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1 **High resilience of seed dispersal webs highlighted by the**

2 **experimental removal of the dominant disperser**

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20

SUMMARY

The pressing need to conserve and restore habitats in the face of on-going species loss [1, 2], requires a better understanding of what happens to communities when species are lost or reinstated [3, 4]. Theoretical models show that communities are relatively insensitive to species loss [5, 6], however they disagree with field manipulations showing a cascade of extinctions [7, 8] and have seldom been tested under field conditions [e.g. 9]. We experimentally removed the most abundant seed-dispersing ant species from seed-dispersal networks in a Mediterranean landscape, replicating the experiment in three types of habitat, and then compared these communities to un-manipulated control communities. Removal did not result in large-scale changes in network structure. It revealed extensive structural plasticity of the remaining community, which rearranged itself through rewiring, while maintaining its functionality. The remaining ant species widened their diet breadth in a way that maintained seed dispersal, despite the identity of many interactions changing. The species interaction strength decreased; thus the importance of each ant species for seed dispersal became more homogeneous, thereby reducing the dependence of seed species on one dominant ant species. Compared to the experimental results, a simulation model which included rewiring considerably overestimated the effect of species loss on network robustness. If community-level species loss models are to be of practical use in ecology or conservation, they need to include behavioural and population responses and they need to be routinely tested under field conditions; doing this would be to the advantage of both empiricists and theoreticians.

RESULTS

We documented 2146 ant-seed interactions from the 36 plots established in three habitat types along a decreasing gradient of ecological complexity (complex Montado forest, grazed forest and cereal fields). In each habitat type we sampled six control plots, and six experimental plots. *Messor barbarus* dominated the networks in the 18 control plots (complex Montado: 67%, grazed forest: 65%, cereal field: 67% of the interactions). The removal of M*. barbarus* in experimental plots was performed by treating trails and nest entrances with a formicidade. Eleven ant species (2 to 7 species per plot) were recorded carrying seeds of 150 plant species (5 to 28 species per plot), establishing 401 unique ant-seed interactions (Supplemental Data Set). Species and interactions have different levels of sampling completeness at the plot scale, being very high for ant species, high for seed species and medium for interactions (96%, 61 % and 41% respectively, Table S1, Supplemental Experimental Procedures). Detection proportion however, was similar in control and experimental plots (Table S1). We tested the effect of the removal of *M. barbarus* on ant and seed species richness, network structure and seed dispersal. We then compare our empirical results to those from a species loss model which predicts the effect of *M. barbarus* removal. Full details and full results of the linear models (LMs), and the generalized linear models (GLMs) for plot-level statistics, or the linear and generalized linear mixed models (LMMs and GLMMs) for multiple seed or ant species per plot are available in Supplemental Experimental Procedures and Supplemental Results.

Effect of removing of *M. barbarus* on the number of seeds dispersed, seed species richness and network architecture.

The effect of *M. barbarus* removal on the number of seeds dispersed differed between habitats, with no significant change in the complex Montado and the cereal habitats (Tukey test, p = 0.153 and p = 0.965, respectively), but a significant decrease in the grazed forest (Tukey test, p < 0.001) (Figure 1A; Table S2). The species richness of seeds dispersed was unaffected by the removal of *M. barbarus* (Figure 1B; Table S3).

To determine whether the removal of *M. barbarus* affected the structure of the networks, six network descriptors [10–12] were calculated for each plot: 1) network specialization [13]; 2) interaction evenness [10]; 3) vulnerability [14]; 4) connectance [15]; 5) interaction strength asymmetry [16] and 6) network robustness [17] (Supplemental Results, Table S4 and references therein). In addition, species richness and species evenness for both ants and plants were calculated for each plot, making ten variables in total. A permutational multivariate analysis of variance (PerMANOVA) was used to test for differences in all ten variables between the experimental and control plots, and between the three habitats. There was no significant differences in either case, nor an interaction effect (Pseudo-$F_{1,30}$ = 0.298, p = 0.626; Pseudo-$F_{2,30}$ = 1.537, p = 0.219; and Pseudo-$F_{2,30}$ = 1.621, p = 0.205 respectively).

GLMs for individual variables showed that habitats differed significantly in terms of seed evenness, interaction evenness, connectance, and interaction strength asymmetry (Figure 2, Table S3). The significant differences were always between the two forested habitats and the cereal fields: between complex Montado and cereal fields (interaction evenness and connectance),

4

96   and between grazed forest and cereal fields (all the four variables) (Figure 2,

97   Table S3). Interaction strength asymmetry was negatively affected by the

98   removal of ants (LM, p = 0.03; Figure 2, Table S3), i.e. the dependence

99   imbalance between the two levels of interacting species was reduced in

100 experimental plots. Network specialization, vulnerability, and network

101 robustness were unaffected by habitat and treatment (Table S3). Ant species

102 richness and evenness was unchanged by the removal of *M. barbarus* and by

103 habitat (Table S3); thus, the loss of *M. barbarus* was offset by the movement of

104 other ant species into the experimental plots. The power of the models fitted to

105 the variables in the PerMANOVA was medium to large (0.53 to 0.99; 0.72 ±

106 0.05) [18]. The minimum effect size, relative to the control, detectable at a

107 significance level of 5%, and power values of 0.80 and 0.95 were modest (2.2%

108 ± 3.0 and 5.4% ± 5.5 respectively, Table S5, Supplemental Experimental

109 Procedures).

110   We calculated the mean number of unique ant-seed interactions in the

111 networks, and this was unaffected by habitat and treatment (Table S2).

112 However, when calculated for species other than *M. barbarus*, allowing us to

113 ask how these species changed their diet following the removal of *M. barbarus,*

114 there were significantly more unique interactions between these ant species

115 and seed species in experimental than in control plots (GLM, p < 0.001; Table

116 S2), this effect being unrelated to habitat.

117   Diet breadth was significantly greater in experimental than in control plots

118 (GLMM, p < 0.001; Table S2), thus in the absence of *M. barbarus*, the

119 remaining ant species expanded their dietary range, and a greater number of

120 seed species was taken by each ant species. Differences were also found

121 between the habitats: significantly more seed species were taken by ants in

122 cereal fields than in complex Montado (GLMM, p = 0.025; Figure 1C; Table S2).

123   To understand how the relative dependency of the network on each ant

124 species changed in response to the removal of *M. barbarus*, we calculated the

125 mean difference in species interaction strength [16] between the ant species

126 with the highest score and the rest of the ant community, i.e. how much seed

127 dispersal functioning is dependent on the ant species with the highest strength.

128 The difference in species interaction strength was significantly smaller (LMM, p

129 < 0.001), decreasing in all habitats when *M. barbarus* was removed, and this

130 effect was significantly greater in grazed forest (Tukey, p < 0.001) than in the

131 other habitats (Figure 1D, Table S2).

132

133 **Effect of removing of *M. barbarus* on seed dispersal**

134 We measured how frequently the different seed species were dispersed by ants

135 by counting the number of plots where each seed was dispersed – i.e.

