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Moving apart together

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1	Moving apart together: co-movement of a symbiont community
2	and their ant host, and its importance for community assembly
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20 **DECLARATIONS**

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33 ABSTRACT

34

Background: Species interactions may affect spatial dynamics when the movement of one species is determined by the presence of another one. The most direct species-dependence of dispersal is vectored, usually cross-kingdom, movement of immobile parasites, diseases or seeds by mobile animals. Joint movements of species should, however, not be vectored by definition, as even mobile species are predicted to move together when they are tightly connected in symbiont communities.

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42 Methods: We studied concerted movements in a diverse and heterogeneous community of
43 arthropods (myrmecophiles) associated with red wood ants. We questioned whether joint44 movement strategies eventually determine and speed-up community succession.

45

46 **Results:** We recorded an astonishingly high number of obligate myrmecophiles outside red 47 wood ant nests. They preferentially co-moved with the host ants as the highest densities were 48 found in locations with the highest density of foraging red wood ants, such as along the network 49 of ant trails. These observations suggest that myrmecophiles resort to the host to move away 50 from the nest, and this to a much higher extent than hitherto anticipated. Interestingly, 51 functional groups of symbionts displayed different dispersal kernels, with predatory 52 myrmecophiles moving more frequently and further from the nest than detritivorous 53 myrmecophiles. We discovered that myrmecophile diversity was lower in newly founded nests 54 than in mature red wood ant nests. Most myrmecophiles, however, were able to colonize new nests fast suggesting that the heterogeneity in mobility does not affect community assembly. 55

56

57 **Conclusions**: We show that co-movement is not restricted to tight parasitic, or cross-kingdom 58 interactions. Movement in social insect symbiont communities may be heterogeneous and 59 functional group-dependent, but clearly affected by host movement. Ultimately, this co-

60 movement leads to directional movement and allows a fast colonisation of new patches, but 61 not in a predictable way. This study highlights the importance of spatial dynamics of local and 62 regional networks in symbiont metacommunities, of which those of symbionts of social insects 63 are prime examples.

64

Key words: ant guest, co-dispersal, community coexistence, host-parasite, inquiline,
metacommunity, spatial structure, succession

67

69 **BACKGROUND**

70 Species interact via local and regional interactions in spatially structured networks (1,2). 71 Dispersal is a central instigator of community assembly and species coexistence in these 72 networks when it affects species interactions across space (3). Dispersal is a three-stage 73 process (4) comprising departure, transfer and settlement decision making. The importance of 74 interspecific interactions has been especially documented for departure (5) and settlement (6), 75 but it is equally important for transfer. This is particularly evident for vectored dispersal, where 76 the transport of one species directly depends on another one, usually cross-Kingdom. 77 Organisms or their propagules can thus be passively transported by other organisms as 78 observed in zoochory and ectoparasitism (7.8). Highly advanced symbionts, for instance 79 lichens, coral-dinoflagellate associations and some ant-symbiont associations (9-12) also 80 passively co-disperse with their host as joint propagules.

81

82 Many organisms do not passively hitchhike, but actively follow other species guided by sensory 83 cues provided by other species. This strategy is present in diverse groups encompassing 84 microbes that use fungal networks as highways (13), fishes in coral reefs (14,15) and birds 85 that form foraging associations with other birds (16,17) or co-forage with mammals (18). Ultimately, these actively following organisms may co-disperse with other organisms and co-86 87 colonize new sites, and thereby have strong ecological and evolutionary implications (13,19) 88 for the structure and functioning of metacommunities (20). Heterospecific attraction for 89 instance leads to substantial deviations from predicted coexistence processes under strict 90 competition (21).

91

92 If we aim to understand species dynamics in realistic metacommunities, we need to collect 93 information beyond emigration probabilities and study the distance decay of movement. Such 94 data are typically summarised in the form of the movement kernels that represent the 95 frequency or probability distribution of movement distance in relation to the place where

96 individuals were born and had their home range. The shape of these kernels is known to be 97 condition dependent. That is, intraspecific interactions such as avoidance of crowding or kin 98 competition may affect these kernels (22). It will eventually determine the colonisation of new 99 patches within the network, but also range expansion capacities (23). In classic competition 100 models, the moments of these kernels can influence the prevalence and weight of spatial 101 coexistence mechanisms (24).

102

103 Ant nests house a diverse assemblage of arthropod species, so-called myrmecophiles (25). 104 These myrmecophiles span different functional groups, ranging from detritivores, scavengers, 105 brood predators and species that prey on other myrmecophiles (26). Ant-myrmecophile 106 associations have been an exquisite model to study different facets of symbiosis (27,28) and 107 are increasingly explored in a community context (29-31). This approach enables the comparison of disparate trait syndromes in co-habiting symbionts and are essential to 108 understand their coexistence and the underlying community assembly rules (32). From the 109 110 perspective of the symbionts, ant nests are spatially distinct patches in a hostile environment, 111 with age of the nest and the associated community structure determining its suitability in terms 112 of fitness. Ant symbiont networks are thus spatially structured, and to some degree spatially 113 heterogeneous (33,34), opening avenues for all metacommunity dynamics to act (35). The 114 behaviour of myrmecophiles outside the nest and colonization events are poorly addressed. 115 There are few anecdotal observations of myrmecophiles outside permanent ant nests (10,36-116 40), but myrmecophiles are typically found in ant nests or at nest entrances. Therefore, it is 117 generally assumed that myrmecophiles mostly reside in these nests and only leave the nest 118 at specific events to colonize new nests (10). Several lab studies demonstrated that 119 myrmecophiles can follow their host by running on the chemical pheromone trails of the ant 120 host (41–45). Yet, it is unknown whether the trail network of the host facilitate the movement of the symbionts outside the nest and initiate co-dispersal of ants and myrmecophiles towards 121 122 new nests in a natural setting. In addition to running, many myrmecophiles possess wings and

may leave the nest by flying. Specific lineages of myrmecophiles such as mites may also travel
outside the nest attached to the host (phoresy) and some are even carried by the host (25).

