# Cadmium ingestion does not disrupt the social transmission of the microbiome in juveniles of a subsocial insect

Running title : No cadmium effect on juvenile earwigs' microbiome

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## Abstract

Symbiotic microbes are essential for host health and fitness. In family-living species, these microbes are often acquired through vertical transmission from parents and horizontal transmission from siblings. However, it is unclear how environmental stressors, such as chemical pollution, influence these contributions to the juvenile microbiome. Here, we tested the independent and interactive effects of social environment and cadmium ingestion - a common heavy metal pollutant - on the microbiome of juvenile European earwigs. We reared juveniles either alone, with siblings or with siblings plus the mother, exposed them to cadmium at 0, 25 or 100mg/L, and analysed their microbiome composition and diversity. Our results showed that both social environment and cadmium independently influenced the phylogenetic beta-diversity of the juvenile microbiome although neither factor nor their interaction affected alpha-diversity. Moreover, we found that observed specific bacterial taxa in the social groups, including the overabundance of putatively pathogenic (*Serratia*) and mutualistic (*Lactobacillus*) symbionts were more abundant in juveniles reared with family members than isolated ones. Overall, our findings suggest that while both social environment and cadmium shape the juvenile microbiome, family does not amplify the effects of chemical exposure. This highlights the resilience of microbial transmission within families, even under environmental stress.

Key-words: heavy metal; facultative social life; Parental care; Microbial heritability; Social microbiome

## Introduction

Most animals harbour microorganisms that are essential to their health and fitness. These microorganisms play crucial roles in hosts' biological processes, such as aiding in nutrient acquisition (Berlanga & Guerrero, 2016; Calderón-Cortés et al., 2012), regulating the immune system (Horak et al., 2020), supporting development (Girard et al., 2023; Y. Wang & Rozen, 2017) and providing protection against pathogens (Muhammad et al., 2019; Shukla et al., 2018). The acquisition of these microorganisms is, therefore, essential for the host, especially for juveniles that are born or hatched with an underdeveloped microbiome (Videvall et al., 2019) or that have lost a large part of their microbiome during development, such as through moulting in insects (Cheutin, Boucicot, et al., 2024a; Manthey et al., 2022; Schmidt & Engel, 2021).

In group-living species, high nest fidelity and high frequency of social interactions between group members are often considered key facilitating the acquisition of microorganisms by juveniles (Onchuru et al., 2018). In family groups, this acquisition occur through tow main pathways. The first pathway is vertical transmission through parental care -a widespread phenomenon across animal taxa (Trumbo, 2012). Parental care typically allows the transfer of microorganisms and community of microorganisms from parents to offspring through behaviours such as trophallaxis and coprophagy (Gurevich et al., 2020; Hosokawa et al., 2012). The second pathway involves horizontal transmission between juveniles. This may occur through the expression of cooperative behaviour between siblings or through passive processes involving fecal production and consumption by family members (Lombardo, 2008; Onchuru et al., 2018; Powell et al., 2014). Together these vertical and horizontal pathways are important drivers of the juvenile microbiome (Onchuru et al., 2018). However, the relative contribution of each pathway during family life, and the influence of external factors on these contributions, remain largely unexplored.

Multiple external factors can directly or indirectly influence the efficiency of vertical and horizontal microbial transfers during family life, the ability of parents and siblings to perform these transfers and, more generally, the composition of the juvenile microbiome (Bright & Bulgheresi, 2010; Hosokawa & Fukatsu, 2020; Sachs et al., 2011). Among these, human-induced chemical pollution is a prime and timely candidate for investigation. This type of pollution, typically caused by the proliferation of pesticides and other chemicals due

to agriculture, urbanisation and industry (Johnson & Munshi-South, 2017; Seto et al., 2012), is increasing worldwide and is known to affect the composition of microorganisms present in water, soil, plants and animals (Bell & Tylianakis, 2016; Martiny et al., 2023). By altering the diversity of microorganisms available to hosts in their environment (Schmidt & Engel, 2021), these changes can directly alter their microbiome community, as it has been reported in wild juvenile passerines (Maraci et al., 2022; Teyssier et al., 2018). These chemicals can also have toxic effects on the exposed hosts, altering both their behaviour and microbiome composition (Teyssier et al., 2018, 2020), and thus their ability to vertically transfer important microorganisms to their offspring. Exposure to anthropogenic chemical pollution may therefore have both direct effects on the juvenile microbiome and alter the relative contribution of vertical and horizontal transfer of microorganisms to the juveniles. However, these effects remain poorly understood.

In this study, we investigated the effects of social environment and exposure to a chemical product (cadmium) on the microbiome of juvenile European earwig *Forficula auricularia*. Cadmium is a highly toxic heavy metal, which alters the behaviour, cognition, development, survival or fecundity of many organisms ranging from mammals to insects (e.g., Arito et al., 1981; Gustin et al., 2018; Honorio et al., 2021). However, a few studies suggest that these effects are not universal in animals, such as in the European earwig, where it has limited effects on the behaviour, survival and development of both adults and juveniles (Honorio, Depierrefixe, et al., 2023; Honorio, Moreau, et al., 2023). The European earwig is an insect where mothers and juveniles live in groups for about two weeks after egg hatching, during which mothers provide multiple forms of care to their juveniles – called nymphs (Kölliker, 2007; Kölliker & Vancassel, 2007). Interestingly, family life is facultative in this species (Honorio et al., 2024). Nymphs rapidly become mobile after hatching, can forage for themselves and are able to live alone (Falk et al., 2014; Meunier & Kölliker, 2012; Van Meyel & Meunier, 2022). Mothers and nymphs express grooming, trophallaxis and coprophagy behaviours (Falk et al., 2014), which are known to be the main routes of microbial transfer in insects (Onchuru et al., 2018; Y. Wang & Rozen, 2017). The earwig microbiome can be influenced by the presence of the mother in early life stages and is highly variable between individuals (Cheutin, Boucicot, et al., 2024; Cheutin, Leclerc, et al., 2024;

Greer et al., 2020; Van Meyel et al., 2021). However, the role of the microbiome or its potential disruption in the face of chemical pollution remains unknown.