136 occurrence, and subtracting occurrence in control plots from occurrence in

137 experimental plots to give an occurrence difference. Removing *M. barbarus* had

138 no consistent effect upon occurrence, increasing in complex Montado, but

139 decreasing in grazed forest and cereal fields (Figure 1E, Table S2). While the

140 system showed considerable variation in seed identity, there was very little

141 variation in the seed species richness. In the experimental plots, 38 plant

142 species absent from control plots were sampled, but in the control plots, 40

143 species absent from the experimental plots were sampled, of which 28 were

144 dispersed only by *M. barbarus*. Looking exclusively at seed species dispersed

145 by *M. barbarus* the number of seeds of each plant species dispersed was

146   positively affected by *M. barbarus* removal from experimental plots (GLMM, p <

147   0.001; Table S2). The rarest plant species appeared to be those most strongly

148   affected by the removal, with 67% of the species lost recorded once or twice

149   (Figure S1). However a randomization test indicated that this difference could

150   be accounted by a sampling effect (Supplemental Experimental Procedures).

151

152   **Comparing the empirical data to mathematical simulations of species**

153   **removal**

154   We simulated *in silico* the effect of removing *M. barbarus* from the plots by

155   removing the species and its interactions from the control plot datasets, while

156   allowing some degree of rewiring [19]: resources from *M. barbarus* were made

157   available to the remaining species, following Carvalheiro *et al.* [20]

158   (Supplemental Experimental Procedures). We looked specifically at two

159   variables: seed species richness, which provides information on the integrity of

160   the seed dispersal service after removal; and robustness, which measures

161   future responses of the networks to further species loss.

162   Our model overestimated the impact of removing *M. barbarus* from the

163   networks (Figure 3). The model predicted its removal would result in a 40%

164   reduction in the number of seed species dispersed (i.e. richness); the empirical

165   removal revealed a rather different outcome: increased seed species richness

166   in the complex Montado experimental plots, and a much smaller than predicted

167   decrease in experimental plots in the other two habitat types (Figure 4A). For

168   robustness, the simulations again predicted a large decrease due to removal of

169   *M. barbarus*, whereas either a small increase (grazed forest) or small decreases

170   were observed in the experimental plots (Figure 4B). Differences between the

171 simulation and both control and experimental plots were highly significant for

172 seed species richness and robustness (GLMMs for both variables, $p < 0.001$,

173 Table S6).

174

175 **DISCUSSION**

176

177 The removal of the most abundant ant species in the network did not result in

178 large-scale changes in the structural properties of the ant-seed dispersal

179 network. Indeed, the number of seeds dispersed was only significantly reduced

180 in grazed forest. Furthermore, the only network variable to change due to

181 removal was interaction strength asymmetry, which increased following the

182 removal of *M. barbarus*. This result is even more striking because *M. barbarus*

183 clearly dominated control plots by transporting ca. 65% of the seeds dispersed.

184 The networks were structurally resilient and, following the removal of the

185 dominant species, the remaining ant species compensated this loss via

186 changes in behaviour. New ant species moved into the community, the

187 remaining ant species dispersed more seed species, and the dependence of

188 plants on the different ant species was homogenized. The identity of some of

189 the interactions within the networks changed however: rare plant species were

190 the most affected by removal of *M. barbarus*, but this is mostly likely

191 consequence of a sampling effect.

192

193 **Limitations**

194 There are two main limitations to our approach. Firstly, seed dispersal only truly

195 occurs when a seed reaches a new place, escaping predation or becoming

8

196 unviable, and generates a new individual [21]. Harvester ants are very effective

197 seed collectors, but actually disperse as few as 0.1% of the seeds they gather

198 [22]. However, these rare dispersal events have the potential to shape seedling

199 recruitment in habitats characterized by harsh germination conditions, and high

200 rates of seed death [23–25]; both of these likely to occur in Montado. Secondly,

201 observations took place over two months, and thus we observed behavioural

202 plasticity rather than population changes. However, this response provides a

203 fast acting buffer to any changes to the community.

204

205 **The effect of removal of *Messor barbarus* on the seed-dispersal network**

206 The number of interactions remained fairly constant because the remaining ant

207 species assumed the role of *M. barbarus*. Simulations of species loss in other

208 mutualistic networks – pollination networks – suggest that they cope surprisingly

209 well to species loss. In pollination networks, the rate of loss due to linked

210 extinctions is linear, rather than showing precipitous decreases, even when the

211 most linked species go extinct first [6]. However, field manipulations of

212 pollination systems suggest that simulations may seriously underestimate the

213 impact of species loss; for example, losing a single pollinator species can impair

214 the reproductive outcome of plants [9]. Brosi and Briggs [9] pointed out that the

215 role of species in ecosystem functions is dynamic; they change their

216 interactions depending on the presence of other competitors. However, these

217 authors considered only the impact of removing one pollinator species on one

218 plant species, so the community-wide impact of their manipulation remains

219 unknown. Our community approach allows the detection of compensatory

220 effects at the scale of the network, and of both losses and gains in a wide range

9

221 of species. We found that most of the seed species lost from the networks were

222 rare, but that these rare species were replaced by other rare species. However,

223 the observed change in species occurrence was a sampling effect. The seeds

224 of 28 rare species were dispersed by *M. barbarus* and the association between

225 a dominant, highly generalized species and rare species is seen in other

226 mutualistic networks that are characterized by a highly nested architecture [26].

227   Habitat type affected seed dispersal with consistent differences between

228 both forested habitats and the cereal fields (Figure 2). Canopy cover is an

229 important driving factor in the stability of host-parasitoid networks in forests, and

230 a reduction in cover may increase spatiotemporal homogenization [10]. The

231 significantly lower interaction evenness in our canopy-free plots than in our

232 forest plots is an effect also observed by Tylianakis et al. [27].

233   Our results fit within the general concepts of resilience and robustness,

234 albeit some subtle distinctions. Highly resilient networks return to their original

235 state after perturbation, as defined by the identity of their interactions [3, 28, 29].

236 This is clearly not the case with our system, as the identity of the interactions is

237 different after the perturbation. Similarly, while we are working in the general

238 field of robustness (recording the response of the community to species loss),

239 the term network robustness refers to network structure rather than function [5,

240 6, 30]. Our ant-seed dispersal system is characterized by a high level of

241 structural plasticity [19] which allows extensive rewiring. However, what we

242 observe goes beyond this, as rewiring does not necessarily imply the continuity

243 of function, a network could rewire, but function could be diminished. What we

244 observed was a highly resilient community that shows considerable structural

245 plasticity while maintaining functionality, via an increase in diet breath of the

10

246 remaining ant species. However, we do not know whether this structural

247 plasticity is sustainable.

248

249 **The simulated species removal**

250 The species loss simulations seriously overestimated the effect of species loss

251 on seed dispersal. Models predicted the loss of the dispersal service to rare

252 species in the community, but failed to predict that other rare species would be

253 dispersed instead. Moreover, the simulations overestimated the impact of

254 species loss on network robustness. These results emphasize a real need to

255 develop models that predict more accurately the outcome of perturbations.

