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Local, environmental and trace metal effects on gut microbiota diversity in urban feral pigeons

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ABSTRACT

Nowadays, understanding the biotic responses to the enhanced urbanization need to encompass not the only the physiological and phenotypic features but also the related microbiota of wildlife animals. One of main threats in urban ecosystems is the chemical pollution. Thus, we have explored whether the cloacal microbiota of feral pigeons (*Columba livia*) is impacted by both their geographical foraging area, and metal exposure in an urban context. First, pigeons were captured in 4 specific areas of Paris (France) and placed in captivity. By applying a 16SrRNA metabarcoding approach, we observed that the gut microbiota diversity was structured according to the capture sites, with strong variation of Actinobacteria, Bacilli and Clostridia, that could be linked to the granivorous or low-protein diets. Subsequently, we experimentally exposed these pigeons to zinc and/or lead (two-factor cross design) during 90 days in a non-urban environment, but no impact on the composition nor diversity of pigeon gut microbiota, has been observed after 45 and 90 days of metal exposures. However, the composition and diversity significantly differed from the microbiota at the capture period, with the emergence of taxa belonging to *Corynebacterium* and *Bifidobacterium* in captive conditions. These data highlight a strong impact of the lifestyles (captivity in non-urban environment) on the gut microbiota composition. In parallel, we hypothesized that the diet and the local environment might have smoothed the impact of the metal exposure for pigeons that could quickly change the structure of their gut microbiota. Our findings shed light on the effects of urban pollution and environment on bird communities, that can be extended to their gut microbiota causing potential additive or synergic negative effects to host organisms and populations.

1. Introduction

Nowadays, human activity, such as resources exploitation, impact of atmosphere composition and reallocation of terrestrial surface, constitutes one of the major sources of threats for biodiversity (Lewis and Maslin, 2015). One manifestation of the intensification of these activities is the growing consolidation of human populations in urban areas. This urbanization leads to a change in land use and the exposure of organisms to various forms of pollution such as trace elements (TEs). Such TEs are mainly metals (zinc and lead) found in the environment in low concentrations, accumulated in several body tissues of organisms (Frantz et al., 2012; Valverde et al., 2024) and known to be toxic (Komarnicki, 2000; Sánchez-Chardi et al., 2009; Chatelain et al., 2016a). Specifically, Chatelain et al. (2016) showed detrimental effects of TEs on the physiology of feral pigeons (*Columba livia*) and their reproductive parameters in urban areas. Moreover, TE exposure can affect the ecological interactions, through contamination within body tissues being transmitted. Moreover, TE effects even biomagnified, along the trophic network through predation (Chouvelon et al., 2019). In a different way, the presence of intestinal parasites may accumulate the pollution and may participate to the detoxication of the host, thus reducing the negative effects of pollutants on their hosts (Molbert et al., 2020; Jeantet et al., 2023).

Recently, it was also suggested that extensive urbanization and exposure to TE might significantly impact gut microbial diversity, causing dysbiosis in human, mice and chicken (Forouzandeh et al., 2021; Yang et al., 2021; Liao et al., 2022). Gut microbial activities sustain multiple essential functions such as host digestion, nutrient

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synthesis (Ramakrishna, 2013; Grond et al., 2018), and protection against pathogens (Fukuda et al., 2011), and are involved in host immune functions (Round and Mazmanian, 2009; Belkaid and Hand, 2014), life history traits (Sison-Mangus et al., 2015) and even behavior (Heijtz et al., 2011; Ezenwa et al., 2012). In parallel, gut microbiota is strongly affected by the diet and anthropogenic perturbations (Li et al., 2021, 2022; Colin et al., 2022). Indeed, the relative abundance of several taxa is mainly controlled by the diet regime, age and physiological state of the hosts (Waite and Taylor, 2015), and can be impacted by habitats (Bletz et al., 2016), geographical location (Linnenbrink et al., 2013) ambient temperature and season (Janiga et al., 2007; Maurice et al., 2015). Although affected by both the host's and environmental conditions, the gut microbiota might be more sensitive to the latter (Teyssier et al., 2018), and thus might be a relevant physiological trait to explore facing anthropogenic perturbations.

Although several studies have shown that the imbalance of gut microbiota could be indicative of stress responses (Colin et al., 2022; Liu et al., 2022), only a few studies have investigated experimentally how the urban life-style and TE exposure could affect the composition and diversity of avian gut microbiota. Thus, in this study, we experimentally tested whether the cloacal microbiota of urban feral pigeons (*Columba livia*) was impacted by both their geographical foraging area, urban lifestyle and TE exposure. To achieve this, pigeons were captured in Paris (France) and placed in captivity in non-urban area. First, we compared the gut microbiota diversity of pigeons according to their capture sites. Subsequently, we experimentally exposed pigeons to zinc and/or lead (two-factor cross design) during 90 days, and we compared how the TE exposure as well as captive conditions could alter their gut microbiota diversity. We first expected that the gut microbiota in terms of species richness and composition differed among the different geographical sites and the different conditions (urban versus captivity). Second, we expected that TE exposure altered the species richness and changed the composition of gut microbiota.

2. Materials and methods

2.1. Urban pigeons capture

A total of 69 urban feral pigeons were captured with nets baited with corn from January 29th to February 4th, 2019 (d0), from 14 sites in Paris city, France (see Table S1 and Fig. S1 for capture areas). These sites were grouped within 4 urban groups corresponding to a local environment (below 800m) which is the main environment experienced by pigeons (Frantz et al., 2012): Site gr1 (Gare Montparnasse), Site gr2 (Place Monge), Site gr3 (Place d'Italie) and Site gr4 (Porte d'Italie). Accordingly, distances between each site ranged from 1,33 km–3.79 km (Table S1). Individuals were first sexed visually based on morphological criteria (size, appearance of the caruncle and iridescent reflection of the neck). We confirmed or corrected sex by behavioral observation specific to each sex, such as "bow-coo" for males, (Fabricius and Jansson, 1963). Pigeons were immediately transferred after the capture to the CEREEP (Centre d'Ecologie Expérimentale et Prédictive-Ecotron) located in a rural area (Saint-Pierre-lès-Nemours, France) and placed in 8 outdoor aviaries. They were identified individually with colored rings. With a total of 38 males and 31 females, we placed 9 pigeons in 6 aviaries (5 males and 4 females), 8 pigeons in one aviary (4 males and 4 females) and 7 pigeons in one aviary (4 males and 3 females). They were fed ad libitum with a mixture of corn, wheat and peas coming from the local farmers cooperative. The aviaries contained a drinking-trough and a basin used as a bath, as well as a nesting area and bamboo perches. Birds were released at capture site at the end of the experiment. All experiments were carried out in strict accordance with the recommendations of the 'European Convention for the Protection of vertebrate Animals used for Experimental and Other Scientific Purposes' and were conducted under the authorization of the French authority (authorization APAFIS#17554-201811161046635v2).

