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1 **With or without you: Gut microbiota does not predict aggregation behavior in**
2 **European earwig females**

3

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10

11 **Abstract**

12 The reasons why some individuals are solitary and others gregarious are the subject
13 of ongoing debate as we seek to understand the emergence of sociality. Recent
14 studies suggest that the expression of aggregation behaviors may be linked to the gut
15 microbiota of the host. Here, we tested this hypothesis in females of the European
16 earwig. This insect is ideal for addressing this question, as adults both naturally vary
17 in the degree to which they live in groups and show inter-individual variation in their
18 gut microbial communities. We video-tracked 320 field-sampled females to quantify
19 their natural variation in aggregation and then tested whether the most and least
20 gregarious females had different gut microbiota. We also compared the general
21 activity, boldness, body size and body condition of these females and examined the
22 association between each of these traits and the gut microbiota. Contrary to our
23 predictions, we found no difference in the gut microbiota between the most and least
24 gregarious females. There was also no difference in activity, boldness and body
25 condition between these two types of females. Independent of aggregation, gut
26 microbiota was overall associated with female body condition, but not with any of our
27 other measurements. Overall, these results demonstrate that a host's gut microbiota is
28 not necessarily a major driver or a consequence of aggregation behavior in species
29 with inter-individual variation in group living and call for future studies to investigate the
30 determinants and role of gut microbiota in earwigs.

31 **Keywords:** Dermaptera; Group living; Insect; Microbial community; Social evolution

32 **Introduction**

33 Aggregation is one of the most basic and common forms of group living in nature
34 (Vulinec 1990). This phenomenon can be found in almost all animal taxa, where it
35 reflects either a simple tolerance between individuals seeking the same environmental
36 parameters (*e.g.*, nutritional resources, shelter availability, light) or an active need to
37 look for conspecific and heterospecific individuals (Camazine 2003; Jeanson and
38 Deneubourg 2007; Broly et al. 2013). In recent decades, many biotic and abiotic factors
39 have been shown to influence whether individuals should live in groups or alone
40 (Krause and Ruxton 2002). For instance, harsh environmental conditions are expected
41 to favor group living if it increases protection from extreme temperatures, improves
42 foraging efficiency and enhances defense against predators (Krause and Ruxton
43 2002). Conversely, several parameters are expected to inhibit the evolution of group
44 living, such as its inherently higher risk of visibility to predators (Lindström 1989),
45 reduced per capita access to resources (*e.g.*, food, nesting material, mating partners),
46 higher levels of inter-individual competition, expression of aggressive behavior and
47 sexual harassment (Mosser and Packer 2009; Elwood and Stolzenberg 2020), and
48 increased risk of encountering and transmitting parasites and pathogens (Deere et al.
49 2021; Lucatelli et al. 2021; Ritchie et al. 2021; Lindsay et al. 2023).

50 Recent data suggest that the reasons why some individuals are solitary and
51 others gregarious may be the consequence or cause of the microbial communities that
52 live in their guts, *i.e.*, their gut microbiota (Hosokawa et al. 2008; Lombardo 2008;
53 Johnson and Foster 2018; Onchuru et al. 2018). There are four possible reasons that
54 have been proposed to explain this association. First, this association could be the
55 result of physiological changes due to the presence of an altered microbiota in the host.
56 The gut microbiota is often essential in the expression of a wide range of host functions,

57 including metabolism, development, cognition, nutrition, and immunity (Sekirov et al.
58 2010; Littman and Pamer 2011; McFall-Ngai et al. 2013). Suffering from an alteration
59 in the gut microbial community could therefore make individuals less active and less
60 likely to seek out conspecifics. Second, this association could be the consequence of
61 host behavioral alterations, including social behavior, due to the presence of an altered
62 microbiota in the host (Vuong et al. 2017; Valdes et al. 2018; Sherwin et al. 2019).
63 Since the gut microbiota often shape the chemical signatures of its host, its alteration
64 may cause profound changes making the host them either less attractive to
65 conspecifics, as reported in cockroaches and locusts (Dillon et al. 2000; Wada-
66 Katsumata et al. 2015), or more prone to receive aggression from conspecifics, as
67 reported in ants and termites (Matsuura 2001; Teseo et al. 2019). This change in social
68 behavior may also be due to perturbations in the gut-brain axis of hosts with altered
69 gut microbiota, which induces abnormal social behaviors, such as reduced sociability
70 and increased social avoidance in rats (González-Miguéns et al., 2020; Wirth et al.,
71 1998) or social dysfunctions including autism-spectrum disorders (Sgritta et al. 2019)
72 and schizophrenia (Zhu et al. 2020) in humans. Third, the gut microbiota could be
73 linked to group living if hosts need to obtain gut microbes from conspecifics (Hosokawa
74 et al. 2008; Lombardo 2008; Nalepa 2015). The need for specific gut microbes could
75 encourage a host to become more gregarious to acquire them from conspecifics, for
76 instance through feces consumption, mouth-to-mouth or mouth-to-anus contacts with
77 other group members (Kopanic et al. 2001; Onchuru et al. 2018; Nalepa 2020). Finally,
78 the link between gut microbiota and group living could be a strategy of manipulation of
79 the host by the microbes. Through the gut-brain axis, gut microbes could manipulate
80 their host to promote the expression of social behaviors, thereby increasing their
81 success in reaching new hosts (Klein 2003; Onchuru et al. 2018). Several lines of

82 argument suggest, however, that the evolution of such behavioral manipulation
83 requires conditions that are often difficult to meet in nature (Johnson and Foster 2018).
84 Irrespective of the cause of this association, all these data suggest that individuals with
85 high or low levels of group living should have different gut microbial communities.
86 However, the occurrence of such a difference remains poorly examined, particularly in
87 species where individuals can be both solitary and social (but see McFrederick et al.
88 2014; Rubin et al. 2018; Raulo et al. 2021).

89 In this study, we investigated whether individuals that remain close to
90 (gregarious) or distant from (solitary) their conspecifics have distinct gut microbial
91 communities in the European earwig. This insect is ideal to address this question.
92 Female and male adults naturally vary in the degree to which they live in groups: some
93 are solitary, while others live in groups from a few to several hundred members (Lamb
94 and Wellington 1975; Sauphanor and Sureau 1993; Raveh et al. 2014; Weiß et al.
95 2014; Kramer and Meunier 2016a). Moreover, a recent study reported inter-individual
96 variation in the gut microbial communities of earwig females (Van Meyel et al. 2021).
97 However, the link between these two parameters remained untested. Here, we set up
98 a non-invasive video-tracking system to quantify (for the first time) natural variation in
99 the aggregation behavior of 320 field-sampled females over three days and then
100 compared the gut microbial communities of the most and least gregarious females.
101 Although males also express aggregation behavior, we focused on females because
102 we only had information on the presence of inter-individual variation in the gut
103 microbiota of female earwigs (Van Meyel et al. 2021). To ensure that the potential
104 association between gut microbiota and aggregation levels was not a by-product of
105 other life-history traits and that the time spent in the laboratory did not interfere with
106 our measurements, we also compared the general activity, boldness and body

107 condition of the tested females throughout their time in the laboratory and examined
108 the association between each of these traits and gut microbiota.

109

110 **Material and Methods**

111 *Animal sampling and laboratory rearing*

112 We field sampled 1000 *F. auricularia* females and males in a peach orchard near
113 Valence, France (Lat 44.9772790, Long 4.9286990) at the end of June 2022. All these
114 individuals were sampled as mated adults and belonged to *F. auricularia* species “A”
115 (Wirth et al. 1998; González-Miguéns et al. 2020). Upon collection, the 1000 individuals
116 were first mixed in a single plastic container and then randomly distributed among four
117 other plastic containers of 100 females and 100 males each (called “test containers”)
118 and two additional plastic containers of 100 females each (called “attraction
119 containers”). The test container held females for which we then measured the
120 aggregation level, general activity, boldness, fresh weight, and gut microbial diversity
121 (see below). The attraction containers held females that served as stimuli for
122 measuring aggregation levels. All plastic containers were grounded with moistened
123 sand and cardboard shelters. Throughout the experiment, the animals were fed with
124 carrot chunks *ad libitum*, which were changed twice a week. Containers were
125 maintained under a controlled 12:12 light-dark cycle at 20°C and 18°C, respectively
126 (Meunier et al. 2012). All measurements were proceeded three days after laboratory
127 conditions had been set up.

128

129 *Aggregation measurements*

130 To investigate the association between the level of aggregation of a female and her
131 gut microbial diversity, we first measured the aggregation level of 320 females from
132 the “test containers” (**Fig S1**). To this end, we used 3D-printed arenas consisting of
133 four linearly aligned circular chambers (diameter 4 cm) (Fig S1 ; Van Meyel et al.,
134 2022). Three of the chambers were connected by 0.5 cm wide corridors allowing
135 earwigs to move between chambers. The fourth chamber (called the “attraction
136 chamber”) had a reduced corridor of 0.15 cm to prevent animals from reaching the
137 other chamber while allowing the circulation of odors and antennal contacts between
138 individuals on both sides (Van Meyel and Meunier 2022).

