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Contrasting indirect effects of an ant host on prey–predator interactions of symbiotic arthropods

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1 **Contrasting indirect effects of an ant host**
2 **on prey-predator interactions of symbiotic arthropods**
3

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18

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20 and DB analyzed the data. TP wrote the manuscript; other authors provided editorial advice.
21

22 **Abstract**

23 Indirect interactions occur when a species affects another species by altering the density
24 (density-mediated interactions) or influencing traits (trait-mediated interactions) of a third
25 species. We studied variation in these two types of indirect interactions in a network of red
26 wood ants and symbiotic arthropods living in their nests. We tested whether the ant workers
27 indirectly affected survival of a symbiotic prey species (*Cyphoderus albinus*) by changing the
28 density and/or traits of three symbiotic predators, i.e. *Mastigusa arietina*, *Thyreosthenius*
29 *biovatus* and *Stenus aterrimus*, provoking respectively low, medium and high ant aggression.

30 An ant nest is highly heterogeneous in ant worker density and the number of aggressive
31 interactions towards symbionts increase with worker density. We therefore hypothesized that
32 varying ant density could indirectly impact prey-predator interactions of the associated
33 symbiont community.

34 Ants caused trait-mediated indirect effects in all three prey-predator interactions, by affecting
35 the prey-capture rate of the symbiotic predators at different worker densities. Prey capture rate
36 of the highly and moderately aggressed spider predators *M. arietina* and *T. biovatus* decreased
37 with ant density, whereas the prey capture rate of the weakly aggressed beetle predator *S.*
38 *aterrimus* increased. Ants also induced density-mediated indirect interactions as high worker
39 densities decreased the survival rate of the two predatory spider species.

40 These results demonstrate for the first time that a host can indirectly mediate the trophic
41 interactions between associated symbionts. In addition, we show that a single host can induce
42 opposing indirect effects depending on its degree of aggression towards the symbionts.

43

44

45 **Introduction**

46 Community dynamics are propelled by a complex set of antagonistic, neutral and mutualistic
47 interactions. Direct interactions as found in symbiosis and predation have been traditionally
48 studied to understand the structuring of communities. However, theoretical and empirical
49 evidence increasingly show that indirect interactions are abundant and strongly shape
50 community dynamics as well (Strauss 1991; Wootton 1994; Guimarães et al. 2017). Indirect
51 interactions between two species arise when the effect of one species on another is mediated
52 by a third species. Indirect effects can be subdivided into two categories. Density-mediated
53 indirect effects arise when a species indirectly affects another by changing the survival of a
54 third species (Abrams et al. 1996). A typical example is a predator that indirectly promotes the
55 growth of plants by decreasing the density of herbivores (Hebblewhite et al. 2005). Trait-
56 mediated indirect effects, on the other hand, emerge when a species affects another species
57 by altering the foraging behavior, refuge use, physiology or other traits of a third species
58 (Abrams et al. 1996; Werner and Peacor 2003).

59

60 Indirect interactions are at the heart of food web dynamics by mediating both consumptive and
61 competitive interactions (Abrams et al. 1996; Werner and Peacor 2003). A diverse array of key
62 ecological processes such as trophic cascades, trait-mediated cascades and competition are
63 induced by indirect interactions (Abrams et al. 1996; Levine et al. 2017). Ultimately, these types
64 of interactions have a structuring role in communities and may promote species coexistence
65 (van Veen et al. 2005; Levine et al. 2017).

66

67 While the study of indirect interactions is tightly linked with food web networks, a cumulative
68 number of studies also recorded them in host-symbiont networks (Dunn et al. 2012; Guimarães
69 et al. 2017). Symbionts are species that live in close association with a host on which they may
70 have beneficial, neutral or adverse effects. Indirect effects between species may govern to a
71 large degree coevolution in mutualistic networks (Guimarães et al. 2017). Guimarães et al.