2.2. TE exposure experiment

To experimentally test the impact of zinc and lead exposure on the gut microbiota diversity,

4 different exposure treatments (one by aviary) were implemented: a lead treatment (10 ppm in tap water; Sigma-Aldrich, St. Louis, MO, USA; 2 aviaries; n = 17); a zinc treatment (100 ppm in tap water; 2 aviaries; n = 18), a lead and zinc treatment (10 ppm of lead and 100 ppm of zinc in tap water; 2 aviaries; n = 16) and a non-exposed metal treatment (tap water only; 2 aviaries, n = 18). These concentrations were chosen to mimic the concentrations to which pigeons are exposed in urban areas based on the concentrations found in the city of Paris (Chatelain et al. 2016). Treated water was added to drinking-troughs. Tap water used as a control does not contain significant amount of lead or zinc as previously reported (shift from 24.34 ppm to 0.71 ppm of lead and from 328 ppm to 89 ppm of zinc in the feathers, after one year of drinking this tap water, Chatelain et al., 2014). More generally, this captive condition in a rural environment guarantees that the TEs the pigeons ingested are only from our treatment and not environmental derived. Pigeons had unlimited access to water which was replaced twice a week. Individuals were allocated to the different aviaries and treatments to balance sex, mass and level of melanin. The effects of lead and zinc exposure on microbiota were then investigated at 45 (d45) and 90 days (d90) after the beginning of the treatment (February 11th, 2019, one week after the capture). This timing in the design was chosen based on our previous studies reporting significant effects of lead and zinc after 6 weeks of exposure on several physiological traits in pigeons (immune system and reproduction Chatelain et al., 2016a, 2016b). A total of 9 pairs (4 from the non-metal exposed group, 3 from the lead treatment, 1 from zinc treatment and 1 from lead and zinc treatment) laid at least one egg over the course of the experiment. This reproductive status was balanced among the treatments and, therefore, did not confound our treatments. Note that all eggs were systematically removed just after laying.

2.3. Individual physiological parameters

Morphological parameters and coloration were measured for each captured pigeon. At d0 (day of capture), wing size (±1 mm) was measured with a ruler. Pigeons' weight were determined at d0, d45 (45 days after the exposure) and d90 (90 days after the exposure) with a Pesola Newton scale to the nearest 5 g. Then, with the wing sizes and the body masses, a scaled body mass index (BMI) was calculated according to Peig and Green (2010). To estimate the melanin coloration of the individuals, a photograph of the right wing of the pigeons was taken at d0. The percentage of dark pixels on the wing was calculated using the Gimp image software (v 2.10; see Jacquin et al. 2011 for the method).

2.4. Cloacal sampling

A total of 207 cloacal samples were collected at d0, d45 and d90, right after the capture (See Table S1). Cloacal contents were sampled following the method described in Teyssier et al. (2018). Briefly, before sampling, the external area of the cloaca was rinsed with Ethanol 96% (Sigma-Aldrich). Then, phosphate buffer (concentration, 200 μL) was gently injected and re-drawn using micro-pipetting. At each capture site (d0 in urban area) or sampling sessions (d45 or d90), sixteen three negative samples were also performed for possible contamination (one for each site at d0 (n = 14) and one for each session d45 and d90 (n = 2)), tubes containing only the saline solution were left opened during sampling and preparation. The samples were transported at −20 °C to the lab, and stored until molecular analyses.

2.5. DNA extraction

Total DNA was successfully extracted from 187 of the 207 cloacal samples (57 at d0, 65 at d45 and d90, see Table S1) following Bestion

et al. (2017), using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Venlo, Netherlands). The nucleic acids were quantified using a spectrophotometer (Nanodrop). The DNA was stored at −20 °C until analysis. For the failed 20 cloacal samples and the sixteen negative sampling controls, DNA was not detectable and thus did not follow the whole procedure.

2.6. PCR amplification and high-throughput sequencing

To amplify the V5-V6 region of the 16S rRNA fragment, the primer set 785-F (5′-TCT GGA TTA GAT ACC CTG GTA GT-3′) and 1080-R (5′-CA CGA CAC GAG CTG ACG-3′) was used (Fliegerova et al., 2014). PCR amplification (final volume of 25 μL) was performed using 2.5U of DreamTaq polymerase (ThermoFisher Scientific), 1.25 μL of Bovine Serum Albumin (BSA, 2 mg mL^{-1}, ThermoFisher Scientific), dNTP mix (10 mM), 2 μL of MgCl2 (25 mM), 1 μl of each primers (10 mM), with the following thermal conditions: 95 °C for 10 min; 25 cycles of 95 °C for 45 s, 55 °C for 1 min, 72 °C for 30 s; and a final extension of 10 min at 72 °C. PCR was performed in triplicates and pooled. The library preparation (including TAG addition) and sequencing (Illumina MiSeq 250 bp paired-end, v3 chemistry) were handled by the @Bridge INRAE platform (Jouy-en-Josas, France, http://abridge.inra.fr/index.php?lang=fr). A total of four negative controls (2 PCR amplification (H_2O) and 2 sequencing controls) were also added.

2.7. Bioinformatic analysis

A total of 185 samples and the 4 negative sequencing controls (see Tables S2 and 57 at d0, 64 at d45 and d90), were successfully sequenced, and reads presented a Phred Quality Score above a threshold of 30 (FastQC software; https://www.bioinformatics.babraham.ac.uk/projects/fastqc).

Data were processed through the FROGS pipeline (Find Rapidly OTU with Galaxy Solution, release 4.1.0) implemented on a galaxy instance (release 21.05) (https://galaxy.migale.inra.fr/) (Escudié et al., 2018). After the prep-process (merging overlap, size and without N), a total of 6 319 734 assembled reads were analyzed. Reads were clustered using the SWARM algorithm (2.1.5) that uses a single linkage clustering on sequences, using the fastidious option by clustering nearly identical amplicons iteratively with an aggregation distance of 1, as recently recommended (FROGS 3.2 version release 2021). Chimera were then removed using VSEARCH (Rognes et al., 2016). The low abundance ASVs (<5 reads) were removed from the dataset (Filtering step, see Table S2). For the four negative controls, 48 to 172 paired-ends reads were assembled, corresponded to 29 to 63 ASVs, with low abundance (<5 reads), and were thus not included in the following analysis. Thus, at the sample level, datasets were rarefied to the lowest number of reads determined according to the filtering step (2751 reads, see Table S2). Rarefaction curves were plotted (Fig. S2). Taxonomy affiliation of each ASV was performed using the blastn + tool (version 2.10, Camacho et al., 2009) against the SILVA 138-16S pintail 100 database (Pruesse et al., 2007), and only the best BLAST hits with the same score are reported.