125

126 Red wood ants (Formica rufa group) form a group of dominant central-place foraging ants in 127 temperate forests (46). Their large nests contain an aboveground mound of organic thatch and 128 a network of underground galleries (46). Red wood ants (RWAs) move in a directed way 129 through the landscape using trail networks. The highest densities of foraging workers outside 130 the nest can be found on and near these trails. The trails connect the nest with trees, where 131 they tend aphids for honeydew. Red wood ant nests may also cooperate and share resources 132 via inter-nest trails (47). A diverse community of arthropods lives in strict association with 133 RWAs. These myrmecophiles are typically beetles, but other arthropod groups such as spiders 134 and springtails are also represented (48). Most of them live permanently in the nest, as all life 135 stages are intranidal. We only recorded a handful of individuals outside the ant nests so far, in 136 spite of hours of observations during the past years (49). Other species have an alternating 137 life cycle with one stage in the nest and the other outside the nest (49). The main functional 138 trophic groups that can be found in the community are predatory species that feed on other 139 living myrmecophiles, scavengers that feed on prey and ant brood and detritivores that mainly 140 feed on organic nest material and fungi (26).

141

Here, we first investigated and compared the frequency and characteristics of the mobility of macrosymbionts associated with the nests of RWAs on the forest floor. We compared the mobility of the different functional groups in the myrmecophile community. We also tested whether the symbiont community showed directed movement by co-moving with their host along the routes with the highest density of workers. Second, we studied the colonization of newly founded RWA nests by the symbionts and linked these with the observed speciesspecific patterns in symbiont mobility.

150 **METHODS**

151 Study sites and study organisms

152 Our research was performed at two study sites in the North of Belgium, i.e. de Sint-Sixtusbossen, West-Vleteren (site WV, 50.885622° 2.698785°) and de Hoge Dijken, 153 154 Oudenburg (site OB, 51.173453°, 3.052895°). The WV site holds a polydomous (= multiple 155 mounds/nests) colony of Formica rufa Linnaeus, 1761 distributed over 48 nests (counted in 156 2019). Polydomous organization is widespread in red wood ants (RWAs) (46). The 157 polydomous colony is spread over different clusters of nests which are lined along the southern 158 edge of deciduous forest fragments (Additional file 1: Fig. S1). Mounds in the same forest 159 fragment cooperate, exchange food, brood and workers via trails running between the nests. 160 Nests do not interact with nests of other forest fragments, because of physical barriers (e.g., 161 road). Every nest mound contains multiple queens (pers. observations TP). The RWA species 162 in OB is Formica polyctena Förster, 1850. Formica rufa and F. polyctena are closely related 163 and may hybridize (50). Their nest structure, behaviour and supported myrmecophilous fauna 164 is similar in north west Belgium (48,51). The nests in OB (total of 30 nests) are more scattered 165 as the canopy of the forest fragment is more open (Additional file 1: Fig. S1). Additionally, 166 some nests can be found in an adjoining meadow. The social organization in the OB site is 167 less clear than in WV. It is unknown whether the nests operate independently or exchange 168 resources. No aggression between the mounds was recorded, but clear inter-nest trail 169 networks are absent in this site.

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171 Spatial distribution of myrmecophiles outside the host nest and underlying drivers

We assessed the spatial distribution of RWA myrmecophiles outside the nest and identified the predictors of the observed patterns. The spatial patterns were assessed using a series of pitfall traps. The densities of workers around ant nests and on the trails are extremely high, which makes classic accumulation pitfalls with a preservative not workable. Therefore, we opted for a pitfall where the ants can easily crawl out, but the myrmecophiles not. We used a 177 plastic box (Sunware Q-Line Box: 27x8.4x9 cm, volume: 1.3 L) with a 1 cm layer of moist 178 plaster on the bottom (Fig. S2a-c). The sides were too slippery for the myrmecophiles to 179 escape from, but ants could easily climb out of these boxes. The rectangular pitfalls were 180 positioned with their long side perpendicular to the direction away from the nest to maximize 181 capture efficiency (see Fig. 1, Fig. S2b and video in Additional file 3). The pitfalls were buried 182 so that their top rim was level with the surface of the soil. We covered pitfalls with a plastic roof 183 to prevent rain falling in. The roof was positioned 2 cm above the opening of the pitfalls by 184 attaching plastic caps in the corners of the roof. Soil and organic material also fell in the pitfalls 185 (came by the wind or the ants passing by), which provided an ideal temporary habitat for the 186 myrmecophiles (Additional file 3). This study was done entirely at the WV-site, where all nests 187 are lined along the forest edge (Additional file 1, Fig. S1). We focused on the distribution of 188 myrmecophiles around twenty pairs of nests formed out of 24 nests. The distance between the 189 nests of each pair greatly varied (range: 1.2 m - 51.2 m). For each pair of nests, we installed 190 seven pitfalls. One pitfall was placed at the midpoint between each pair of nests along the 191 forest edge ('edge pitfall') (Fig. 1). These pitfalls assessed movement of myrmecophiles along 192 the shortest path to the other nest of the pair and were often positioned on an inter-nest trail. 193 Movement along this trajectory was expected to be the preferred direction. We compared this 194 movement direction with the perpendicularly orientated movement away from the forest edge 195 towards the inner forest. Therefore, we placed for each nest of the nest pair a pitfall ('forest 196 pitfall) on a line segment originating from the nest and perpendicular to the shortest inter-nest 197 path. We positioned these pitfalls in such a way that a nest was equidistant from the edge and 198 forest pitfall (Fig. 1). Next, we positioned a pitfall just outside each nest of the nest pair 199 ('periphery pitfall', periphery = 0 m). The peripheral zone was discernible from the actual nest 200 by the lack of nest openings and organic material. These pitfalls were not aligned with the other 201 extranidal pitfalls to avoid trapping myrmecophiles before they could reach other extranidal 202 pitfalls. We also burrowed a pitfall inside every nest ('intranidal pitfall') of a focal pair of nests 203 (Fig. 1).