72 (2017) showed that large and nested mutualistic networks are expected to have more indirect
73 effects than small and modular networks shaped by specialized mutualisms. Empirical studies
74 on indirect interactions in host-symbiont networks typically reported that these interactions
75 could alter the competitive outcome of two susceptible hosts (Hambäck et al. 2006; van
76 Nouhuys and Kraft 2012) or of two competing parasites (Waage and Davies 1986; Bush and
77 Malenke 2008; Lebrun et al. 2009). Host-mediated competition between two parasites was for
78 example found in two feather-feeding lice in which the magnitude of competition was
79 dependent on the defense (preening) of the host bird (Bush and Malenke 2008). Indirect effects
80 can also mediate trophic interactions rather than competitive interactions in this type of
81 networks as demonstrated in symbiont communities associated with pitcher plants (terHorst
82 2010).

83

84 Red wood ants (*Formica rufa* group) also form such host-symbiont networks. These
85 widespread ants are keystone arthropods in European forest ecosystems (Gösswald 1989;
86 Stockan et al. 2016). They exert wide-range effects on the forest fauna and flora and drive
87 biogeochemical processes (Wardle et al. 2011). The above ground part of a red wood ant nest
88 is a conspicuous mound of organic thatch (Rosengren et al. 1987), which is tightly regulated
89 and provides an ideal habitat for a diverse community of associated symbionts, known as
90 myrmecophiles (Parmentier et al. 2014). The majority of these red wood ant myrmecophiles
91 directly interact with their host by feeding on their eggs and larvae and stealing prey carried
92 into the nest (Parmentier et al. 2016a). In addition, many red wood ant myrmecophiles hunt on
93 smaller myrmecophiles in the red wood ant nest microcommunity (Parmentier et al. 2016a).
94 Ants also directly interact with their myrmecophile guests. If detected, ants try to deter or hurt
95 them by showing aggression. Red wood ants show a highly variable degree of aggression
96 towards myrmecophiles ranging from almost complete ignorance to heavy persecution,
97 depending on the identity of the myrmecophile species (Parmentier et al. 2016b). In addition,
98 the aggression response will be more prominent in densely crowded chambers in the nest,
99 such as the chambers with brood, compared to the nest periphery, because of an increase in

100 aggressive events and fewer opportunities to hide. Interestingly, myrmecophiles show
101 preferences for particular nest locations and corresponding worker densities (Parmentier et al.
102 2016b), likely reflecting their tolerance to different degrees of ant aggression.

103 We thus anticipate the density of red wood ant workers to indirectly affect the survival of a
104 myrmecophilous prey species by altering the behavior or survival of its myrmecophilous
105 predator. Red wood ants vary greatly in their degree of aggression towards different
106 myrmecophilous predators. The prey capture rate of those predators is as a result expected to
107 change differently with increasing worker densities. We compared the effect of worker density
108 on the prey capture rate among three different myrmecophilous predators, i.e. the rove beetle
109 *Stenus aterrimus* Erichson, 1839 and the spiders *Thyreosthenius biovatus* (O. Pickard-
110 Cambridge, 1875) and *Mastigusa arietina* (Thorell, 1871). They provoke respectively low,
111 medium and high levels of ant aggression (Parmentier et al. 2016b). The myrmecophilous
112 springtail *Cyphoderus albinus* Nicolet, 1842 was used as the shared prey of the three
113 myrmecophilous predators (Fig. 1). The increase of aggressive events caused by increased
114 worker densities will likely change the behavior of persecuted myrmecophilous predators,
115 whereas the behavior of myrmecophilous predators that provoke little or no aggression is
116 expected not to alter (Fig. 1). Therefore we hypothesized that increasing worker densities
117 would have the strongest negative effect on the prey capture rate of the myrmecophilous
118 predator that provoked the highest degree of aggression.