139 The measurement of each aggregation score started by placing a randomly
140 selected female from the two test containers in the circular chambers at one end of the
141 system, and three females taken randomly from the two attraction containers in the
142 attraction chamber at the other end of the system (we alternated the orientation of the
143 system between trials). All these females had access to the food source until they were
144 used in the 3D printed arenas. To avoid injuries during animal handling, all these
145 individuals were previously anaesthetized with CO₂ for 30 s. One hour later (to allow
146 recovery after anesthesia), we started to record whether the focal female was in the
147 chamber next to the group of females (yes or no) and then repeated this measurement
148 by taking pictures every hour for 72 hours using infrared cameras and the software
149 pylon Viewer v5.1.0 (Basler©, Ahrensburg, Germany). For each female, we thus
150 calculated an aggregation score, which was defined as the total number of pictures in
151 which a female was in the chamber next to the group of females for 72 hours. No
152 female was used as both a focal and a non-focal individual. We measured the
153 aggregation score of 66 females per week for five consecutive weeks (10 died during
154 the experiment). Note that the aggregation score (AS) calculated over 24h was

155 consistent over the three days of the experiment (Linear models; ASday2~ASday1:
156 $F_{1,310} = 175,52$, Adjusted $R^2 = 0.358$, $P < 0.001$; ASday3~ASday1: $F_{1,310} = 52.85$, $P <$
157 0.001 , Adjusted $R^2 = 0.132$; and ASday3~ASday2: $F_{1,310} = 206.29$, $P < 0.001$, Adjusted
158 $R^2 = 0.387$) and did not change over the five weeks of the experiment (ASday1: $F_{4,310}$
159 $= 1.25$, $P = 0.292$; ASday2: $F_{4,310} = 0.08$, $P = 0.990$; ASday3: $F_{4,310} = 0.25$, $P = 0.912$;
160 all interactions $P > 0.141$) (Fig S2A).

161 At the end of the 72 hours, we isolated each tested female in a Petri dish (5
162 cm diameter) lined with moistened sand for further measurements (see below), while
163 we returned the three attractor females to the attraction containers. To avoid cross-
164 contamination with biological material between trials (e.g., due to chemical signatures
165 or feces), we grounded each plastic arena with a paper sheet that was renewed at
166 each trial and cleaned the glass plates with ethanol between each trial.

167 For the rest of the experiment, we selected the 20 females (out of 320) with
168 the lowest aggregation scores (AS from 0 to 15, $n = 20$; later called low-aggregation
169 females) and the 20 females with the highest aggregation scores (AS from 49 to 72, n
170 $= 20$; later called high-aggregation females) calculated over 72 hours. These females
171 were evenly distributed over the five weeks of the experiment, i.e. 4 females per week
172 and category (Fig S2B).

173

174 *Behavioral and morphological measurements*

175 We then measured the general activity and boldness of the 40 high- or low-aggregation
176 females (**Fig S1**). At the end of the aggregation test (i.e. in the early afternoon), we
177 gently transferred each female to a circular arena (diameter 7.8 cm) held between two
178 glass sheets and maintained on an infrared light table. We then video-recorded
179 females' activity for 15 minutes in the dark and under infrared light (BASLER BCA

180 1300, Germany; Media Recorder v4.0, Noldus Information Systems, Netherlands) – as
181 this species is nocturnal and lucifugous. We defined the level of general activity as the
182 total distance walked by a female during these 15 minutes (Merleau et al. 2022). This
183 distance was automatically extracted from our videos using the video-tracking software
184 EthoVision XT 16 (Noldus Information Technology®, Wageningen, Netherlands). We
185 measured boldness using the same videos and the same software by extracting the
186 time spent by each female in the central area (diameter 5.8 cm) of the tested arena.
187 This measurement of boldness is standard in rodents (Archer 1973; Stratton et al.
188 2021) and applies to earwigs, as they are highly thigmotactic and typically avoid open
189 areas (Rankin and Palmer 2009). At the end of the behavioral measurements, we
190 analyzed the 72 pictures sampled per females to select the four and four females (of
191 the given session/week) with the least and highest aggregation scores, respectively.

192 The day after (morning), we measured the body condition of the 40 females
193 by first anaesthetizing each of them with CO₂ and then measuring their eye distance
194 and fresh weight (Thesing et al. 2015). Using these two measurements, we calculated
195 the initial body condition for each female based on the “scaled mass index” (Kramer
196 and Meunier 2016b). In brief, this index standardizes body mass at a fixed value of a
197 linear body measurement based on the scaling relationship between these measures
198 (Peig and Green 2010). Accordingly, this index indicates which mass a particular
199 female would have at the average eye distance. We measured eye distance to the
200 nearest 0.01 mm using a binocular scope coupled to the *Leica Application Suite*
201 software (Leica Biosystems®, Wetzlar, Germany). We weighed each female using an
202 isoCaL Quintix® precision balance to the nearest 0.01 mg (Sartorius®, Göttinger,
203 Germany). Note that the 8 females from the last week were dissected before weight
204 measurement and were thus not included in this measurement.

205

206 *Gut microbiota collection and diversity metrics*

207 Immediately after morphological measurement (*i.e.*, less than 24 hours after the last
208 aggregation score recording), we extracted the gut of the 40 selected females to
209 investigate whether high or low aggregation females had different gut microbial
210 communities. The remaining females (and males) were used for other experiments not
211 shown in this study. Following the protocol detailed in Van Meyel *et al.* (2021), each
212 female was CO₂ anaesthetized and dissected under sterile conditions. We immediately
213 flash-freeze each gut with liquid nitrogen and stored them individually in Eppendorf
214 tubes at -80°C until DNA extraction. Total genomic DNA was extracted using the
215 NucleoMag® Tissue extraction kit (Macherey-Nagel™, Düren, Germany) and the V3-
216 V4 region of the 16S rRNA gene was amplified with the prokaryotic primers 343F (5'-
217 ACGGRAGGCAGCAG – 3') and 784R (5'- TACCAGGGTATCTAATC – 3') (Muyzer *et*
218 *al.* 1993) using the Taq Polymerase Phusion® High-Fidelity PCR Master Mix with GC
219 buffer (Qiagen, Hilder, Germany). Final amplicon products were sequenced using
220 2x250bp Illumina MiSeq technology at the Bio-Environment platform (University of
221 Perpignan, France). Blanks were involved at each step but were not sequenced as
222 they were all negatives (*i.e.*, the PCR never amplified any DNA) (**Fig S3**). The obtained
223 libraries were processed through the DADA2 pipeline (v1.24.0) (Callahan *et al.* 2016)
224 and converted into Amplicon Sequence Variants (ASVs) (Glassman and Martiny 2018)
225 that were managed using the Phyloseq package v1.40.0 (McMurdie and Holmes
226 2013). All samples were rarefied at 10 180 sequences (*i.e.*, the minimum sample sum
227 of the dataset that was enough to reach the species richness maxima for an index
228 coverage of Good = 99,97%; **Fig S4**) for a final dataset comprising 1 593 different
229 ASVs from 19 low- and 20 high-aggregation females (one sample did not amplify) (**Fig**

230 **S3**). Amplification and bioinformatic procedures are detailed in the Supplementary
231 Material.

232 For each sample, we calculated taxonomic and phylogenetic alpha diversity with
233 the Shannon and the Hill1 indices ($q = 1$) (Chao et al. 2010). We then measured the
234 taxonomic and phylogenetic dissimilarities computed on relative abundances of ASVs
235 between pairs of gut microbiota using Bray-Curtis and Weighted Unifrac distances,
236 respectively (Lozupone and Knight 2005). The community homogeneity (*i.e.* microbial
237 dispersion) within low- and high-aggregation status is calculated for each beta diversity
238 metric used with the function `betadisper` from the package *vegan* v2.6.2 (Hartig, 2022).

239

240 *Statistical analyses*

241 We conducted all statistical analyses using R v4.2.0 (R Core Team 2022) loaded with
242 *car* v3.1 (Fox et al. 2019), *lme4* v1.1.29 (Bates et al. 2015), *vegan* v2.6.2 (Oksanen et
243 al. 2022) and *ape* v5.6.2 (Paradis and Schliep 2019). We ran six independent statistical
244 models where the response variable was either Shannon (Linear Model, LM), Hill1
245 (LM), Bray-Curtis dispersion (LM), Weighted Unifrac dispersion (LM), Bray-Curtis
246 dissimilarity matrix (Permutational Multivariate Analyses of Variances; PERMANOVA)
247 or Weighted Unifrac dissimilarity matrix (PERMANOVA). In each of these models, we
248 entered the same 5 explanatory variables, namely aggregation status (high or low),
249 general activity, boldness, body condition (*i.e.*, scaled body mass index) and sampling
250 week. We first included the interaction between sampling week and each variable in
251 the different models and then, where appropriate, removed it after model simplification
252 by AIC comparison. The PERMANOVA on the beta-diversity dissimilarity matrices
253 included 999 permutations and was run using the `adonis2` function from the package
254 *vegan* v2.6.2 (Oksanen et al. 2022). We performed post-hoc pairwise Adonis tests

255 between sampling week when it was significant in the PERMANOVA. For each
256 statistical model, we checked that all assumptions were fulfilled using the *DHARMA* R
257 package v0.4.6 (Hartig 2022). There was no correlation between the continuous
258 variables entered in each statistical model (Pearson tests; $P > 0.05$ and $|R| \leq 0.28$ for
259 all pairs).