2.8. Statistical analyses

All statistical analyses were performed using R (4.2.3, and 3.3.6) (R Development Core Team, http://www.R-project.org). The Phyloseq (1.36.0, McMurdie and Holmes, 2013) and Microbiome (1.14.0, Lahti and Shetty (2012–2019)) packages were used to describe community composition and diversity. Plots were drawn using the packages ggplot2 (3.4.2, Wickham, 2016) and Phyloseq. Alpha-diversity (species richness, Simpson and Evenness indices) was calculated. Analysis of variance followed by Tukey HSD post-hoc test, was used to determine significant differences between capture group. Difference in the relative abundances of the major taxonomical classes was also determined by analysis of variance, followed by Tukey HSD post-hoc test. A non-metrical dimensional scaling (NMDS), based on the Bray Curtis dissimilarity matrix was used for the ordination of the gut microbial communities. As similar pictures were obtained with both Unifrac and Jaccard dissimilarities (see Fig. S3), only the Bray-Curtis dissimilarities was presented and discussed in the main text. Differences according to the local foraging area (formula: Capture_Group, at d0 in urban conditions), as well as the captive and the metal-exposed conditions were statistically tested using permutational PERMANOVA (9999 permutations, p-value<0.01). A post-hoc pairwise test (999 permutations, and Bonferroni as the p-value correction method with ADONIS and pairwise ADONIS (vegan 2.6.2, Oksanen et al., 2022) and presented in Tables S3 and S4. Under the factor "Treatment", "Urban" refers to local conditions at d0, "Captive" to captive rural conditions (d45 and d90) without metal exposure, and "Lead", "Zinc" and "Zinc-Lead" refer to captive conditions with metal exposure (d45 and d90). Under the factor "Treatment-Date", "Urban-d0" refers to local conditions at d0 day, "Captive-d45 and Captive-d90" to captive conditions at d45 and d90 days, without metal exposure, and Lead-d45, Lead-d90, Zinc-d45, Zinc-d90 and Zinc-Lead-d45 and Zinc-Lead-d90 refer to captive conditions with metal exposure at d45 and d90 days. Here, under the factor "Habitat", "Urban" refers to the local conditions, while "Captive" refers to the captive conditions, whatever the metal exposure or not.

As no difference were observed for the microbial communities after 45 and 90 days of metal exposure (see Table S4 and Fig. S6); results for the two timepoints were regrouped only for the taxonomical analysis in Fig. 2B and C.

The ASVs that contributed the most to the dissimilarity among communities, were determined using the SIMPER function (vegan 2.6.2, Oksanen et al., 2022) with the criterion of up to 70% of the cumulative explained dissimilarity (with an adjusted p-value <0.1). A differential expression analysis of these ASVs was performed using DESeq2 (1.32.0, Love et al., 2014; padj <0.01).

2.9. Nucleotide read accession numbers

All the nucleotide reads have been deposited to the SRA database (Sequence Reads Archive) under the accession number PRJNA1122576 (https://www.ncbi.nlm.nih.gov/bioproject/).

3. Results

3.1. Urban geographical impact on the pigeon's gut microbiota

We detected no significant differences in the specific richness of the microbiota among the capture sites (mean of 116 taxa, Fig. 1A). However, we found higher Simpson and Evenness indexes for the capture sites gr3 (p-value = 0.04) and gr4 (p-value = 0.004) as compared to gr1 and gr2 (Tables S3A and B). This result highlights that although the species numbers were similar, the community structure was different with fewer ASVs being highly dominant within the microbial communities of gr3 and gr4 as compared to gr1 and gr2 (Fig. 1A). In parallel, the ordination (Fig. 1B) showed a distinction for the capture sites along the first axis (PERMANOVA, p-value = 0.0004, R2 = 0.97), but no significant differences according to the sex, the weight nor the BMI (Fig. S4A, Table S3C). When focusing on the taxonomic composition (Fig. 1C, and S5), the three main class detected were Bacilli (26.5–74%), Actinobacteriota (11–34%), Clostridia (6.7–23.5%), with significantly different higher abundances of Actinobacteria taxa (p-value = 0.002) and Clostridiae (p-value = 0.01) and a lower abundances of Bacilli taxa (p-value = 0.028) in the capture sites gr2, 3 and 4 as compared to gr1 (Tables S3E and F). Using a similarity percentage analysis, the few ASVs, that statistically explained the dissimilarity between capture sites, were picked out, and their differential abundance was measured (Fig. 1D). The abundances of theses taxa were significantly different (p-value<0.01) between capture sites gr1 and the 3 others. Taxa belonging to