We reared 900 earwig juveniles either isolated (alone), with siblings or in a family group (with siblings and the mother) and exposed them to one of three concentrations of cadmium (0, 25 and 100 $mg.L^{-1}$) for the entire duration of family life, i.e. 14 days (Meunier, 2024). We then extracted the whole microbiome from a subset of 90 nymphs (10 per combination of social environment and cadmium concentration) and used 16S rRNA metabarcoding to analyse their alpha- and beta-diversity, as well as identify potential treatment-specific bacterial taxa. If microbial transfers occur between siblings (*i.e.,* horizontal) and/or between mother and nymphs during family life (*i.e.,* vertical), we predict greater microbial diversity in nymphs living in group (sibling and family groups) compared to isolated nymphs, and that some microbial taxa will be specific to nymphs reared with mothers. If cadmium alters the microbiome composition of the hosts, we predict a lower microbial diversity in nymphs treated with cadmium, and that some microbial taxa will be specific to nymphs that have ingested cadmium. Finally, if cadmium ingestion alters the social transmission of the microbiome to the nymphs, we predict that the effects of the group composition will depend on the cadmium treatment - or vice versa. For example, if social interactions enhance the diversification of the host microbiome, we predict that there would be a greater loss of microbial diversity due to cadmium ingestion in isolated compared to group-living nymphs.

## Material and methods

### Laboratory rearing and experimental social environment

This study involved the offspring of 30 females collected in a peach orchard near Valence, France (Lat 44.9772790, Long 4.9286990). All these individuals belonged to *Forficula auricularia* Linneaus, 1758, also called *Forficula auricularia* clade A (González-Miguéns et al., 2020). Just after collection, these females were reared under standard laboratory conditions until oviposition and egg hatching (Meunier et al., 2012). In brief, we maintained the group of females with males for four months to allow uncontrolled mating (Sandrin et al., 2015) and then isolated each female in individual containers to allow egg production. At isolation,

about 50 days before oviposition, each female received 50 mg of a standard laboratory-made diet (*i.e.,* mostly composed of pollen, cat food, carrots, agar (see details in Kramer et al., (2015) without preservative acids – not useful in this study), covered by one of three concentrations of cadmium (see details below). The food (including cadmium) was changed twice a week until oviposition. At that time, we removed the food as mothers typically stop their foraging activity to care for their eggs (Kölliker, 2007).

One day after egg hatching, we randomly assigned 30 nymphs per family (mean clutch size per family ± SD = 51.6 ± 12.7 nymphs) to three experimental groups: family group (10 nymphs), sibling group (10 nymphs), and isolated nymphs (10 nymphs). The family group consisted of 10 nymphs reared with their mother, the sibling group consisted of 10 nymphs reared together without their mother, and the isolated group consisted of single nymphs reared in isolation. We then provided each experimental group with 50 mg of the standard laboratory-made diet described above, mixed with one of the three concentrations of cadmium. Each mother and her nymphs received the same cadmium treatment throughout the experiment. We maintained these experimental groups in Petri dishes lined with moistened sand at 20-18°C under a 12:12h light:dark cycle. To standardise the space available per individual, we maintained the family and sibling groups in large Petri dishes (diameter 9 cm, height 2 cm), and the isolated nymphs in small Petri dishes (diameter 3.6 cm, height 1 cm). Fourteen days later, which marks the end of family life under natural conditions (Meunier et al., 2012), one nymph per experimental group and cadmium concentration was transferred to an Eppendorf tube and stored at -80°C until the extraction of the genomic material. Hence, a total of 30 nymphs were obtained for each concentration of cadmium, comprising 10 that were raised in a family group, 10 that were raised with siblings only, and 10 that were raised in isolation. This experiment ended at this point.

**Cadmium exposure**

We exposed the 30 mothers and their nymphs to cadmium using the protocol and concentrations presented in Honorio, Moreau, et al., (2023). In particular, we applied 10 µL of each cadmium solution directly to the 50 mg of food provided to tested individuals. We chose cadmium exposure via ingestion because this is the main source of cadmium exposure in nature (H. Zhang & Reynolds, 2019). We used cadmium concentrations of either

161 0, 25, or 100 mg.L-1 (corresponding to 0, 5, 20 mg.kg$^{-1}$ of food, respectively), which were
162 obtained by diluting cadmium chloride powder (Sigma-Aldrich #202908, CAS number:
163 10108-64-2, purity: 99.99%) in MilliQ water. We chose these concentrations to cover both
164 those found in the food chain and soil (Butt et al., 2018; FitzGerald & Roth, 2015; H. Zhang &
165 Reynolds, 2019) and those known to induce behavioural and microbial changes after
166 ingestion in other species (Dési et al., 1998; Rothman et al., 2019; Su et al., 2021). A previous
167 study confirmed that this method of exposure ensures the uptake of cadmium by earwig
168 females (Honorio et al, *submitted*; (Honorio, Moreau, et al., 2023)).
169

170 **Genomic extraction and 16S amplification**

171 The 90 nymphs were individually isolated in 2mL Eppendorf tube and immediately stored at
172 -80°C until the extraction of both external and internal DNA material (i.e., whole individual
173 body) using the NucleoMag® Tissue extraction kit (Macherey-Nagel™, Düren, Germany). We
174 then amplified the hypervariable region V3-V4 of the 16S rRNA gene using the primers 343F
175 (5′ - ACGGRAGGCAGCAG - 3′) and 784R (5′- TACCAGGGTATCTAATC – 3′) (Muyzer et al., 1993)
176 coupled with platform-specific Illumina linkers. PCR reactions were processed using the Taq
177 Polymerase Phusion® High-Fidelity PCR Master Mix with GC buffer following the
178 manufacturer's instructions (Qiagen, Hilder, Germany). Each PCR reaction involved an initial
179 step of 30 sec denaturation at 98°C, followed by 22 amplification cycles (10 sec denaturation
180 at 98°C; 30 sec annealing at 61.5°C; 30 sec extension at 72°C), and ended with a final step of
181 10 min extension at 72°C. Each reaction was checked for the probe specificity and
182 amplification success with an electrophoresis migration. Extraction and amplification steps
183 involved several blank controls to confirm that samples were not contaminated by
184 environmental microorganisms. As blanks were all negative, we did not send them for
185 sequencing (Figure S1). Samples were amplified in duplicate and equally pooled for a final
186 product of 30 µL which was further sequenced using 2x250bp Illumina MiSeq technology at
187 the Bio-Environnement platform (University of Perpignan, France). Finally, two of the 90
188 nymphs used for genomic extraction were removed from the study as one did not amplify
189 (isolated nymphs) and one did not respect the experimental condition (the sibling group was
190 only constituted by two nymphs instead of 10). Both samples were exposed to 100 mg.L$^{-1}$ Cd.