204 The pitfalls were left for one week and then emptied in a large tray in the field. Myrmecophiles 205 were counted and identified to species (beetles following (52,53) spiders following (54)) and 206 also the number of F. rufa workers in the pitfall (including the individuals on the inner walls) 207 was counted. RWA networks are relatively stable over the season, and therefore the number 208 of ants in the pitfall at the time of sampling is a good proxy for the general ant activity at that 209 location. Pitfalls were emptied and ants were counted between 11h and 15h to minimize effects 210 of temperature on the activity of the ants. Pitfalls which were positioned on or near trails were 211 visited by much more workers than pitfalls away from them. We grouped the myrmecophilous 212 species Monotoma angusticollis and Monotoma conicicollis as Monotoma, because they can 213 only be distinguished using a stereomicroscope. We used the same type of pitfalls with roofs 214 to assess the diversity in the nests. As ants gradually fill the pitfall with nest material, these 215 boxes had to be emptied sooner to avoid that the myrmecophiles could escape. We emptied 216 these boxes every 1-2 days and kept the myrmecophiles apart to avoid double counting. After 217 a week, we sampled these boxes a last time and the myrmecophiles that were caught during 218 the week were put back in their nest of origin. In this way, the intranidal sampling effort was 219 similar to the extranidal sampling effort.

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221 The two forest and common edge pitfall were sampled three times (7-day interval between 222 resampling), resulting in nine pitfalls per distance level. The peripheral pitfalls were also 223 sampled three times. Sampling of peripheral, forest and edge pitfalls was organized in 9 time 224 periods: first 5 pairs of nests were checked at 01/07, 08/07 and 15/07/2019, the following 7 225 pairs of nests at 22/07, 29/07 and 05/08/2019 and the last 8 pairs of nests at 12/08, 19/08 and 226 26/08/2019. Intranidal pitfalls were only tested once, after the third replicate of each set of 227 nests. A total of 279 pitfalls were emptied (24 intranidal, 75 peripheral, 60 edge and 120 forest 228 pitfalls). Ants and myrmecophiles were put back after each sampling approximately two meters 229 from the pitfall to avoid that they would directly fall back in the pitfall. We moistened the plaster 230 if needed and put the empty pitfall back in place and did the same sampling protocol for the 231 next replicate.

232 Spatial distribution of myrmecophiles outside the nest

233 In a first analysis, we plotted the distribution of myrmecophiles (abundances and proportion of 234 pitfalls with individuals) along the spatial gradient outside the nest. Next, we compared 235 myrmecophiles and the different functional groups in their tendency to leave the nest, by 236 dividing per species the average number of individuals in an extranidal pitfall (> 0 m) by the 237 average number of individuals in a nest pitfall. The higher the ratio, the higher the tendency to 238 leave the nest. Some myrmecophiles may often leave the nest, but stay very close to the nest 239 entrances. To differentiate this with the tendency to leave the nest, we divided per species the 240 average number of individuals in a peripheral pitfall (0 m) by the average number of individuals 241 in a nest pitfall. We calculated these ratios for each time period, resulting in nine extranidal 242 and nine peripheral estimates per species. Overall differences among the myrmecophiles in 243 the tendency to leave the nest or to occur at the periphery were assessed using a non-244 parametric Kruskal-Wallis test, with myrmecophile species as independent predictor. Pairwise 245 comparisons in the tendency to leave the nest or to occur at the periphery between the 246 myrmecophile species were tested using Pairwise Wilcoxon Rank Sum Tests with the 247 Benjamini-Hochberg correction for multiple testing (55).

In addition, we wanted to test whether myrmecophile species differ in their long-distance movement. For each myrmecophile species, we selected the individuals in the upper decile of the distance distribution outside the nest (periphery not included). Overall differences in longdistance movement among the top movers of the myrmecophiles were tested using a nonparametric Kruskal-Wallis test, with myrmecophile species as independent predictor. Post hoc pairwise comparisons were performed by Pairwise Wilcoxon Rank Sum Tests with the Benjamini-Hochberg correction (55).