119 **Material & Methods**

120 *Study system and sampling*

121 Myrmecophiles, ant workers, and organic nest material were repeatedly collected in a colony
122 of the red wood ant *Formica rufa* Linnaeus, 1761 in Boeschepe, France, from June until
123 September 2017. This supercolony was headed by many queens (polygynous) and consisted
124 of multiple mounds which constantly exchanged workers and resources (polydomous).
125 Myrmecophiles were collected by spreading out nest material into a large plastic tray. The
126 white springtail *Cyphoderus albinus* is an obligate ant symbiont that may reach high densities
127 in ant nests (Parmentier et al. 2015). Ant workers pay little or no attention to its presence
128 (Parmentier et al. 2016b). This springtail is likely to be the principal prey for many
129 myrmecophilous predators and was therefore used as the myrmecophilous prey in the
130 experiment (Parmentier et al. 2016a). We selected three predators which were obligate ant
131 symbionts as well. *Stenus aterrimus* is a rove beetle specialized to capture springtails (Koerner
132 et al. 2012). Ants show weak rates of aggression (aggression in 13% of encounters; see
133 Parmentier et al. 2016a) towards this species and are distributed throughout the nest
134 (Parmentier et al. 2016b). The two other selected predators were the spiders *Thyreosthenius*
135 *biovatus* and *Mastigusa arietina*. The former can be found throughout the nest and is
136 occasionally attacked (aggression in 24% of encounters), whereas the latter is strongly
137 persecuted (aggression in 73% of encounters) and is restricted to the sparsely occupied
138 periphery of the nest (Parmentier et al. 2016b). The three tested myrmecophilous predators
139 predate on the springtail and interact with their host by kleptoparasitism and brood predation
140 (so far only recorded for *T. biovatus* and *M. arietina*, Parmentier et al. 2016a). The ants do not
141 predate on the myrmecophilous predators and prey, but only negatively interact with them by
142 an aggression response.

143 Ant workers were kept in a container with a plaster-filled bottom and given access to water and
144 sugar water ad libitum prior to the experiment. The myrmecophilous prey *C. albinus* was
145 cultured in a container with organic nest material and some hundreds of workers of the

146 supercolony. Myrmecophilous predators were individually placed in small snap-lid containers
147 (diameter 4.5 cm) filled with moistened plaster in the lab. Next, we offered to all predators three
148 *C. albinus* prey to level out differences in feeding status. Subsequently, the beetle *S. aterrimus*
149 and the spider *M. arietina* were starved for a period of 4 days and the spider *T. biovatus* for 10
150 days. We opted for a longer starvation period in *T. biovatus*, as the proportion of surviving prey
151 in presence of *T. biovatus* starved for four days (mean = 0.92, $N = 10$) was close to prey
152 survival without predators. Starving *T. biovatus* for a period of 10 days considerably increased
153 its prey capture rate. Nest material was stored in a freezer (-21 °C) to kill tiny and hidden
154 organisms such as mites, and which could potentially serve as additional prey for the
155 myrmecophilous predators. We reconciled the extensive search effort to find the relatively rare
156 predators with the need for a high number of replicates, by reusing the predators in subsequent
157 trials. Before using a predator again, it was first re-isolated, fed with three individuals of *C.*
158 *albinus* prey and starved as before. All spider individuals were female.

159 *Experiment: Prey-predator interactions under different worker densities*

160 We aimed with this experiment to test the effect of increasing density of ant workers on the
161 prey capture success of symbiotic predators (Fig. 1). The experiment was done in plastic
162 containers (diameter 5 cm, height = 5.5 cm) with a 1-cm bottom of plaster of Paris. The side of
163 the container was coated with fluon to prevent ants and myrmecophiles to climb on. The
164 container was closed with a lid in which there was a hole (diameter 2 cm) covered with fine
165 mesh. This prevented myrmecophiles from escaping by rare flying (*S. aterrimus*) or jumping
166 events (*C. albinus*). Approximately 5 mL of the defrosted organic material was then added to
167 the container together with 5 fully grown individuals of *C. albinus* collected in the stock colony.
168 The nest material and plaster was moistened. As we wanted to test prey-predation under
169 differing worker densities, we assigned 0 (0W: control), 5 (5W: low density), 15 (15W: medium
170 density) or 45 (45W: high density) ant workers to a container. Finally, one starved predator
171 individual of *S. aterrimus*, *T. biovatus* or *M. arietina* was introduced (Fig. 2). The introduction
172 of the predator in the container was approximately 30 min. later than the prey giving the latter

173 time to hide. After 48h, the number of surviving prey individuals out of 5 was recorded. To test
174 the effect of predation, we need to control for natural mortality of the prey. Therefore we
175 counted the number of prey out of 5 that survived without predators under the 4 levels of worker
176 density (number of replicates: 0W = 37, 5W = 39, 15W = 37, 45W = 40). All containers were
177 kept at 20-22 °C in complete darkness. The experiment was conducted from July until October
178 2017. The number of trials for each treatment is summarized in Table 1. We did not test the
179 effect of 45 workers on the prey capture rate and survival of *M. arietina*. In 4 preliminary tests
180 with this species, all individuals died within a couple of hours. The spider is expected not to
181 survive these stressful conditions, which was already suggested by another lab experiment
182 (Parmentier et al. 2016b). As it was difficult to obtain large numbers of *M. arietina*, we chose
183 to allocate the spiders only to 0W, 5W and 15W to assess their prey capture rate.

