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

Marie-Charlotte Cheutin[*#1], Romain Honorio[*#1,2], Joël Meunier[1]

[1] Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS, University of Tours, Tours, France
[2] Laboratoire d'Ethologie Expérimentale et Comparée UR 4443 (LEEC), Université Sorbonne Paris Nord, 93430 Villetaneuse, France

*Corresponding author
Correspondence: mccheutin@gmail.com
# These authors contributed equally to this work

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.

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

Genomic extraction and 16S amplification

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

Bioinformatic process

We used the DADA2 algorithm v1.24.0 (Callahan et al., 2016) in R software v4.2.0 (R Core Team, 2024) to process sequences. Libraries were first trimmed for primers and reads were truncated at 240 base pairs (both forward and reverse), filtered with their quality profiles, cleaned, dereplicated and inferred into Amplicon Sequence Variants (ASVs) (Glassman & Martiny, 2018). The resulting chimeras were removed and taxonomic assignment using the SILVA reference

database (release 138) (Quast et al., 2012). Sequences were aligned using the MAFFT program (Katoh, 2002) and entered into a phyloseq object using the *phyloseq* package v1.40.0 (McMurdie & Holmes, 2013). We inferred the phylogenetic tree associated with these sequences using the FastTree 2 tool (Price et al., 2010) and the *Phangorn* package v2.8.1 (Schliep, 2011). We removed 2 211 sequences that belonged to 65 ASVs that were assigned as mitochondrial sequences or not assigned at least at the Phylum level. We also removed ASVs carried by only a few individuals to minimize noise from statistically exaggerated inter-individual variability. This removal (which represents 1.5% of the sequences in the initial dataset) followed a standard method that preserves rare sequences while filtering out statistically random sequences (i.e., unique sequences present in only few individuals) without relying on subjective or arbitrary occurrence and abundance (Magurran & Henderson, 2003). Specifically, we calculated an index of dispersion (*i.e.,* the variance to mean ratio, VMR) for each ASV. We then tested whether these indices followed a Poisson distribution, checking if they fall within the 2.5 and 97.5% confidence limits of the Chi^2 distribution (Krebs, 1999) and we preserved the ASVs with index values outside these confidence limits (Figure S2).

Microbial diversity and indicators

We tested the effects of social environment and cadmium ingestion on the alpha- and beta-diversity of the resulting microbiome of each host nymph. To calculate alpha-diversity, we first rarefied all samples to 11 637 sequences corresponding to the threshold at which rarefaction curves reach their stationary phases (Cameron et al., 2021; Figure S3). The 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%).

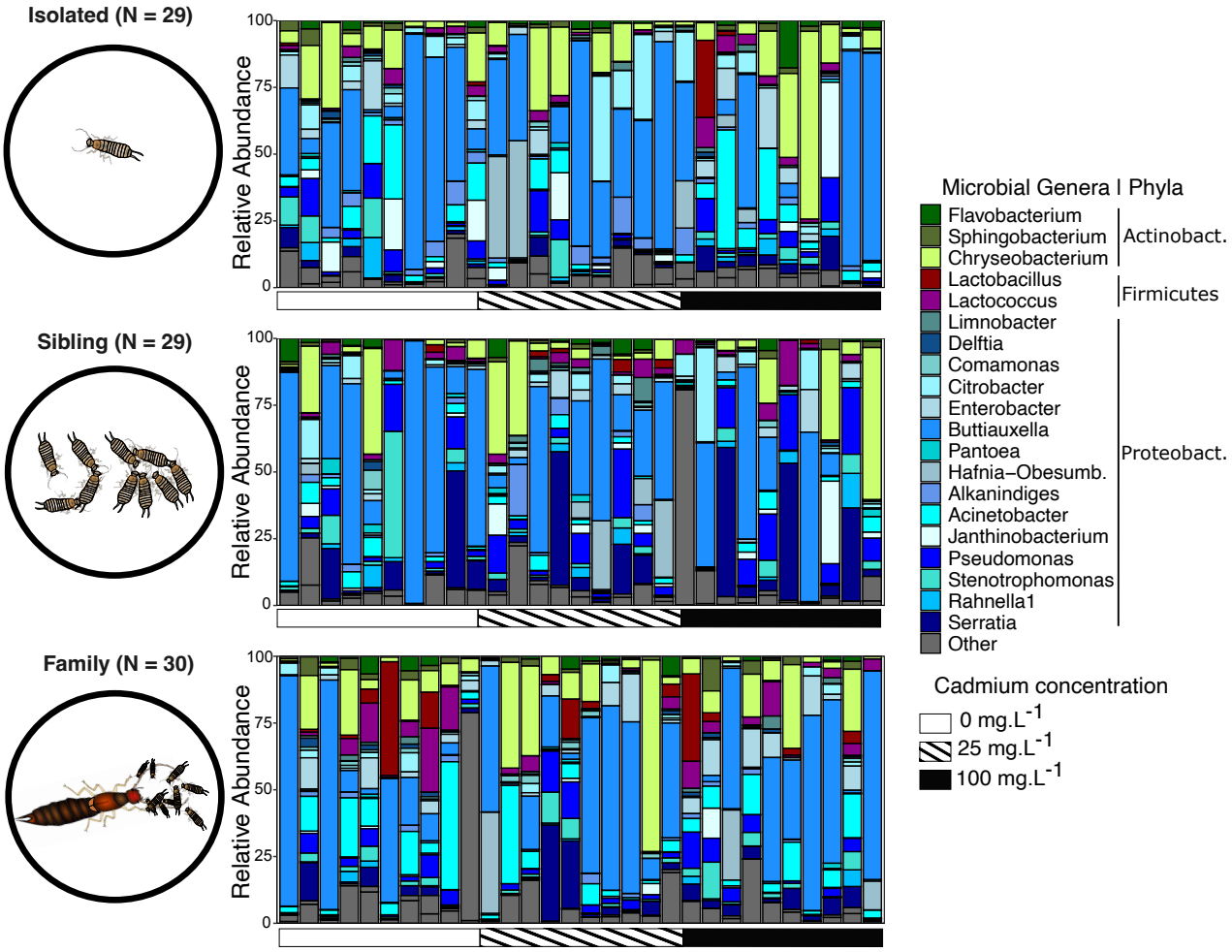
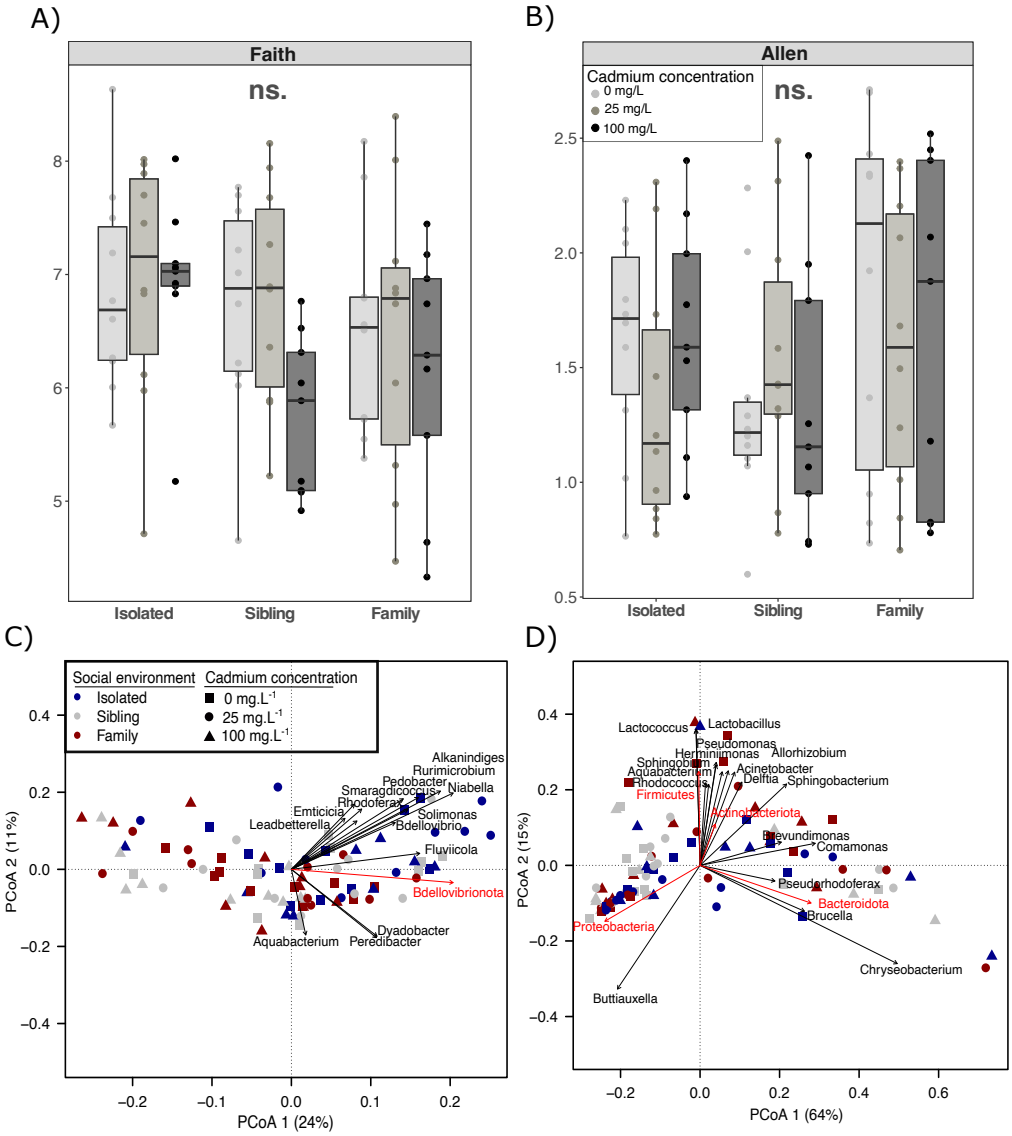