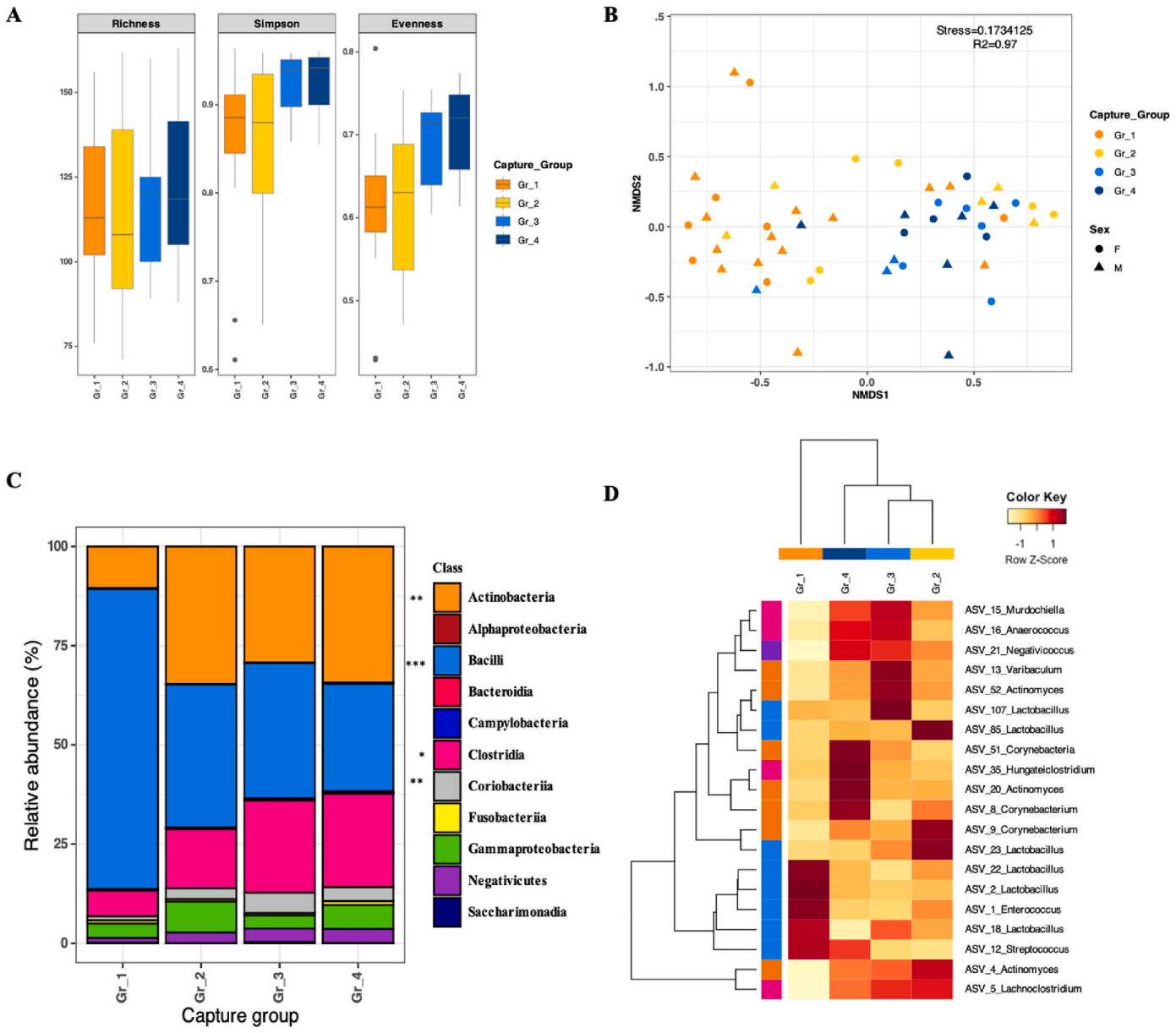

Fig. 1. A, B, C and D. Local urban impact on the gut microbiota
A) α-diversity metrics based on Richness (observed ASV) Simpson and Evenness indices. Data are presented as boxplot with mean values ± standard deviation (n = 57), according to the capture group.
B) Community structure of the gut microbiota, based on Bray-Curtis dissimilarity, according to the capture group (color) and sex (triangle and dot).
C) Taxonomic composition of the local urban gut microbiota. Major taxa (>0.1% of the dataset) are presented at the class taxonomic level, and expressed as % of the total read abundance; significant differences between capture groups are highlighted by asterisks (p-value significance, "0 '***' 0.001 '**' 0.01 '*').
D) Heatmap of the selected AVS explaining the dissimilarity between capture groups microbiota's communities, presented at the class taxonomic level, and expressed as % of the total read abundance; significant differences between capture groups were determined by SIMPER and DESeq analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the Bacilli class, mainly *Lactobacillus*, were up-represented in capture site gr1, while taxa belonging to the Clostridia class (3 over 4) were down-represented in capture group gr1. Most of the taxa from Actinobacteria, mainly *Corynebacterium* and *Actinomyces*, showed equal patterns or up-represented in capture sites gr2, 3 and 4. Moreover, at the class level, the ratios Clostridia:Bacilli and Actinobacteria:Bacilli were significantly lower for the gr1 (respectively 0.09 and 0.15) as compared to other sites, respectively ranging from 0.37 to 0.88 for Clostridia: Bacilli ratio and ranging from 0.85 to 1.28 for Actinobacteria:Bacilli ratios (Table S3G). In contrast, the ratio Actinobacteria:Clostridia did not differ among the capture sites (Table S3G). Note that no sex effects were detected on the alpha and beta diversity nor on taxa relative abundances (Tables S3A and C).

3.2. *Effects of metal exposure and captivity on the pigeon gut microbiota*

The metal exposure did not significantly impact the physiological body traits of the pigeons (Fig. S4B), nor the diversity indexes (Fig. S7). But we detected significant differences between individuals in their urban foraging area (at d0, labelled "urban", Fig. 2A, and Tables S4A and B) and individuals in captive conditions without metal-exposure (labelled "captive"), and with metal-exposure (labelled "Zinc", "Lead", and "Zinc_lead"). The first axis (PERMANOVA, p-value = 0.0003) of the NMDS analysis significantly discriminated between the urban areas (d0)

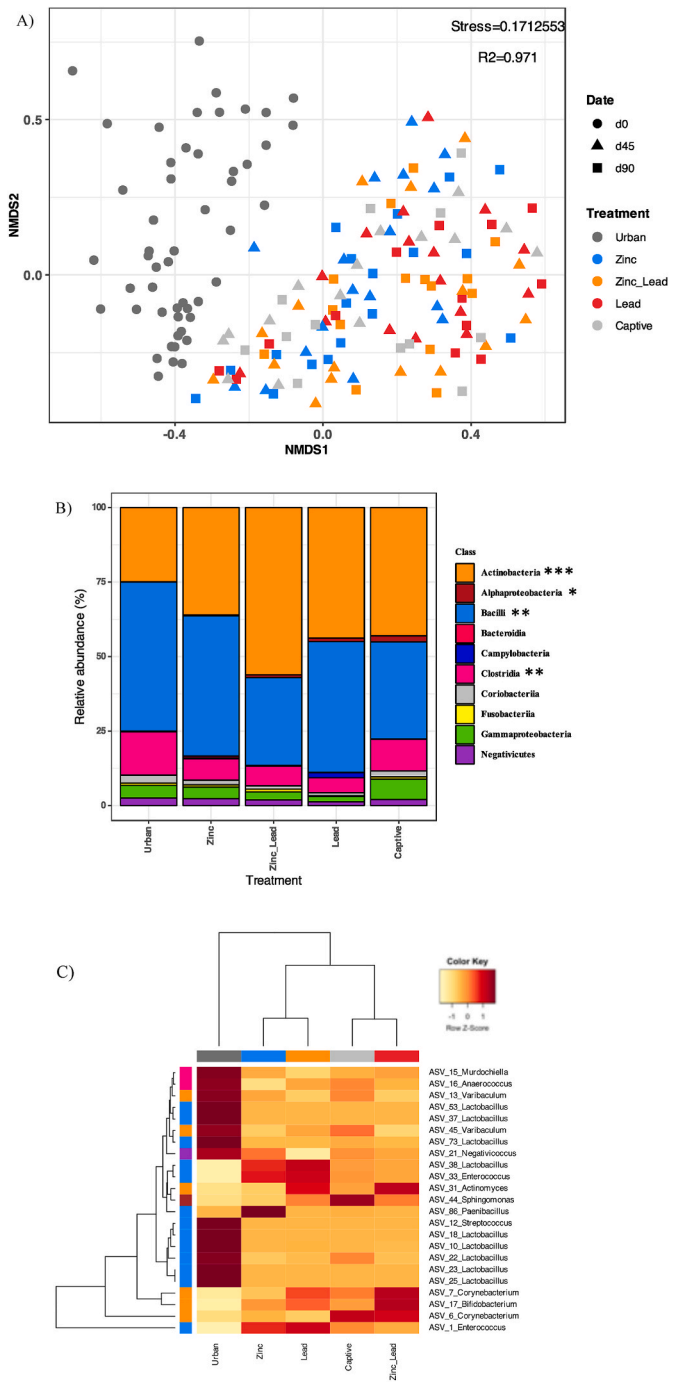

Fig. 2. A, B and C: Impact of the captivity and metal exposure on the gut microbiota