Factors affecting the spatial distribution of myrmecophiles outside host nests

First, we assessed whether the distribution of individual myrmecophile species (i) is inversely related to the distance away from the nearest nest (ii) and positively affected by higher numbers of foraging RWA workers at a given distance outside the nest. The highest number 259 of foraging workers outside the nest is found on and near trails. A positive correlation between 260 ant activity/density and myrmecophile density outside the nest does not automatically imply 261 that the ants affect the movement directions of the myrmecophiles. This association can be 262 the consequence of movement in similar directions away from the nest (for example to shared 263 food patches). However, the distribution of resources outside the nest is very homogeneous 264 for myrmecophiles and hence no directed movement is expected. By contrast, RWAs do show 265 very directed movement outside the nest and preferentially move towards food patches and other nests using trails (46). In addition, many lab experiments clearly showed that 266 267 myrmecophiles follow the pheromone trails of their host (41–45). As such, we expect that the 268 directed movement of myrmecophiles overlapping with the preferred RWA routes, can be 269 explained by the myrmecophiles making use of the host ants and its pheromone network to 270 move outside the nest. Note that myrmecophiles caught outside the nest are not necessarily dispersing to another nest, but may forage as well. For this first set of analyses, we only 271 focused on the peripheral pitfalls (0m, N = 75) and the forest pitfalls (N = 120) and did not test 272 273 the directionality of movement (forest vs edge). Per myrmecophile species, we modelled 274 number of individuals found in the pitfalls against the predictors distance from the nearest nest, 275 density of RWA workers in the pitfall and intranidal density in the nearest nest. The latter 276 covariate was included as the number of emigrants was expected to be positively correlated 277 with the intranidal densities. We also included the interaction between distance and density of 278 RWA workers as a predictor. As the models showed overdispersion, we used a negative 279 binomial generalized linear mixed-effect model with poisson error distribution and log link 280 function (glmer.nb function, R package lme4). The nearest nest of a pitfall and the sampling 281 period were modelled as random factors. We ran these models for the following species: 282 Thyreosthenius biovatus, Stenus aterrimus, Thiasophila angulata, Lyprocorrhe anceps, 283 Notothecta flavipes, Pella humeralis and Cyphoderus albinus. The other species were sparsely 284 recorded outside the nest, so that no model could be fitted. The predictors distance from the 285 nearest nest and intranidal density were square root transformed. Density of RWA workers 286 was incorporated either as a continuous (the square root of the number of workers) or a 287 categorical factor (high density: > 20 workers, low density \leq 20 workers) in separate models 288 (i.e. two models per species). From the full model, we removed with the drop1 function fixed 289 factors which removal did not significantly reduce the explanatory power of the model (56). In 290 addition, we fitted a similar generalized mixed model to explain total species richness (sum of 291 all myrmecophile species) along the forest spatial gradient. Here we opted for a glmer rather 292 than a glmer.nb as there was no overdispersion. RWA density, distance from the nearest nest 293 and species richness of the nearest nest (square root transformed) were modelled as 294 covariates, sampling period and nearest nest as random factors.

295

296 Second, we assessed whether the myrmecophile community preferentially moved along the 297 shortest path to another nest (edge direction). Myrmecophiles travelling along the forest edge 298 follow the shortest path to the nearest nest (the location of all nests is known), as all nests are 299 lined along the forest edge (Fig. 1, Additional file 1, Fig. S1). Myrmecophiles caught in the edge 300 pitfalls between two nests could originate from either of the adjoining nests when they were 301 moving between these nests, whereas peripheral and forest pitfalls mainly capture 302 myrmecophiles from the nearest nest (Fig. 1). To make the sampling effort of the forest pitfalls 303 comparable with the edge pitfalls, we pooled the total number of species over the two inner 304 forest pitfalls per pair of nests. As such, for each pair of nests, we obtained one data point with 305 myrmecophiles caught in the forest and one along the edge at the same distance away from 306 the nests (Fig. 1). Because of the positioning of the nests, the focus here is on nest pairs rather 307 than individual nests. Sampling was replicated three times for each pair of nests. Note that we 308 did not include the data of the peripheral pitfalls (at distance 0 m) in these analyses, as 309 directionality of movement could otherwise not be tested.

We modelled the predictors directionality of movement (edge vs forest), distance to the nearest nest and density of RWA workers to predict the response variable species richness (total number of myrmecophile species) using a generalized linear mixed-effect model with Poisson error distribution and log link function. We also included the intranidal species richness pooled over a pair of nests as a fourth covariate. Pair of nests and sampling period were modelled as

random factors. From the full model, we removed with the drop1 function fixed factors which
removal did not significantly reduce the explanatory power of the model (56). We performed
LR-tests to assess the significance of the fixed effects in the reduced species richness model.

We validated all models by analyzing their residuals in the DHARMa package (57), but no issues were identified. Significance of the predictors was estimated with a χ^2 Wald (type 3) test using the function Anova (car package).

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322 Colonization dynamics of myrmecophiles

323 To examine the colonization dynamics of RWA myrmecophiles, we compared the diversity and 324 identity of supported myrmecophiles between well-established, mature nests ("old nests") and newly founded nests ("new nests"). The distribution of RWA mounds in the study sites have 325 326 been intensely surveyed for the last 20 years (49). Therefore, we have a clear idea of the age 327 of the mounds in these sites. We selected old (2 sites: OB: N = 4, WV: N = 8) nests which were 328 older than five years (mean surface: 4.94 m² ± SE 0.46). Newly founded nests (2 sites: OB: N 329 = 8, WV: N = 7) arise during spring and were smaller (mean surface: 1.83 m² ± SE 0.32). Sampling was during summer, so these nests were younger than half a year at the time of 330 331 sampling (Fig. S2d). To avoid lasting damage to the small, new nests, we used non-invasive 332 pitfalls in this experiment (Fig. S2e). They consisted of a plastic 0.5 L pot (height 7 cm) with a 333 1 cm plaster bottom and a top opening (diameter 11 cm). The pitfall was filled with wood chips 334 (Pinus maritima, commercially available DCM bark). The myrmecophiles could enter the pitfall 335 through the top opening or through four circular openings (diameter: 1.5 cm) that were made 336 at 90° in the lower part of the pot. In contrast to the pitfalls used in the previous experiment, 337 myrmecophiles were able to exit the pitfall and myrmecophiles were here thus not accumulated 338 over time. We placed a pitfall deep inside the nest with the top rim level with the interface 339 between the aboveground organic material mound and the underground earth nest. The pitfalls 340 were left for two weeks in the nest and then checked for myrmecophiles in a large tray in the 341 field. Afterwards, myrmecophiles were put back in the nest and the pitfall with wood chips was

re-installed. Every nest was resampled four to six times, with a 14-days interval between each
resampling. Sampling took place between the end of June and end of August, either in the
summer of 2018 or 2019. Note that colonization here can occur through running on the ground,
but also by flying or passive transport (see carrying of *Clytra quadripunctata* by the host (39).