191

192 **Bioinformatic process**

193 We used the DADA2 algorithm v1.24.0 (Callahan et al., 2016) in R software v4.2.0 (R Core
194 Team, 2024) to process sequences. Libraries were first trimmed for primers and reads were
195 truncated at 240 base pairs (both forward and reverse), filtered with their quality profiles,
196 cleaned, dereplicated and inferred into Amplicon Sequence Variants (ASVs) (Glassman &
197 Martiny, 2018). The resulting chimeras were removed and taxonomic assignment using the
198 SILVA reference database (release 138) (Quast et al., 2012). Sequences were aligned using
199 the MAFFT program (Katoh, 2002) and entered into a phyloseq object using the *phyloseq*
200 package v1.40.0 (McMurdie & Holmes, 2013). We inferred the phylogenetic tree associated
201 with these sequences using the FastTree 2 tool (Price et al., 2010) and the *Phangorn* package
202 v2.8.1 (Schliep, 2011). We removed 2 211 sequences that belonged to 65 ASVs that were
203 assigned as mitochondrial sequences or not assigned at least at the Phylum level. We also
204 removed ASVs carried by only a few individuals to minimize noise from statistically
205 exaggerated inter-individual variability. This removal (which represents 1.5% of the
206 sequences in the initial dataset) followed a standard method that preserves rare sequences
207 while filtering out statistically random sequences (i.e., unique sequences present in only few
208 individuals) without relying on subjective or arbitrary occurrence and abundance (Magurran
209 & Henderson, 2003). Specifically, we calculated an index of dispersion (*i.e.,* the variance to
210 mean ratio, VMR) for each ASV. We then tested whether these indices followed a Poisson
211 distribution, checking if they fall within the 2.5 and 97.5% confidence limits of the $Chi^2$
212 distribution (Krebs, 1999) and we preserved the ASVs with index values outside these
213 confidence limits (Figure S2).

214

215 **Microbial diversity and indicators**

216 We tested the effects of social environment and cadmium ingestion on the alpha- and beta-
217 diversity of the resulting microbiome of each host nymph. To calculate alpha-diversity, we
218 first rarefied all samples to 11 637 sequences corresponding to the threshold at which
219 rarefaction curves reach their stationary phases (Cameron et al., 2021; Figure S3). The
220 rarefaction involved the removal of one sample of family nymphs (100 $mg.L^{-1}$ of Cd), which

contained fewer reads than the threshold. We then calculated the observed richness, the Shannon entropy, and their respective equivalents in terms of phylogenetic diversity using the Faith and Allen indices. For beta-diversity, we calculated the Jaccard distance between samples with ASVs presence/absence and its quantitative equivalent Bray-Curtis. As for alpha-diversity, we also used their phylogenetic equivalents with the Unifrac metrics for qualitative (unweighted) and quantitative (weighted) distances (Chao et al., 2010; Yang et al., 2021). The dissimilarities between samples were projected into a Principal Coordinates Analysis (PCoA), which we plotted on the first two axes. The significant microbial genera correlated along these axes were projected through the PCoA using the function *envfit()* from the package *vegan* v2.6-4 (Oksanen et al., 2022). Alpha- and beta-diversity were also calculated at genus level by merging all ASVs in their belonging genus using the function *tax_glom()* from the *phyloseq* package (McMurdie & Holmes, 2013).

**Statistics**

All statistical analyses were performed using R v4.3.4 (R Core Team, 2024). First, we analysed the alpha-diversity of nymph microbiome using four linear mixed models (LMER) with the *lme4* v1.1-31 (Bates et al., 2015) and analyses of variances (Anova) were run using *car* version packages v3.1-1 (Fox & Weisberg, 2019). In these models, we entered either the observed richness, Shannon entropy, Faith or Allen indices as a response variable, and social environment (isolated, sibling or family nymphs) and cadmium concentration (discrete; 0, 25 or 100 $mg.L^{-1}$) as explanatory variables. We also included the interaction between these two factors to test whether cadmium ingestion affects the social transmission, or wether being in a specific social context modulates the effect of the cadmium ingestion on the microbiome. To control for the same origin of each social environment, we also entered the mother identity as a random effect in each model. When necessary, we conducted post-hoc pairwise comparisons using the estimated marginal means via the *emmeans* package v1.8.0 (Lenth, 2022). The $R^2$ corresponding to each pairwise comparison was calculated with the *MuMIn* package v1.47.1 (Bartoń, 2022). Second, we analysed the beta-diversity of nymph microbiome using four permutational analyses of variances (PERMANOVAs). In these models, we entered either Jaccard, Bray-Curtis, unweighted and weighted Unifrac metrics as response variables, and social environment, cadmium concentration and their

interaction as explanatory variables. We specified the origin of the mother as strata to control for the effects of this variable. We conducted post-hoc pairwise comparisons using the *pairwiseAdonis* package v0.4.1 (Martinez Arbizu, 2017). Overall, we ran these eight models on diversity indices calculated on both ASVs and Genus levels. All models were simplified after AICc comparisons by removing the non-significant interactions. Finally, to identify bacterial indicators of both the social environment and the cadmium concentration, we used the *DESeq2* package v1.36-0 (Love et al., 2014). Each raw counts were clustered into bacterial genera, normalized by its geometric mean, and then compared to a negative binomial distribution. The estimated coefficient (Log2FoldChange) for each genus in each sample group was transformed into a Z-statistic, compared to a normal distribution, and then corrected for multiple comparisons.

We checked all model assumptions using the *DHARMa* package (Hartig, 2022) and the homoscedasticity of the variances between groups of each variable with the function *betadisper* from the *vegan* package v2.6-4 (Oksanen et al., 2022). To fulfil model assumptions, we log-transformed alpha proxies (*i.e.,* Observed richness, Shannon, Faith and Allen indices) as response variable. All p-values were adjusted for multiple testing with False Discovery Rate method (FDR) and Benjamini-Hochberg correction (Haynes, 2013).

## Results

### Overall microbial composition of the nymphs

Overall, the microbiome of the tested nymphs included 2 075 719 sequences (98.52% abundance of the initial dataset) representing 590 different ASVs (31.45% richness of the initial dataset; Figure S2). While Proteobacteria largely dominated the microbiome (78.7%), the Bacteroidota represented 15.2%, and the Firmicutes 5.43%. Finally, the microbiome also contained other bacterial phyla at a low level, such as Actinobacteriota, Bdellovibrionota and Patescibacteria (all < 1 %). The dominant genera (Figure 1) were *Buttiauxella* (Proteobacterales, Enterobacteriacae) (32.1 % of all reads), *Chryseobacterium* (Bacteroidota, Weeksellaceae) (11.5%), *Acinetobacter* (Proteobacteria, Moraxellaceae) (6.33%), *Serratia* (Proteobacteria, Yersiniaceae) (6.31%) and *Pseudomonas* (Proteobacteria, Pseudomonadaceae) (5.34%).