A) Community structure of the gut microbiota, based on Bray-Curtis dissimilarity, according to the local or exposure treatment, and the date. B) Major taxa (>0.1% of the dataset) are presented at the class taxonomic level, and expressed as % of the total read abundance; significant differences between capture groups are highlighted by asterisks (p-value significance, "0 '***' 0.001 '**' 0.01 '*'), C) Heatmap of the AVS explaining the dissimilarity between the bacterial communities, presented at the class taxonomic level, and expressed as % of the total read abundance; significant differences between urban, captive with and without metal-exposure groups were determined by SIMPER and DESeq analysis.

and the captive conditions, whatever the metal exposure, while none of the two axes discriminated among TE exposure treatments (p-value from 0.2 to 1, Table S4B). Difference in their composition was also depicted by higher abundances of Actinobacteria taxa (p-value <0.0001) and lower abundances of Bacilli (p-value = 0.007) and Clostridia (p-value 0.008) in captivity as compared to urban foraging areas (Fig. 2B, Fig. S8, and Tables S4C and D). At the class level, the ratio Actinobacteria: Clostridia and Actinobacteria:Bacilli were significantly higher for the captive condition: 4.02 to 8.31 and 0.82 to 2 respectively compared to 1.66 and 0.48 for the urban condition (Table S4E). In contrast, the ratio Clostridia:Bacilli did not differ among captive and urban conditions (Table S4E). Moreover, the ASV, explaining most of the dissimilarity between urban and captive control conditions, displayed clear different abundances (p-value<0.01): most of the taxa from Actinobacteria, belonging to *Corynebacterium* and *Bifidobacterium*, were up-represented in captive conditions, while the taxa belonging to the Lactobacillales (Bacilli) were up-represented in the urban condition (Fig. 2C).

4. Discussion

In order to explore the impact of the urban environment and metal exposure on the avian gut microbiota, we experimentally exposed feral pigeons to zinc and lead. We also profited of this study to describe the pigeon gut microbiota in Paris and examine how the urban environment impacts the species richness and composition of gut microbiota by comparing gut microbiota among 4 geographical sites and between individuals sampled in urban areas (at d0) and after 45 and 90 days of captivity (d45 and d90).

4.1. Pigeon gut microbiota composition

Whatever the capture sites or the TE exposure, the main phyla detected in all the gut microbiota of the feral pigeons were Firmicutes (including both Clostridia and Bacilli, 36–66%), Actinobacteriota (26–57%), and Proteobacteria (3–9%), which all have been documented as major phyla in other avian taxa (Grond et al., 2018; Wang et al., 2022). Despite cloacal sampling being handy and non-invasive for hosts (Knutie and Gotanda, 2018), this method might have introduced bias in the study. First, it might not fully reflect the composition of the gut or other intestinal segments (Diaz-Carrasco et al., 2019). Second, some taxa might be preferentially found in the cloacal zone (Bestion et al., 2017; Lee et al., 2020), such as members of the *Lactobacillus* genus.

Interestingly, such a high abundance of Actinobacteria has also been documented in several avian microbiota, such as in wild seabirds and in granivorous birds (Columbiformes and Passeriformes). The high abundances of this taxon in gut microbiota have been reported to be associated with a granivorous diet (Garcia-Amado et al., 2018; Grond et al., 2019). In contrast, Bacteroidetes only represented a small fraction of the gut microbiota (0.27–0.90%), while it usually represents 5–35% in previous studies (reviewed in Waite and Taylor, 2015). Bacteroidetes are generally associated with the digestion of proteins (Zhang et al., 2020; Liu et al., 2022) and complex carbohydrates, including starch (Garcia-Amado et al., 2018). However, in agreement with our study, the low proportion of this phylum has been once reported for neotropical birds, granivorous Columbiformes and Passeriformes, parrots, pet-birds and several temperate birds (Garcia-Amado et al., 2018).

4.2. Differences among geographical foraging area

As expected, the composition of pigeon gut microbiota significantly differed among the different capture sites in Paris. These differences were even detected without considering site gr3, for which the distances among sample subsites were sometimes larger than the distances to the site gr2. At the class level, differences were revealed through variations in Actinobacteria, Clostridiae and Bacilli abundances, notably lower Clostridia:Bacilli and Actinobacteria:Bacilli ratios highlighting the

overdominance of Bacilli within the capture site gr1 as compared to the 3 other sites. Although environmental bacteria could colonize the gut microbiota by direct contact with the surrounding substrates (Sullam et al., 2012; Teyssier et al., 2018), diet is one of the driving parameters as it is the main interface between the gut and the host's environment (Wu et al., 2011). Urban areas can provide very different trophic resources and food types, it is therefore likely that differences in gut microbiota may be largely mediated by the diet. Herein, the different capture sites corresponded to distinct home ranges or foraging areas. Although the feral pigeons do move around residential areas, they typically have a smaller home range at a local scale (Frantz et al., 2012). Cohorts sharing the same home range show less variation in terms of abundance (numbers of individuals) and usually consist of regular visitors. It has been shown that changes in microbiota diversity were much more associated with local scale in urbanized area (Linnenbrink et al., 2013; Teyssier et al., 2018) and highlight the importance of the direct environment in which organisms are living. City parks are known to be refuges for organisms living in an urban environment and could provide a number of crucial resources to their inhabitants (Lin et al., 2012). Specifically, variation in the abundance of *Lactobacillus* spp., was highlighted in the differentiation between capture sites. *Lactobacillus* spp. is often related to the host feed efficiency and has been known to be a beneficial commensal for both humans and animals for years (Li et al., 2017; McCormack et al., 2017; Valeriano et al., 2017; Levkovich et al., 2013). In birds, *Lactobacillus* spp. is associated with reducing pathogen abundance through competitive exclusion, and with fermentation of lactate and plant-derived molecules, as well as benefiting health through improving body condition (Arun et al., 2021; Garcia-Mazcorro et al., 2021). It is possible that the plant-based diet of pigeons could provide better substrates for *Lactobacillus* spp., thus giving them an advantage (Stojanov et al., 2020; Grond et al., 2019). The pigeons of the site gr1 (encompassing the main station "Montparnasse" and the city park "Luxembourg") hosted higher abundances of *Lactobacillus* spp. and could be more prone to eat plants than pigeons from the other sites. However, to better appraise how the type of diet may explain the differences of gut microbiota observed in different geographic sites, experimental approach involving food quality manipulation is now required. Differences in the gut microbiota could be due to differences in TE across foraging areas, which can be highly variable in pigeons across small scales (Frantz et al., 2012). However, our experimental approach suggests that this is not the case in the present study. Other pollutants that can vary across urban environments, such as noise pollution, ca affect avian gut microbiotas (Berlow et al., 2022), and could have contributed to the detected differences in pigeon gut microbiotas across capture sites.