We constructed a negative binomial generalized linear mixed-effect model to predict total species richness in a nest as a function of the fixed effects nest age (old vs new), connectivity (the number of mature nests within a 100 m radius) and site (OB or WV). The first order interactions between the predictors were also modelled. Nest identity was included as a random variable as nests were resampled (4-6 times). From the full models, we removed terms using the drop1 function (56). We validated this model by analyzing its residuals in the DHARMa package (57). We did not identify residual problems.

353 All statistical analyses were performed using R (version 3.4.2).

355 **RESULTS**

356 Spatial distribution of myrmecophiles outside the host nest

357 Myrmecophiles abundant outside host nest, but mobility is functional group-specific

We recorded 3436 obligate myrmecophiles belonging to 17 species (two Monotoma species 358 359 were grouped) at the periphery and outside the nest of their Formica rufa host. The distribution 360 of myrmecophiles was related to the functional role in the community. Predatory species and, 361 to a lesser extent, scavengers were more mobile and had a higher tendency to reside outside 362 the nest than detritivorous species. The spider Thyreosthenius biovatus and the beetles 363 Monotoma and Clytra quadripunctata were present in most nests and reached high densities 364 in the pitfalls (Fig. 2, Table 1). The rove beetles Stenus aterrimus, Lyprocorrhe anceps and 365 Notothecta flavipes occurred in a higher percentage of pitfalls at the periphery than inside the 366 nest (Fig. 2, Table 1). Most species were captured in a lower percentage of pitfalls and in lower 367 abundances with increasing isolation from the host nest (Fig. 2), but this pattern was not 368 present in the rove beetle Pella humeralis. This beetle was also atypical in the myrmecophile 369 community as it almost exclusively occurred outside the nest.

370 Myrmecophile species greatly differed in their tendency to occur at the periphery of the nest (Kruskal-Wallis test, chi-squared = 45.39, df = 11, P < 0.001, Fig. 3a, Additional file 1: Fig. S3, 371 372 Post hoc differences Additional file 2: Table S1). Stenus aterrimus and Q. brevis tend to occur 373 more often at the periphery than other species. Similarly, the average number of individuals in 374 an extranidal pitfall divided by the average number of individuals in a nest pitfall was greatly 375 different among the myrmecophile species (Kruskal-Wallis test, chi-squared = 54.705, df = 11, 376 *P* < 0.001, Fig. 3b, Additional file 1: Fig. S3, Post hoc differences Additional file 2: Table S2). 377 The detritivores Monotoma, C. albinus and C. quadripunctata had a very low tendency to leave 378 the nest (Fig. 3b, Additional file 1: Fig. S3). Pella humeralis displayed the highest tendency to 379 occur outside a nest (Fig. 3b, Additional file 1: Fig. S3). Myrmecophile species differed in the 380 average distance travelled by the individuals at the upper 10% of their distance distribution (Kruskal-Wallis test, chi-squared = 79.83, df = 11, P < 0.001, Fig. 3c, Post hoc differences 381

Additional file 2: Table S3). The predatory myrmecophiles *S. aterrimus*, *T. biovatus* had individuals that forage at a very large distance from host nests (Figs. 3c,4, Additional file 1: Fig. S3), whereas the detritivorous species *Monotoma*, *C. albinus* and *C. quadripuncta* only travelled low to moderate distances (Fig. 3c, Additional file 1: Fig. S3).

386 **Co-movement of myrmecophiles and foraging red wood ants**

Myrmecophile species richness decreased away from the host nest (Fig. 4a, Table 2). 387 388 Myrmecophile species richness was higher when more ants were present at a given distance 389 outside the nest (host density as a categorical or continuous factor in Table 2, as a categorical 390 factor in Fig. 4a). This implies that the myrmecophile community prefers to co-move with 391 foraging host workers. This co-movement was clearly present in the predatory species T. 392 biovatus and S. aterrimus, and in the scavenging species T. angulata, L. anceps and N. 393 flavipes, as their individual distribution was positively correlated with the distribution of the host 394 workers outside the nest (Fig. 4b, Table 2). The density of the detritivorous springtail C. albinus 395 outside the nest was not correlated with higher host densities. Unlike other myrmecophiles, 396 the density of *P. humeralis* increased away from the nest (Fig. 4b, Table 2). The number of 397 individuals/species found outside the nest positively correlated with the number of 398 myrmecophilous individuals found in a nest (or number of species in case of the species 399 richness model) in multiple models (Table 2). Finally, a higher number of species was found 400 towards the inner forest than along the forest edge (Table 2, P < 0.001).

401

402 Colonization dynamics of myrmecophiles

Newly founded nests supported fewer myrmecophile species than old nests (glmer.nb, df=1, $\chi^2 = 50.3$, P < 0.001, Fig. 5). The difference in number of species between old and new nests (OB site: Post-hoc Tukey test: P < 0.001; WV-site Post-hoc Tukey test: P = 0.09) was higher in the site OB than in the WV-site (Fig. 5). Nest connectivity positively affected species richness, both in new and old nests (glmer.nb, df=1, $\chi^2 = 7.8$, P = 0.005). There was a lower likelihood to find myrmecophiles in new nests. The proportion of new and old nests colonized by each species is given in Fig. 6. The density of myrmecophile populations, and especially in the OB-site, was mostly lower in new nests (for each species, bar lengths proportional to mean abundance in Fig. 6). However, almost all myrmecophile species were able to colonize new nests in the first months after they were founded (Fig. 6). Only *Dinarda maerkelii, Quedius brevis* and *Mastigusa arietina* were not recorded in the new nests, but these species were also caught in very low numbers in old nests.

415

417 **DISCUSSION**

We found a remarkably high number of intranidal ant symbionts or myrmecophiles outside their host nest. We showed that these tightly connected ant symbiont communities are also connected during movement, by following the movement of their shared host. There was heterogeneous variation among symbiont groups which was linked to their functional role in the community. Assembly processes in new patches could not be directly connected to these differences in mobility.