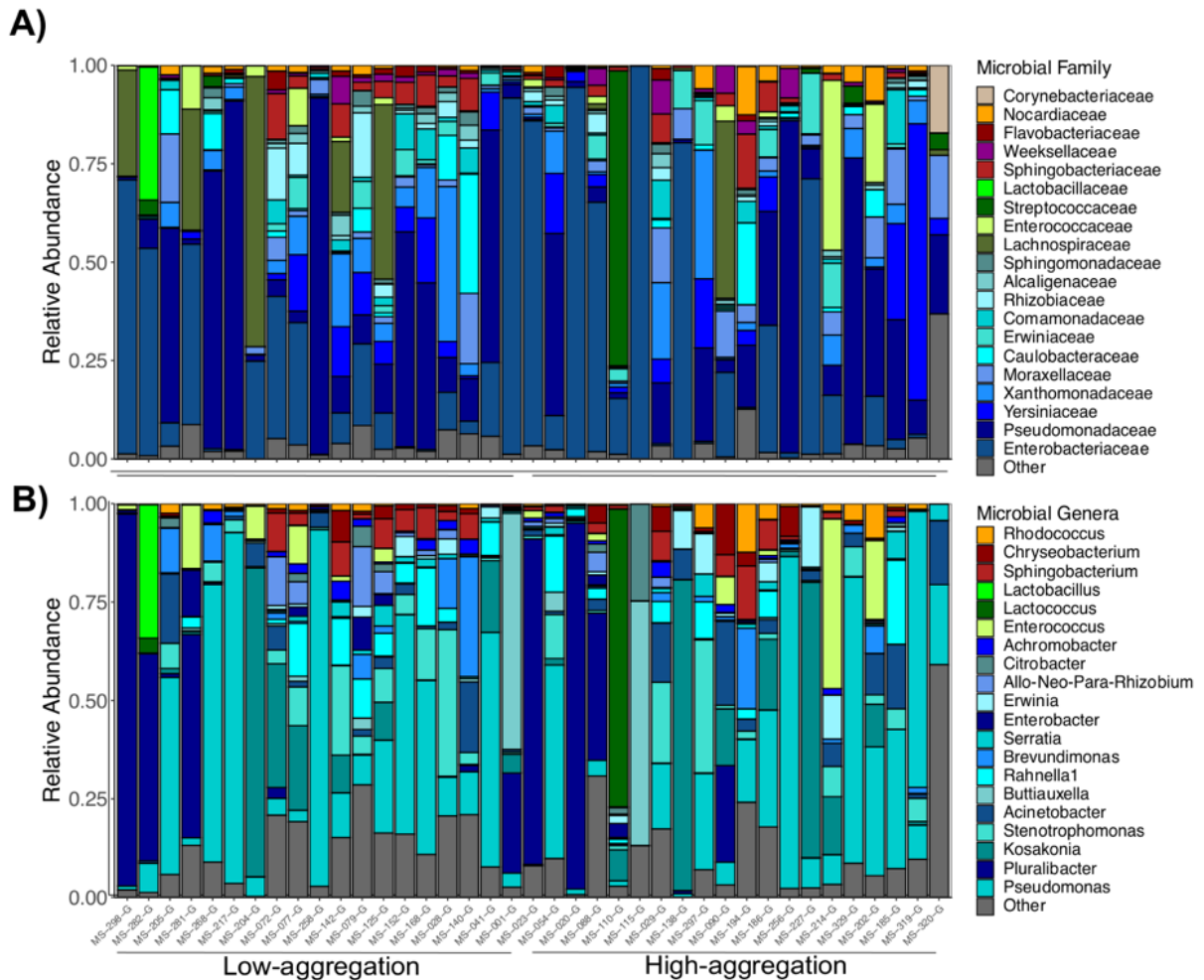
260 To identify potential microbial discriminants of females with high or low-
261 aggregation status, we used a Linear discriminant analysis with Effect Size (LEfSe,
262 Segata et al., 2011) based on microbial Genera conducted on the Galaxy platform.

263 Finally, to test whether high- and low-aggregation females differ in their other
264 life-history traits, we performed four t-tests on the general activity, boldness, and body
265 condition (*i.e.*, scaled body mass index).

266

267 **Results**

268 Overall, the gut microbiota was highly variable between females. It was predominantly
269 composed of Proteobacteria (79.4%) - of which 50% belonged to the
270 Enterobacteriaceae and Pseudomonadaceae families - and Firmicutes (12.2%),
271 including Lachnospiraceae (6.1%) and Enterococcaceae (2.6%) (**Figure 1**) with five
272 dominant bacterial genera (*Pseudomonas*, *Pluralibacter*, *Kosakonia*,
273 *Stenotrophomonas* and *Acinetobacter*) that jointly reach 50% of the gut composition
274 (**Figure 1**).

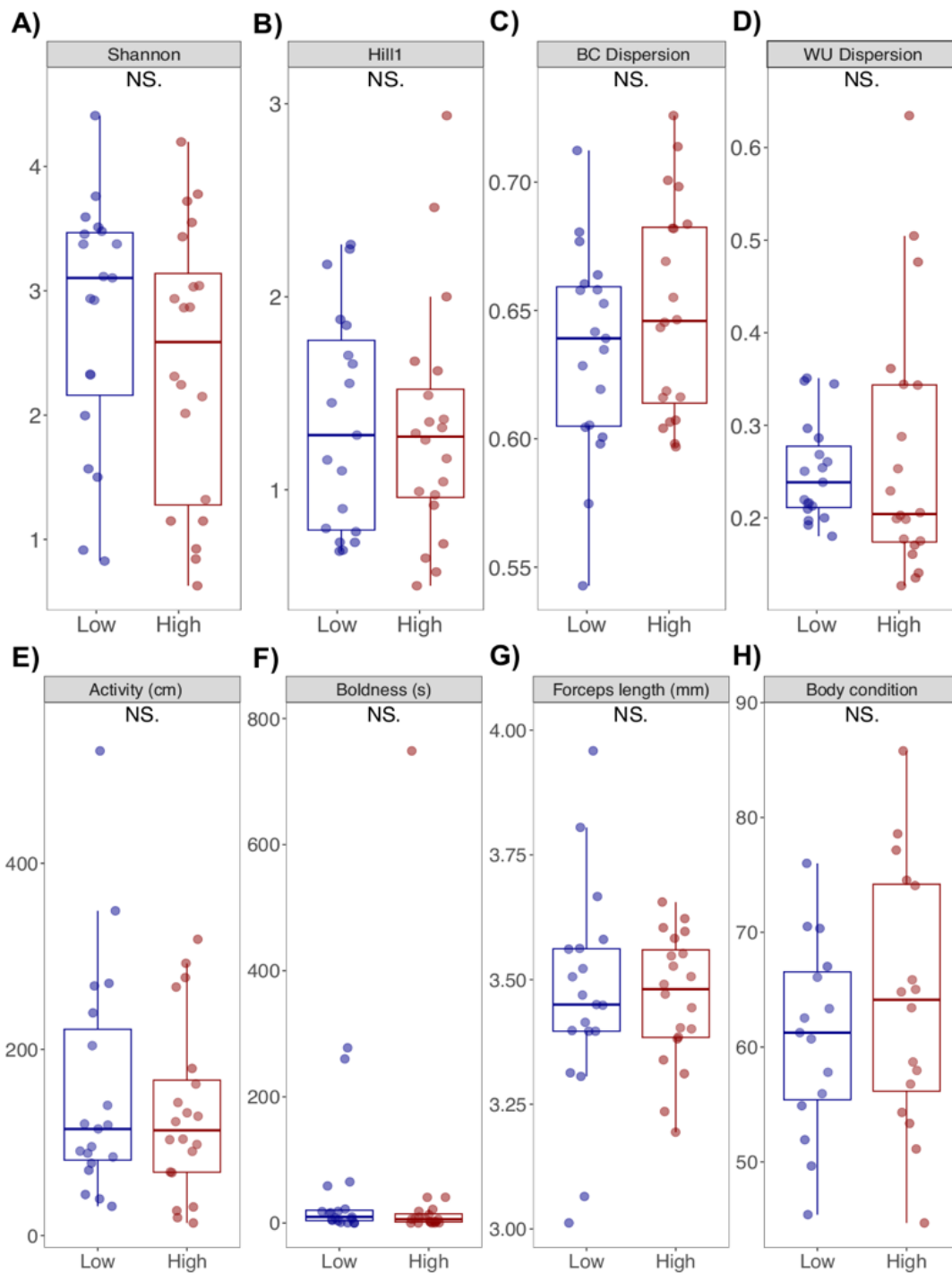


275

276 **Figure 1: Gut microbial community for the 19 low- and 20 high-aggregation**
 277 **females. A) Gut microbial community at family level (top 20) and B) at genus level**
 278 where hosts are individually ranked according to their score in an ascending order.
 279 Microbial taxa are ordered in function of their relative abundance by phylum where blue
 280 taxa belong to Proteobacteria, green to Firmicutes, red to Bacteroidota and
 281 orange/taupe to Actinobacteriota.