256 Although we assumed that some rewiring would occur (distributing shared seed

257 resources among the remaining species), our simulations remained unrealistic.

258 In real communities, mechanisms of compensation following the loss of a

259 competitive species induce reshuffling of the interactions between other

260 species, which may provide better resistance to disturbance to the system as a

261 whole [31]. This could occur at both the individual level (short-term behavioral

262 responses) and the species level (longer term population responses).

263

264 **CONCLUSION**

265

266 This is one of the few studies in which the effect of species removal from an

267 ecological network is tested in a replicated field experiment at the level of the

268 whole community. We observed a remarkable degree of resilience and

269 restructuring in our seed dispersal networks, enabling seed dispersal to

270 continue, despite a huge experimental perturbation. The simulation models,

11

271   however, provided a poor prediction of our experimental findings, emphasizing

272   the need for better models if these are to be of practical use. The incorporation

273   of behavioral and population responses is critically needed in this context.

274   Closer collaboration between field ecologists and theoreticians would improve

275   the likelihood of this, as large-scale, well replicated, ambitious field experiments

276   are needed, alongside new theoretical approaches.

277

278   **AUTHOR CONTRIBUTIONS**

279   ST collected the data, performed modelling work, undertook analysis and wrote

280   the first draft, JR advised on field logistics and analysis, IV advised on the

281   statistics and revisions to the manuscript, JM designed the study, ST, JR and

282   JM contributed substantially to manuscript revisions.

283

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373

FIGURE LEGENDS

**Fig. 1:** A) Number of seeds recorded as being dispersed, and B) seed species richness (B), means ± SEM (n = 36 plots). C) Diet breadth of ants other than *M. barbarus*, means ± SEM (114 observations of ten ant species, in 36 plots). D) Species interaction strength difference in ant species, between the most abundant species in the network and the remaining species, means ± SEM (31 observations of ten ant species), in control plots (no removal of *M. barbarus*) and experimental plots (removal of *M. barbarus*) in three habitats. E) Seed species occurrence difference, mean difference ± SEM (220 observations from 150 plants species), bars above the line show that the number of plots where seed species were dispersed was higher in the experimental plots; bars below the line show that the number of plots where seed species were dispersed was higher in the control plots), in the three habitats sampled in control plots (no removal of the ant *M. barbarus*) and experimental plots (with removal of *M. barbarus*). Different letters (a, b) indicate significant differences between habitats. * Significant effect of treatment within habitat. Details of the statistical analysis and outcomes can be found in Supplemental Experimental Procedures and Tables S2 and S3.

Fig. 2: Response variables used in the univariate LMs showing differences between the three habitats, means ± SEM (n = 36 plots): A) Seed species evenness, B) Interaction evenness, C) Connectance, and Interaction strength asymmetry. Only variables with significant differences are shown (different letters (a, b, c) indicate significant differences between habitats). Details of the

16

statistical analysis and outcomes can be found in the Supplemental

Experimental Procedures and Table S3.

Fig. 3: Control, empirical removal, and simulated ant—seed dispersal networks, from plots chosen as representative of the different treatments and habitats. Each species is represented by a rectangle, seeds at the bottom level and ants at the top level; the widths of the rectangles are proportional to the species' abundance in each plot. The size of each triangle connecting ants and seeds represents the frequency of interactions in the each plot. *M. barbarus* interactions are shown in white in the control plots; this species was removed from the empirical removal and simulated removal plots.

