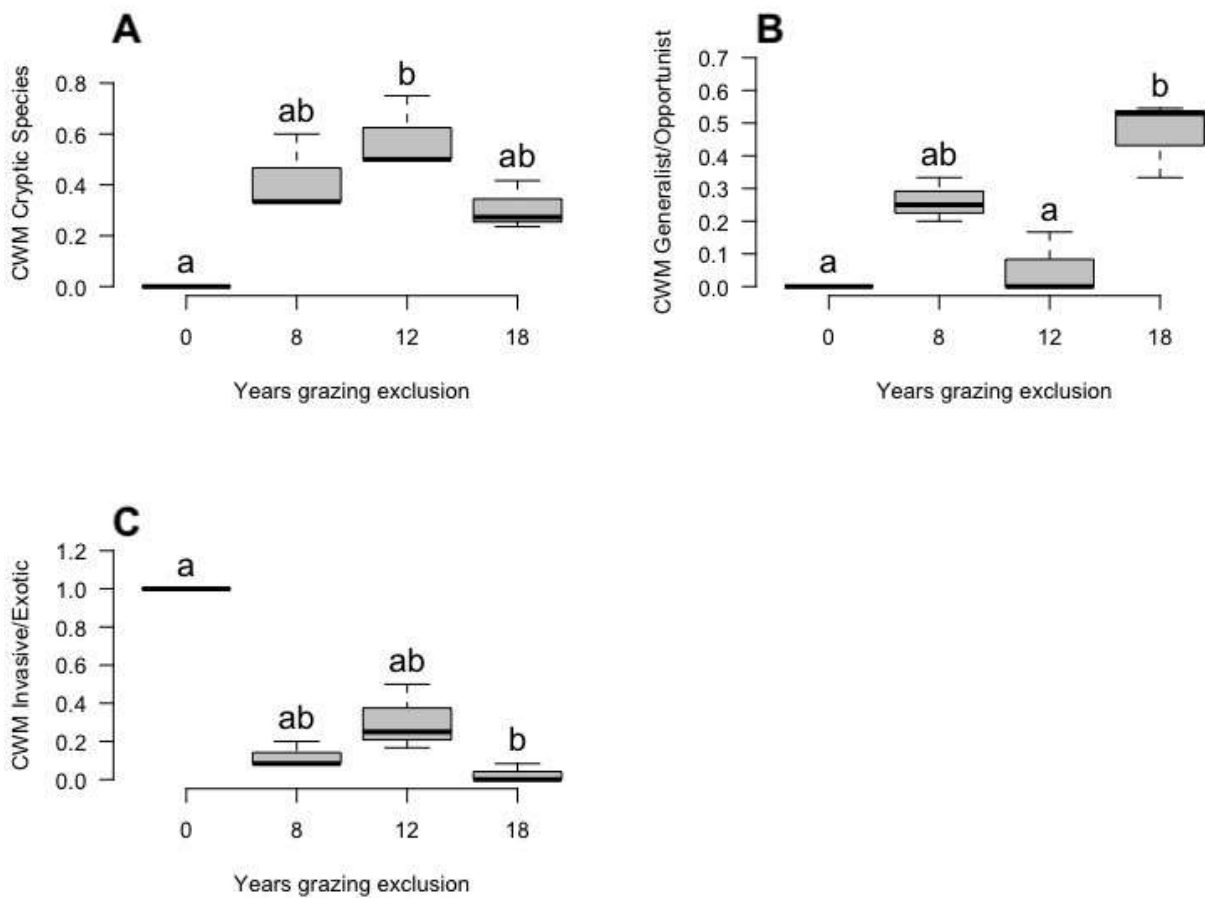
226 Species richness significantly differed between the four sites (Fig 2; Kruskal-Wallis: $\chi^2 = 9.46$, $P =$
227 0.02).



228
229 **Figure 2.** Ant species richness along a chronosequence of grazing exclusion. Boxplots represent the mean and
230 standard deviation, for the four sampling sites. Different letters represent significant differences in the means assessed
231 by Dunn's post-hoc analysis.

232
233 The PERMANOVA test on ant community composition showed a significant difference along
234 the post-grazing succession ($df = 3$, $F = 8.16$, $p < 0.001$). The majority of native ant species were
235 associated with the sampling plots with longer time since grazing exclusion, whereas the invasive ant
236 species was on the opposite side at the grazed site (Fig S1 and S2; correlations (Spearman, $P < 0.05$)
237 between CWMs and RaoQ and ordination are in Table S3).