282

283 We found no evidence that the 39 low-aggregation and high-aggregation
 284 females carried distinct gut microbial communities. Regarding the alpha diversity, there
 285 was no difference in terms of Shannon (LR $\chi^2_1 = 2.77$, P = 0.110) and Hill1 (LR $\chi^2_1 =$
 286 0.65, P = 0.430) indices (**Figure 2; Table 1**).

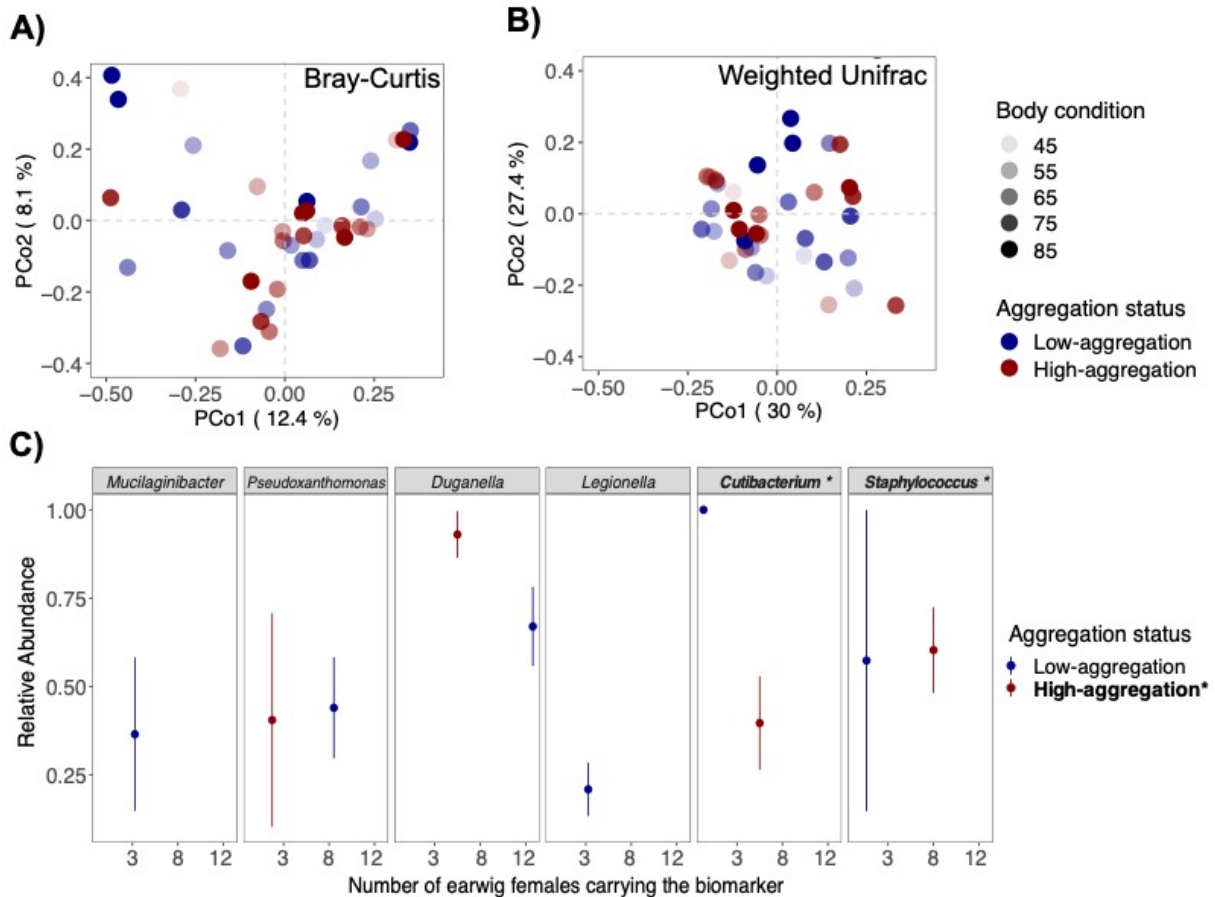


288 **Figure 2: Gut microbiota and life history traits between low- (blue) vs high-**
 289 **aggregation (red) earwig females. A-B) Differences in microbial alpha diversity for**
 290 **the Shannon and Hill1 indices, for the C-D) beta-dispersion within groups of**
 291 **aggregation levels based on Bray-Curtis and Weighted Unifrac dissimilarity matrices**
 292 **and for E-H) four life history traits of which general activity, boldness, and body**
 293 **condition. Boxplots represent the median (middle bar) and the interquartile range (box)**
 294 **with whiskers extending to 1.5 times the interquartile range. NS. Non-significant.**

295

296 Regarding the beta diversity, there was also no difference in terms of dispersion
297 **(Figure 2)** and community composition for Bray-Curtis (LR $\chi^2_1 = 2.53$, P = 0.125 ;
298 PERMANOVA R² = 0.02, P = 0.978) and Weighted Unifrac (LR $\chi^2_1 = 0.22$, P = 0.643 ;
299 PERMANOVA R² = 0.03, P = 0.335) distances **(Figure 3; Table 1)**. This apparent lack
300 of association between aggregation status and alpha/beta diversity indices was
301 constant throughout the experiment (all interactions between aggregation status and
302 sampling week of females; P > 0.151). Finally, even though the LEfSe algorithm based
303 on all bacterial Genera identified four potential biomarkers (p < 0.05; LDA scores (log
304 10) > 2.5) for low-aggregation females (*Mucilaginibacter*, *Pseudoxanthomonas*,
305 *Duganella* and *Legionella*) and two (*Cutibacterium* and *Staphylococcus*) for high-
306 aggregation females, these biomarkers were found in only 1 (5%) to 12 (63%) females
307 per category and could thus not be successfully used to separate the two categories
308 **(Figure 3)**. There was also no difference between low- and high-aggregation females
309 in terms of general activity (t₃₇ = -1.05, P = 0.300), boldness (t₃₇ = 0.116, P = 0.909),
310 and body condition (t₃₇ = 0.904, P = 0.374; **Figure 2**).

311 In addition to aggregation, alpha and beta diversity indices were not associated
312 with females' general activity and boldness **(Table 1)**. However, the beta diversity in
313 term of microbial composition was significantly associated with female body condition
314 for both Bray-Curtis (PERMANOVA: R²= 0.05, P = 0.017; **Figure 3** and **Table 1**) and
315 Weighted Unifrac (PERMANOVA: R²= 0.13, P = 0.001) distances **(Table 1; Figure 3)**.
316 This association was mainly due to changes in the ratio between Proteobacteria and
317 Firmicutes: Proteobacteria were more abundant in low body condition hosts, while
318 Firmicutes (enriched with Lachnospiraceae and Enterococcaceae families) were more
319 abundant in high body condition hosts **(Fig S5)**.



321

322 **Figure 3: Gut microbial beta-diversity visualized through a 2D Principal**
 323 **Coordinates Analysis (PcoA) and microbial genera biomarkers. A)** Ordination
 324 computed from the comparison per pair of samples based on the Bray-Curtis and **B)**
 325 its phylogenetic equivalent Weighted Unifrac distance, showing the microbial
 326 dissimilarity between low- (blue) and high- (red) aggregation individuals, respective to
 327 their body condition (represented with different circle sizes) proxied by the Scaled Mass
 328 Index. **C)** Microbial Genera discriminants resulting from the LefSe analysis (LDA score
 329 (\log_{10}) > 2.5) with the proportion of females carrying the discriminant and its respective
 330 relative abundance in low- (in blue) and high-aggregation female microbiota (in red).
 331 Discriminants for the high-aggregation individuals are marked in bold with a star. The
 332 others are discriminated in the low-aggregation individuals. Points represent the
 333 median of the relative abundance and lines extend to the upper (top line) and lower
 334 (bottom line) values.

335

336 Finally, the five weeks of laboratory rearing had no effect on the gut microbiota
337 of the tested females in terms of alpha diversity (*i.e.*, Shannon and Hill1 index values;
338 **Table 1**). Although there was an effect of the sampling week on beta diversity (Bray-
339 Curtis distances: **Table 1**; $R^2 = 0.13$, $P = 0.023$), this effect was due to a sampling
340 effect rather than a linear homogenization of the microbial community during laboratory
341 rearing. This is supported by the facts that females sampled in first and last weeks had
342 a similar gut bacterial composition (Pairwise Adonis $R^2 = 0.08$, $P = 0.231$), while all
343 other dates differed from each other (Pairwise Adonis in **Table 2**), and because gut
344 microbial dispersion for both Bray-Curtis and Weighted Unifrac dissimilarity indices did
345 not change between sampling dates (Bray-Curtis $F_4 = 0.89$, $P = 0.442$; Weighted
346 Unifrac $F_4 = 0.25$, $P = 0.907$; **Table 1**).