Fig. 4: Comparison of the empirical removal of *M. barbarus* and its simulated removal, in three habitats: A) the empirical and simulated differences (%) to control plots in dispersed seed species richness, and B) the empirical and simulated differences to control plots in network robustness (53 observations from two types of networks). Bars above the x-axis show that the parameter was greater in the presence of *M. barbarus* than in its absence, bars below the x-axis show the opposite. Details of the statistical analysis and outcomes can be found in Supplemental Experimental Procedures and Table S6.
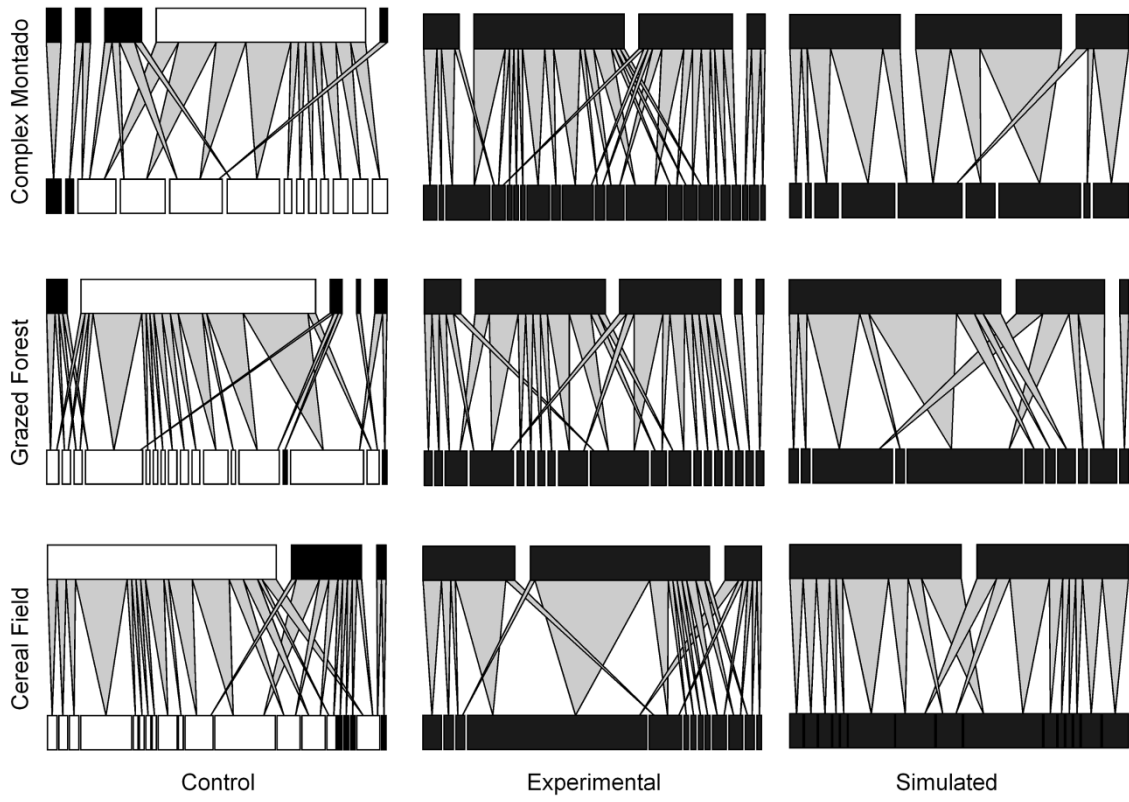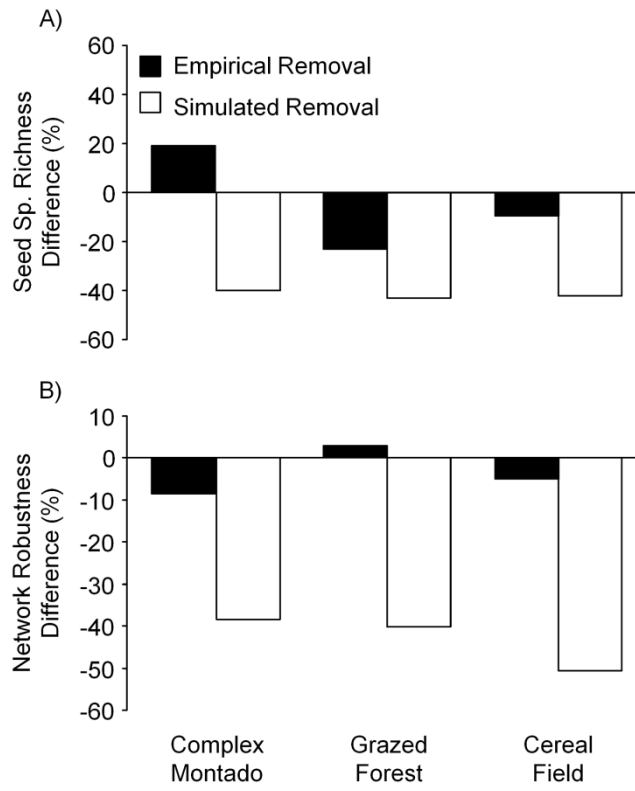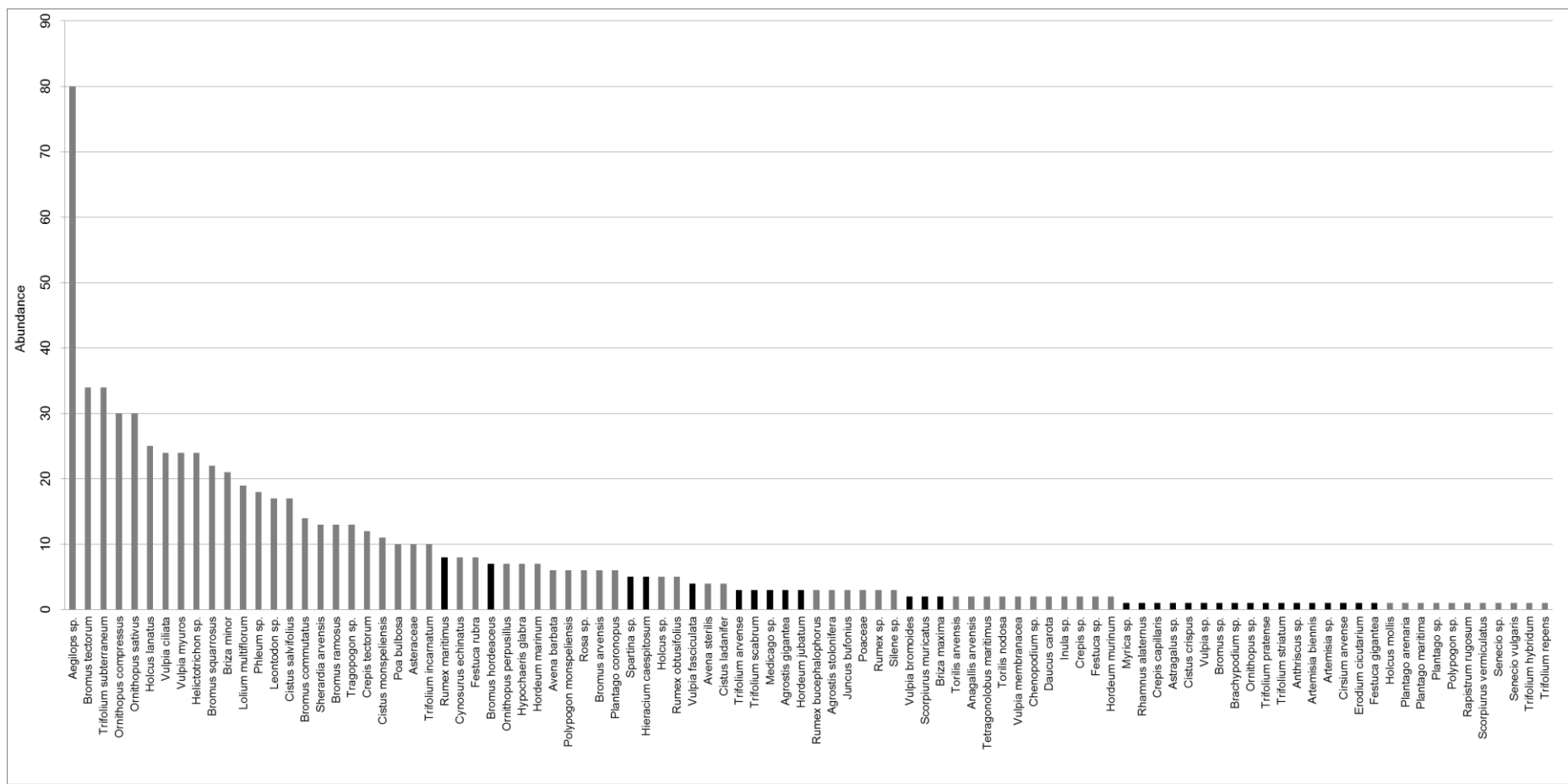
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Fig. 1