238 Most ant species belonged to the Generalist/Opportunist group (N = 9). Seven and five species
239 were listed in the Cryptic Species and in the Hot Climate Specialist/Open Habitat group, respectively;
240 whereas the Cold Climate Specialist/Shade Habitat group accounted for two species and the
241 Invasive/Exotic and Specialist Predator groups had a single species each. The Invasive/Exotic group,
242 which accounted for only the invasive Argentine ant species (*Linepithema humile*), showed the
243 highest incidence in all sites, except in the 18 years-excluded site, where the Generalist/Opportunist
244 was the most abundant functional group. The CWM of continuous functional traits did not
245 significantly increase along the post-grazing succession (data not plotted). Whereas, the CWM of
246 four out of six groups, namely the Generalist/Opportunist (Kruskal-Wallis: $\chi^2 = 9.97$, $P = 0.01$), the
247 Invasive/Exotic (Kruskal-Wallis: $\chi^2 = 9.82$, $P = 0.02$), the Cryptic species (Kruskal-Wallis: $\chi^2 = 8.93$,
248 $P = 0.03$) and the Hot Climate Specialist/Open Habitat (Kruskal-Wallis: $\chi^2 = 8.23$, $P = 0.04$) groups,
249 significantly differed along the post-grazing succession (Fig 3). The 18 years-excluded site had higher
250 percentage of the Generalist/Opportunist species and lower of the Invasive/Exotic group compared
251 to the 0 years-excluded site, whereas the Cryptic Species group differed between the 12 and the 0
252 years-excluded sites.

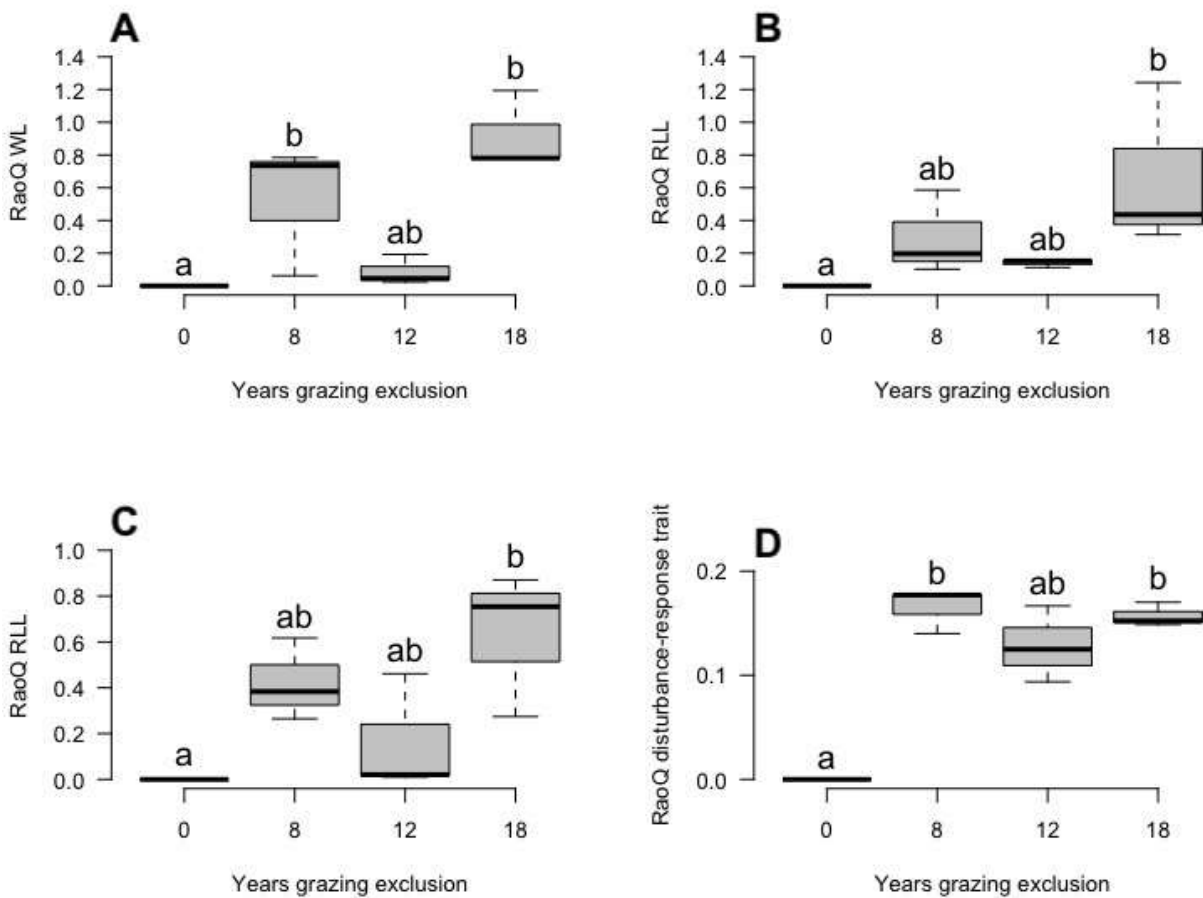


253

254 **Figure 3.** Changes in functional structure (Community-Weighted-Mean) of: A) the Cryptic species group, B) the
 255 Generalist/Opportunist group and the C) Invasive/Exotic group along the post-grazing succession. Boxplots represent
 256 the mean and standard deviations for the four sampling sites. In each graph, the different letters represent significant
 257 differences in the means assessed by Dunn's post-hoc analysis.