4.3. Effect of trace metals exposure and captive conditions

Indeed, using an experimental approach in a controlled environment, we showed that exposure to lead and zinc, specific contaminants of the urban environment, did not result in structural changes within pigeon gut microbiota. Our results contrast with previous studies reporting that trace metal exposure modifies the microbiota by enhancing *Bacteroidetes* relative abundance and reducing the *Firmicutes* and *Proteobacteria* ones (Zhai et al., 2017). One explanation resides in the fact that pigeons in our study were captured in Paris, and had therefore experienced metal exposition in their natural urban habitat. As we maintained metal exposure for metal exposed birds in our experiment, we would have expected change in microbiota over the time only for the non-metal exposed birds. Lead and Zinc concentrations used in this study correspond to the natural range found in Paris (Chatelain et al., 2016), and appeared not apply long enough (over 90 days) to detect differences in the gut microbiotas. Indeed, ingested metals can accumulate in pigeon feathers and organs overtime (Kekkonen et al., 2012;Reid et al., 2012) and can be released into the blood stream after the environmental exposure. Consequently, their microbiota might have been shaped by past metal exposure and the time required for removing all the trace metal was not long enough to induce modifications in the non-metal exposed birds.

Herein, the captive environment where the pigeons were placed, including abiotic and biotic factors (diet, stress, social group structure, energetic demands), might have smoothed the impact of the TE exposure (Bensch et al., 2023). For example, life in captivity or the density of host populations sharing the same living space could have modified the host-microbiota relationship. Accordingly, a study on pika demonstrated through an experimental approach that α-diversity was positively correlated with the host population density. Within high-density groups, gut bacterial communities were more similar between individuals than in groups with fewer individuals (Li et al., 2016).

Effectively, we observed differences in gut microbiota composition between pigeons captured in Paris (urban) and the same pigeons after being placed in captivity. Our results show that changes in environmental conditions in pigeons, such as those related to the present captivity, can change the structure of the gut microbiota. Once more, one hypothesis would be that diet is a major factor modulating the composition of gut microbiota. In fact, in urban areas, pigeons mainly use food sources of anthropogenic origin, which considerably broadens the spectrum of the composition of food consumption with a high protein content, compared to granivorous food with low protein and high starch contents. In contrast, in captivity, they have been fed with a less diverse granivorous diet (Ciminari et al., 2005). In addition, and contrary to urban areas, the quality and quantity of food remain relatively constant in captivity (Anderies et al., 2007). Herein, segregation between urban and captive gut microbiota relied mainly on the decrease of Lactobacillus in captive conditions, and a change in Actinobacteria: Bacilli and Actinobacteria:Clostridia ratios, and these variations F:B ratio could be regarded as a dysbiosis (Colin et al., 2022; Stojanov et al., 2020).

Alternatively, the differences observed between urban and captive conditions could be due to changes in ambient temperatures over the course of the experiment, either due to natural temporal or environmental variations. Indeed, cities have a buffering effect on temperature ranges, due to the heat island effect (Rizwan et al., 2008), and fluctuations (Shochat et al., 2006). Accordingly, differences in microbiota according to ambient temperatures and season have been reported in numerous studies in wild species (Janiga et al., 2007; Maurice et al., 2015). Firmicutes are generally more abundant at lower ambient temperatures, while Bacteroidetes are more abundant at higher temperatures (Dietz et al., 2022).

5. Conclusion

In conclusion, differences in pigeon gut microbiota were observed according to the capture sites within Paris, and could be related to possible different trophic resources and food types but not TE exposure. A shift in gut microbiota composition was detected after a few weeks in captivity, illustrating a strong impact of lifestyle on the gut microbiota. Our finding shed light on the effects of urban environment on bird communities, that can be extended to their gut microbiota.

CRediT authorship contribution statement

Clarence Schmitt: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julien Gasparini:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Héloïse Moullec:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Laurence Walch:** Writing – original draft, Methodology, Investigation, Data

curation. **Mathieu Leroux-Coyau:** Resources, Investigation, Formal analysis, Data curation. **Julie Leloup:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121263.

Data availability

Data will be made available on request.

References

Anderies, J.M., Katti, M., Shochat, E., 2007. Living in the city: resource availability, predation, and bird population dynamics in urban areas. J. Theor. Biol. 247 (1), 36–49. https://doi.org/10.1016/j.jtbi.2007.01.030.

Arun, K.B., Madhavan, A., Sindhu, R., Emmanual, S., Binod, P., Pugazhendhi, A., Sirohi, R., Reshmy, R., Kumar Awasthi, M., Gnansounou, E., Pandey, A., 2021. Probiotics and gut microbiome – Prospects and challenges in remediating heavy metal toxicity. J. Hazardous Materials 420, 126676. https://doi.org/10.1016/j.jhazmat.2021.126676.

Belkaid, Y., Hand, T.W., 2014. Role of the microbiota in immunity and inflammation. Cell 157, 121–141. https://doi.org/10.1016/j.cell.2014.03.011.

Bensch, H.M., Tolf, C., Waldenström, J., et al., 2023. Bacteroidetes to Firmicutes: captivity changes the gut microbiota composition and diversity in a social subterranean rodent. Anim microbiome 5, 9. https://doi.org/10.1186/s42523-023-00231-1.

Berlow, M., Wada, H., Derryberry, E.P., 2022. Experimental exposure to noise alters gut microbiota in a captive songbird. Microb. Ecol. 84, 1264–1277. https://doi.org/10.1007/s00248-021-01924-3.

Bestion, E., Jacob, S., Zinger, L., Di Gesu, L., Richard, M., White, J., Cote, J., 2017. Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. Nature Ecol. Evol. 1, 161. https://doi.org/10.1038/s41559-017-0161.

Bletz, M., Goedbloed, D., Sanchez, E., et al., 2016. Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. Nat. Commun. 7, 13699. https://doi.org/10.1038/ncomms13699.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+ : architecture and applications. BMC Bioinf. 10, 421. https://doi.org/10.1186/1471-2105-10-421.

Chatelain, M., Gasparini, J., Jacquin, L., Frantz, A., 2014. The adaptive function of 440 melanin-based plumage colouration to trace metals. Biol. Lett. 10, 20140164–20140441. https://doi.org/10.1098/rsbl.2014.0164, 20140164.

Chatelain, M., Frantz, A., Gasparini, J., Leclaire, S., 2016a. Experimental exposure to trace metals affects plumage bacterial community in the feral pigeon. J. Avian Biol. 47, 521–529. https://doi.org/10.1111/jav.00857.

Chatelain, M., Gasparini, J., Frantz, A., 2016b. Do trace metals select for darker birds in urban areas? An experimental exposure to lead and zinc. Glob. Change Biol. 22, 2380–2391. https://doi.org/10.1111/gcb.13170.

Chouvelon, T., Strady, E., Harmelin-Vivien, M., Radakovitch, O., Brach-Papa, C., Crochet, S., Knoery, J., et al., 2019. Patterns of trace metal bioaccumulation and trophic transfer in a phytoplankton-zooplankton-small pelagic fish marine food web. Mar. Poll. Bull. 146, 1013–1030. https://doi.org/10.1016/j.marpolbul.2019.07.047.

Ciminari, M.E., Del Valle Moyano, G., Chediak, J.G., Cavedies-Vidal, E., 2005. Feral pigeons in urban environments: dietary flexibility and enzymatic digestion? Rev. Chil. Hist. Nat. https://doi.org/10.4067/S0716-078X2005000200011.