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Fig. 2

Fig. 3

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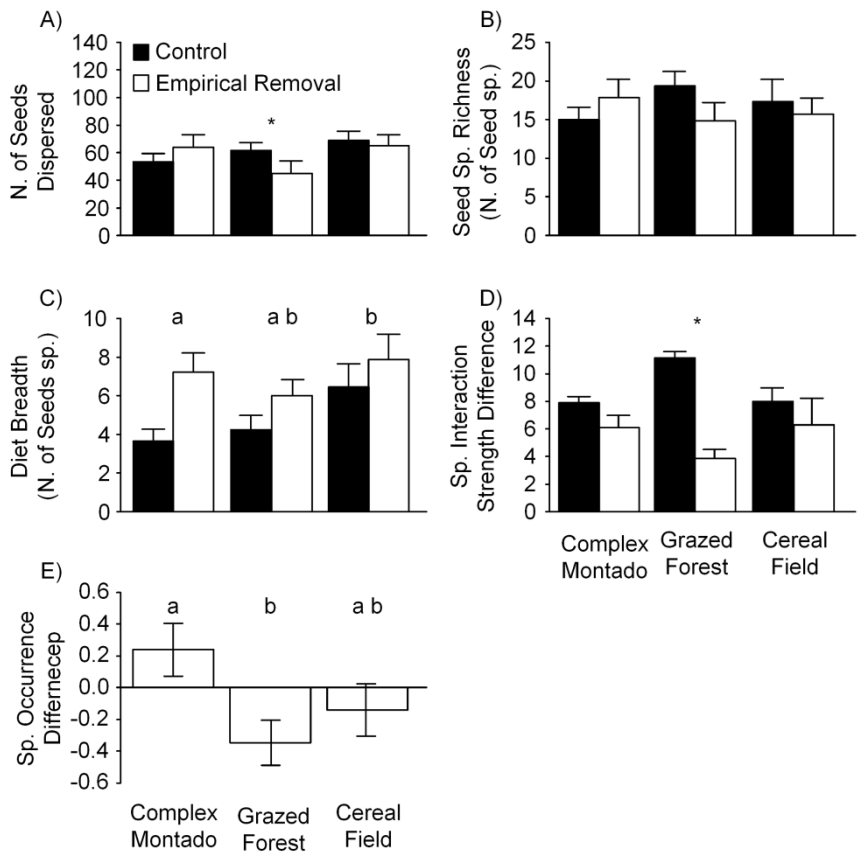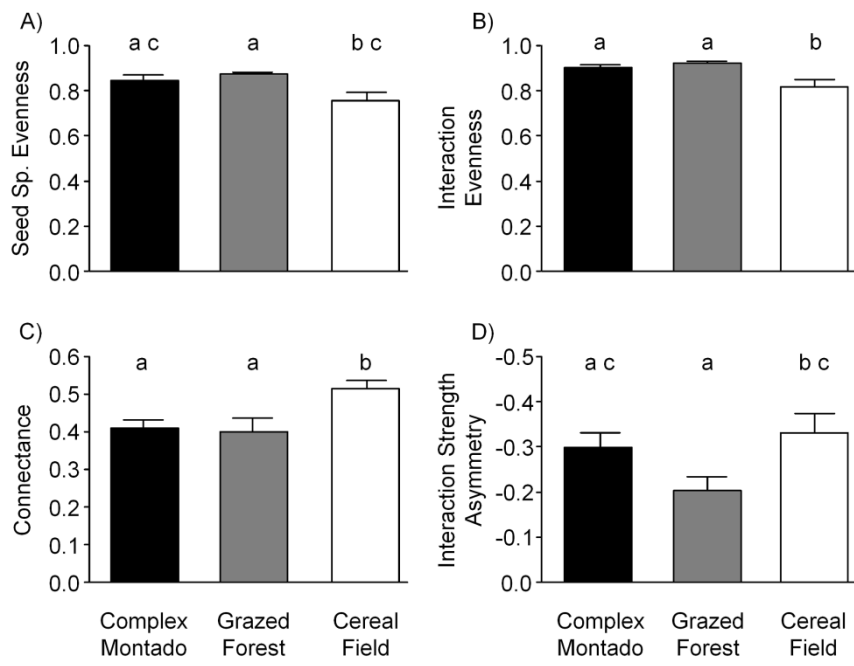
Fig. 4

Figure S1 - Distribution of the abundance of seeds dispersed by *Messor barbarus* in control plots. Species lost from the experimental plots are shown in black.

	Control	Treatment	Overall
Ant species	96% ± 2.39	97% ± 1.79	96% ± 1.50
Seed species	63% ± 5.48	59% ± 4.17	61% ± 3.46
Interactions	43% ± 4.63	39% ± 4.23	41% ± 3.16

Table S1 – Mean detection percentage (± SEM) of ant species, seed species and interactions between species, in control plots, experimental plots, and overall. Detection percentage was calculated as the proportion of the estimated number of species/interactions recorded (based on Chao 2 estimator) (see Supplemental Experimental Procedures).

3

	Response variable and model structure	Parameter	Estimate ± SE	P
Effect of removing of *M. barbarus* on network architecture and species diversity	Number of seeds dispersed: GLM; log-link, Poisson errors	Intercept	3.977 ± 0.056	< 0.001
		Habitat (GF)	0.145 ± 0.076	0.057
		Habitat (CF)	0.255 ± 0.074	< 0.001
		Plot (Experimental)	0.182 ± 0.076	0.016
		GF:Experimental	- 0.501 ± 0.110	< 0.001
		CF:Experimental	- 0.240 ± 0.104	0.021
		X^2 = 41.479, 5 df, p < 0.001		
	Unique interactions (all ants): GLM; log-link, Poisson errors	Both variables	> 0.05	
		X^2 = 6.414, 3 df, p = 0.093		
	Unique interactions (no *M. barbarus*): GLM; log-link, Poisson errors	Intercept	2.283 ± 0.100	< 0.001
		Habitat (GF)	0.091 ± 0.110	0.408
		Habitat (CF)	- 0.188 ± 0.119	0.112
		Plot (Experimental)	0.512 ± 0.096	< 0.001
		X^2 = 35.138, 3 df, p < 0.001		
	Diet breadth: GLMM; log-link, Poisson errors	Intercept	0.980 ± 0.246	< 0.001
		Habitat (GF)	0.032 ± 0.140	0.818
		Habitat (CF)	0.327 ± 0.146	0.025
		Plot (Experimental)	0.425 ± 0.118	< 0.001
		X^2 = 15.421, 3 df, p = 0.001		
	Species interaction strength difference: LMM; identity link, normal errors	Intercept	8.412 ± 0.737	< 0.001
		Habitat (GF)	3.084 ± 0.812	< 0.001
		Habitat (CF)	- 0.020 ± 0.963	0.983
		Plot (Experimental)	- 1.817 ± 0.865	0.045
		GF:Experimental	- 5.654 ± 1.122	< 0.001
		CF:Experimental	- 0.090 ± 1.371	0.948
		X^2 = 15.421, 5 df, p < 0.001		

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14   (Cont.)

	Response variable and model structure	Parameter	Estimate ± SE	P
Effect of removal of *M. barbarus* on seed dispersal	Difference in occurrence: LMM; identity link, normal errors	Intercept	0.239 ± 0.160	0.137
		Habitat (GF)	- 0.586 ± 0.221	0.009
		Habitat (CF)	-0.380 ± 0.226	0.095
		$X^2 = 7.125$, 2 df, p = 0.028		
	Seeds dispersed by *M. barbarus*: GLMM; log-link, Poisson errors	Intercept	1.144± 0.145	< 0.001
		Plot (Experimental)	0.264 ± 0.049	< 0.001
		$X^2 = 28.962$, 1 df, p < 0.001		