Figure 1 - Microbiome composition of nymphs from isolated, sibling and family treatments. Barplots represent the individual composition for the top 20 microbial genera. Taxa belonging to Proteobacteria are in blue, Firmicutes in red/purple, and Bacteroidota in green. The gray contrasted horizontal bar 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

289 significantly correlated along the PCoA axes (P < 0.01) and tended to project in the direction of
290 isolated nymph's microbiomes along the first PCoA axis (Figure 2B).



291

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

304

305 Second, cadmium ingestion marginally affected unweighted Unifrac dissimilarities at ASV level
306 (R^2 = 0.041, F = 1.88, P_{adj} = 0.053). Nymphs exposed to 100 mg.L^{-1} cadmium had a different
307 microbial structure than nymphs exposed to 0 mg.L^{-1} (R^2 = 0.035, F = 2.04, P_{adj} = 0.023) and 25

mg.L^{-1} (R^2 = 0.038, F = 2.21, P_{adj} = 0.018), whereas there was no difference 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 last results must be taken with caution as dispersion (i.e., homogeneity) of microbiomes regarding the unweighted Unifrac distances differed between the three cadmium concentrations with highest distance from the centroid in the concentration 25 mg.L^{-1} (P_{adj} = 0.026). Finally, we did not detect any effect of the interaction between social environment and cadmium ingestion, either for alpha diversity (all P_{adj} ≥ 0.123) or beta diversity (all P_{adj} ≥ 0.392; Table 1).

Table 1 - Effects of social environment, cadmium ingestion, and their interaction on the alpha-diversity and beta-diversity of nymph microbiomes at both ASV and Genus levels. Statistical values from linear models with random effects (LMERs) or PERMANOVAs conducted on each index. P-values were corrected for multiple testing according to False Discovery Rate (FDR) correction. The models were simplified by removing the interaction as it was not significant for both alpha (all P_{adj} ≥ 0.136) and beta diversity (all P_{adj} ≥ 0.169). Significant results (P_{adj} ≤ 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