347

348 **Discussion**

349 The reasons why some individuals are solitary and others gregarious are the subject
350 of ongoing debate as we seek to understand the emergence of sociality (Onchuru et
351 al. 2018; Biedermann et al. 2021). In this study, we investigated whether the gut
352 microbial communities of a host could explain natural variation in the expression of
353 aggregation behaviors using females of the European earwig. Contrary to our
354 predictions, we found that neither the diversity, dispersion nor composition of gut
355 bacterial communities was associated with the high or low levels of adult aggregation.
356 We also found no association between female's gut microbiota, general activity and
357 boldness. Finally, we showed that the beta diversity of the gut microbiota overall
358 reflected female body condition, while the five weeks of laboratory rearing did not
359 homogenize or alter its composition.

360 Over the past few decades, a growing number of studies have reported
361 differences in the gut microbiota of social and less-social individuals (Vuong et al. 2017;

362 Valdes et al. 2018; Sherwin et al. 2019), suggesting that the microbiota may (at least
363 partly) explain the propensity of a host to live in a group, or might be predicted by the
364 ability of the group to share their symbionts (Hosokawa et al. 2008; Raulo et al. 2021).
365 Our results are at odds with this prediction: we did not detect any difference in the gut
366 microbiota of the most and least gregarious earwig females, both in terms of alpha and
367 beta diversities. This apparent lack of association between gut microbiota and levels
368 of aggregation first suggests that poorly gregarious earwig females are not moribund
369 individuals expressing abnormal social behaviors (Desbonnet et al. 2014; Buffington
370 et al. 2016; Vuong et al. 2017; Valdes et al. 2018; Sherwin et al. 2019). This is
371 consistent with our data showing no association between gut microbiota and both
372 female activity and boldness, as well as with previous results showing that altering the
373 gut microbiota of earwig mothers with antibiotics does not alter their expression of
374 maternal care (Van Meyel et al. 2021). Second, our results suggest that the most
375 gregarious females are not those lacking specific symbionts that can only be acquired
376 through group living (Kopanic et al. 2001; Nalepa 2015; Onchuru et al. 2018). This
377 highlights that many of the social behaviors known to be expressed in earwig groups
378 such as coprophagy, trophallaxis and allo-grooming may not only evolve and function
379 to exchange these microbes (Boos et al. 2014; Falk et al. 2014; Körner et al. 2016).
380 Finally, the apparent lack of association between gut microbiota and aggregation
381 supports the view that host sociality is unlikely to reflect a manipulative strategy by gut
382 microbes to improve their chances of reaching new hosts (Johnson & Foster, 2018).
383 Taken together, our results suggest that the drivers of aggregation in earwig females
384 may depend on parameters independent of their own gut microbiota, including the
385 direct costs and benefits of group living in terms of conflict, cooperation and
386 reproduction (Krause and Ruxton 2002).

387 Our data then show that the gut microbiota of earwigs did not homogenize
388 during the five weeks of rearing in the laboratory and that, overall, it reflected female
389 body conditions. The lack of effect of laboratory rearing on gut microbiota contrasts
390 with studies in other insects (Staudacher et al. 2016; Waltmann et al. 2019; Tinker and
391 Ottesen 2021), and suggests that sharing a standard environment for five weeks is not
392 long enough to induce major microbiota turnover in earwig females. Conversely, the
393 reported association between gut microbiota and host body condition is consistent with
394 findings in several animals (Haro et al. 2016; Chun et al. 2020; Hernández-Gómez et
395 al. 2020; Sugden et al. 2020) of which insects (Moret and Schmid-Hempel 2009;
396 McMullen et al. 2020; Greenwood et al. 2021). However, the fact that this link is based
397 on beta rather than alpha diversity is more surprising. It highlights the potential
398 challenge faced by female earwigs to change of body condition, as it would require
399 them to achieve a major turnover in the composition of their gut microbial communities.
400 Such major turnover often occurs during offspring development, particularly during
401 molting events where the gut microbiota can be completely changed (Manthey et al.
402 2022; Girard et al. 2023; Querejeta et al. 2023). Whether this is the case in the
403 European earwigs is still unknown. More generally, future work is needed to investigate
404 whether and how such turnover can occur in this species, or whether it is impossible
405 and female body condition is determined early in development.

406 The absence of any clear link between gut microbiota and female behaviors
407 such as aggregation, activity, or boldness, together with the limited impact of gut
408 microbiota changes caused by antibiotics on maternal care (Van Meyel et al. 2021),
409 calls into question the role of gut microbiota in the European earwig. Most of the taxa
410 we found in the earwig gut microbiota are environmental bacteria that are known to be
411 abundant in both the soil and the gut of many arthropods. When present in the gut of

412 arthropods, they often play a major role in the host biology. For instance, some
413 bacterial strains that belong to the genera *Serratia*, *Pseudomonas* and *Rhanella*
414 metabolize and reduce the concentrations of monoterpenes in the Bark beetle (Boone
415 et al. 2013; Xu et al. 2016). Similarly, *Enterobacter*, *Rhanella* and *Pantoea* often
416 perform atmospheric N-fixation and participate in cellulose breakdown to provide
417 nutrition in the red turpentine beetle (Morales-Jiménez et al. 2009). Whether these
418 bacteria also play an important role in the biology of the European earwig remains to
419 be tested. However, a recent study suggests that they may not: the lifelong ingestion
420 of antibiotics by earwig females did not alter 30 proxies for their physiology,
421 reproduction and longevity (Van Meyel et al. 2021). This may suggest that earwig
422 biology is independent of their gut microbiota, as reported in the three Lepidoptera
423 *Danaus chrysippus*, *Ariadne merione* and *Choristoneura fumiferana* (Hammer et al.
424 2017; Phalnikar et al. 2019; Ravenscraft et al. 2019). In the Lepidoptera, it has been
425 suggested that this is because individuals do not have to rely on specific bacteria to
426 obtain critical nutrients from their food sources (Chaturvedi et al. 2017; Hammer et al.
427 2017). As the European earwig is omnivorous, this may also be the case for this
428 species (Blubaugh 2023).

429 To conclude, our study suggests that the gut microbiota of a European earwig
430 female cannot account alone for her tendency to be in either close or far proximity to a
431 group of female conspecifics. This result emphasizes that the gut microbiota of a host
432 is not necessarily a major driver nor a consequence of its social behavior, and stresses
433 that natural variation in the expression of social behavior is not necessarily linked to
434 an alteration in the community of bacteria residing in the gut of the host, the presence
435 or absence of particular gut microbes in the host, or specific gut microbial communities
436 in the focal individual. However, generalizing our results to the gut microbiota as a

437 whole should be done with caution, as we didn't take into account non-bacterial
438 components of the microbiota (*e.g.*, fungi, protozoa, viruses) that might play a role in
439 their behavior (Hughes et al. 2011; Gasque et al. 2019). Similarly, we cannot rule out
440 the possibility that our statistical power did not allow us to detect more subtle
441 differences between the most and least gregarious females, and that these differences
442 might emerge with the development of more sensitive approach to determine bacterial
443 species/strain from ASV. Nevertheless, our results provide a first empirical
444 investigation of the link between gut microbiota and aggregation behavior in a
445 facultatively group-living insect and highlight that such a link may not be universal
446 among insects. Moreover, our study calls for further systematic analyses on the
447 existence of a core microbiome in the European earwig, and the development of
448 gnotobiotic systems with microbe-depleted hosts. This will help us to test and
449 understand the specific functions of gut bacteria at the different life stages of the host,
450 and their contribution to the fitness of the species.

451

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464

465 **Data availability**

466 Analyses reported in this article can be reproduced using the data provided by Cheutin,
467 M-C. Leclerc, B. and Meunier, J. (Forthcoming 2024). With or without you: Gut
468 microbiota does not predict aggregation behavior in European earwig females
469 [Dataset]. Dryad. <https://doi.org/10.5061/dryad.z8w9ghxmm>. Libraries for each sample
470 are also deposited in the NCBI Sequence Read Archive (SRA) under BioProject
471 accession no. PRJNA936136.

472

473 **References**

474

- 475 Archer J. 1973. Tests for emotionality in rats and mice: A review. *Animal Behaviour*.
476 21(2):205–235. doi:10.1016/S0003-3472(73)80065-X.
- 477 Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models
478 using **lme4**. *J Stat Soft*. 67(1). doi:10.18637/jss.v067.i01.
479 <http://www.jstatsoft.org/v67/i01/>.
- 480 Biedermann PHW, Rohlf M, McMahon DP, Meunier J. 2021. Editorial: Microbial
481 drivers of sociality – from multicellularity to animal societies. *Front Ecol Evol*.
482 9:752906. doi:10.3389/fevo.2021.752906.
- 483 Blubaugh CK. 2023. An omnivore vigour hypothesis? Nutrient availability strengthens
484 herbivore suppression by omnivores across 48 field sites. *Journal of Animal*
485 *Ecology*. 92(3):751–759. doi:10.1111/1365-2656.13890.
- 486 Boone CK, Keefover-Ring K, Mapes AC, Adams AS, Bohlmann J, Raffa KF. 2013.
487 Bacteria associated with a tree-killing insect reduce concentrations of plant defense
488 compounds. *J Chem Ecol*. 39(7):1003–1006. doi:10.1007/s10886-013-0313-0.
- 489 Boos S, Meunier J, Pichon S, Kölliker M. 2014. Maternal care provides antifungal
490 protection to eggs in the European earwig. *Behavioral Ecology*. 25(4):754–761.
491 doi:10.1093/beheco/aru046.