15   **Table S2 (related to Figure 1)** – Results for the Linear Mixed Models (LMMs), Generalized Linear
16   Models (GLMs) and Generalized Linear Mixed Models (GLMMs) comparing different response variables
17   between control and treatment plots, and among the three habitat types. For ease of interpretation, models
18   only included the interaction between treatment and habitat when this reduced the AIC: no other model
19   simplification was used. *P*-values are derived from *t*-tests for models with normal errors and *z*-tests for
20   models with Poisson errors. X^2 test was performed on deviance of the final model against that of a null
21   model. GF – Grazed Forest, CF – Cereal Fields. Parameter estimates for habitat are relative to complex
22   Montado.
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	Response variable and model structure	Parameter	Estimate ± SE	P
Effect of removing of *M. barbarus* on network architecture and species richness	Ant species richness: GLM; log-link, Poisson errors	Both variables		> 0.05
		$X^2 = 4.052$, 3 df, p = 0.256		
	Seed species richness: GLM; log-link, Poisson errors	Both variables		> 0.05
		$X^2 = 0.856$, 3 df, p = 0.836		
	Network specialization: LM; identity link, normal errors	Both variables		> 0.05
		Adjusted $R^2 = -0.006$, $F_{3,32} = 0.931$, p = 0.437		
	Interaction evenness: LM; identity link, normal errors	Intercept	0.906 ± 0.655	< 0.001
		Habitat (GF)	0.696 ± 0.674	0.223
		Habitat (CF)	- 0.786 ± 0.674	0.007
		Plot (Experimental)	0.226 ± 0.655	1.000
		Adjusted $R^2 = 0.302$ $F_{3,32} = 9.349$, p = 0.002		
	Vulnerability: LM; identity link, normal errors	Both variables		> 0.05
		Adjusted $R^2 = 0.024$, $F_{3,32} = 4.459$, p = 0.297		
	Connectance: LM; identity link, normal errors	Intercept	0.388 ± 5.950	< 0.001
		Habitat (GF)	7.826 ± 5.280	0.616
		Habitat (CF)	2.776 ± 5.280	0.009
		Plot (Experimental)	5.634 ± 5.950	0.281
		Adjusted $R^2 = 0.233$, $F_{3,32} = 4.538$, p = 0.009		
	Interaction strength asymmetry: LM; identity link, normal errors	Intercept	- 0.338 ± - 0.029	< 0.001
		Habitat (GF)	0.073 ± - 0.037	0.041
		Habitat (CF)	- 0.024 ± - 0.037	0.511
		Plot (Experimental)	0.064 ± - 0.029	0.030
		Adjusted $R^2 = 0.234$, $F_{3,32} = 4.562$, p = 0.009		

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	Response variable and model structure	Parameter	Estimate ± SE	P
Effect of removing of *M. barbarus* on network architecture and species richness	Network robustness: LM; identity link, normal errors	Both variables		> 0.05
		Adjusted R^2 = - 0.037, $F_{3,32}$ = 0.587, p = 0.628		
	Ant evenness: LM; identity link, normal errors	Both variables		> 0.05
		Adjusted R^2 = 0.181, $F_{5,30}$ = 5.119, p = 0.049		
	Seed evenness: LM; identity link, normal errors	Intercept	1.162 ± 2.670	< 0.001
		Habitat (GF)	2.791 ± 2.495	0.480
		Habitat (CF)	1.866 ± 2.495	0.023
		Plot (Experimental)	3.140 ± 2.670	0.543
		Adjusted R^2 = 0.185, $F_{3,32}$ = 3.655, p = 0.023		

Table S3 (related to Figure 1 and Figure 2) – Results for the GLMs and LMs of variables included in the univariate models: network specialization, interaction evenness, vulnerability, connectance, interaction strength asymmetry, robustness, ant evenness, seed evenness, ant species richness, and seed richness. Variables were entered transformed or untransformed, whichever applies. Model fitting and presentation of results is as described for Supplemental Table S1. Parameter estimates for Habitat are relative to complex Montado (CM). GF – Grazed Forest, CF – Cereal Fields.

Variable	Description
Interaction Specialization	A network level measure for specialization, based on the Shannon diversity index, and calculated as the deviation from be the minimum specialization expected given the matrix. Interaction Specialization ranges from 1 (total specialization) to 0 (no specialization). This index has the advantage of not being affected by network size or sampling intensity, delivering reliable and robust comparisons [S1].
Interaction Evenness	This index is the Shannon index for interactions, using the total number of realised interactions as the denominator [S2, S3]. It has been demonstrated that habitat disturbance affects Interaction Evenness negatively [S2], although this may be a mathematical consequence of losses in abundance of plants or animals [S4].
Vulnerability	Originally defined as the weighted mean number of predators per prey [S3]; in the present work adapted to ant species per seed species.
Connectance	An unweighted measure of the fill of the network, calculated by dividing the number of observed links by the total number of possible links (C=L/(IJ). It has been shown that Connectance has a negative relationship with species diversity [S5], though this could be a probabilistic artefact due to a combination of sampling effort with species abundance, in networks of different sizes [S4]. In food webs, Connectance promotes an increase in Robustness to secondary extinctions, and they are more susceptible to "attacks" (extinction of the most connected nodes) than to "errors" (random loss of nodes) [S6]. In mutualistic networks, Connectance may promote persistence (number of species remaining after disturbance) of the assemblage but is detrimental to its resilience (speed of recovery) [S5].
Interaction Strength Asymmetry	Also an indicator of specialization between the levels of the web, measuring dependence asymmetry in the overall interactions [S7]. Singleton species are assigned disproportional influence, but bipartite package's version of this metric removes all singleton species in order to avoid such influence [S8]. Negative values imply higher dependency in the lower levels of the network.
Network Robustness	Robustness gives a measure of robustness to the loss of species. It is rooted on the rationale that if a given proportion of species becomes extinct (primary extinctions) from one level of a network, species on other levels that depend on them will be eliminated as a consequence (secondary extinctions). This can be depicted by a extinction curve characterized by its slope - extinction slope [S9]. This was improved by Burgos *et al.* [S10] who proposed a simple single parameter: the area under the curve (AUC) of extinction.

44 **Table S4** – Description of the network variables entered in the PerMANOVA and LM models (see
45 Supplemental Experimental Procedures).

Variables	Minimum effect size (%) detectable at 0.80 power	Minimum effect size (%) detectable at 0.95 power
Ant species richness	2.7	9.2
Seed species richness	0.8	1.1
Network specialization	< 0.1	0.6
Interaction Evenness	< 0.1	< 0.1
Vulnerability	6.2	16.8
Connectance	8.3	11.1
Network Robustness	21.3	31.2
Ant evenness	12.1	33.5
Seed evenness	8.1	12.0

Table S5 - The smallest effect sizes (control vs experimental) detectable using our experimental design, assuming a significance level of 0.05 and a power of 0.80 and 0.95 (see Supplemental Experimental Procedures). Values are calculated as the percent change in the treatment plots relative to the control, for the network metrics that were not found to respond significantly to *M. barbarus* removal (see Table S2).