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

326 Out of a total of 84 bacterial genera that formed the nymph's microbiome, 18 and 7 were
327 statistical indicators of the social environment and the cadmium concentration, respectively. Of
328 these, 13 and 5 bacterial genera showed a |log2fold change| > 2 (*i.e.,* for which the abundance is
329 at least 4 times different), respectively. Among these bacteria, *Janthinobacterium* (Proteobacteria,
330 Oxalopacteraceae), *Kosakonia* (Proteobacteria, Enterobacteriaceae), *Pedobacter* (Bacteroidota,
331 Sphingobacteriaceae), *Rurimicrobium* (Bacteroidota, Chitinophagaceae) were more abundant in
332 isolated nymphs than in sibling or family nymphs (Figure 3; Table S1). In contrast, the bacterial
333 genera *Duganella* (Proteobacteria, Oxalobacteraceae), *Cellvibrio* (Proteobacteria,
334 Cellvibrionaceae) and *Serratia* (Proteobacteria, Yersiniaceae) dominated the sibling nymphs.
335 *Rhodococcus* (Actinobacteria, Nocardiaceae), *Lactobacillus* (Firmicutes, Lactobacillaceae),
336 *Paracoccus* (Proteobacteria, Rhodobacteraceae), *Tsukamurella* (Actinobacteria,
337 Tsukamurellaceae), *Erwinia* (Proteobateria, Erwiniaceae) and *Sphingobium* (Proteobacteria,
338 Sphingomonadaceae) were the most abundant bacteria in family nymphs (Figure 3; Table S1).
339 Regarding cadmium ingestion, we detected a striking increase in *Duganella* (Proteobacteria,
340 Oxalobacteraceae), *Hafnia-Obesumbacterium* (Proteobacteria, Hafniaceae), *Erwinia*
341 (Proteobateria, Erwiniaceae), *Nubsella*, *Pedobacter* (Bacteroidota, Sphingobacteriaceae) and
342 *Rurimicrobium* (Bacteroidota, Chitinophagaceae) in nymphs exposed to 25 $mg.L^{-1}$ of Cd compared
343 to nymphs exposed to 0 or 100 $mg.L^{-1}$ of Cd (Figure 4; Table S1). None of these bacteria were
344 represented as discriminant of both parameters (*i.e.,* social environment and cadmium ingestion),
345 suggesting that the effect of the social environment does not influence (or is not influenced) by the
346 cadmium ingestion.

347

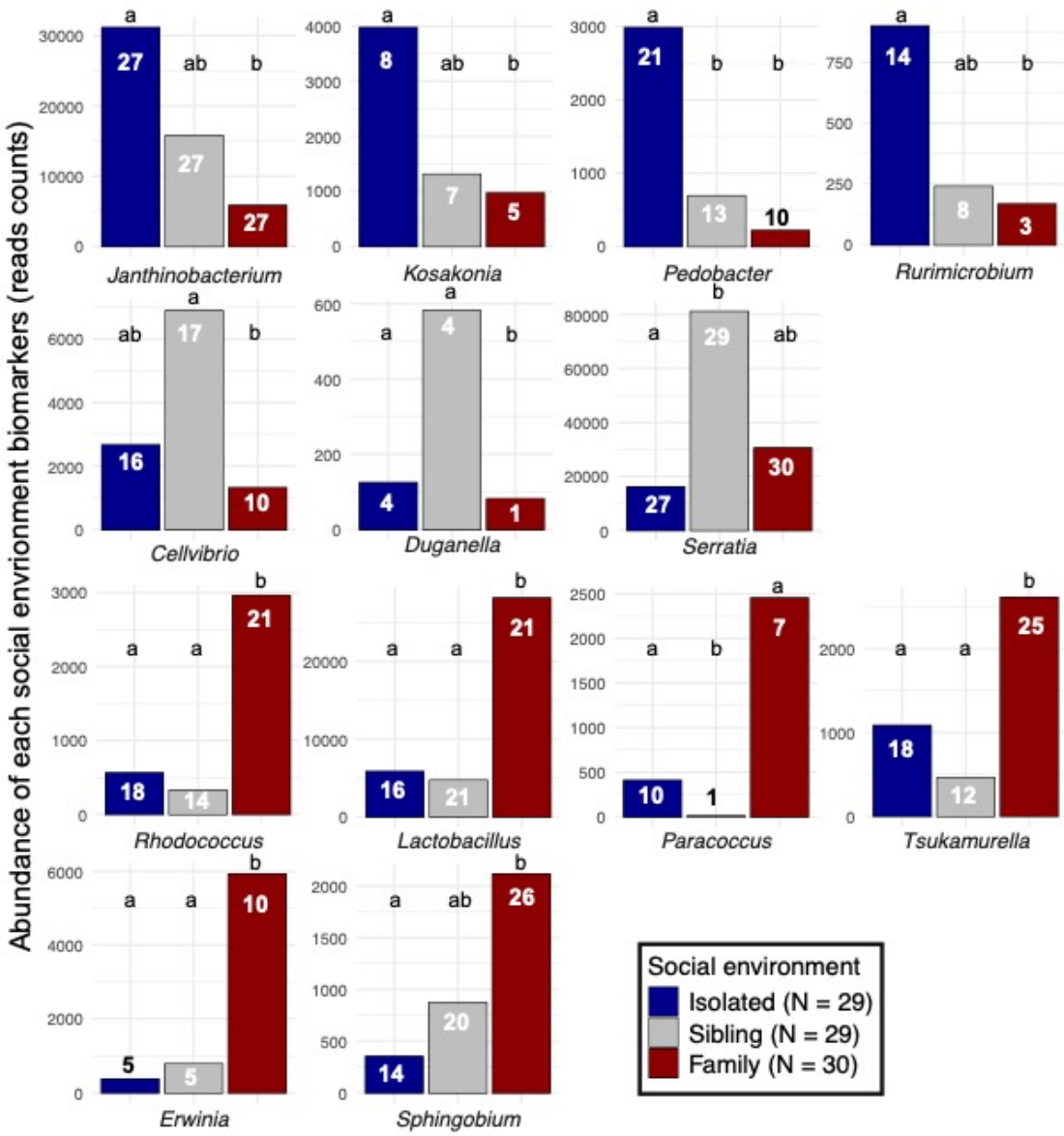


Figure 3 - Abundance of each bacterial genus indicators related to isolated, sibling or family nymphs. Indicators are delineated using a DESeq2 approach ($P < 0.05$) for which the significant contrast showed a |log2fold change| > 2. The prevalence for each genus is labelled in the bar of the corresponding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