492 Broly P, Deneubourg J-L, Devigne C. 2013. Benefits of aggregation in woodlice: a
493 factor in the terrestrialization process? *Insect Soc.* 60(4):419–435.
494 doi:10.1007/s00040-013-0313-7.

495 Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M.
496 2016. Microbial reconstitution reverses maternal diet-induced social and synaptic
497 deficits in offspring. *Cell.* 165(7):1762–1775. doi:10.1016/j.cell.2016.06.001.

498 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016.
499 DADA2: High-resolution sample inference from Illumina amplicon data. *Nat*
500 *Methods.* 13(7):581–583. doi:10.1038/nmeth.3869.

501 Camazine S, editor. 2003. Self-organization in biological systems. Princeton studies in
502 complexity. Princeton, NJ: Princeton Univ. Press.

503 Chao A, Chiu C-H, Jost L. 2010. Phylogenetic diversity measures based on Hill
504 numbers. *Phil Trans R Soc B.* 365(1558):3599–3609. doi:10.1098/rstb.2010.0272.

505 Chaturvedi S, Rego A, Lucas LK, Gompert Z. 2017. Sources of variation in the gut
506 microbial community of *Lycaeides melissa* Caterpillars. *Sci Rep.* 7(1):11335.
507 doi:10.1038/s41598-017-11781-1.

508 Chun JL, Ji SY, Lee SD, Lee YK, Kim B, Kim KH. 2020. Difference of gut microbiota
509 composition based on the body condition scores in dogs. *J Anim Sci Technol.*
510 62(2):239–246. doi:10.5187/jast.2020.62.2.239.

511 Deere JR, Schaber KL, Foerster S, Gilby IC, Feldblum JT, VanderWaal K, Wolf TM,
512 Travis DA, Raphael J, Lipende I, et al. 2021. Gregariousness is associated with
513 parasite species richness in a community of wild chimpanzees. *Behav Ecol*
514 *Sociobiol.* 75(5):87. doi:10.1007/s00265-021-03030-3.

515 Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. 2014. Microbiota is
516 essential for social development in the mouse. *Mol Psychiatry.* 19(2):146–148.
517 doi:10.1038/mp.2013.65.

518 Dillon RJ, Vennard CT, Charnley AK. 2000. Exploitation of gut bacteria in the locust.
519 *Nature.* 403(6772):851–851. doi:10.1038/35002669.

520 Elwood RW, Stolzenberg DS. 2020. Flipping the parental switch: from killing to caring
521 in male mammals. *Animal Behaviour.* 165:133–142.
522 doi:10.1016/j.anbehav.2020.05.001.

523 Falk J, Wong JWY, Kölliker M, Meunier J. 2014. Sibling cooperation in earwig families
524 provides insights into the early evolution of social life. *The American Naturalist.*
525 183(4):547–557. doi:10.1086/675364.

526 Fox J, Weisberg S, Fox J. 2019. An R companion to applied regression. 3rd ed.
527 Thousand Oaks, Calif: Sage.
528 <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.

529 Gasque SN, Van Oers MM, Ros VI. 2019. Where the baculoviruses lead, the
530 caterpillars follow: baculovirus-induced alterations in caterpillar behaviour. *Current*
531 *Opinion in Insect Science*. 33:30–36. doi:10.1016/j.cois.2019.02.008.

532 Girard M, Luis P, Valiente Moro C, Minard G. 2023. Crosstalk between the microbiota
533 and insect postembryonic development. *Trends in Microbiology*. 31(2):181–196.
534 doi:10.1016/j.tim.2022.08.013.

535 Glassman SI, Martiny JBH. 2018. Broadscale ecological patterns are robust to use of
536 Exact Sequence Variants versus Operational Taxonomic Units. Tringe SG, editor.
537 *mSphere*. 3(4):e00148-18. doi:10.1128/mSphere.00148-18.

538 González-Miguéns R, Muñoz-Nozal E, Jiménez-Ruiz Y, Mas-Peinado P, Ghanavi HR,
539 García-París M. 2020. Speciation patterns in the *Forficula auricularia* species
540 complex: cryptic and not so cryptic taxa across the western Palaearctic region.
541 *Zoological Journal of the Linnean Society*. 190(3):788–823.
542 doi:10.1093/zoolinnean/zlaa070.

543 Greenwood MP, Hull KL, Brink-Hull M, Lloyd M, Rhode C. 2021. Feed and host
544 genetics drive microbiome diversity with resultant consequences for production
545 traits in mass-reared black soldier fly (*Hermetia illucens*) larvae. *Insects*.
546 12(12):1082. doi:10.3390/insects12121082.

547 Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N. 2017. Caterpillars lack a
548 resident gut microbiome. *Proc Natl Acad Sci USA*. 114(36):9641–9646.
549 doi:10.1073/pnas.1707186114.

550 Haro C, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P,
551 Delgado-Lista J, Quintana-Navarro GM, Landa BB, Navas-Cortés JA, Tena-
552 Sempere M, et al. 2016. Intestinal microbiota is influenced by gender and body mass
553 index. Sanz Y, editor. *PLoS ONE*. 11(5):e0154090.
554 doi:10.1371/journal.pone.0154090.

555 Hartig F. 2022. DHARMa: Residual diagnostics for hierarchical (multi-Level / mixed)
556 regression models. R package version 046. [https://CRAN.R-](https://CRAN.R-project.org/package=DHARMa)
557 [project.org/package=DHARMa](https://CRAN.R-project.org/package=DHARMa).

558 Hernández-Gómez O, Byrne AQ, Gunderson AR, Jenkinson TS, Noss CF, Rothstein
559 AP, Womack MC, Rosenblum EB. 2020. Invasive vegetation affects amphibian skin

560 microbiota and body condition. PeerJ. 8:e8549. doi:10.7717/peerj.8549.

561 Hosokawa T, Kikuchi Y, Shimada M, Fukatsu T. 2008. Symbiont acquisition alters
562 behaviour of stinkbug nymphs. Biol Lett. 4(1):45–48. doi:10.1098/rsbl.2007.0510.

563 Hughes DP, Andersen SB, Hywel-Jones NL, Himaman W, Billen J, Boomsma JJ. 2011.
564 Behavioral mechanisms and morphological symptoms of zombie ants dying from
565 fungal infection. BMC Ecol. 11(1):13. doi:10.1186/1472-6785-11-13.

566 Jeanson R, Deneubourg J. 2007. Conspecific attraction and shelter selection in
567 gregarious insects. The American Naturalist. 170(1):47–58. doi:10.1086/518570.

568 Johnson KV-A, Foster KR. 2018. Why does the microbiome affect behaviour? Nat Rev
569 Microbiol. 16(10):647–655. doi:10.1038/s41579-018-0014-3.

570 Klein SL. 2003. Parasite manipulation of the proximate mechanisms that mediate
571 social behavior in vertebrates. Physiology & Behavior. 79(3):441–449.
572 doi:10.1016/S0031-9384(03)00163-X.

573 Kopanic RJ, Holbrook GL, Sevala V, Schal AC. 2001. An adaptive benefit of facultative
574 coprophagy in the German cockroach *Blattella germanica*: Coprophagy in the
575 German cockroach. Ecological Entomology. 26(2):154–162. doi:10.1046/j.1365-
576 2311.2001.00316.x.

577 Körner M, Diehl JMC, Meunier J. 2016. Growing up with feces: benefits of allo-
578 coprophagy in families of the European earwig. BEHECO.:arw113.
579 doi:10.1093/beheco/arw113.

580 Kramer J, Meunier J. 2016a. Kin and multilevel selection in social evolution: a never-
581 ending controversy? F1000Res. 5:776. doi:10.12688/f1000research.8018.1.

582 Kramer J, Meunier J. 2016b. Maternal condition determines offspring behavior toward
583 family members in the European earwig. BEHECO. 27(2):494–500.
584 doi:10.1093/beheco/arv181.

585 Krause J, Ruxton GD. 2002. Living in groups. Oxford ; New York: Oxford University
586 Press (Oxford series in ecology and evolution).

587 Lamb RJ, Wellington WG. 1975. Life history and population characteristics of the
588 European earwig, *Forficula auricularia* (Dermaptera: Forficulidae), at Vancouver,
589 British Columbia. Can Entomol. 107(8):819–824. doi:10.4039/Ent107819-8.

590 Lindsay RJ, Holder PJ, Talbot NJ, Gudelij I. 2023. Metabolic efficiency reshapes the
591 seminal relationship between pathogen growth rate and virulence. Ecology Letters.
592 26(6):896–907. doi:10.1111/ele.14218.

593 Lindström Å. 1989. Finch flock size and risk of hawk predation at a migratory stopover

594 site. *The Auk*. 106(2):225–232.

595 Littman DR, Pamer EG. 2011. Role of the commensal microbiota in normal and
596 pathogenic host immune responses. *Cell Host & Microbe*. 10(4):311–323.
597 doi:10.1016/j.chom.2011.10.004.