	Response variable and model structure	Parameter	Estimate ± SE	P
Comparing the empirical data to mathematical simulations of species removal	Seed richness: GLMM; log-link, Poisson errors	Intercept	2.308 ± 0.090	< 0.001
		Habitat (GF)	0.077 ± 0.088	0.381
		Habitat (CF)	0.065 ± 0.089	0.468
		Plot (Control)	0.492 ± 0.094	< 0.001
		Plot (Experimental)	0.425 ± 0.095	< 0.001
		$X^2 = 31.999$, 2 df, p < 0.001		
	Robustness: LMM; identity link, normal errors	Intercept	0.155 ± 0.013	< 0.001
		Habitat (GF)	0.007 ± 0.014	0.625
		Habitat (CF)	- 0.017 ± 0.014	0.226
		Plot (Control)	0.113 ± 0.014	< 0.001
		Plot (Experimental)	0.104 ± 0.014	< 0.001
		$X^2 = 49.023$, 4 df, p < 0.001		

51 **Table S6 (related to Figure 4)** – Results for the GLMM and LMM for dispersed seed species richness
52 and network robustness, respectively. Models were simplified as described for Table S1. Parameter
53 estimates for Plot are relative to Predicted (predicted values from the simulation models).
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Supplemental Experimental Procedures

Field site, study system and data collection

The study was conducted in the Portuguese Montado, which is an agro-sylvo-pastoral system, largely dominated by two species of evergreen oaks, *Quercus suber* and *Q. rotundifolia,* with a diverse shrubby and herbaceous understory. The area is subject to the Mediterranean climate of long and dry summers, and has high diversity of both plant and animal species. The field experiments were conducted on a 1700 ha farm (N38° 42' 12.708", W-8° 19' 29.1396"). The Montado is a matrix of three habitats, all three of which were present at the field site: 1) complex Montado forest, used to harvest the bark of *Q. suber* (cork oak); the structure of the habitat is diverse, and it has trees and well-developed shrub and herbaceous layers; 2) grazed forest, used mainly for livestock grazing (sheep, pigs, or cows); the habitat is simplified, and it has a reduced shrub layer and a simplified herbaceous community; and 3) cereal fields, characterized by a very low density of trees, a complete absence of shrubs and, with the exception of the crop and annual weeds, no herbaceous layer.

We replicated our experiment in each of the three habitats, to determine whether our results were context-dependent or could be generalized across different habitats. We chose an ant-seed dispersal community for manipulation, and used observations of ants carrying seeds as a proxy for dispersal; the latter is the usual approach in this field [S11, S12]. In each of the three habitats, six control and six experimental plots were chosen (a total of 36 plots); each plot included a nest entrance of the most abundant ant species in this area (*M. barbarus*, Linnaeus 1767: Formicidae: *Messor*). Ant species in the *Messor* genus are found all over the world, especially in the Palearctic, being *M. barbarus* the most studied species of them [S13]. *M. barbarus* is a very common seed harvester in Mediterranean grasslands and scrublands [S14, S15], with an important role on the composition and structure of the plant communities of these habitats [S16] The plots were 10 m by 10 m in size and at least 30 m apart (further than the maximum distance seeds are transported by large individual ants [S17]). Control and experimental plots were assigned haphazardly avoiding clustering of plots of either type (i.e. they were not paired spatially). To remove *M. barbarus* from experimental plots, the nest entrances and trails leading to them were treated with a formicide (Deltamethrin, a synthetic pyrethroid). Nests were checked every other day, and re-treated if necessary, until ant activity ceased after about five weeks. Nests were monitored throughout the field season for any further activity and retreated if necessary. The application of the formicide was highly targeted and is unlikely to have affected other ant species, as these do not use the trails and entrances of *M. barbarus*.

Ants and seeds were sampled twice between the beginning of August and mid-September 2012, from 0730h to 1330h, by a team of two people. Ant activity was low in the afternoons due to high temperatures; ants are most active at temperatures of between 25 and 30ºC [S18, S19], and the afternoon temperature at the field site exceeded 35 ºC. Each plot was searched for interactions – an ant of any species carrying a seed – for two hours each day. Two plots were sampled each day, one experimental and one control and in order to sample ant species with different activity periods, searches alternated hourly between the two plots. Both the ants and their seeds were collected for identification. Ant identification was carried out using a guide to the ants of Portugal [S20] and confirmed by an ant taxonomist (see Acknowledgements); identification of seeds to plant species (71.4%), or to the lowest taxonomic level possible (27.6% and 1.0% to genus and family levels, respectively, and henceforth referred as species for simplification), was carried out using a reference collection from the field, along with identification manuals [S21, S22] and two online resources [S23, S24]. The seed-dispersal network for each plot, showing all recorded interactions, was visualized and analyzed using bipartite package in R [S25]. Analysis was based on these 36 networks and on the data used to construct them.

Sampling completeness and species/interactions detection

To estimate the effectiveness of species and interaction detection, we estimated the total numbers of ant species, seed species, and interactions present in all 36 plots, comparing these estimates to our observed values. A non-parametric estimator – Chao 2 was used, which is based on the proportion of unique species relative to the proportion of duplicate species (species/interactions collected in a single sample and in two samples, respectively) [S26, S27]. The software EstimateS 9.1.0 [S28] was used to calculate expected richness. Sampling completeness was calculated by dividing the observed richness by the estimated total richness. Detection of ant species was very high and close to the expected richness (96%), high for seed species (61%) and relatively low for interactions (41%) (Table S1). Critically, sampling completeness was independent of treatment, with the detection proportion in the control and experimental plots always within 4% of one another (Table S1).

Effect of removing of *M. barbarus* on the number of seeds dispersed, network architecture and seed/ant species richness.

To test effect of removing *M. barbarus* on the number of seeds dispersed at each habitat we performed for each habitat a separate generalized linear model (GLM). To determine the effect of *M. barbarus* removal on the architecture of the networks, six widely used network variables [e.g. S2, S29, S30] were calculated for each plot (see Table S4). Four of the six network variables provide information about how generalized the seed dispersal process is: 1: network specialization (based on interaction diversity [S1]), 2: interaction evenness (uniformity of link distribution [S2]), 3: vulnerability (number of ant species per seed species [S3]), and 4: connectance (proportion of realized links [S31]). The fifth variable, interaction strength asymmetry, indicates how balanced the network is, and measures overall dependence and the direction of the asymmetry between the two levels [S7]. Finally, network robustness evaluates the ability of the network to cope with extinctions, and measures its response to species loss [S10]. As well as these six network variables, species richness and evenness for both ants and plants were calculated for each plot, making 10 variables in total.

A permutational multivariate analysis of variance (PerMANOVA [S32]) was used to test for overall differences in the ten variables between the experimental and control plots and between the three habitats. This is a non-parametric multivariate test that makes use of dissimilarity matrices, and through a series of permutations calculates p values drawn from F statistics [S32]. Although its power is lower than a parametric counterpart, such as a MANOVA, it is robust to multivariate heterogeneity of multivariate dispersion, and makes no assumptions about multivariate normality [S32, S33].

We explored the effect of treatment and habitat on each individual on each individual variable. Separate linear models (LMs), were used to test the effect of treatment and habitat on the individual variables, except for ant and plant species richness for which GLMs were used. Residuals were plotted and checked for departures from normality, and variables were transformed (log or power transformations) to ensure the best fit to normality; variables were back-transformed for the presentation of results. Linear Models (LMs), General Linear Models (GLMs), and the linear and generalized linear mixed models (LMMs and GLMMs) were fitted using R software [S34], using packages lme4 [S35] and nlme [S36] for the latter two. Tables S2, S3 and S6 provide full details of the different models used, including link functions and error distributions, model refinement and full results. Models always contained the main effects, but for ease of interpretation, interaction terms were only included if they reduced the AIC. Pairwise differences between habitats and habitat-treatment combinations were assessed using Tukey tests, with R's multcomp package.