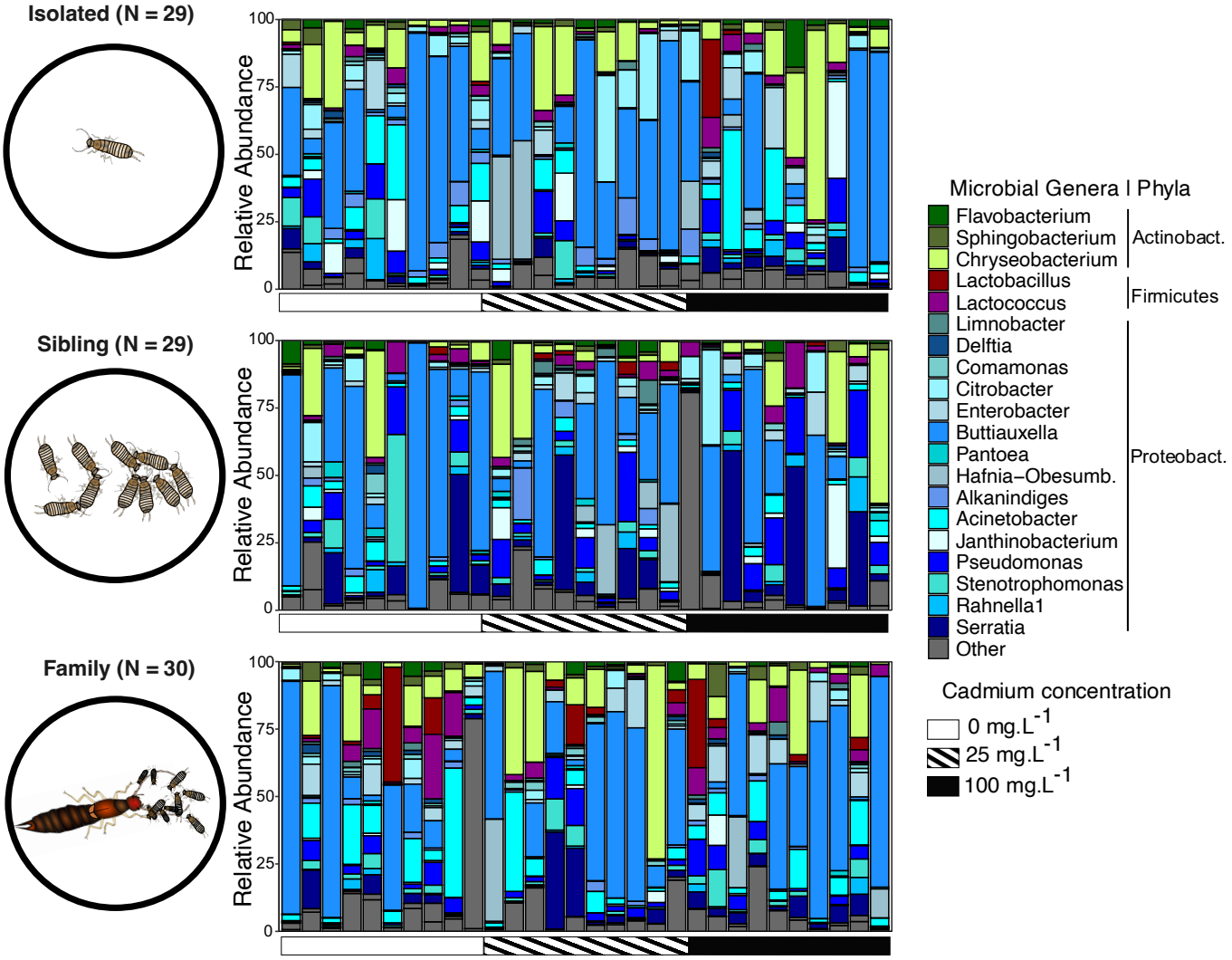


**Figure 1: Microbiome composition of nymphs from isolated, sibling and family treatments.** Barplots represent the individual composition for the top 20 microbial genera. Taxa belonging to Proteobacteria are in blue, Firmicutes in red/purple, and Bacteroidota in green. The gray contrasted horizontal bar represent the cadmium concentration for exposed nymphs. N indicates the number of nymphs used per social environment.

**Effects of social environment and cadmium on microbial diversity**

Social environment and cadmium ingestion had independent effects on a some indices of beta diversity, but no effect on alpha diversity. First, the social environment affected the unweighted Unifrac dissimilarities at both ASV level ($R^2$ = 0.063, F = 2.90, $P_{adj}$ = 0.016) and Genus level ($R^2$ = 0.070, F = 3.28, $P_{adj}$ = 0.016). This effect showed that nymphs exhibited microbiome structures that were specific of their social environment (Table 1, Figure 2B): isolated vs family treatments (ASV $R^2$ = 0.066, F = 3.10, $P_{adj}$ = 0.001; Genus $R^2$ = 0.075, F = 4.61, $P_{adj}$ = 0.001), isolated vs sibling treatments (ASV $R^2$ = 0.039, F = 2.25, $P_{adj}$ = 0.018; Genus $R^2$ = 0.047, F = 2.73, $P_{adj}$ = 0.022), and family vs sibling treatments (ASV $R^2$ = 0.038, F = 2.25, $P_{adj}$ = 0.014; Genus $R^2$ = 0.038, F = 2.23, $P_{adj}$ = 0.029). These results were consistent with the first two axes of the PCoA associated with the unweighted Unifrac distances where microbial structures of isolated nymphs were seemingly projected in the opposite direction of other samples along the first axis (Figure 2B). Microbial taxa such as bacteria belonging to the Phylum Bdellovibrionota and bacterial genera *Niabella* (Bacteroidota, Chitinophagaceae), *Rurimicrobium* (Bacteroidota, Chitinophagaceae), *Pedobacter* (Bacteroidota, Sphingobacteriaceae) or *Alkanindiges* (Proteobacteria, Moraxellaceae) were significantly correlated along the PCoA axes ($P < 0.01$) and tended to project in the direction of isolated nymph's microbiomes along the first PCoA axis (Figure 2B).