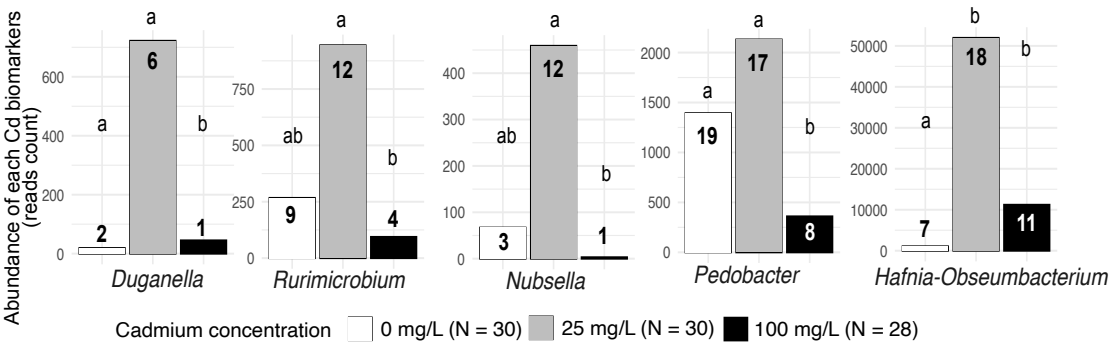


Figure 4 - Abundance of each bacterial genus indicators related to cadmium concentration. Indicators are delineated using a DESeq2 approach ($P < 0.05$) for which the significant contrast showed a |log2fold change| > 2. The prevalence for each genus is labelled in the bar of the corresponding cadmium 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 Meyel et al., 2021), and future experimental and transcriptomic studies are needed to investigate the potential function of these bacteria in this species.

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

In addition to the social environment, we found that exposure to a chemical pollutant also plays an important role in the diversity of the nymph microbiome. First, we showed that the unweighted Unifrac distances of the microbiome were more altered in nymphs exposed to the highest (100mg.L^{-1}) compared to intermediate (25 mg.L^{-1}) or control (0 mg.L^{-1}) doses of cadmium. This effect is in line with the typical effects of cadmium on microbial communities reported in a taxonomically wide range of animals (Li et al., 2021; Rothman et al., 2019; Trevors et al., 1986; S. Zhang et al., 2015; Y. Zhang et al., 2016), although it should be treated with caution here due to the relatively high level of dispersal in our dataset. Second, we found that cadmium ingestion at the intermediate concentration was significantly associated with the presence or absence of five bacterial taxa. Of these, only one (*Hafnia-Obesumbacterium*) has been reported in animal microbiomes (S. Zhang et al., 2023). This bacterium is a pathogen in many species and its abundance increases under stressful conditions (Janda & Abbott, 2006). For example, *Hafnia-Obesumbacterium* is dominant in the gut microbiomes of tadpole *Rana chensinensis* and crayfish *Procambarus clarkii*, respectively exposed to lead pollution (S. Zhang et al., 2023) and acidic conditions (Z. Wang et al., 2024). As such, our data suggest that this genus is the only bacterium sensitive to cadmium ingestion in juveniles of the European earwig. The other four bacterial taxa specific to the intermediate dose of cadmium probably reflect their good development on medium contaminated with low levels of cadmium and their passive environmental uptake by earwig nymphs in contact with this food. This is because the growth of *Pedobacter* and *Duganella* is known to be typically favoured in cadmium-enriched soils (Cheng et al., 2022; Stout et al., 2010; H. Wang et al., 2023).

Overall, this study shows that the access to a social environment, the type of group members and the exposure to a chemical pollutant can all influence the composition and diversity of the microbiome in juveniles of the European earwig. Interestingly, we show that these effects do not interact with each other, *i.e.* varying the access to a social environment or changing the composition of that AssociaEthologie ymph microbiome, and vice versa. As anthropogenic environmental changes continue to accelerate globally (Johnson & Munshi-South, 2017; Seto et al., 2012), these results demonstrate that the ability to naturally switch between solitary and social life (as found in the European earwig nymphs, Meunier, 2024) does not necessarily confer an advantage in mitigating the negative effects of chemical contaminants (at least) on the host microbiome. Future studies are now needed to better understand the role of the earwig microbiome on its fitness and

population dynamics (Cheutin, Leclerc, et al., 2024; Van Meyel et al., 2021), and to determine whether the reported effects of social environment and cadmium intake on its diversity can alter these parameters.

Data availability

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

Acknowledgements

We thank Charlotte Lécureuil for her comments on this manuscript. We also thank all members of the EARWIG group at IRBI for their help with animal rearing and setup installation, and the Bio-environment platform of the University of Perpignan (France) for providing the MiSeq Illumina. Finally, we thank Armand Guillermin and Stéphanie Drusch of the INRAE unité expérimentale Recherche Intégrée in Gotheron for giving us access to their orchards for earwig field sampling. The graphical abstract was made with BioRender application.

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

Funding information

This work was funded by the Région Centre-Val de Loire (Project DisruptCare; n°2021 00149474) and by a research grant from the Agence Nationale de la Recherche (ANR-20-CE02-0002).

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.

Reference

Arito, H., Sudo, A., & Suzuki, Y. (1981). Aggressive behavior of the rat induced by repeated administration of cadmium. Toxicology Letters, 7(6), 457–461. https://doi.org/10.1016/0378-4274(81)90093-X

Bartoń, K. (2022). MuMIn: Multi-Model Inference. https://CRAN.R-project.org/package=MuMIn

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67(1). https://doi.org/10.18637/jss.v067.i01

Bell, T., & Tylianakis, J. M. (2016). Microbes in the Anthropocene: Spillover of agriculturally selected bacteria and their impact on natural ecosystems. Proceedings of the Royal Society B: Biological Sciences, 283(1844), 20160896. https://doi.org/10.1098/rspb.2016.0896

Berlanga, M., & Guerrero, R. (2016). The holobiont concept: The case of xylophagous termites and cockroaches. Symbiosis, 68(1–3), 49–60. https://doi.org/10.1007/s13199-016-0388-9

Biswas, S., & Paul, D. (2021). Isolation and characterization of Rhodococcus qingshengii, a cellulolytic bacteria from Cnaphalocrocis medinalis (Lepidoptera: Crambidae) gut. Environmental and Experimental Biology, 19(4). https://doi.org/10.22364/eeb.19.19

Bright, M., & Bulgheresi, S. (2010). A complex journey: Transmission of microbial symbionts. Nature Reviews Microbiology, 8(3), 218–230. https://doi.org/10.1038/nrmicro2262