Colin, Y., Molbert, N., Berthe, T., et al., 2022. Dysbiosis of fish gut microbiota is associated with helminths parasitism rather than exposure to PAHs at environmentally relevant concentrations. Sci Reports 12, 11084. https://doi.org/10.1038/s41598-022-15010-2.

Diaz Carrasco, J.M., Casanova, N.A., Fernández Miyakawa, M.E., 2019. Microbiota, gut health and chicken productivity: what is the connection? Microorganisms 7, 374. https://doi.org/10.3390/microorganisms7100374.

Dietz, M.W., Matson, K.D., Versteegh, M.A., van der Velde, M., Parmentier, H.K., Arts, J. A.J., Salles, J.F., Tieleman, B.I., 2022. Gut microbiota of homing pigeons shows summer-winter variation under constant diet indicating a substantial effect of temperature. Anim Microbiome 13 (1), 64. https://doi.org/10.1186/s42523-022-00216-6.

Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., et al., 2018. FROGS: find, rapidly, OTUs with galaxy solution. Bioinformatics 34, 1287–1294. https://doi.org/10.1093/bioinformatics/btx791.

Ezenwa, V.O., Gerardo, N.M., Inouye, D.W., Medina, M., Xavier, J.B., 2012. Animal behavior and the microbiome. Science 338, 198–199. https://doi.org/10.1126/science.1227412.

Fabricius, E., Jansson, A.M., 1963. Laboratory observations on the reproductive behaviour of the pigeon (*Columba livia*) during the pre-incubation phase of the breeding cycle. Anim. Behav. 11, 534–547.

Fliegerova, K., Tapio, I, Bonin, A., Mrazek, J., Callegari, M.L., Bani, P., Bayat, A., et al., 2014. Effect of DNA extraction and sample preservation method on rumen bacterial population. Anaerobe 29, 80. https://doi.org/10.1016/j.anaerobe.2013.09.015, 4.

Forouzandeh, A., Blavi, L., Abdelli, N., Melo-Duran, D., Vidal, A., Rodriguez, M., et al., 2021. Effects of dicopper oxide and copper sulfate on growth performance and gut microbiota in broilers. Poult. Sci. 100, 101224. https://doi.org/10.1016/j.psj.2021.101224.

Frantz, A., Pottier, M.A., Karimi, B., Corbel, H., Aubry, E., Haussy, C., Gasparini, J., Castrec-Rouelle, M., 2012. Contrasting levels of heavy metals in the feathers of urban pigeons from close habitats suggest limited movements at a restricted scale. J Envir Poll. https://doi.org/10.1016/J.ENVPOL.2012.04.003.

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., et al., 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469, 543–547. https://doi.org/10.1038/nature09646.

Garcia-Amado, M.A., Shin, H., Sanz, V., et al., 2018. Comparison of gizzard and intestinal microbiota of wild neotropical birds. PLoS One 133, e0194857. https://doi.org/10.1371/journal.pone.0194857.

Grond, K., Sandercock, B.K., Jumpponen, A., Zeglin, L.H., 2018. The avian gut microbiota: community, physiology and function in wild birds. Journal Avian Biol 49 (11), e01788. https://doi.org/10.1111/jav.01788.

Grond, K., Perreau, J.M., Loo, W.T., Spring, A.J., Cavanaugh, C.M., et al., 2019. Longitudinal microbiome profiling reveals impermanence of probiotic bacteria in domestic pigeons. PLoS One 14 (6), e0217804. https://doi.org/10.1371/journal.pone.0217804.

Heijtz, R.D., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., Pettersson, S., Eijtz, R., 2011. Normal gut microbiota modulates brain development and behavior. Proc Nat Acad Sciences. https://doi.org/10.1073/pnas.1010529108.

Janiga, M., Sedlárová, A., Rigg, R., et al., 2007. Patterns of prevalence among bacterial communities of alpine accentors (*Prunella collaris*) in the Tatra Mountains. J. Ornithol. 148, 135–143. https://doi.org/10.1007/s10336-006-0104-2.

Jeantet, A., Sandmeyer, L., Campech, C., et al., 2023. The "parasite detoxification hypothesis": lead exposure potentially changes the ecological interaction from parasitism to mutualism. Ecotoxicology 32, 666–673. https://doi.org/10.1007/s10646-023-02678-z.

Knutie, S.A., Gotanda, K.M., 2018. A non-invasive method to collect fecal samples from wild birds for microbiome studies. Microb. Ecol. 76, 851–855. https://doi.org/10.1007/s00248-018-1182-4.

Komarnicki, G.J.K., 2000. Tissue, sex and age specific accumulation of heavy metals (Zn, cu, Pb, cd) by populations of the mole (*Talpa europaea* L.) in a central urban area. Chemosphere 41, 1593–1602. https://doi.org/10.1016/S0045-6535(00)00018-7.

Lee, S.-J., Cho, S., La, T.-M., Lee, H.-J., Lee, J.-B., Park, S.-Y., et al., 2020. Comparison of microbiota in the cloaca, colon, and magnum of layer chicken. PLoS One 15 (8), e0237108. https://doi.org/10.1371/journal.pone.0237108.

Levkovich, T., Poutahidis, T., Smillie, C., Varian, B.J., Ibrahim, Y.M., Lakritz, J.R., et al., 2013. Probiotic bacteria induce a 'glow of health'. PLoS One 8 (1), e53867. https://doi.org/10.1371/journal.pone.0053867.

Lewis, S., Maslin, M., 2015. Defining the anthropocene. Nature 519, 171–180. https://doi.org/10.1038/nature14258.

Li, H., Qu, J., Li, T., Li, J., Lin, Q., Li, X., 2016. Pika population density is associated with the composition and diversity of gut microbiota. Frontiers Microbiol. https://doi.org/10.3389/fmicb.2016.00758.

Li, Z., Wang, W., Liu, D., Guo, Y., 2017. Effects of *Lactobacillus acidophilus* on gut microbiota composition in broilers challenged with *Clostridium perfringens*. PLoS One 12 (11), e0188634. https://doi.org/10.1371/journal.pone.0188634.

Li, M., Qin, Y., Wang, C., Wang, K., Deng, Z., Xu, W., Xiang, P., Ma, L.Q., 2021. Total and bioaccessible heavy metals in cabbage from major producing cities in Southwest China: health risk assessment and cytotoxicity. Royal Soc. Chem. Adv. 11, 12306–12314. https://doi.org/10.1039/D1RA01440D.

Li, M., Wang, F., Wang, J., Wang, A., Yao, X., Strappe, P., et al., 2022. Starch acylation of different short-chain fatty acids and its corresponding influence on gut microbiome and diabetic indexes. Food Chem. 389, 133089. https://doi.org/10.1016/j.foodchem.2022.133089.