598 Lombardo MP. 2008. Access to mutualistic endosymbiotic microbes: an
599 underappreciated benefit of group living. *Behav Ecol Sociobiol*. 62(4):479–497.
600 doi:10.1007/s00265-007-0428-9.

601 Lozupone C, Knight R. 2005. UniFrac: a new phylogenetic method for comparing
602 microbial communities. *Appl Environ Microbiol*. 71(12):8228–8235.
603 doi:10.1128/AEM.71.12.8228-8235.2005.

604 Lucatelli J, Mariano-Neto E, Japyassú HF. 2021. Social interaction, and not group size,
605 predicts parasite burden in mammals. *Evol Ecol*. 35(1):115–130.
606 doi:10.1007/s10682-020-10086-6.

607 Manthey C, Johnston PR, Nakagawa S, Rolff J. 2022. Complete metamorphosis and
608 microbiota turnover in insects. *Molecular Ecology*.:mec.16673.
609 doi:10.1111/mec.16673.

610 Matsuura K. 2001. Nestmate recognition mediated by intestinal bacteria in a termite,
611 *Reticulitermes speratus*. *Oikos*. 92(1):20–26. doi:10.1034/j.1600-
612 0706.2001.920103.x.

613 McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE,
614 Dubilier N, Eberl G, Fukami T, Gilbert SF, et al. 2013. Animals in a bacterial world,
615 a new imperative for the life sciences. *Proc Natl Acad Sci USA*. 110(9):3229–3236.
616 doi:10.1073/pnas.1218525110.

617 McFrederick QS, Wcislo WT, Hout MC, Mueller UG. 2014. Host species and
618 developmental stage, but not host social structure, affects bacterial community
619 structure in socially polymorphic bees. *FEMS Microbiol Ecol*. 88(2):398–406.
620 doi:10.1111/1574-6941.12302.

621 McMullen JG, Peters-Schulze G, Cai J, Patterson AD, Douglas AE. 2020. How gut
622 microbiome interactions affect nutritional traits of *Drosophila melanogaster*. *Journal*
623 *of Experimental Biology*. 223(19):jeb227843. doi:10.1242/jeb.227843.

624 McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive
625 analysis and graphics of microbiome census data. Watson M, editor. *PLoS ONE*.
626 8(4):e61217. doi:10.1371/journal.pone.0061217.

627 Merleau L-A, Larrigaldie I, Bousquet O, Devers S, Keller M, Lécureuil C, Meunier J.

628 2022. Exposure to pyriproxyfen (juvenile hormone agonist) does not alter maternal
629 care and reproduction in the European earwig. *Environ Sci Pollut Res.*
630 29(48):72729–72746. doi:10.1007/s11356-022-20970-z.

631 Meunier J, Wong JWY, Gómez Y, Kuttler S, Röllin L, Stucki D, Kölliker M. 2012. One
632 clutch or two clutches? Fitness correlates of coexisting alternative female life-
633 histories in the European earwig. *Evol Ecol.* 26(3):669–682. doi:10.1007/s10682-
634 011-9510-x.

635 Morales-Jiménez J, Zúñiga G, Villa-Tanaca L, Hernández-Rodríguez C. 2009.
636 Bacterial community and nitrogen fixation in the red turpentine beetle, *Dendroctonus*
637 *valens* LeConte (Coleoptera: Curculionidae: Scolytinae). *Microb Ecol.* 58(4):879–
638 891. doi:10.1007/s00248-009-9548-2.

639 Moret Y, Schmid-Hempel P. 2009. Immune responses of bumblebee workers as a
640 function of individual and colony age: senescence versus plastic adjustment of the
641 immune function. *Oikos.* 118(3):371–378. doi:10.1111/j.1600-0706.2008.17187.x.

642 Mosser A, Packer C. 2009. Group territoriality and the benefits of sociality in the African
643 lion, *Panthera leo*. *Animal Behaviour.* 78(2):359–370.
644 doi:10.1016/j.anbehav.2009.04.024.

645 Muyzer G, de Waal EC, Uitterlinden AG. 1993. Profiling of complex microbial
646 populations by denaturing gradient gel electrophoresis analysis of polymerase chain
647 reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol.* 59(3):695–
648 700. doi:10.1128/aem.59.3.695-700.1993.

649 Nalepa CA. 2015. Origin of termite eusociality: trophallaxis integrates the social,
650 nutritional, and microbial environments: Origin of termite eusociality. *Ecol Entomol.*
651 40(4):323–335. doi:10.1111/een.12197.

652 Nalepa CA. 2020. Origin of mutualism between termites and flagellated gut protists:
653 transition from horizontal to vertical transmission. *Front Ecol Evol.* 8:14.
654 doi:10.3389/fevo.2020.00014.

655 Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O’Hara R,
656 Solymos P, Stevens M, Szoecs E, et al. 2022. vegan: community ecology package.
657 <https://CRAN.R-project.org/package=vegan>.

658 Onchuru TO, Javier Martinez A, Ingham CS, Kaltenpoth M. 2018. Transmission of
659 mutualistic bacteria in social and gregarious insects. *Current Opinion in Insect*
660 *Science.* 28:50–58. doi:10.1016/j.cois.2018.05.002.

661 Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and

662 evolutionary analyses in R. *Bioinformatics*. 35:526–528.

663 Peig J, Green AJ. 2010. The paradigm of body condition: a critical reappraisal of
664 current methods based on mass and length: The paradigm of body condition.
665 *Functional Ecology*. 24(6):1323–1332. doi:10.1111/j.1365-2435.2010.01751.x.

666 Phalnikar K, Kunte K, Agashe D. 2019. Disrupting butterfly caterpillar microbiomes
667 does not impact their survival and development. *Proc R Soc B*.
668 286(1917):20192438. doi:10.1098/rspb.2019.2438.

669 Querejeta M, Hervé V, Perdereau E, Marchal L, Herniou EA, Boyer S, Giron D. 2023.
670 Changes in bacterial community structure across the different life stages of black
671 soldier fly (*Hermetia illucens*). *Microb Ecol*. 86(2):1254–1267. doi:10.1007/s00248-
672 022-02146-x.

673 R Core Team. 2022. R: A language and environment for statistical computing.
674 <https://www.R-project.org/>.

675 Rankin SM, Palmer JO. 2009. Dermaptera. In: *Encyclopedia of Insects*. Elsevier. p.
676 259–261.

677 Raulo A, Allen BE, Troitsky T, Husby A, Firth JA, Coulson T, Knowles SCL. 2021.
678 Social networks strongly predict the gut microbiota of wild mice. *The ISME Journal*.
679 15(9):2601–2613. doi:10.1038/s41396-021-00949-3.

680 Raveh S, Vogt D, Montavon C, Kölliker M. 2014. Sibling aggregation preference
681 depends on activity phase in the European Earwig (*Forficula auricularia*). Fusani L,
682 editor. *Ethology*. 120(8):776–782. doi:10.1111/eth.12249.

683 Ravenscraft A, Kish N, Peay K, Boggs C. 2019. No evidence that gut microbiota
684 impose a net cost on their butterfly host. *Mol Ecol*. 28(8):2100–2117.
685 doi:10.1111/mec.15057.

686 Ritchie KL, Vredenburg VT, Chaukulkar S, Butler HM, Zink AG. 2021. Social group
687 size influences pathogen transmission in salamanders. *Behav Ecol Sociobiol*.
688 75(10):136. doi:10.1007/s00265-021-03057-6.

689 Rubin BER, Sanders JG, Turner KM, Pierce NE, Kocher SD. 2018. Social behaviour
690 in bees influences the abundance of *Sodalis* (Enterobacteriaceae) symbionts. *R Soc*
691 *open sci*. 5(7):180369. doi:10.1098/rsos.180369.

692 Sauphanor B, Sureau F. 1993. Aggregation behaviour and interspecific relationships
693 in Dermaptera. *Oecologia*. 96(3):360–364. doi:10.1007/BF00317506.

694 Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C.
695 2011. Metagenomic biomarker discovery and explanation. *Genome Biol*. 12(6):R60.

696 doi:10.1186/gb-2011-12-6-r60.

697 Sekirov I, Russell SL, Antunes LCM, Finlay BB. 2010. Gut microbiota in health and
698 disease. *Physiological Reviews*. 90(3):859–904. doi:10.1152/physrev.00045.2009.

699 Sgritta M, Dooling SW, Buffington SA, Momin EN, Francis MB, Britton RA, Costa-
700 Mattioli M. 2019. Mechanisms underlying microbial-mediated changes in social
701 behavior in mouse models of autism spectrum disorder. *Neuron*. 101(2):246-259.e6.
702 doi:10.1016/j.neuron.2018.11.018.

703 Sherwin E, Bordenstein SR, Quinn JL, Dinan TG, Cryan JF. 2019. Microbiota and the
704 social brain. *Science*. 366(6465):eaar2016. doi:10.1126/science.aar2016.