Butt, A., Qurat-ul-Ain, Rehman, K., Khan, M. X., & Hesselberg, T. (2018). Bioaccumulation of cadmium, lead, and zinc in agriculture-based insect food chains. Environmental Monitoring and Assessment, 190(12), 698. https://doi.org/10.1007/s10661-018-7051-2

Calderón-Cortés, N., Quesada, M., Watanabe, H., Cano-Camacho, H., & Oyama, K. (2012). Endogenous plant cell wall digestion: A key mechanism in insect evolution. Annual Review of Ecology, Evolution, and Systematics, 43(1), 45–71. https://doi.org/10.1146/annurev-ecolsys-110411-160312

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

Cameron, E. S., Schmidt, P. J., Tremblay, B. J.-M., Emelko, M. B., & Müller, K. M. (2021). Enhancing diversity analysis by repeatedly rarefying next generation sequencing data describing microbial communities. Scientific Reports, 11(1), 22302. https://doi.org/10.1038/s41598-021-01636-1

Chao, A., Chiu, C.-H., & Jost, L. (2010). Phylogenetic diversity measures based on Hill numbers. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1558), 3599–3609. https://doi.org/10.1098/rstb.2010.0272

Cheng, X., Cao, X., Tan, C., Liu, L., Bai, J., Liang, Y., & Cai, R. (2022). Effects of four endophytic bacteria on cadmium speciation and remediation efficiency of Sedum plumbizincicola in farmland soil. Environmental Science and Pollution Research, 29(59), 89557–89569. https://doi.org/10.1007/s11356-022-21711-y

Cheutin, M.-C., Boucicot, M., & Meunier, J. (2024). Microbiome turnover during offspring development varies with maternal care, but not moult, in a hemimetabolous insect. Peer Community Journal, 4, e67. https://doi.org/10.24072/pcjournal.446

Cheutin, M.-C., Leclerc, B., & Meunier, J. (2024). With or without you: Gut microbiota does not predict aggregation behavior in European earwig females. Behavioral Ecology, 35(3), arae022. https://doi.org/10.1093/beheco/arae022

Cheutin, M.-C., Villéger, S., Hicks, C. C., Robinson, J. P. W., Graham, N. A. J., Marconnet, C., Restrepo, C. X. O., Bettarel, Y., Bouvier, T., & Auguet, J.-C. (2021). Microbial shift in the enteric

bacteriome of coral reef fish following climate-driven regime shifts. Microorganisms, 9(8), 1711. https://doi.org/10.3390/microorganisms9081711

Davidov, Y., Friedjung, A., & Jurkevitch, E. (2006). Structure analysis of a soil community of predatory bacteria using culture-dependent and culture-independent methods reveals a hitherto undetected diversity of Bdellovibrio -and-like organisms. Environmental Microbiology, 8(9), 1667–1673. https://doi.org/10.1111/j.1462-2920.2006.01052.x

Delalibera, I., Handelsman, J., & Raffa, K. F. (2005). Contrasts in cellulolytic activities of gut microorganisms between the wood borer, Saperda vestita (Coleoptera: Cerambycidae), and the bark beetles, Ips pini and Dendroctonus frontalis (Coleoptera: Curculionidae). Environmental Entomology, 34(3), 541–547. https://doi.org/10.1603/0046-225X-34.3.541

Dési, I., Nagymajtényi, L., & Schulz, H. (1998). Behavioural and neurotoxicological changes caused by cadmium treatment of rats during development. Journal of Applied Toxicology, 18(1), 63–70. https://doi.org/10.1002/(SICI)1099-1263(199801/02)18:1<63::AID-JAT475>3.0.CO;2-Z

Diehl, J. M. C., Körner, M., Pietsch, M., & Meunier, J. (2015). Feces production as a form of social immunity in an insect with facultative maternal care. BMC Evolutionary Biology, 15(1), 40. https://doi.org/10.1186/s12862-015-0330-4

Diehl, J. M. C., & Meunier, J. (2018). Surrounding pathogens shape maternal egg care but not egg production in the European earwig. Behavioral Ecology, 29(1), 128–136. https://doi.org/10.1093/beheco/arx140

Falk, J., Wong, J. W. Y., Kölliker, M., & Meunier, J. (2014). Sibling cooperation in earwig families provides insights into the early evolution of social life. The American Naturalist, 183(4), 547–557. https://doi.org/10.1086/675364

Figueiredo, A. R. T., & Kramer, J. (2020). Cooperation and conflict within the microbiota and their effects on animal hosts. Frontiers in Ecology and Evolution, 8, 132. https://doi.org/10.3389/fevo.2020.00132

FitzGerald, R. E., & Roth, N. (2015). Cadmium in mineral fertilizers – human and environmental risk update. https://doi.org/10.13140/RG.2.2.26451.17442

Fox, J., & Weisberg, S. (2019). An R companion to applied regression (Third). Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

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

Glassman, S. I., & Martiny, J. B. H. (2018). Broadscale ecological patterns are robust to use of Exact Sequence Variants versus Operational Taxonomic Units. mSphere, 3(4), e00148-18. https://doi.org/10.1128/mSphere.00148-18

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

Greer, J. A., Swei, A., Vredenburg, V. T., & Zink, A. G. (2020). Parental care alters the egg microbiome of maritime earwigs. Microbial Ecology, 80(4), 920–934. https://doi.org/10.1007/s00248-020-01558-x

Grimont, P. A. D., Grimont, F., & Lysenko, O. (1979). Species and biotype identification of Serratia strains associated with insects. Current Microbiology, 2(3), 139–142. https://doi.org/10.1007/BF02605870

Gurevich, Y., Lewin-Epstein, O., & Hadany, L. (2020). The evolution of paternal care: A role for microbes? Philosophical Transactions of the Royal Society B: Biological Sciences, 375(1808), 20190599. https://doi.org/10.1098/rstb.2019.0599

Gustin, K., Tofail, F., Vahter, M., & Kippler, M. (2018). Cadmium exposure and cognitive abilities and behavior at 10 years of age: A prospective cohort study. Environment International, 113, 259–268. https://doi.org/10.1016/j.envint.2018.02.020

Haack, F. S., Poehlein, A., Kröger, C., Voigt, C. A., Piepenbring, M., Bode, H. B., Daniel, R., Schäfer, W., & Streit, W. R. (2016). Molecular keys to the Janthinobacterium and Duganella

spp. Interaction with the plant pathogen Fusarium graminearum. Frontiers in Microbiology, 7. https://doi.org/10.3389/fmicb.2016.01668