Liao, J., Liu, Y., Yi, J., Li, Y., Li, Q., Li, Y., et al., 2022. Gut microbiota disturbance exaggerates battery wastewater-induced hepatotoxicity through a gut-liver axis. Sci. Total Environ. 809, 152188. https://doi.org/10.1016/j.scitotenv.2021.152188.

Lin, T., Coppack, L., Lin, Q.X., Kulemeyer, C., Schmidt, A., Behm, H., Luo, T., 2012. Does avian flight initiation distance indicate tolerance towards urban disturbance? Ecol Indicators 15 (1), 30–35. https://doi.org/10.1016/j.ecolind.2011.09.018.

Linnenbrink, M., Wang, J., Hardouin, E.A., Künzel, S., Metzler, D., Baines, J.F., 2013. The role of biogeography in shaping diversity of the intestinal microbiota in house mice. Mol. Ecol. 22, 1904–1916. https://doi.org/10.1111/mec.12206.

Liu, S., Luo, H., Wang, M., Wang, Q., Duan, L., Han, Q., Sun, S., Wei, C., Jin, J., 2022. Microbiome analysis reveals the effects of black soldier fly oil on gut microbiota in pigeon. Front. Microbiol. 13, 998524. https://doi.org/10.3389/fmicb.2022.998524.

Maurice, C.F., Knowles, C.S., Ladau, J., Pollard, K.S., Fenton, A., Pedersen, A.B., et al., 2015. Marked seasonal variation in the wild mouse gut microbiota. ISME J. 9, 2423–2434. https://doi.org/10.1038/ismej.2015.53.

McCormack, U.M., Curiao, T., Buzoianu, S.G., Prieto, M.L., Ryan, T., Varley, P., et al., 2017. Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. Appl. Environ. Microbiol. 83, 15. https://doi.org/10.1128/AEM.00380-17.

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.

Molbert, N., Alliot, F., Leroux-Coyau, M., Médoc, V., Biard, C., Meylan, S., Jacquin, L., Santos, R., Goutte, A., 2020. Potential benefits of acanthocephalan parasites for chub hosts in polluted environments. Env Science Tech 54, 5540–5549. https://doi.org/10.1021/acs.est.0c00177.

Peig, J., Green, A.J., 2010. The paradigm of body condition : a critical reappraisal of current methods based on mass and length : the paradigm of body condition. Func Ecol. 6, 1323–1332. https://doi/10.1111/j.1365-2435.2010.01751.x.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glockner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucl. Acids Res. 35 (21), 7188–7196. https://doi.org/10.1093/nar/gkm864.

Ramakrishna, B.S., 2013. Role of the gut microbiota in human nutrition and metabolism: role of the gut microbiota. Jour Gastr Hepatol 28, 9–17. https://doi/10.1111/jgh.12294.

Reid, C., McInnes, K., McLelland, J.M., Gartrell, B.D., 2012. Anthropogenic lead (Pb) exposure in populations of a wild parrot (kea Nestor notabilis). New Zealand J. Ecology 36, 56–63.

Rizwan, A.M., Leung, Y.C., Dennis, L.Y.C., Chunho Liu, C., 2008. A review on the generation, determination and mitigation of Urban Heat Island. Journal Env Sciences 1, 120–128. https://doi.org/10.1016/S1001-0742(08)60019-4.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. Vsearch : a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi/10.7717/peerj.2584.

Round, J.L., Mazmanian, S.K., 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9, 313–323. https://doi.org/10.1038/nri2515.

Sánchez-Chardi, A., Peñarroja-Matutano, C., Borrás, M., Nadal, J., 2009. Bioaccumulation of metals and effects of a landfill in small mammals Part III: structural alterations. Environ. Res. 109, 960–967. https://doi.org/10.1016/j.envres.2009.08.004.

Shochat, E., Paige, S., Stanley, W., Faeth, H., 2006. Future directions in urban ecology. Trends Ecol. Evol. 12, 661–662. https://doi.org/10.1016/j.tree.2006.09.007.

Sison-Mangus, M.P., Mushegian, A.A., Ebert, D., 2015. Water fleas require microbiota for survival, growth and reproduction. ISME J. 9, 59–67. https://doi.org/10.1038/ismej.2014.116.

Stojanov, S., Berlec, A., Štrukelj, B., 2020. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms 8, 1715.

Sullam, K.E., Essinger, S.D., Lozupone, C.A., O'Connor, M.P., Rosen, G.L., et al., 2012. Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Mol. Ecol. 21, 3363–3378. https://doi.org/10.1111/j.1365-294X.2012.05552.x.

Teyssier, A., Rouffaer, L.O., Hudin, N.S., 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. Sci Total Env 612, 1276–1286. https://doi.org/10.1016/j.scitotenv.2017.09.035.

Valeriano, V.D.V., Balolong, M.P., Kang, D.K., 2017. Probiotic roles of Lactobacillus sp in swine: insights from gutmicrobiota. J. Appl. Microbiol. 122 (3), 554–567. https://doi.org/10.1111/jam.13364.

Valverde, I., Defosseux, I., Le Bot, T., Jouvion, G., Le Barzic, C., Arné, P., Gasparini, J., 2024. Effect of urbanization on the trace element concentrations in the kidney, liver and spines of the European hedgehog (*Erinaceus europaeus*). Sci Total Env 914, 169944. https://doi.org/10.1016/j.scitotenv.2024.169944.

Waite, D.W., Taylor, M.W., 2015. Exploring the avian gut microbiota : current trends and future directions. Front. Microbiol. 3, 666–673. https://doi.org/10.3389/fmicb.2015.00673.

Wang, B., Zhong, H., Liu, Y., Ruan, L., Kong, Z., Mou, X., Wu, L., 2022. Diet drives the gut microbiome composition and assembly processes in winter migratory birds in the Poyang Lake wetland, China. Front. Microbiol. 13, 973469. https://doi.org/10.3389/fmicb.2022.973469.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. ISBN 978-3-319-24277-4.

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S.A., et al., 2011. Linking long-term dietary patterns with gut microbial enterotypes. Science 334, 105–108. https://doi.org/10.1126/science.1208344.

Yang, Y., Du, L., Shi, D., Kong, C., Liu, J., Liu, G., Li, X., Ma, Y., 2021. Dysbiosis of human gut microbiome in young-onset colorectal cancer (2021. Nat. Commun. 12, 6757. https://doi.org/10.1038/s41467-021-27112-y.

Zhai, Q., Li, Z., Yu, L., Xiao, Y., Feng, S., Wu, J., Zhao, J., et al., 2017. Effects of subchronic oral toxic metal exposure on the intestinal microbiota of mice. Sci Bulletin 62, 831–840. https://doi.org/10.1016/j.scib.2017.01.031.

Zhang, L., Jiang, X., Li, A., Waqas, M., Gao, X., Li, K., et al., 2020. Characterization of the microbial community structure in intestinal segments of yak (*Bos grunniens*). Anaerobe 61, 102115. https://doi.org/10.1016/j.anaerobe.2019.102115.