184

185 *Statistical analyses*

186 Myrmecophile survival under different levels of worker density

187 In some trials, predators were dead before the end of the 48h test period. This could be
188 explained by natural mortality, starvation, through increased stress or inflicted injuries by the
189 ants (Parmentier 2016). The effect of ant worker density on predator survival was compared
190 by calculating the proportion of trials in which the predator survived. Here, we only based on
191 trials in which the predator was used for the first time so that the exposure time to the ants was
192 comparable. We compared pairwise the effect of different levels of ant density on predator
193 survival by employing Fisher exact tests and subsequently controlled the false discovery rate
194 of the corresponding P-values using the Benjamini-Hochberg (BH) procedure (Benjamini and
195 Hochberg 1995).

196 To test the effect of ant density on the proportion of surviving *Cyphoderus albinus* in absence
197 of predators, a quasibinomial error distributed (to account for overdispersion) GLM was used

198 with logit link function. We assessed the significance of these baseline data with a likelihood-
199 ratio test using the 'Anova' function of the R-package car (Fox and Weisberg 2011).

200 Prey-predator interactions under different worker densities

201 Capture success under different worker densities was based on trials where the predators
202 survived until the end. One trial was excluded, because the predator was visibly hurt at the
203 end and which may have affected its prey capture success. In all other trials, predators showed
204 normal behavior at the end. For each predator separately, we modelled the proportion of
205 surviving prey under different densities of ant workers using a GLMM (generalized linear mixed
206 model) with binomial error distribution and logit link function. Treatment (= different levels of
207 worker ant density: 0 workers, 5 workers, 15 workers and 45 workers) was included as a fixed
208 factor. To account for re-using predator individuals in subsequent trials (max. 4 trials per
209 individual), we included predator identity as a random factor. This factor was nested in
210 treatment as an individual was always exposed to the same treatment level (i.e. identical
211 worker ant density) in all of its replicates. We also added an observation level random factor
212 to correct for overdispersion (Browne et al. 2005). The GLMMs were run using the 'glmer'
213 function in R-package lme4 (Bates et al. 2015) and significance was tested using a likelihood-
214 ratio test implemented in the 'Anova' function of the R-package car (Fox and Weisberg 2011).
215 Subsequently, the different levels of ant density in these GLMM-models were pairwise
216 compared with the glht function of the 'multcomp' package (Hothorn et al. 2008). Within each
217 series of pairwise tests, we controlled the false discovery rate using the BH-procedure.

218

219 **Results**

220 *Myrmecophile survival under different levels of worker density*

221 The prey *Cyphoderus albinus* was rarely noticed by the host ant and freely foraged in the nest
222 material. Survival of this species was correspondingly very high after 48h across all treatments.
223 It was seemingly not affected by the presence of ants, ranging from low to high density of ant
224 workers (quasibinomial GLM, $\text{Chisq} = 3.60$, $P = 0.31$; Fig. 3). Survival rate of the predator
225 *Stenus aterrimus* was high and equal across different levels of ant density (P -values of 6 BH
226 corrected Fisher Exact tests $P = 1.00$, proportions survivals see Table 1). Survival of the
227 predator *Thyreosthenius biovatus* declined with increasing worker density (Table 1, Fig. 3), but
228 the 6 pairwise BH corrected Fisher Exact tests were not significantly different. Survival of the
229 spider *Mastigusa arietina* steeply declined with increasing worker density (Table 1, Fig. 3).
230 Survival at medium worker densities (15 workers) was significantly lower than survival without
231 ants (BH corrected Fisher Exact test $P = 0.002$) and survival at low (5 workers) worker densities
232 (BH corrected Fisher Exact test $P = 0.029$).