Hartig, F. (2022). DHARMa: Residual diagnostics for hierarchical (multi-Level / mixed) regression models. R Package Version 0.4.6. https://CRAN.R-project.org/package=DHARMa

Haynes, W. (2013). Benjamini–Hochberg Method. In W. Dubitzky, O. Wolkenhauer, K.-H. Cho, & H. Yokota (Eds.), Encyclopedia of Systems Biology (pp. 78–78). Springer New York. https://doi.org/10.1007/978-1-4419-9863-7_1215

Honorio, R., Cheutin, M. –C., Pasquier, L., De Wever, S., Perdereau, E., Villalta, I., Lécureuil, C., & Meunier, J. (2024). The European earwig: A model species for studying the (early) evolution of social life. Insectes Sociaux. https://doi.org/10.1007/s00040-024-00985-0

Honorio, R., Depierrefixe, P., Devers, S., Rouelle, M., Meunier, J., & Lécureuil, C. (2023). Effects of cadmium ingestion on reproduction and maternal egg care in the European earwig. Animal Behaviour, 195, 1–8. https://doi.org/10.1016/j.anbehav.2022.10.008

Honorio, R., Jacquier, L., Doums, C., & Molet, M. (2021). Disentangling the roles of social and individual effects on cadmium tolerance in the ant Temnothorax nylanderi. Biological Journal of the Linnean Society, 134(4), 823–834. https://doi.org/10.1093/biolinnean/blab116

Honorio, R., Moreau, S., Lécureuil, C., & Meunier, J. (2023). Cadmium does not affect post-hatching maternal care or early offspring development in earwigs. Behavioral Ecology, 34(3), 399–407. https://doi.org/10.1093/beheco/arad011

Horak, R. D., Leonard, S. P., & Moran, N. A. (2020). Symbionts shape host innate immunity in honeybees. Proceedings of the Royal Society B: Biological Sciences, 287(1933), 20201184. https://doi.org/10.1098/rspb.2020.1184

Hosokawa, T., & Fukatsu, T. (2020). Relevance of microbial symbiosis to insect behavior. Current Opinion in Insect Science, 39, 91–100. https://doi.org/10.1016/j.cois.2020.03.004

Hosokawa, T., Hironaka, M., Mukai, H., Inadomi, K., Suzuki, N., & Fukatsu, T. (2012). Mothers never miss the moment: A fine-tuned mechanism for vertical symbiont transmission in a subsocial insect. Animal Behaviour, 83(1), 293–300. https://doi.org/10.1016/j.anbehav.2011.11.006

Hosokawa, T., Kikuchi, Y., & Fukatsu, T. (2007). How many symbionts are provided by mothers, acquired by offspring, and needed for successful vertical transmission in an obligate insect–bacterium mutualism? Molecular Ecology, 16(24), 5316–5325. https://doi.org/10.1111/j.1365-294X.2007.03592.x

Janda, J. M., & Abbott, S. L. (2006). The Genus Hafnia: From Soup to Nuts. Clinical Microbiology Reviews, 19(1), 12–28. https://doi.org/10.1128/CMR.19.1.12-28.2006

Johnson, M. T. J., & Munshi-South, J. (2017). Evolution of life in urban environments. Science, 358(6363), eaam8327. https://doi.org/10.1126/science.aam8327

Katoh, K. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30(14), 3059–3066. https://doi.org/10.1093/nar/gkf436

Kölliker, M. (2007). Benefits and costs of earwig (Forficula auricularia) family life. Behavioral Ecology and Sociobiology, 61(9), 1489–1497. https://doi.org/10.1007/s00265-007-0381-7

Kölliker, M., & Vancassel, M. (2007). Maternal attendance and the maintenance of family groups in common earwigs (Forficula auricularia): A field experiment. Ecological Entomology, 32(1), 24–27. https://doi.org/10.1111/j.1365-2311.2006.00831.x

Kramer, J., Thesing, J., & Meunier, J. (2015). Negative association between parental care and sibling cooperation in earwigs: A new perspective on the early evolution of family life? Journal of Evolutionary Biology, 28(7), 1299–1308. https://doi.org/10.1111/jeb.12655

Krebs, C. (1999). Ecological methodology (2nd ed.). Harper and Row.

Lazzeri, A. M., Mangia, N. P., Mura, M. E., Floris, I., Satta, A., & Ruiu, L. (2020). Potential of novel food-borne Lactobacillus isolates against the honeybee pathogen Paenibacillus larvae. Biocontrol Science and Technology, 30(9), 897–908. https://doi.org/10.1080/09583157.2020.1769556

Lenth, R. (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. https://CRAN.R-project.org/package=emmean

Li, X.-D., Xin, L., Rong, W.-T., Liu, X.-Y., Deng, W.-A., Qin, Y.-C., & Li, X.-L. (2021). Effect of heavy metals pollution on the composition and diversity of the intestinal microbial community of a pygmy grasshopper (Eucriotettix oculatus). Ecotoxicology and Environmental Safety, 223, 112582. https://doi.org/10.1016/j.ecoenv.2021.112582

Lombardo, M. P. (2008). Access to mutualistic endosymbiotic microbes: An underappreciated benefit of group living. Behavioral Ecology and Sociobiology, 62(4), 479–497. https://doi.org/10.1007/s00265-007-0428-9

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Magoga, G., Piombo, C., Locatelli, D. P., Limonta, L., & Montagna, M. (2023). The microbiota of Idaea inquinata developing on dry herbs. Entomologia Experimentalis et Applicata, 171(10), 796–805. https://doi.org/10.1111/eea.13347

Magurran, A. E., & Henderson, P. A. (2003). Explaining the excess of rare species in natural species abundance distributions. Nature, 422(6933), 714–716. https://doi.org/10.1038/nature01547

Manthey, C., Johnston, P. R., Nakagawa, S., & Rolff, J. (2022). Complete metamorphosis and microbiota turnover in insects. Molecular Ecology, mec.16673. https://doi.org/10.1111/mec.16673

Maraci, Ö., Corsini, M., Antonatou-Papaioannou, A., Jünemann, S., Sudyka, J., Di Lecce, I., Caspers, B. A., & Szulkin, M. (2022). Changes to the gut microbiota of a wild juvenile passerine in a multidimensional urban mosaic. Scientific Reports, 12(1), 6872. https://doi.org/10.1038/s41598-022-10734-7

Martinez Arbizu, P. (2017). pairwiseAdonis: Pairwise Multilevel Comparison using Adonis.