The number of unique ant-seed interactions, in each network was calculated; in the control plots it was calculated for all ant species collectively and then for all species minus *M. barbarus*. To test the effects of *M. barbarus* removal and habitat on the number of unique ant-seed interactions, we used a GLM.

To quantify ant diet breadth, we calculated the number of seed species taken by each ant species other than *M. barbarus* in each plot. We investigated variation in ant diet breadth by using a GLMM with treatment and habitat as fixed effects. Plot and ant species were included as random effects to control for potential pseudo-replication given the multiple observations (ant species) from the same plot, and for differences in the ant species among plots.

We used species interaction strength to quantify the overall dependence of plants on each of the ant species. Species interaction strength measures how important a species at one level of the network is to the species at another level, and is calculated as the sum of the dependencies of each species [S7]. In the context of our ant—seed system, we used species interaction strength to quantify the overall dependence of plants (all species) on each of the ant species. To understand how the relative dependency of the network on each ant species changed in response to the removal of *M. barbarus*, we calculated the mean difference in species interaction strength between the ant species with the highest score and the rest of the ant community, in each habitat for each species. The greater this difference, the greater the dependence of plant species on a single species of ant, and this demonstrates the degree of its dominance in relation to the rest of the community. Differences in species interaction strength in plots with and without *M. barbarus* and between habitats were tested with a LMM, entering ant species as a random effect.

The number of seeds recorded as being dispersed in each plot was compared between treatments (control vs. experimental) and habitats by using a GLM.

Power analysis

The level of replication of our networks across the system (6 plots per treatment per habitat, n = 36) is above the common level of replication typical in most empirical network studies [e.g. S37–S41], and in line with the best replicated empirical networks [e.g. S2, S42]. However non-significant differences between treatments (control vs experimental plots) could still be due to insufficient replication. Consequently, we performed a post-hoc power analysis to estimate the smallest treatment effect (difference between treatment and control) that could be detected using our experimental design. This was done for the non-significant statistical results from the univariate analysis of network metrics (see Table S3 for variables under analysis), following the procedure by Johnson et al. [S43]. This method is based on 1000 simulations of the dataset used to fit the model, using parameters estimated from the original model as a base to recreate the dataset. We re-ran the simulation using a range of possible effect sizes to find the smallest value that would be detected at a significance level of 5% and adopting power values of 0.80, which is commonly adopted in ecology, and 0.95, which ensures that as much emphasis is placed on Type II errors (our primary concern here) as for Type I errors [S44]. The results (Table S5) suggest that we would have been able to detect treatment effects relative to the control of < 0.01% to 21.3% (2.2% ± 3.0), with a power of 0.80, and of < 0.01% to 33.5% (5.4% ± 5.5), with a power of 0.95, for the nine variables, confirming the ability of our experiment to detect large changes in network structure, and increasing the confidence in our conclusion that network metrics are little affected by removal of *M. barbarus*.

Effect of removing of *M. barbarus* on seed dispersal

We measured how frequently the different seed species were dispersed by ants, and how this was affected by *M. barbarus* removal. For each plant species in each habitat, its occurrence in control plots (i.e. the number of plots where seed species were recorded as being dispersed) was subtracted from its occurrence in experimental plots, calling this variable "occurrence difference". We used a LMM to test whether this difference varied between habitats, entering plant species as a random effect.

Finally, we looked exclusively at the seed species dispersed by *M. barbarus*, and tested the effect of *M. barbarus* removal on the overall mean number of dispersed seeds per plant species, by using a GLMM, entering plant species and plot as random effects to control for multiple observations from the same plot, and differences in plant species composition among the plots.

Randomization test to determine whether observed differences in the dispersal of rare seed species is a sampling effect.

We used a permutational MANOVA, based on 10 000 permutations, to identify differences in the overall seed species composition among habitats, and between treatments. The permutational MANOVA showed that the overall seed community was significantly different among habitats (p < 0.001), but not between treatment and control (0.72).

However, the observed differences in the dispersal of rare species could be a sampling effect, and for this reason we used a randomization test. We tested whether the number of seed species unique to either control (40 species) or experimental (38 species) plots (a total of 78 species, henceforth referred to as "unique species" as opposed to the other 72 species found in both control and treatment plots) was significantly greater than that expected by chance (i.e. exceeding a potential sampling effect). We repeatedly randomised the data, and each time the number of unique species was counted and compared to the observed number (78).

The randomization test worked in the following way: 1) the ant-seed interactions were summarised in each of the 36 plots, giving the number of seeds of each species; 2) within each of the three habitats separately (to account for the large inter-habitat differences in the overall seed species list), the 12 plots were randomly reallocated to give six 'control' and six 'treatment' plots (i.e. just switching the labels around); 3) the randomised data sets from the three habitats were combined and the overall number of unique species calculated. The process was run 10 000 times to build up a frequency distribution for the number of unique species. The observed number of unique species in the field data (78) was then compared to this frequency distribution, and the number of simulations that produced >78 unique species counted.

Across the simulations, the mean number of unique species to either control or treatment plots was 74.9, with more than 78 unique species occurring in 1291 simulations, giving a p-value of 0.13 i.e. the observed number of unique species was not significantly greater than that expected by chance, suggesting that 78 unique species was consistent with a sampling effect.

Comparing the empirical data to mathematical simulations of species removal

For comparison with the empirical data, we simulated the effect of removing *M. barbarus* from the plots by removing the species and its interactions from the control plot datasets, i.e. by instigating an *in silico* extinction. Given the efficiency with which ants locate and gather newly available resources [S14, S45], we assumed that the seeds we observed being dispersed by *M. barbarus* would be taken by other ant species in the plots. Therefore, seeds made available by the removal of *M. barbarus* (i.e. the seeds they dispersed in the control plots) were allocated to the remaining species in the network (7 ant species, ranging from 1- 6) in proportion to their abundance in each plot. Following the approach of Carvalheiro *et al.* [S46], we used the proviso of an ant species only being allocated a seed species if it had been observed taking a seed of this species. Carvalheiro *et al.* [S46] accounted for both saturated and unsaturated resources, but we assumed that the remaining ant community was unsaturated, and that each of the remaining species added more workers to the community to collect the additional seeds. Mathematically, the process follows the equation:

$$A_i = O_i + R \times ((O_i) \div \sum_{i=1}^{n}(O_i))$$

where A_i is the predicted amount of seeds of a remaining seed species following removal of *M. barbarus*, and dispersed by each ant species ; O_i is the original quantity of a remaining seed taken by an ant species; and R is the quantity of seeds taken originally by *M. barbarus*. Plants whose seeds were solely moved by *M. barbarus* are lost from the network, thus reflecting the impact that the removal of this species has on the seed dispersal process. In reality, we don't know how many seeds remain in the environment as we only have data on those observed being carried by an ant. However, we are interested in the ant-seed dispersal community and so we assume that we have data on all the seeds that are ant dispersed. We looked specifically at the variables seed species richness and network robustness. Both seed species richness and network robustness were compared between treatments (control, empirical removal, and simulated removal) using a GLMM. Network was modeled as a random effect to account for a lack of independence between control and simulated networks, the latter being derived from the control network in each plot.

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