705 Staudacher H, Kaltenpoth M, Breeuwer JAJ, Menken SBJ, Heckel DG, Groot AT. 2016.
706 Variability of bacterial communities in the moth *Heliothis virescens* indicates
707 transient association with the host. Abdo Z, editor. *PLoS ONE*. 11(5):e0154514.
708 doi:10.1371/journal.pone.0154514.

709 Stratton JA, Nolte MJ, Payseur BA. 2021. Evolution of boldness and exploratory
710 behavior in giant mice from Gough Island. *Behav Ecol Sociobiol*. 75(4):65.
711 doi:10.1007/s00265-021-03003-6.

712 Sugden S, Sanderson D, Ford K, Stein LY, St. Clair CC. 2020. An altered microbiome
713 in urban coyotes mediates relationships between anthropogenic diet and poor
714 health. *Sci Rep*. 10(1):22207. doi:10.1038/s41598-020-78891-1.

715 Teseo S, van Zweden JS, Pontieri L, Kooij PW, Sørensen SJ, Wenseleers T, Poulsen
716 M, Boomsma JJ, Sapountzis P. 2019. The scent of symbiosis: gut bacteria may
717 affect social interactions in leaf-cutting ants. *Animal Behaviour*. 150:239–254.
718 doi:10.1016/j.anbehav.2018.12.017.

719 Thesing J, Kramer J, Koch LK, Meunier J. 2015. Short-term benefits, but
720 transgenerational costs of maternal loss in an insect with facultative maternal care.
721 *Proc R Soc B*. 282(1817):20151617. doi:10.1098/rspb.2015.1617.

722 Tinker KA, Ottesen EA. 2021. Differences in gut microbiome composition between
723 sympatric wild and allopatric laboratory populations of omnivorous cockroaches.
724 *Front Microbiol*. 12:703785. doi:10.3389/fmicb.2021.703785.

725 Valdes AM, Walter J, Segal E, Spector TD. 2018. Role of the gut microbiota in nutrition
726 and health. *BMJ*:k2179. doi:10.1136/bmj.k2179.

727 Van Meyel S, Devers S, Dupont S, Dedeine F. 2021. Alteration of gut microbiota with
728 a broad-spectrum antibiotic does not impair maternal care in the European earwig.
729 *Peer Community in Evolutionary Biology*.

730 Van Meyel S, Devers S, Meunier J. 2022. Earwig mothers consume the feces of their
731 juveniles during family life. *Insect Science*. 29(2):595–602. doi:10.1111/1744-
732 7917.12941.

733 Van Meyel S, Meunier J. 2022. Costs and benefits of isolation from siblings during
734 family life in adult earwigs. *Animal Behaviour*. 193:91–99.
735 doi:10.1016/j.anbehav.2022.09.003.

736 Vulinec K. 1990. Collective security: Insect aggregations as a defense. In: *Insect*
737 *Defenses: adaptive mechanisms and strategies of prey and predators*. Albany (NY):
738 State University of New York Press: David L. Evans and Justin O. Schmidt. p. 251–
739 288.

740 Vuong HE, Yano JM, Fung TC, Hsiao EY. 2017. The microbiome and host behavior.
741 *Annu Rev Neurosci*. 40(1):21–49. doi:10.1146/annurev-neuro-072116-031347.

742 Wada-Katsumata A, Zurek L, Nalyanya G, Roelofs WL, Zhang A, Schal C. 2015. Gut
743 bacteria mediate aggregation in the German cockroach. *Proc Natl Acad Sci USA*.
744 112(51):15678–15683. doi:10.1073/pnas.1504031112.

745 Waltmann A, Willcox AC, Balasubramanian S, Borrini Mayori K, Mendoza Guerrero S,
746 Salazar Sanchez RS, Roach J, Condori Pino C, Gilman RH, Bern C, et al. 2019.
747 Hindgut microbiota in laboratory-reared and wild *Triatoma infestans*. Gürtler RE,
748 editor. *PLoS Negl Trop Dis*. 13(5):e0007383. doi:10.1371/journal.pntd.0007383.

749 Weiß C, Kramer J, Holländer K, Meunier J. 2014. Influences of relatedness, food
750 deprivation, and sex on adult behaviors in the group-living Insect *Forficula*
751 *auricularia*. Fusani L, editor. *Ethology*. 120(9):923–932. doi:10.1111/eth.12261.

752 Wirth T, Le Guellec R, Vancassel M, Veuille M. 1998. Molecular and reproductive
753 characterization of sibling species in the European earwig (*Forficula auricularia*).
754 *Evolution*. 52(1):260–265. doi:10.1111/j.1558-5646.1998.tb05160.x.

755 Xu L-T, Lu M, Sun J-H. 2016. Invasive bark beetle-associated microbes degrade a
756 host defensive monoterpene: RTB-associated microbes in α -pinene degradation.
757 *Insect Science*. 23(2):183–190. doi:10.1111/1744-7917.12255.

758 Zhu F, Guo R, Wang W, Ju Y, Wang Q, Ma Q, Sun Q, Fan Y, Xie Y, Yang Z, et al.
759 2020. Transplantation of microbiota from drug-free patients with schizophrenia
760 causes schizophrenia-like abnormal behaviors and dysregulated kynurenine
761 metabolism in mice. *Mol Psychiatry*. 25(11):2905–2918. doi:10.1038/s41380-019-
762 0475-4.

763

764 **Table 1: Relationships between gut microbiota diversity and life history traits.**
765 Alpha-diversity (*i.e.*, Shannon and Hill1 index values) relationships are computed as
766 response variable in LMs. Influence on beta-diversity (*i.e.*, microbial community
767 dispersion and dissimilarities between samples calculated with Bray-Curtis and
768 Weighted Unifrac metrics) are tested with LMs and PERMANOVAs (999
769 permutations), respectively. The host activity, boldness, body condition proxied with
770 the scaled mass index and sampling week are tested as explanatory variables in each
771 statistical model. All interactions were tested and removed after AICc comparisons.
772 Significant effects ($P \leq 0.05$) are indicated in bold.

| | Aggregation status | Activity | Boldness | Body condition | Sampling week |
|--|------------------------------------|------------------------------------|------------------------------------|---|---|
| Alpha diversity (Linear Models) | | | | | |
| Shannon | LR $\chi^2_1= 2.77$, P = 0.110 | LR $\chi^2_1= 2.74$, P = 0.112 | LR $\chi^2_1= 0.54$, P = 0.471 | LR $\chi^2_1= 0.03$, P = 0.870 | LR $\chi^2_1= 1.44$, P = 0.257 |
| Hill1 | LR $\chi^2_1= 0.65$, P = 0.430 | LR $\chi^2_1= 3.32$, P = 0.082 | LR $\chi^2_1= 0.05$, P = 0.827 | LR $\chi^2_1= 1.28$, P = 0.269 | LR $\chi^2_1= 2.14$, P = 0.122 |
| Beta-dispersion (Linear Models) | | | | | |
| Bray-Curtis | LR $\chi^2_1= 2.53$, P = 0.125 | LR $\chi^2_1= 0.55$, P = 0.466 | LR $\chi^2_1= 0.08$, P = 0.774 | LR $\chi^2_1= 2.91$, P = 0.101 | LR $\chi^2_1= 2.16$, P = 0.120 |
| Weighted Unifrac | LR $\chi^2_1= 0.22$, P = 0.643 | LR $\chi^2_1= 0.14$, P = 0.709 | LR $\chi^2_1= 0.65$, P = 0.428 | LR $\chi^2_1= 3.21$, P = 0.08 | LR $\chi^2_1= 0.06$, P = 0.981 |
| Dissimilarity matrices (PERMANOVAs) | | | | | |
| Bray-Curtis | $R^2 = 0.02$, P = 0.978 | $R^2 = 0.03$, P = 0.317 | $R^2 = 0.03$, P = 0.284 | $R^2 = 0.05$, P = 0.015 | $R^2 = 0.13$, P = 0.023 |
| Weighted Unifrac | $R^2 = 0.03$, P = 0.335 | $R^2 = 0.04$, P = 0.174 | $R^2 = 0.01$, P = 0.931 | $R^2 = 0.13$, P = 0.001 | $R^2 = 0.12$, P = 0.176 |

773

774 **Table 2: Pairwise comparisons of the gut microbiota composition between**
 775 **sampling weeks.** Comparisons are made on dissimilarity distances based on the
 776 Bray-Curtis metric of the gut microbiota for each of the five sampling weeks (*i.e.*, Run1
 777 to the Run 5). Significant p-values ($P \leq 0.05$) are indicated in bold.

778

| | Run 1 | Run 2 | Run 3 | Run 4 |
|-------|-----------------------------|-----------------------------|-----------------------------|----------------------|
| Run 2 | R2 = 0.08, P = 0.311 | - | - | - |
| Run 3 | R2 = 0.09, P = 0.44 | R2 = 0.10, P = 0.014 | - | - |
| Run 4 | R2 = 0.11, P = 0.004 | R2 = 0.10, P = 0.047 | R2 = 0.10, P = 0.031 | - |
| Run 5 | R2 = 0.08, P = 0.245 | R2 = 0.10, P = 0.058 | R2 = 0.11, P = 0.009 | R2 = 0.07, P = 0.288 |

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