Martiny, J. B. H., Martiny, A. C., Brodie, E., Chase, A. B., Rodríguez-Verdugo, A., Treseder, K. K., & Allison, S. D. (2023). Investigating the eco-evolutionary response of microbiomes to environmental change. Ecology Letters, 26(S1). https://doi.org/10.1111/ele.14209

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE, 8(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Meunier, J. (2024). The biology and social life of Earwigs (Dermaptera). Annual Review of Entomology, 69(1), annurev-ento-013023-015632. https://doi.org/10.1146/annurev-ento-013023-015632

Meunier, J., & Kölliker, M. (2012). Parental antagonism and parent–offspring co-adaptation interact to shape family life. Proceedings of the Royal Society B: Biological Sciences, 279(1744), 3981–3988. https://doi.org/10.1098/rspb.2012.1416

Meunier, J., Wong, J. W. Y., Gómez, Y., Kuttler, S., Röllin, L., Stucki, D., & Kölliker, M. (2012). One clutch or two clutches? Fitness correlates of coexisting alternative female life-histories in the European earwig. Evolutionary Ecology, 26(3), 669–682. https://doi.org/10.1007/s10682-011-9510-x

Muhammad, A., Habineza, P., Ji, T., Hou, Y., & Shi, Z. (2019). Intestinal microbiota confer protection by priming the immune system of red palm weevil Rhynchophorus ferrugineus Olivier (Coleoptera: Dryophthoridae). Frontiers in Physiology, 10, 1303. https://doi.org/10.3389/fphys.2019.01303

Muratoğlu, H., Sezen, K., & DemiRbağ, Z. (2011). Determination and pathogenicity of the bacterial flora associated with the spruce bark beetle, Ips typographus (L.) (Coleoptera: Curculionidae: Scolytinae). Turkish Journal of Biology. https://doi.org/10.3906/biy-0902-12

Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, 59(3), 695–700. https://doi.org/10.1128/aem.59.3.695-700.1993

Nowak, A., Szczuka, D., Górczyńska, A., Motyl, I., & Kręgiel, D. (2021). Characterization of Apis mellifera gastrointestinal microbiota and lactic acid bacteria for honeybee protection—A review. Cells, 10(3), 701. https://doi.org/10.3390/cells10030701

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., … Weedon, J. (2022). vegan: Community ecology package. https://CRAN.R-project.org/package=vegan

Onchuru, T. O., Javier Martinez, A., Ingham, C. S., & Kaltenpoth, M. (2018). Transmission of mutualistic bacteria in social and gregarious insects. Current Opinion in Insect Science, 28, 50–58. https://doi.org/10.1016/j.cois.2018.05.002

Powell, J. E., Martinson, V. G., Urban-Mead, K., & Moran, N. A. (2014). Routes of acquisition of the gut microbiota of the honey bee Apis mellifera. Applied and Environmental Microbiology, 80(23), 7378–7387. https://doi.org/10.1128/AEM.01861-14

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 – Approximately maximum-likelihood trees for large alignments. PLoS ONE, 5(3), e9490. https://doi.org/10.1371/journal.pone.0009490

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team. (2024). R: A language and environment for statistical computing (Version 4.3.4) [Computer software]. https://www.R-project.org/

Raulo, A., Allen, B. E., Troitsky, T., Husby, A., Firth, J. A., Coulson, T., & Knowles, S. C. L. (2021). Social networks strongly predict the gut microbiota of wild mice. The ISME Journal, 15(9), 2601–2613. https://doi.org/10.1038/s41396-021-00949-3

Rodrigues, J. L. M., Silva-Stenico, M. E., De Souza, A. N., Lopes, J. R. S., & Tsai, S. M. (2006). In situ probing of Xylella fastidiosa in honeydew of a xylem sap-feeding insect using 16S rRNA-targeted fluorescent oligonucleotides. Environmental Microbiology, 8(4), 747–754. https://doi.org/10.1111/j.1462-2920.2005.00958.x

Rothman, J. A., Leger, L., Kirkwood, J. S., & McFrederick, Q. S. (2019). Cadmium and selenate exposure affects the honey bee microbiome and metabolome, and bee-associated bacteria show potential for bioaccumulation. Applied and Environmental Microbiology, 85(21), e01411-19. https://doi.org/10.1128/AEM.01411-19

Sachs, J. L., Skophammer, R. G., & Regus, J. U. (2011). Evolutionary transitions in bacterial symbiosis. Proceedings of the National Academy of Sciences, 108(supplement_2), 10800–10807. https://doi.org/10.1073/pnas.1100304108

Sandrin, L., Meunier, J., Raveh, S., Walser, J.-C., & Kölliker, M. (2015). Multiple paternity and mating group size in the European earwig, Forficula auricularia. Ecological Entomology, 40(2), 159–166. https://doi.org/10.1111/een.12171

Schliep, K. P. (2011). phangorn: Phylogenetic analysis in R. Bioinformatics, 27(4), 592–593. https://doi.org/10.1093/bioinformatics/btq706

Schmidt, K., & Engel, P. (2021). Mechanisms underlying gut microbiota–host interactions in insects. Journal of Experimental Biology, 224(2), jeb207696. https://doi.org/10.1242/jeb.207696

Seto, K. C., Güneralp, B., & Hutyra, L. R. (2012). Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. Proceedings of the National Academy of Sciences, 109(40), 16083–16088. https://doi.org/10.1073/pnas.1211658109

Shukla, S. P., Plata, C., Reichelt, M., Steiger, S., Heckel, D. G., Kaltenpoth, M., Vilcinskas, A., & Vogel, H. (2018). Microbiome-assisted carrion preservation aids larval development in a burying beetle. Proceedings of the National Academy of Sciences, 115(44), 11274–11279. https://doi.org/10.1073/pnas.1812808115

Stout, L. M., Dodova, E. N., Tyson, J. F., & Nüsslein, K. (2010). Phytoprotective influence of bacteria on growth and cadmium accumulation in the aquatic plant Lemna minor. Water Research, 44(17), 4970–4979. https://doi.org/10.1016/j.watres.2010.07.073