233

234 *Prey-predator interactions under different worker densities*

235 To compare prey capture rate among predators and worker densities, we here only included
236 trials in which the predator survived till the end (48h-period) (Table 1). Natural mortality was
237 very low and constant across all 4 levels of ant density (see above). Therefore, we directly
238 compared the effect of the predator on the survival of the prey under different worker densities
239 without controlling for natural mortality. All three predators efficiently captured the prey *C.*
240 *albinus* in absence of ants. Indeed, the proportion of surviving prey *C. albinus* in absence of
241 ants and predators was reduced from $0.97 \pm 95\% \text{ CI } [0.94-0.99]$ to $0.46 [0.40-0.52]$, $0.54 [0.48-$
242 $0.60]$, and $0.41 [0.35-0.47]$ in presence of *S. aterrimus*, *T. biovatus*, and *M. arietina*, resp.
243 Survival of *C. albinus* in presence of the spiders *T. biovatus* and *M. arietina* was significantly
244 higher with increasing ant density (GLMM_{*T. biovatus*}: $\text{Chisq} = 28.27$, $P < 0.001$; GLMM_{*M. arietina*}:

245 Chisq = 31.61, $P < 0.001$), indicating a lower capture rate of both spiders in higher ant
246 densities. BH controlled post hoc tests on the survival of *C. albinus* with *T. biovatus* indicated
247 that *C. albinus* survival without ants and with low ant density was significantly higher than in
248 the treatments with medium and high ant densities (Table 2). Higher worker density thus
249 negatively affected prey capture success of the two spiders (Fig. 3). In contrast, survival of *C.*
250 *albinus* with *S. aterrimus* was significantly higher in the absence and low densities of ants
251 compared to medium and high worker densities (GLMM: Chisq = 31.96, $P < 0.001$, Table 2),
252 reflecting a higher prey capture rate of the beetle in medium and high worker densities.

253

254 **Discussion**

255 While it is widely accepted that symbionts can impose strong antagonistic to mutualistic direct
256 effects on their partners (Thrall et al. 2007) it is predicted that indirect interactions may be
257 equally pervasive in host-symbiont networks (e.g. Lebrun et al. 2009, van Nouhuys and Kraft
258 2012). These indirect effects may mediate competitive and trophic interactions between
259 different symbionts or change the competitive outcome of different hosts. Here, we showed
260 that indirect effects can radically alter trophic interactions between symbionts in a host-ant
261 community. We surprisingly found that the host caused contrasting indirect effects on the
262 survival of the prey symbiont *Cyphoderus albinus* by inducing different responses in its
263 predators. The symbiotic predator species responded differently to increasing worker densities
264 and their response was tightly linked with the level of aggression they provoked in the host
265 colony. The prey capture rate of the heavily persecuted predator *Mastigusa arietina* declined
266 sharply, the poorly attacked spider *Thyreosthenius biovatus* became moderately less
267 successful, whereas the tolerated rove beetle *Stenus aterrimus* even captured a higher
268 proportion of *C. albinus* prey with increasing worker densities of their red wood ant host.

269

270 Increasing ant densities differently altered the prey capture rate of the three symbiotic
271 predators. The contrasting indirect effects of the ants on the symbiotic prey here are trait-
272 mediated as they arise from changes in the behavior of the symbiotic predators, which in turn
273 affect the survival of the myrmecophilous prey. Worker density in ant nests is highly
274 heterogeneous, typically low at the periphery and high in the brood chambers (Hölldobler and
275 Wilson 1990). The frequency of aggressive interactions towards symbionts will be much higher
276 in densely crowded chambers. Consequently, the center of the nest may not be a safe
277 microhabitat for the myrmecophilous predators *T. biovatus* and *M. arietina*, which is underlined
278 by their increased mortality risk in the crowded chambers. The increasing harassment with
279 higher ant densities is likely to promote hiding behavior in the two spiders, a typical behavior
280 seen in many animals to avoid predation or injury risks (Blanchard et al. 1990, Cooper and