424 The majority of ant species are central-place foragers which construct permanent nests (58). 425 Myrmecophiles obligately living in the nest of these ants are only sporadically reported outside 426 the host nest (10,36–40,59) and are typically collected by opening the nest. Red wood ant 427 (RWA) myrmecophiles of this study have been occasionally recorded outside the nest using 428 pitfalls or hand capture: T. angulata (38,60), T. biovatus (61–63), P. hoffmannseggii (64,65), 429 N. flavipes (66,67), S. aterrimus: (67), A. talpa (67), Q. brevis (66), M. paykulli (68), but they 430 have always been reported in very small numbers (max. five individuals) (cfr. their large 431 densities inside RWA nests (69)). The large number of records outside the nest, and including 432 all members of the studied community, here is therefore unexpected and very novel. The 433 records of myrmecophiles associated with other permanent ant nests often seemed to be 434 linked to specific events in the host colony life cycle (e.g. 10). Some myrmecophile species 435 were recorded when they followed their host colony moving to a new nest site (39). Flying 436 Paussus beetles are captured using light pitfalls and in increased numbers at the beginning of 437 the rains, coinciding with the host's nuptial flights (70). The high extranidal mobility found in 438 the RWA myrmecophile community, by contrast, was found all summer and probably spans 439 from spring to autumn. It should be noted that high mobility is known in the peculiar group of 440 myrmecophiles associated with nomadic army ants. These ants do not construct permanent 441 nests and are almost incessantly on the move (71). Consequently, there is a high selection 442 pressure on the associated myrmecophile fauna to keep pace with the very mobile host colony.

They mainly achieve this by running independently among the moving ants on the trails or byphoretic transport (71,72).

445 Species in the myrmecophile community greatly differed in their tendency to exit the nest and 446 the distance they travelled away from the host nest. The extranidal mobility was strongly 447 correlated with their functional role. Detritivorous species were more restricted to the host nest 448 than predatory species. Moreover, leaving individuals of detritivores stayed closer to the nest 449 than those of predatory species. Differential mobility among competing species may result in 450 a competition-colonization trade-off, which promotes the community assembly of regular 451 metacommunities (73), but also of symbiont communities (e.g. 58). However, species that 452 compete for the same food sources in the myrmecophile system tend to have similar degrees 453 of mobility. It is unclear whether the high mobility of predatory species is translated into superior 454 dispersal capacities. It is possible that the predatory species leave the nest to hunt for prey 455 and return. The rove beetle Pella humeralis showed a deviating spatial distribution. It was 456 rarely found in or near the nest, but was the dominant myrmecophile at greater distances away 457 from the nest. Other studies showed that this species and congeners frequently dwell around 458 ant nests (58,75).

459 Organisms move non-randomly in the landscape and they often prefer certain routes to move 460 from one patch to another, as evidenced in insects (76), amphibians (77), birds (78) and 461 mammals (79). Likewise, the myrmecophile community associated with RWAs did show 462 directed movement outside the nest. They preferentially moved along the highest density of 463 ants outside the nest (such as along trails) and avoided the forest edge. Central-place foraging 464 ants often deploy a network of pheromone trails radiating out to food sources (58), and this 465 web of trails is especially well developed in RWAs (47,80). Lab experiments demonstrated that 466 pheromone trails of ants may be followed by symbionts (41-45). Here, we found that RWA 467 myrmecophiles likely exploit these cues to co-move in the landscape in a natural setting. 468 Running among large numbers of workers offers the myrmecophiles protection against 469 predators. The RWA myrmecophiles can flexibly shift between foraging, dispersal or escaping

470 from enemies as they do not co-move attached to a vector species. Ant trails may also guide 471 myrmecophiles to extranidal food sources or lead them to new nests as trails may overlap or 472 connect different nests (47). The denser network of ant trails and the polydomous organization 473 with inter-nest trails in the WV site may have resulted in a faster colonization of newly founded 474 nests compared to the OB site. Movement was also directed away from the forest edges. 475 These edges are characterized by higher temperature fluctuations, higher light levels, reduced 476 moisture and increased predation (81). The higher stress at the edge may explain the 477 preferential movement of the myrmecophiles away from the edge.

478 The process of colonization and succession of new habitat patches (habitat islands) reveals 479 how communities may adapt to fluctuating patch availability and assemble over time. Host-480 symbiont communities provide ideal microcosms to track colonization in natural settings (82). 481 We tracked for the first time colonization of newly emerged ant nests by symbionts. In line with 482 theoretical and empirical studies, we found lower diversity in newly founded nests than in 483 mature nests (83-85). Most myrmecophiles were able to colonize a new nest within the first 484 months, but the lower observed diversity indicate that the associated communities did not 485 reach an equilibrium, yet. The weakly mobile myrmecophiles C. albinus and Monotoma beetles 486 surprisingly colonized most new nests and even reached the highest densities of the newly assembled communities. This discrepancy between extranidal motility and colonization can be 487 488 caused by different processes. A few myrmecophilous species, such as the springtail C. 489 albinus, target other ant hosts scattered over the study site, as well. These species can use 490 nests of other ant species as stepping stones to colonize new RWA nests. This process could 491 explain why C. albinus was able to rapidly colonize even the most isolated new RWA nest (400 492 m away from the nearest RWA nest). Another explanation is that the densities of 493 myrmecophiles in new nests do not reflect the number of successful colonization events. It is 494 possible that a few colonizers may reproduce rapidly. Furthermore, high extranidal mobility as 495 observed in S. aterrimus and T. biovatus may be linked to foraging rather than to dispersing 496 events. Lastly, the community has other modes of dispersing than running. One species, the

497 larvae of the beetle Clytra quadripunctata, may be carried by the host from one nest to another 498 (39). But more crucially, a large part of the community has functional wings. Flying has rarely 499 been recorded in this community (49), and aerial dispersal is probably restricted to a narrow 500 time frame in their life cycle or limited to particular seasonal conditions. This was also 501 suggested by (38) who found that newly emerged Thiasophila beetles associated with RWAs 502 were attracted to light and attempted to fly off. After two weeks, the beetles did no longer show 503 attempts to fly, avoided light and mostly hid in the nest material. Overall, the relative importance 504 of flying dispersal compared to dispersal by walking is unclear in this community.