258

259 All single-traits RaoQs changed along the post-grazing succession. The RaoQ of Weber's length
 260 (Kruskal-Wallis: $\chi^2 = 9.28$, $P = 0.02$) and of the disturbance-response trait (Kruskal-Wallis: $\chi^2 = 7.95$,
 261 $P = 0.04$) differed significantly between the grazed and 18 years-excluded site and between the grazed
 262 site and the 8 years-excluded site (Fig 4). The RaoQ of the RLL (Kruskal-Wallis: $\chi^2 = 8.55$, $P = 0.03$)
 263 and of the REL (Kruskal-Wallis: $\chi^2 = 8.19$, $P = 0.04$) differed significantly between the grazed and
 264 18 years-excluded site. Multi-trait RaoQ increased along the post-grazing succession too, with the 8
 265 years- and the 18 years-excluded sites differing significantly from the grazed site ($P < 0.05$; Fig S3).



267

268 **Figure 4.** Changes in functional diversity (RaoQ) of: A) Weber's length (WL), B) Relative leg length (RLL), C) Relative
 269 eye length (REL) and D) disturbance-response trait along the post-grazing succession. Boxplots represent the mean and
 270 standard deviations for the four sampling sites. In each graph, the different letters represent significant differences in
 271 the means assessed by Dunn's post-hoc analysis.

272

273 Variation for species richness, all CWMs, except for the CWM of the Cryptic species, and
 274 single- and multi-traits RaoQ was explained by years since grazing exclusion and shrub cover (Table
 275 1). More years since grazing exclusion or a higher shrub cover contributed to an increase in CWMs
 276 of native groups and in single- and multi-traits RaoQ, while less years since grazing exclusion
 277 contributed to an increase in the CWM of the Invasive/Exotic group.

278 **Discussion**

279 We found a non-linear increase of ant species richness along the post-grazing succession: the 8 and
280 the 18 years-excluded sites showed a significantly increase in species richness compared to the grazed
281 site, where only one species, namely the invasive Argentine ant, was recorded. However, the
282 occurrence of the Argentine ant in most sampling sites puzzled the response of ant biodiversity to the
283 post-grazing succession. Invasive Argentine ants arrived in Portugal more than one hundred years
284 ago (Silva Dias, 1955) and established along the coast of the Iberian Peninsula (Espadaler and Gómez
285 2003). Our observations concur with previous studies in other ecosystems: the occurrence of invasive
286 ant species may result in a loss of native ant species richness and in a strong taxonomic
287 homogenization (Holway and Suarez 2006; Arnan et al. 2018) and may reduce or even prevent
288 ecosystem processes and services mediated by the native ant community (Gómez and Oliveras 2003).
289 In terms of ant community composition, the one encountered in the 18 years-excluded site differed
290 from those found in the grazed one, with ant community assemblages sharing a similar ant community
291 composition to the findings by Jiménez-Carmona et al. (2020) in an analogous Mediterranean
292 woodland. The measurement of ant functional structure and diversity allowed us to better understand
293 ant biodiversity responses along the post-grazing succession.

294 With regards to functional diversity and functional structure, functional diversity of all traits
295 and the proportion for four disturbance-response groups changed along the succession, although the
296 increase was non-linear. For example, the proportion of the Hot Climate Specialist/Open Habitat and
297 the Generalist/Opportunist group increased in the 8 and in the 18 years-excluded sites, whereas the
298 Invasive/Exotic group showed an opposite trend: it dominated in the grazed site, it was present (at
299 high incidence) in the 8 and 12 years-grazing excluded sites, while it was nearly absent in the 18
300 years-grazing excluded site. The collapse of the native community and biotic homogenization of the
301 grazed site is associated to the numerical and behavioural dominance and faster discovery of food
302 resources of the invasive species (Holway et al. 2002; Holway and Suarez 2006). Once invasive
303 species are established, they represent a secondary source of pressure on native biodiversity, as stated
304 by the “back-seat drivers” hypothesis (Bauer 2012). In the grazed site, livestock grazing and the

305 presence of the invasive ant may have acted synergistically, displacing all native ant species and
306 causing the collapse of the entire ant community.

307 In the 8 and 12 years-excluded sites, the proportion of the Invasive/Exotic group was still high and it
308 may still represent a pressure. However, some native species were able to co-occur with the invasive
309 species, probably benefiting from the absence of cattle and the recovery of vegetation cover and
310 diversity which allowed niche diversification (Cammell et al. 1996).