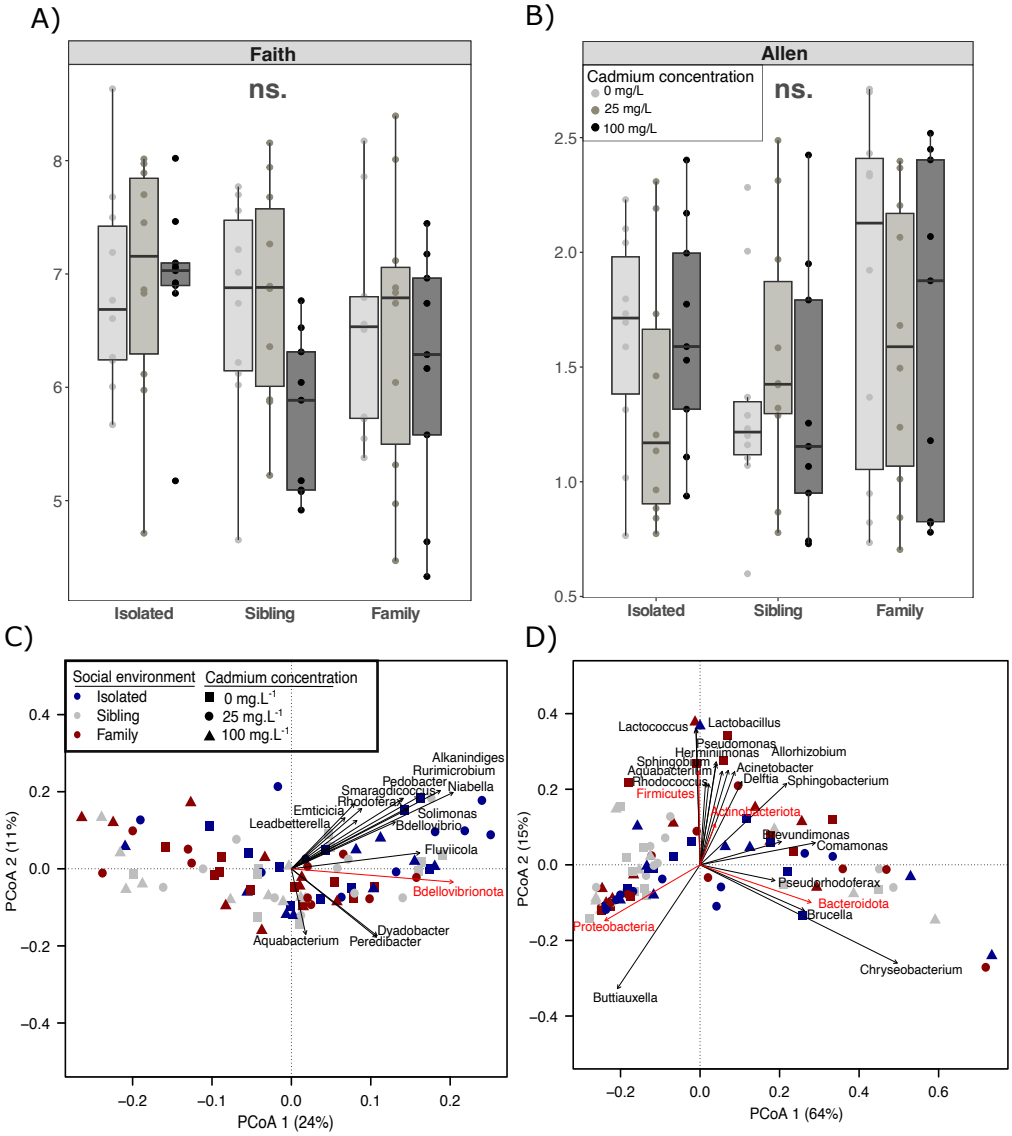


**Figure 2: Alpha and beta-diversity of the nymph microbiomes.** Differences in alpha-diversity where **(A)** the Faith (phylogenetic diversity) and **(B)** the Allen indices were tested according to the social environment and the cadmium concentration. Boxplots show the median (middle bar) and the interquartile range (box) with whiskers representing the 1.5-fold the interquartile range. Non-significant results ($P_{adj} > 0.05$) are labelled by 'ns'. Beta-diversity is based on the (**C**) unweighted and (**D**) weighted Unifrac distances of the ASVs composition between samples. Dissimilarities are visualized by the first two axes of a Principal Coordinates Analyses (PCoA), where each nymph is coloured according to their social environment and shaped by the cadmium concentration. The microbial genera and phyla that are significantly correlated ($P < 0.01$) are projected in black and red, respectively.

319 Second, cadmium ingestion marginally affected unweighted Unifrac dissimilarities at ASV
320 level ($R^2$ = 0.041, F = 1.88, $P_{adj}$ = 0.053). Nymphs exposed to 100 mg.$L^{-1}$ cadmium had a
321 different microbial structure than nymphs exposed to 0 mg.$L^{-1}$ ($R^2$ = 0.035, F = 2.04, $P_{adj}$ =
322 0.023) and 25 mg.$L^{-1}$ ($R^2$ = 0.038, F = 2.21, $P_{adj}$ = 0.018), whereas there was no difference
323 between 0 mg.$L^{-1}$ and 25 mg.$L^{-1}$ ($R^2$ = 0.022, F = 1.29, $P_{adj}$ = 0.188). Note, however, that these
324 last results must be taken with caution as dispersion (i.e., homogeneity) of microbiomes
325 regarding the unweighted Unifrac distances differed between the three cadmium
326 concentrations with highest distance from the centroid in the concentration 25 mg.$L^{-1}$ ($P_{adj}$ =
327 0.026). Finally, we did not detect any effect of the interaction between social environment
328 and cadmium ingestion, either for alpha diversity (all $P_{adj} \geq 0.123$) or beta diversity (all $P_{adj} \geq$
329 0.392; Table 1).
330
331 **Table 1: Effects of social environment, cadmium ingestion, and their interaction on the**
332 **alpha-diversity and beta-diversity of nymph microbiomes at both ASV and Genus levels.**
333 Statistical values from linear models with random effects (LMERs) or PERMANOVAs
334 conducted on each index. P-values were corrected for multiple testing according to False
335 Discovery Rate (FDR) correction. The models were simplified by removing the interaction as
336 it was not significant for both alpha (all $P_{adj} \geq 0.136$) and beta diversity (all $P_{adj} \geq 0.169$).
337 Significant results ($P_{adj} \leq 0.05$) are indicated in bold.