281 Blumstein 2015, see for myrmecophilous beetles in von Beeren et al. 2010), and will eventually
282 decrease their prey capture rate (Abrams et al. 1996; Preisser 2005; Cooper and Blumstein
283 2015). In line with the prediction that the defense behavior of animals will increase with higher
284 levels of risk (Lima and Bednekoff 1999), we found that negative effect of increasing worker
285 density was more pronounced in the spider *M. arietina*. As this species provokes aggression
286 much more frequently and severely than *T. biovatus*, it is expected to show a stronger defense
287 response and its foraging behavior will consequently be more affected. The reduced prey
288 capture rate observed in the spiders could also result from a more timid prey instead of a more
289 timid predator in presence of more ants. But this mechanism is unlikely because the prey's
290 foraging behavior and survival seemed not to be affected by ant density (pers. observations
291 TP). *Stenus aterrimus* is hardly noticed by the ants and could freely forage in the crowded nest
292 chambers. We did a priori not expect a change in its predation rate (Fig. 1), but our results
293 surprisingly showed that the beetle captured more prey in denser nest chambers. No
294 convincing explanation has yet been found to explain this pattern. It is possible that the
295 increased prey capture rate of *S. aterrimus* in densely populated chambers could be driven by
296 a higher encounter rate between prey and predator. Higher ant densities may stimulate either
297 the activity of the predator, prey or even both leading to more prey captures.

298

299 While trait-mediated effects were omniprevalent, we also found evidence of density-mediated
300 indirect effects, which are caused by a lethal effect of increasing ant density on two predators.
301 Survival of *M. arietina* was significantly lower in chambers with higher worker densities. A
302 negative, yet non-significant, trend in survival was detected in *T. biovatus*. As in many other
303 networks, trait-mediated and density-mediated indirect effects act thus simultaneously in our
304 study system (Abrams et al. 1996; Banerji et al. 2015). Reduced survival of the spiders with
305 increasing ant density could be caused by starvation or higher levels of stress. Additionally,
306 crowded nest chambers offer fewer hiding places and could make the spiders more vulnerable
307 to lethal ant attacks. We found no evidence of reduced survival of the beetle *S. aterrimus* in
308 denser nest chambers. It should be noted that the 48h test period was relatively short and we

309 cannot exclude long-term detrimental effects of high worker densities on beetle fitness.
310 Nevertheless, aggression towards the beetles is extremely low, which hints that the long-term
311 impact of living in high worker densities is low for this species.

312

313 Indirect effects can have a major effect on competitive interactions. Plants are well known to
314 mediate competitive interactions between herbivores (Inbar and Gerling 2008; Utsumi et al.
315 2010). Typically, a herbivore induces morphological or chemical defences in the host plant
316 which in turn will hamper the fitness of a competing herbivore (Inbar and Gerling 2008).
317 Likewise, host-mediated interspecific competition in parasite communities has been reported
318 in multiple studies focusing both on micro- and macroparasites (Waage and Davies 1986;
319 Chilcutt and Tabashnik 1997; Bush and Malenke 2008; Lebrun et al. 2009; Ulrich and Schmid-
320 Hempel 2012). Both theoretical (Levine et al. 2017) and empirical work (van Veen et al. 2005)
321 underline the role of indirect effects in host-symbiont network stability and shaping species
322 coexistence. Multiple studies already reported niche partitioning in host-symbiont networks
323 (Proffitt et al. 2007, Hechinger et al. 2009, von Beeren et al. 2010), but did not report indirect
324 effects as a mechanism of the symbiont community assembly so far. We suggest that indirect
325 effects may shape the competitive interactions between the three symbiotic predators in our
326 study system. The host ant differently affected the prey capture rate of competing predators,
327 resulting in a competitive advantage for predators that induce lower aggression by the host
328 than their competitors. The springtail *C. albinus* is generally the most abundant
329 myrmecophilous prey in red wood ant nests. It can be found throughout the nest, but it reaches
330 its highest densities in the center of the nest (Parmentier et al. 2016a, pers. observations TP).
331 Densities of other prey species are low in the core of the nest, but a group of facultative
332 symbionts can be found at the periphery of the nest (unpublished results). These species
333 (mites, flies, spiders ...) mostly live away from ant nests (Robinson and Robinson 2013;
334 Parmentier et al. 2014), but may occupy the nest periphery in high numbers. The spider
335 *Mastigusa arietina* cannot compete with the other predators in the core of the nest. It strictly
336 lives at the periphery of the nest and it is readily killed in dense nest chambers (Parmentier et

337 al. 2016b). *Mastigusa arietina* is likely to have a broader dietary niche than the two other
338 predators as it may feed on the diverse community of facultative symbionts as well. The
339 predators *S. aterrimus* and *T. biovatus*, in contrast to *M. arietina*, can be found throughout the
340 nest (Parmentier et al. 2016b). Based on our results, the beetle *S. aterrimus* will have a strong
341 competitive advantage to *T. biovatus* in the more crowded parts of the nest, because of higher
342 tolerance to high worker densities and an increased predation response.