311 Instead, in the 18 years-grazing excluded site, the Invasive/Exotic functional group was nearly absent,
312 while the Hot Climate Specialist/Open Habitat and the Generalist/Opportunist groups dominated.
313 This last group accounts for dominant species belonging to the genera *Pheidole* and *Tapinoma*, which
314 are characterized by broad adaptations in terms of food preferences, as well as fast recruitment and
315 ability to monopolize food sources (Andersen 1995). Similar to our results, Lindsay and Cunningham
316 (2009) reported an increase in the Generalist Myrmicinae and Opportunists, which is the equivalent
317 group to the Generalist/Opportunist found in the Iberian Peninsula, in grazing excluded sites of grassy
318 woodlands in Australia. Following the “dominance-diversity relationship” (Arnan et al., 2018), which
319 states that communities with native dominant species show a higher species richness compared to
320 communities without them, the occurrence of native dominant species may have promoted ant species
321 richness, as well as trait diversity in the 18 grazing excluded site.

322 Our results evidenced that species richness and trait-based indices responded to both years
323 since grazing exclusion and shrub cover and agree with findings by Azul et al. (2011) in a similar
324 Mediterranean ecosystem. In particular, we found that while years of grazing exclusion or shrub cover
325 explained changes for species richness and most CWMs of the functional groups, both years since
326 grazing exclusion and shrub cover are necessary to describe changes in single- and multi-trait
327 functional diversity. The positive relationship between species richness and years since grazing
328 exclusion contrast with findings by Eldridge et al. (2020), who instead reported a positive relation
329 between ant species richness and grazing. Differences in habitat structure may imply changes in
330 microclimatic and microhabitat conditions and in resources, which may in turn affect ant functional

331 diversity (Andersen 2018; Martello et al. 2018; Nooten et al. 2019). In other words, more years since
332 grazing exclusion may allow ant species to arrive and establish at the sites while changes in shrub
333 cover may promote exploitation on a larger number of ecological niches (higher functional diversity)
334 and ant species to coexist in the same environment (Martello et al. 2018).
335 However, years of post-grazing succession and shrub cover were not related in a linear way and
336 vegetation structure may be influenced by other variables. Furthermore, the drop in the 12 years-
337 excluded site of ant species richness, functional structure of some native functional groups and
338 functional diversity and increase in the proportion of the Cryptic species group may be related to
339 unmeasured characteristics at this site. Cryptic species live on the soil and litter and may show a
340 positive relationship with sites where percentage of leaf litter increases (Andersen 2018; Eldridge et
341 al. 2020). Thus, sampling leaf litter and investigating the relationship between leaf litter and this
342 group at the 12 years-grazing excluded site is needed, as it may be an important variable explaining
343 changes in this functional group (Arcoverde et al., 2018).

344 Concluding, we found that changes in ant biodiversity were primarily explained by two
345 factors, years since grazing exclusion and shrub cover. However, dynamics of ant community along
346 the post-grazing succession remained unclear, as the responses of ant species richness, functional
347 structure and diversity were non-linear along the post-grazing succession and were puzzled by the
348 presence of the invasive Argentine ant species, which led to a taxonomic and functional
349 homogenization of ant community in the grazed site. Thus, long-term monitoring is essential to
350 understand the interactive effects of vegetation recovery, grazing exclusion and invasive ant presence
351 on native ant diversity.

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501 **Table 1** Summary of the best linear models for each response variable (CWMs and single- and multi-
502 traits RaoQ of ant functional traits). Explanatory variables are years of post-grazing succession and
503 shrub cover and their interaction (years post-grazing X shrub cover). For each explanatory variable
504 we indicate the sign of the coefficients and the significance (p-value: . < 0.1; * < 0.05; ** < 0.01; ***
505 < 0.001)

Response Variables							Adj. r ²
	Index	Functional traits	Disturbance-response groups	Years post-grazing	Shrub cover	Years post-grazing X shrub cover	
Species richness				(+)**			0.46
Multi-trait	RaoQ			(+)**	(+)**		0.77
Single-trait	RaoQ	FG		(+)**	(+)*		0.67
				(+)*			0.38
				(+)**	(+)	(+)*	0.63
				(+)*			0.36
CWM	Disturbance-response trait	HCS/OH			(+)*		0.29
					(+)*		0.39
				GO	(+)**		0.49
				IE	(-)***		0.70

506 Abbreviations: FG = disturbance-response trait; WL = Weber's length; RLL = Relative leg length; REL = Relative eye
507 length; HCS/OH = Hot climate specialist/open habitat; CCS/SH = Cold Climate Specialist/Shadow Habitat; GO =
508 Generalist/Opportunist; IE = Invasive/Exotic.