| **Index** | **Social environment** | | | **Cadmium ingestion** | | |
|---|---|---|---|---|---|---|
| **Alpha-diversity (LMERs)** | | | | | | |
| | **LR $X^2_2$** | **P** | **$P_{adj}$** | **LR $X^2_2$** | **P** | **$P_{adj}$** |
| **- ASV level** | | | | | | |
| Observed richness | 0.587 | 0.746 | 0.892 | 1.231 | 0.540 | 0.720 |
| Shannon | 0.141 | 0.932 | 0.962 | 0.371 | 0.831 | 0.892 |
| Faith | 7.581 | 0.023 | 0.123 | 2.129 | 0.345 | 0.552 |
| Allen | 2.609 | 0.271 | 0.551 | 0.358 | 0.836 | 0.892 |
| **- Genus level** | | | | | | |
| Observed richness | 4.054 | 0.132 | 0.453 | 3.550 | 0.170 | 0.453 |
| Shannon | 0.370 | 0.831 | 0.892 | 0.075 | 0.963 | 0.963 |
| Faith | 9.268 | 0.010 | 0.080 | 2.243 | 0.326 | 0.551 |
| Allen | 2.956 | 0.228 | 0.551 | 0.653 | 0.721 | 0.887 |

| Beta-diversity (PERMANOVAs) | | | | | | |
|---|---|---|---|---|---|---|
| | $R^2$ | P | $P_{adj}$ | $R^2$ | P | $P_{adj}$ |
| **- ASV level** | | | | | | |
| Jaccard | 0.026 | 0.161 | 0.453 | 0.027 | 0.389 | 0.556 |
| Bray-Curtis | 0.029 | 0.156 | 0.453 | 0.031 | 0.363 | 0.553 |
| Unweighted unifrac | **0.063** | **0.001** | **0.016** | **0.041** | **0.005** | **0.053** |
| Weighted unifrac | 0.026 | 0.285 | 0.551 | 0.015 | 0.572 | 0.732 |
| **- Genus level** | | | | | | |
| Jaccard | 0.032 | 0.093 | 0.392 | 0.025 | 0.290 | 0.551 |
| Bray-Curtis | 0.035 | 0.098 | 0.392 | 0.027 | 0.304 | 0.551 |
| Unweighted unifrac | **0.070** | **0.001** | **0.016** | 0.040 | 0.019 | 0.122 |
| Weighted unifrac | 0.026 | 0.327 | 0.551 | 0.012 | 0.507 | 0.705 |

**Effect of social environment and cadmium on bacterial genera**

Out of a total of 84 bacterial genera that formed the nymph's microbiome, 18 and 7 were statistical indicators of the social environment and the cadmium concentration, respectively. Of these, 13 and 5 bacterial genera showed a |log2fold change| > 2 (*i.e.,* for which the abundance is at least 4 times different), respectively. Among these bacteria, *Janthinobacterium* (Proteobacteria, Oxalopacteraceae), *Kosakonia* (Proteobacteria, Enterobacteriaceae), *Pedobacter* (Bacteroidota, Sphingobacteriaceae), *Rurimicrobium* (Bacteroidota, Chitinophagaceae) were more abundant in isolated nymphs than in sibling or family nymphs (Figure 3; Table S1). In contrast, the bacterial genera *Duganella* (Proteobacteria, Oxalobacteraceae), *Cellvibrio* (Proteobacteria, Cellvibrionaceae) and *Serratia* (Proteobacteria, Yersiniaceae) dominated the sibling nymphs. *Rhodococcus* (Actinobacteria, Nocardiaceae), *Lactobacillus* (Firmicutes, Lactobacillaceae), *Paracoccus* (Proteobacteria, Rhodobacteraceae), *Tsukamurella* (Actinobacteria, Tsukamurellaceae), *Erwinia* (Proteobateria, Erwiniaceae) and *Sphingobium* (Proteobacteria, Sphingomonadaceae) were the most abundant bacteria in family nymphs (Figure 3; Table S1).