343

344

345 Our results contribute to a deeper understanding of the dynamics occurring in host-symbiont
346 networks. We showed that variable conditions in a host environment determine interaction
347 strength of associated symbionts by inducing density- and trait-mediated indirect effects.
348 Fitness balances of the symbionts may shift with the variation in host conditions. Further
349 research is needed to understand whether the interplay of indirect effects facilitate species
350 coexistence and explain the spatial niche partitioning of the myrmecophile symbiont
351 community.

352

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356

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Tables

Table 1. Number of replicates and number of surviving predators for each treatment.

	0 workers	5 workers	15 workers	45 workers	
<i>Stenus aterrimus</i>					
total different individuals tested	$N = 16$	$N = 28$	$N = 18$	$N = 22$	$N_{\text{tot}} = 84$
total predators survived in first replicate (dead)	16 (0)	26 (2)	17 (1)	21 (1)	
total replicates predator survived (dead)	36 (8)	39 (5)	42 (3)	40 (6)	
<i>Thyreosthenius biovatus</i>					
total different individuals tested	$N = 24$	$N = 32$	$N = 34$	$N = 37$	$N_{\text{tot}} = 129$
total predators survived in first replicate (dead)	23 (1)	26 (6)	27 (7)	27 (10)	
total replicates predator survived (dead)	38 (3)	35 (9)	39 (11)	38 (13)	
<i>Mastigusa arietina</i>					
total different individuals tested	$N = 16$	$N = 24$	$N = 33$		$N_{\text{tot}} = 73$
total predators survived in first replicate (dead)	16 (0)	21 (3)	19 (14)		
total replicates predator survived (dead)	41 (1)	42 (5)	31 (20)		

Figures

Fig. 1. Overview of the ant-symbiont and symbiont-symbiont interactions in the red wood ant microcosm. Black arrows depict prey-predator interactions between myrmecophiles, grey arrows denote aggressive interactions between ant and myrmecophiles. The thickness of the lines corresponds with the provoked level of aggression (proportion of aggressive interactions) as reported in Parmentier et al. (2016a). No effect on the prey capture rate of tolerated (ignored) predators is a priori expected. A medium negative effect on prey capture rate is expected in moderately attacked predators (thin red dotted line), a strong negative effect in heavily persecuted predators (thick red dotted line).

Fig. 2. A. Top view of a container used in the experiments. In this trial, the rove beetle *Stenus aterrimus* (indicated by arrow) is introduced in a container with 45 red wood ant workers. B. The springtail *Cyphoderus albinus* captured by *T. biovatus*. C. The springtail *Cyphoderus albinus* captured by *S. aterrimus*. D. Detailed view of a test container with *Mastigusa arietina* and *Cyphoderus albinus* (indicated by arrows). Note that all trials were conducted in complete darkness.

Fig. 3. Multipanel displaying at 4 densities (0, 5, 15 and 45) of host ant workers: (i) proportional survival of *Cyphoderus albinus* in absence of a myrmecophilous predator (white points) (ii) proportional survival of a myrmecophilous predator (grey points) and (iii) proportional survival of *C. albinus* in presence of the myrmecophilous predator (black points). Data are given in three separate boxes for the three different myrmecophilous predators, i.e. left: *Stenus aterrimus*, middle: *Thyreosthenius biovatus*, right: *Mastigusa arietina*. Survival of prey without a predator was determined in a single series of trials, but is depicted in all three figures as a baseline. Error bars represent 95% confidence intervals. Means of a treatment (i.e. same color code and with same predator) labelled with different letters are significantly different at the $\alpha = 0.05$ level (corrected for multiple testing using Benjamini-Hochberg procedure).