505

506 Conclusions

507 Future research may further elaborate this neat host-symbiont system and address 508 fundamental ecological questions, such as assessing the relative role of local and regional 509 processes in assembling metacommunities, and testing the effect of (co-)dispersal on the 510 stability of the communities and food webs. Much theory on metacommunities and 511 metafoodwebs were derived from the results of lab microcosms, but extending our focus to 512 natural metacommunities, and in which the movement of a species might be directly or 513 indirectly affected by other species, could start to fill the gap in our understanding of the 514 dynamics of realistic metacommunities.

515

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- 721

724 **TABLES**

Table 1. Distribution of the myrmecophiles in the pitfalls (WV site). For each myrmecophile species, the number of captured individuals (N_{ind}) and number of pitfalls with at least one individual ($N_{pitfall}$) are summarized for intranidal pitfalls (N = 24), pitfalls at the periphery (N =728 75), and pitfalls outside the nests (> 0 m, N = 180).

Species	Functional group	Taxon	Intranidal (24 pitfalls)		Intranidal Periphe (24 pitfalls) (75 pitfal		ohery itfalls)	Outside (180 pitfalls)		Total records
			Nind	N _{pitfall}	Nind	Npitfall	Nind	N _{pitfall}		
Stenus aterrimus	predator	Coleoptera (Staphylinidae)	22	10	467	53	152	30	641	
Thyreosthenius biovatus	predator	Araneae (Linyphiidae)	238	23	370	57	189	67	797	
Thiasophila angulata	scavenger	Coleoptera (Staphylinidae)	144	19	496	35	73	28	713	
Lyprocorrhe anceps	scavenger	Coleoptera (Staphylinidae)	45	10	565	49	113	59	723	
Quedius brevis	scavenger	Coleoptera (Staphylinidae)	1	1	16	12	24	10	41	
Dinarda maerkelii	scavenger	Coleoptera (Staphylinidae)	4	4	52	19	22	12	78	
Notothecta flavipes	scavenger	Coleoptera (Staphylinidae)	40	3	94	34	23	18	157	
Amidobia talpa	scavenger	Coleoptera (Staphylinidae)	2	1	25	11	7	5	34	
Leptacinus formicetorum	scavenger	Coleoptera (Staphylinidae)	1	1	0	0	1	1	2	
Myrmetes paykulli	scavenger	Coleoptera (Histeridae)	2	2	3	3	1	1	6	
Pella humeralis	extranidal scavenger	Coleoptera (Staphylinidae)	2	2	14	8	133	64	149	
Monotoma	detritivore	Coleoptera (Monotomidae)	518	19	140	33	6	5	664	
Cyphoderus albinus	detritivore	Collembola (Cyphoderidae)	416	15	184	35	27	15	627	
Spavius glaber	detritivore	Coleoptera (Cryptophagidae)	0	0	5	2	0	0	5	
Platyarthrus hoffmannseggii	detritivore	Isopoda (Platyarthridae)	9	1	51	3	0	0	60	
Clytra quadripunctata	detritivore/parasite	Coleoptera (Chrysomelidae)	286	23	176	31	7	5	469	

729

Table 2. Test results of the factors affecting spatial distribution outside host nests in the WV

site (Type 3 Wald χ^2 tests).

					Host density continuous			Host density categorical (low vs high density)			
	Response variable	model	predictor	Df	effect	χ²	Р	effect	χ²	Р	
Gradier	nt towards the forest interior										
	Total species richness	glmer	distance from nest	1	-	47.5	<0.001	-	54.9	<0.001	
	·	0	host density	1	+	19.4	<0.001	+	39.2	<0.001	
			number of species in the nest	1	+	6.5	0.011	+	10.2	0.001	
			distance from nest x host density	1	+	9.1	0.003				
	Number of Thyreosthenius	glmer.nb	distance from nest	1	-	36.5	<0.001	-	10.2	0.001	
	-	-	host density	1	+	3.9	0.049	+	0.0	0.84	
			number of individuals in the nest	1	+	14.5	<0.001	+	11.9	<0.001	
			distance from nest x host density	1	+	15.5	<0.001	+	15.8	<0.001	
	Number of Stenus	glmer.nb	distance from nest	1	-	25.7	<0.001	-	33.5	<0.001	
		-	host density	1	+	16.0	<0.001	+	26.2	<0.001	
			distance from nest x host density					-	11.8	<0.001	
	Number of Thiasophila	glmer.nb	distance from nest	1	-	31.4	<0.001	-	38.1	<0.001	
		0	host density	1	+	10.6	0.001	+	3.4	0.07	
			number of individuals in the nest	1	+	11.4	<0.001				
	Number of Lyprocorrhe	glmer.nb	distance from nest	1	-	20.7	<0.001	-	23.0	<0.001	
		-	host density	1	+	22.5	<0.001	+	13.4	<0.001	
	Number of Notothecta	glmer.nb	distance from nest	1	-	8.7	0.003	-	11.3	<0.001	
			host density	1	+	18.2	<0.001	+	13.6	<0.001	
			number of individuals in the nest					+	3.2	0.072	
			distance from nest x host density					-	5.6	0.018	
	Number of <i>Pella</i>	glmer.nb	distance from nest	1	+	24.9	<0.001	+	24.9	<0.001	
	Number of Cynhoderus	almer nh	distance from nest	1	_	36.0	~0 001	_	36.0	~0 001	
	Number of Cyphoderus	gimer.no	number of individuals in the nest	1	+	3.7	0.06	+	3.7	0.06	
-				•	•	0.7	3.00	·	0.1	0.00	
Gradier	nt forest vs edge										
	Total species richness	glmer	directionality of movement	1	-	14.7	<0.001		6.1	0.013	
		-	distance from nest	1	+	6.8	0.010	-	8.5	0.003	
			host density	1	+	31.8	<0.001	+	9.7	0.002	