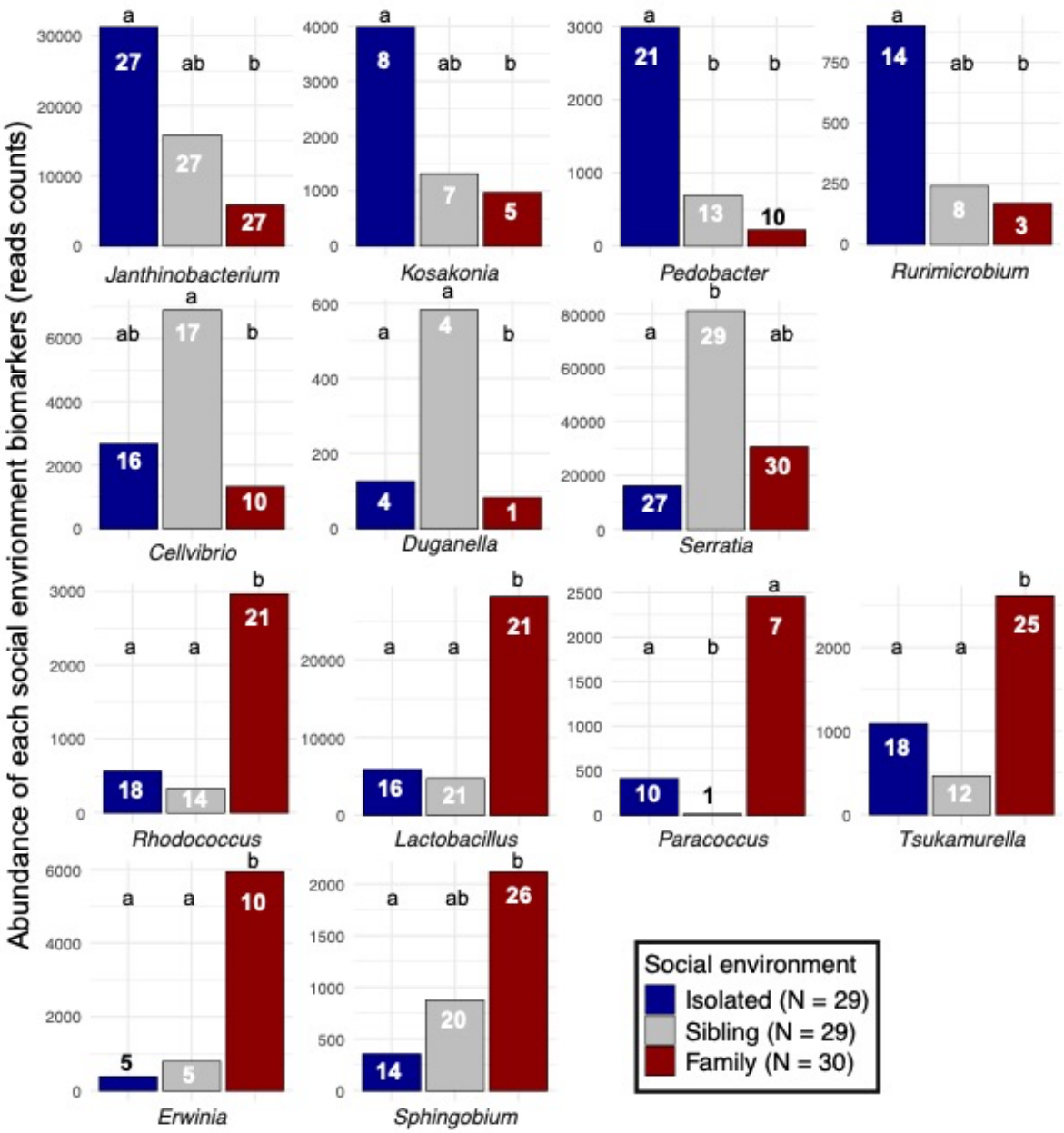


**Figure 3: Abundance of each bacterial genus indicators related to isolated, sibling or family nymphs.** Indicators are delineated using a DESeq2 approach ($P < 0.05$) for which the significant contrast showed a |log2fold change| > 2. The prevalence for each genus is labelled in the bar of the corresponding social environment. Different letters indicate $P < 0.05$.

Regarding cadmium ingestion, we detected a striking increase in *Duganella* (Proteobacteria, Oxalobacteraceae), *Hafnia-Obesumbacterium* (Proteobacteria, Hafniaceae), *Erwinia* (Proteobateria, Erwiniaceae), *Nubsella*, *Pedobacter* (Bacteroidota, Sphingobacteriaceae) and *Rurimicrobium* (Bacteroidota, Chitinophagaceae) in nymphs exposed to 25 mg.L$^{-1}$ of Cd

compared to nymphs exposed to 0 or 100 mg.L$^{-1}$ of Cd (Figure 4; Table S1). None of these bacteria were represented as discriminant of both parameters (*i.e.,* social environment and cadmium ingestion), suggesting that the effect of the social environment does not influence (or is not influenced) by the cadmium ingestion.

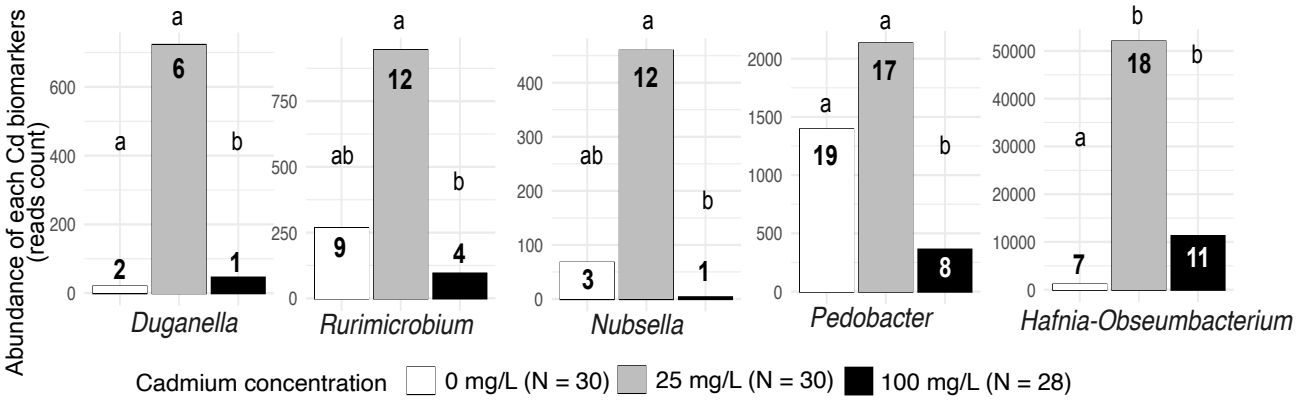


**Figure 4: Abundance of each bacterial genus indicators related to cadmium concentration.** Indicators are delineated using a DESeq2 approach ($P < 0.05$) for which the significant contrast showed a |log2fold change| > 2. The prevalence for each genus is labelled in the bar of the corresponding cadmium concentration. Different letters indicate $P < 0.05$.

## Discussion

Understanding the factors that drive changes in communities of mutualistic and commensal microorganisms in animal hosts is crucial for enhancing our knowledge of host fitness, population dynamics and ecosystem functioning (Martiny et al., 2023). This is particularly true in the context of anthropogenic environmental changes, which are increasingly known to both directly and indirectly shape host-symbiont relationships (Cheutin et al., 2021; Maraci et al., 2022; Teyssier et al., 2018). In this study, we tested the simultaneous effects of the social environment and exposure to a chemical pollutant on the diversity and nature of the microbial communities present in earwig juveniles. Our results first show that the social environment affected the phylogenetic diversity of the microbial communities (*i.e.,* unweighted Unifrac distances) at both the bacterial sequence (*i.e.,* ASV) and genus levels. Our results then show that cadmium ingestion influenced the phylogenetic qualitative composition of the microbiome at the ASV level. Notably, we found that changes in the microbiome induced by the social environment and cadmium ingestion were associated with the turnover of specific bacterial taxa. However, there was no effect of the social

environment and cadmium ingestion on the other indices of alpha-diversity and beta-diversity of the nymph microbiome. Finally, our results show that cadmium ingestion did not affect the social transmission of the microbiome to earwig nymphs.