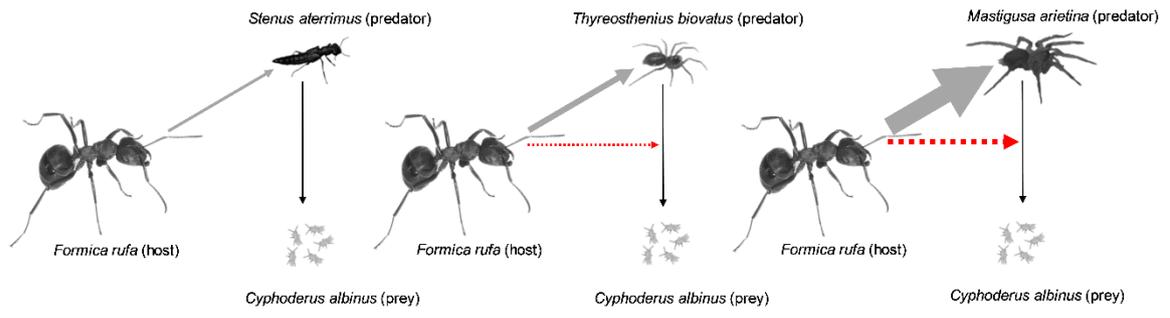


Fig. 1



Fig. 2

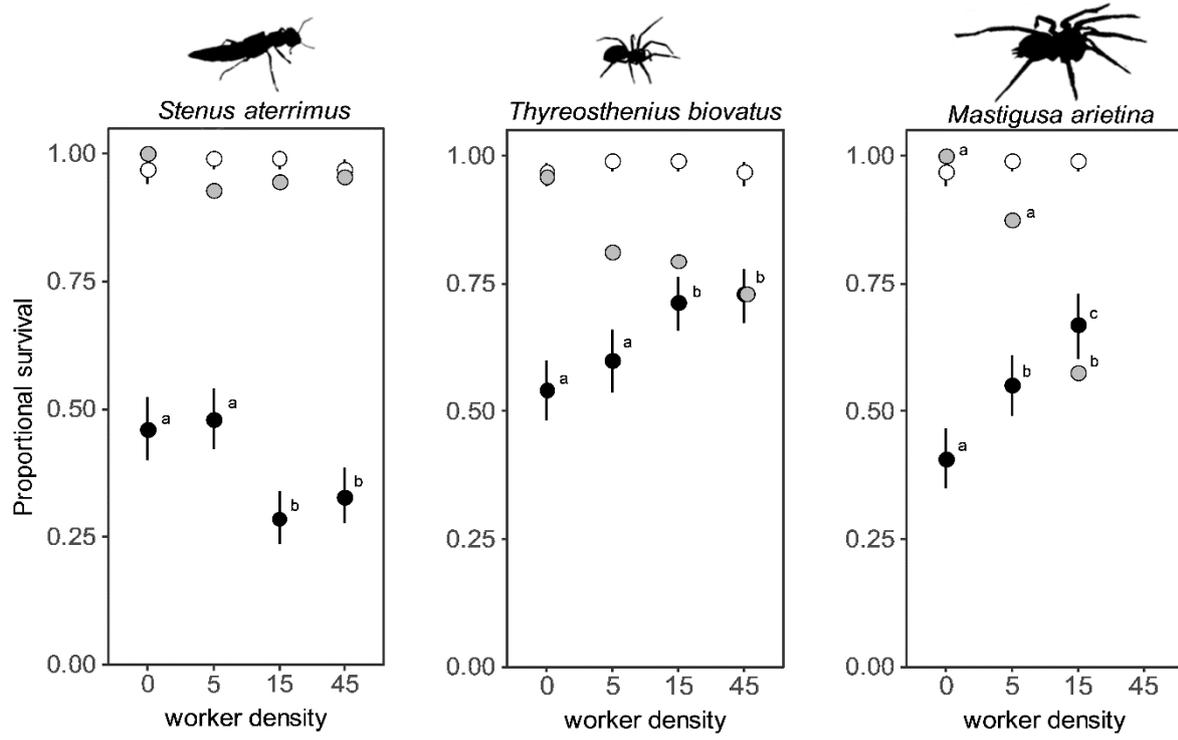


Fig. 3