736 **FIGURE LEGENDS**

737 FIGURE 1. Schematic diagram of the positioning of pitfalls, here around three nests lined along 738 a forest edge. We sampled the myrmecophiles inside a nest with an intranidal pitfall (i) and at the boundary (0 m) of a nest with a periphery pitfall (p). We placed an edge pitfall 739 740 on the midpoint between two nests (along the forest edge direction). The captured 741 myrmecophiles of this pitfall originate from either of the adjoining nests (see arrows). 742 For both nests of this pair, a forest pitfall (f) was installed equidistant from the distance 743 to the midpoint. Myrmecophiles found in this type of pitfall were mainly coming from the 744 nearest nest (see single arrow). A nest which lies between two other nests in a forest 745 fragment was part of two pairs of nests (here pair: nest1-nest2 and pair: nest2-nest3). 746 For such a nest, two forest pitfalls were positioned at different distances: one at the half 747 of the distance between nest 1 and 2 (midpoint distance x_{1-2}), and one at the half of the 748 distance between nest 2 and 3 (midpoint distance x_{2-3}). Distance x varies from 0.6 to 749 25.6 m across the 20 tested nest pairs (distribution nests see Additional file 1: Fig. S1).

750

751 FIGURE 2. Spatial distribution of the 12 most widely distributed myrmecophile species in the 752 community (present in more than 10 pitfalls). The pitfalls along the spatial gradient have been grouped in seven different distance bins: 'intranidal' (*N*pitfalls = 24), 'periphery' 753 754 (0m, Npitfalls = 75) and five distance bins of outside pitfalls ('<3 m': Npitfalls = 54, '3m-6m': Npitfalls = 27, '6m-12m': Npitfalls = 45, '12m-18m': Npitfalls = 45, '>18m': Npitfalls 755 756 = 9). For each distance bin, the proportion of pitfalls with 0, 1, 2-3, 4-5, 6-10, 11-20 and more than 20 individuals of a particular species is indicated with a multicolored stacked 757 bar. The left y-axis shows the proportional distribution of these abundance classes 758 759 along the distance gradient (x-axis). For each species, we also plotted the average 760 abundance \pm SE of individuals in a pitfall per distance bin with black-bordered circles. 761 The y-axis corresponding to these average abundances is given on the right.

FIGURE 3. Tendency of myrmecophiles associated with red wood ants to leave the nest. a)
Tendency of occurring at the periphery of the nest (abundance in a peripheral
pitfall/abundance in an intranidal pitfall) b) Tendency of occurring outside the nest
(abundance in an outside pitfall/abundance in an intranidal pitfall) c) Mean distance
travelled by the 10% top dispersers for each species. Functional groups: P predator, S
scavenger, S* extranidal scavenger, D detritivore, D* detritivore/parasite. Error bars
indicate standard errors. Post hoc differences see Additional file 2: Table S1-3.

770

771 FIGURE 4. Effect plots corresponding to the mixed models in Table 2 (gradient towards forest 772 interior and host density as categorical variable). The plots display the partial effect of 773 distance away from the nest and host ant density on myrmecophile distribution, while 774 other predictors are held fixed: a) total species richness with increasing distance from 775 the nearest nest b) the change in abundance for individual myrmecophile species with increasing distance from the nearest nest (± 95% CI, 100 bootstrap replicates). These 776 777 plots are related to Fig. 2. However, Fig. 2 also includes data from edge pitfalls, does 778 not account for other predictors and its x-axis gives the distance away from the nearest 779 nest in distance bins rather than as a continuous variable.

780

FIGURE 5. Effect plot showing the partial effect of nest age on species richness (± 95% CI)
for site OB and site WV while the predictor connectivity in the model is held fixed. Old
nests hold higher number of species than new nests for a given level of connectivity,
but this effect was clearer in the OB-site.

785

FIGURE 6. Average abundance \pm SE of myrmecophile species found using wood chip pitfalls in new and old nests in the OB ($N_{new} = 8 N_{old} = 4$) and WV-site ($N_{new} = 7 N_{old} = 8$). *Cyphoderus albinus* average abundance per trap given on lower axis, abundances of other myrmecophiles given on the top-axis. Functional groups: P predator, S scavenger, D detritivore, D* detritivore/parasite. The proportion of new and old nests

- 791 where the myrmecophile species was found at least once (each nest was sampled four
- to six times) is given to the right of the average abundance bars.

FIGURES



797 FIGURE 1



800 FIGURE 2



803 FIGURE 3



FIGURE 4





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813 FIGURE 6
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817 Additional files

- 818 Additional file 1: supporting figures S1, S2 and S3
- 819 S1: Map of red wood ant nest distribution in site WV and site OB.
- 820 S2: Overview of the sampling of the myrmecophiles.
- 821 S3: Relative abundances of the 12 most widely distributed myrmecophile species along822 the spatial gradient.
- Additional file 2: supporting tables Table S1-S3 listing the Post-hoc test results related to Fig.
- 824 3.
- Additional file 3: video featuring a pitfall near a wood ant nest.