Our first finding is that nymphs exhibited distinct microbial communities in terms of phylogenetic beta-diversity (unweighted Unifrac metric) depending on whether they lived alone, with siblings or with their family group (siblings plus mother). These results offer two key insights. First, they suggest that living in a group may not only enhance access to new bacterial sources, as reported in other social mammals and insects (Hosokawa et al., 2007; Raulo et al., 2021; Tung et al., 2015; Y. Wang & Rozen, 2017), but also help to prevent colonization by opportunistic environmental taxa. For instance, the phylum Bdellovibrionota (with the two genera *Peredibacter* and *Bacteriovorax*) was more abundant in the isolated treatment compared to both the sibling and whole family treatments. The phylum Bdellovibrionota is typically abundant in the soil and is occasionally present in trace amounts in the gut microbiome of certain insects (Davidov et al., 2006). The proliferation of Bdellovibrionota in isolated nymphs may therefore be due to the lack of access to group members, and the resulting lack of competition from other bacteria that would normally be acquired through contact with family members (Figueiredo & Kramer, 2020). However, further testing is needed to validate this hypothesis. The second key finding is that the composition of the group plays an important role in shaping the nymph's microbiome. Some bacterial taxa were more abundant in nymphs reared in a whole-family group than in nymphs reared with their siblings only. This suggests a maternal transmission of bacteria to nymphs during earwig family life. Interestingly, many of these maternally transmitted bacteria have known beneficial functions in other (social) insects in terms of digestion and immunity. For example, the Proteobacteria *Sphingobium* or *Rhodococcus* present a large variety of catabolic functions such as aromatic hydrocarbons metabolization or cellulose digestion in cockroaches, termites or beetles (Biswas & Paul, 2021; Delalibera et al., 2005; Wenzel et al., 2002). Similarly, the bacterium *Lactobacillus* can protect against the negative effects of pathogens, pesticides and cadmium, as well as help for the fermentation of carbohydrate plants and cereals in bees, honeybees or grasshoppers (Lazzeri et al., 2020; Li et al., 2021; Nowak et al., 2021; Vásquez et al., 2012). To date, the role of the gut microbiome in the fitness of the European earwig remains unclear (Cheutin, Leclerc, et al., 2024; Van

421 Meyel et al., 2021), and future experimental and transcriptomic studies are needed to
422 investigate the potential function of these bacteria in this species.

423 We found that the social environment not only affected the beta-diversity of the
424 nymph microbiome, but was also significantly associated with the presence or absence of
425 13 bacterial taxa (4 taxa were specific to the isolated treatment, 3 taxa to the sibling
426 treatment, and 7 taxa to the family treatment). In isolated nymphs, two of these four taxa
427 (*Kosakonia* and *Janthinobacterium)* are insect mutualists and typically enhance host
428 protection against pathogens. For instance, *Janthinobacterium* has antifungal functions in
429 many insects (Haack et al., 2016; Magoga et al., 2023; Rodrigues et al., 2006), while *Kosakonia*
430 can inhibit the growth of the entomopathogenic strains belonging to the bacteria *Serratia*
431 (Grimont et al., 1979; Muratoğlu et al., 2011) and render mosquitoes and tsetse flies hosts
432 refractory to parasite infections such as *Trypanosoma* and *Plasmodium* (Weiss et al., 2019).
433 The overabundance of these bacteria in isolated nymphs might be an adaptive strategy to
434 compensate for the loss of access to social immunity from siblings and mothers (Diehl et al.,
435 2015; Diehl & Meunier, 2018) and still show a high level of defence against pathogen
436 infection. This hypothesis would also be consistent with our results showing that *Serratia*
437 (an opportunistic entomopathogen) is less abundant in isolated than group-living nymphs
438 (see above).

439 In addition to the social environment, we found that exposure to a chemical pollutant
440 also plays an important role in the diversity of the nymph microbiome. First, we showed that
441 the unweighted Unifrac distances of the microbiome were more altered in nymphs exposed
442 to the highest (100mg.$L^{-1}$) compared to intermediate (25 mg.$L^{-1}$) or control (0 mg.$L^{-1}$) doses
443 of cadmium. This effect is in line with the typical effects of cadmium on microbial
444 communities reported in a taxonomically wide range of animals (Li et al., 2021; Rothman et
445 al., 2019; Trevors et al., 1986; S. Zhang et al., 2015; Y. Zhang et al., 2016), although it should
446 be treated with caution here due to the relatively high level of dispersal in our dataset.
447 Second, we found that cadmium ingestion at the intermediate concentration was
448 significantly associated with the presence or absence of five bacterial taxa. Of these, only
449 one (*Hafnia-Obesumbacterium*) has been reported in animal microbiomes (S. Zhang et al.,
450 2023). This bacterium is a pathogen in many species and its abundance increases under
451 stressful conditions (Janda & Abbott, 2006). For example, *Hafnia-Obesumbacterium* is

452 dominant in the gut microbiomes of tadpole *Rana chensinensis* and crayfish *Procambarus*
453 *clarkii*, respectively exposed to lead pollution (S. Zhang et al., 2023) and acidic conditions (Z.
454 Wang et al., 2024). As such, our data suggest that this genus is the only bacterium sensitive
455 to cadmium ingestion in juveniles of the European earwig. The other four bacterial taxa
456 specific to the intermediate dose of cadmium probably reflect their good development on
457 medium contaminated with low levels of cadmium and their passive environmental uptake
458 by earwig nymphs in contact with this food. This is because the growth of *Pedobacter* and
459 *Duganella* is known to be typically favoured in cadmium-enriched soils (Cheng et al., 2022;
460 Stout et al., 2010; H. Wang et al., 2023).
461 Overall, this study shows that the access to a social environment, the type of group
462 members and the exposure to a chemical pollutant can all influence the composition and
463 diversity of the microbiome in juveniles of the European earwig. Interestingly, we show that
464 these effects do not interact with each other, *i.e.* varying the access to a social environment
465 or changing the composition of that AssociaEthologie ymph microbiome, and vice versa. As
466 anthropogenic environmental changes continue to accelerate globally (Johnson & Munshi-
467 South, 2017; Seto et al., 2012), these results demonstrate that the ability to naturally switch
468 between solitary and social life (as found in the European earwig nymphs, Meunier, 2024)
469 does not necessarily confer an advantage in mitigating the negative effects of chemical
470 contaminants (at least) on the host microbiome. Future studies are now needed to better
471 understand the role of the earwig microbiome on its fitness and population dynamics
472 (Cheutin, Leclerc, et al., 2024; Van Meyel et al., 2021), and to determine whether the reported
473 effects of social environment and cadmium intake on its diversity can alter these
474 parameters.
475

476 **Data availability**

477 The data set and the scripts are available on Zenodo with the url
478 https://zenodo.org/doi/10.5281/zenodo.13380552. The reads for each sample were
479 deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession no.
480 PRJNA1152783.
481

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## Author's contribution

**Marie-Charlotte Cheutin**: Data curation; Formal analysis; Investigation; Methodology; Writing - original draft; and Writing - review & editing. – **Romain Honorio**: Conceptualization; Methodology; Writing - original draft; and Writing - review & editing. **Joël Meunier**: Conceptualization; Funding acquisition; Supervision; Writing - review & editing

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## Conflict of interest

The authors declare that this study had no conflicts of interest.

## Ethics approval statements

Our investigation complies with the current European Directive 2010/63/EU that does not acquire ethical approval on invertebrates. All animals were handled with care until necessary sacrifices.

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