Su, H., Wu, J., Zhang, Z., Ye, Z., Chen, Y., & Yang, Y. (2021). Effects of cadmium stress at different concentrations on the reproductive behaviors of beet armyworm Spodoptera exigua (Hübner). Ecotoxicology, 30(3), 402–410. https://doi.org/10.1007/s10646-021-02365-x

Teyssier, A., Matthysen, E., Hudin, N. S., De Neve, L., White, J., & Lens, L. (2020). Diet contributes to urban-induced alterations in gut microbiota: Experimental evidence from a wild passerine. Proceedings of the Royal Society B: Biological Sciences, 287(1920), 20192182. https://doi.org/10.1098/rspb.2019.2182

Teyssier, A., Rouffaer, L. O., Saleh Hudin, N., Strubbe, D., Matthysen, E., Lens, L., & White, J. (2018). Inside the guts of the city: Urban-induced alterations of the gut microbiota in a wild passerine. Science of The Total Environment, 612, 1276–1286. https://doi.org/10.1016/j.scitotenv.2017.09.035

Trevors, J. T., Stratton, G. W., & Gadd, G. M. (1986). Cadmium transport, resistance, and toxicity in bacteria, algae, and fungi. Canadian Journal of Microbiology, 32(6), 447–464. https://doi.org/10.1139/m86-085

Trumbo, S. (2012). Patterns of parental care in invertebrates. In N. Royle, P. Smiseth, & M. Kölliker, The evolution of parental care (1st ed., pp. 81–93). Oxford University Press.

Tung, J., Barreiro, L. B., Burns, M. B., Grenier, J.-C., Lynch, J., Grieneisen, L. E., Altmann, J., Alberts, S. C., Blekhman, R., & Archie, E. A. (2015). Social networks predict gut microbiome composition in wild baboons. eLife, 4, e05224. https://doi.org/10.7554/eLife.05224

Van Meyel, S., Devers, S., Dupont, S., Dedeine, F., & Meunier, J. (2021). Alteration of gut microbiota with a broad-spectrum antibiotic does not impair maternal care in the European earwig. Journal of Evolutionary Biology, 34(7), 1034–1045. https://doi.org/10.1111/jeb.13791

Van Meyel, S., & Meunier, J. (2022). Costs and benefits of isolation from siblings during family life in adult earwigs. Animal Behaviour, 193, 91–99. https://doi.org/10.1016/j.anbehav.2022.09.003

Vásquez, A., Forsgren, E., Fries, I., Paxton, R. J., Flaberg, E., Szekely, L., & Olofsson, T. C. (2012). Symbionts as major modulators of insect health: Lactic acid bacteria and honeybees. PLoS ONE, 7(3), e33188. https://doi.org/10.1371/journal.pone.0033188

Videvall, E., Song, S. J., Bensch, H. M., Strandh, M., Engelbrecht, A., Serfontein, N., Hellgren, O., Olivier, A., Cloete, S., Knight, R., & Cornwallis, C. K. (2019). Major shifts in gut microbiota during development and its relationship to growth in ostriches. Molecular Ecology, 28(10), 2653–2667. https://doi.org/10.1111/mec.15087

Wang, H., Du, X., Zhang, Z., Feng, F., & Zhang, J. (2023). Rhizosphere interface microbiome reassembly by arbuscular mycorrhizal fungi weakens cadmium migration dynamics. iMeta, 2(4), e133. https://doi.org/10.1002/imt2.133

Wang, Y., & Rozen, D. E. (2017). Gut microbiota colonization and transmission in the burying beetle Nicrophorus vespilloides throughout development. Applied and Environmental Microbiology, 83(9), e03250-16. https://doi.org/10.1128/AEM.03250-16

Wang, Z., Li, J., Zhao, P., Yu, Z., Yang, L., Ding, X., Lv, H., Yi, S., Sheng, Q., Zhang, L., Zhou, F., & Wang, H. (2024). Integrated microbiome and metabolome analyses reveal the effects of low pH on intestinal health and homeostasis of crayfish (Procambarus clarkii). Aquatic Toxicology, 270, 106903. https://doi.org/10.1016/j.aquatox.2024.106903

Weiss, B. L., Maltz, M. A., Vigneron, A., Wu, Y., Walter, K. S., O'Neill, M. B., Wang, J., & Aksoy, S. (2019). Colonization of the tsetse fly midgut with commensal Kosakonia cowanii Zambiae inhibits trypanosome infection establishment. PLOS Pathogens, 15(2), e1007470. https://doi.org/10.1371/journal.ppat.1007470

Wenzel, M., Schonig, I., Berchtold, M., Kampfer, P., & Konig, H. (2002). Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite Zootermopsis angusticollis. Journal of Applied Microbiology, 92(1), 32–40. https://doi.org/10.1046/j.1365-2672.2002.01502.x

Yang, Z., Xu, F., Li, H., & He, Y. (2021). Beyond samples: A metric revealing more connections of gut microbiota between individuals. Computational and Structural Biotechnology Journal, 19, 3930–3937. https://doi.org/10.1016/j.csbj.2021.07.009

Zhang, H., & Reynolds, M. (2019). Cadmium exposure in living organisms: A short review. Science of The Total Environment, 678, 761–767. https://doi.org/10.1016/j.scitotenv.2019.04.395

Zhang, S., Chen, A., Deng, H., Jiang, L., Liu, X., & Chai, L. (2023). Intestinal response of Rana chensinensis larvae exposed to Cr and Pb, alone and in combination. Ecotoxicology and Environmental Safety, 255, 114774. https://doi.org/10.1016/j.ecoenv.2023.114774

Zhang, S., Jin, Y., Zeng, Z., Liu, Z., & Fu, Z. (2015). Subchronic exposure of mice to cadmium perturbs their hepatic energy metabolism and gut microbiome. Chemical Research in Toxicology, 28(10), 2000–2009. https://doi.org/10.1021/acs.chemrestox.5b00237

Zhang, Y., Yu, X., Sun, S., Li, Q., Xie, Y., Li, Q., Zhao, Y., Pei, J., Zhang, W., Xue, P., Zhou, Z., & Zhang, Y. (2016). Cadmium modulates hematopoietic stem and progenitor cells and skews toward myelopoiesis in mice. Toxicology and Applied Pharmacology, 313, 24–34. https://doi.org/10.1016/j.taap.2016.10.016