

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Bioaccumulation of trace elements affects chick body condition and gut microbiome in greater flamingos

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Gillingham, M.A.F., Borghesi, F., Montero, B.K., Migani, F., Bechet, A., Rendon-Martos, M., et al. (2021). Bioaccumulation of trace elements affects chick body condition and gut microbiome in greater flamingos. SCIENCE OF THE TOTAL ENVIRONMENT, 761, 1-17 [10.1016/j.scitotenv.2020.143250].

Availability:

This version is available at: <https://hdl.handle.net/11585/838413> since: 2023-02-10

Published:

DOI: <http://doi.org/10.1016/j.scitotenv.2020.143250>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

Bioaccumulation of trace elements affects chick body condition and gut microbiome in greater flamingos

Mark A.F. Gillingham^{a, *}, Fabrizio Borghesi^b, B.Karina Montero^{a, c}, Francesca Migani^d,
Arnaud Béchet^e, Manuel Rendón-Martos^f, Juan A. Amat^g, Enrico Dinelli^b, Simone
Sommer^a

^a University of Ulm, Institute of Evolutionary Ecology and Conservation Genomics, Albert-Einstein
Allee 11, D-89069 Ulm, Germany

^b Bologna University, Department of Biological, Geological and Environmental Sciences (BiGeA),
Operative Unit of Ravenna, Via Sant'Alberto, 163, 48123 Ravenna, Italy

^c Animal Ecology and Conservation, Hamburg University, Hamburg, Germany

^d Via Fenaria Vecchia, 99, 48123 Ravenna, Italy

^e Institut de Recherche de la Tour du Valat, Le Sambuc, 13200 Arles, France

^f R.N. Laguna de Fuente de Piedra, Consejería de Medio Ambiente y Ordenación del Territorio, Junta
de Andalucía, Apartado 1, E-29520 Fuente de Piedra, Málaga, Spain ^g Department of Wetland
Ecology, Estación Biológica de Doñana, (EBD-CSIC), calle Américo Vespucio s/n, E-41092 Sevilla,
Spain

* Corresponding author at: University of Ulm, Institute of Evolutionary Ecology, and Conservation
Genomics, Albert-Einstein Allee 11, D-89081 Ulm, Germany.

E-mail addresses: mark.gillingham@uni-ulm.de (M.A.F. Gillingham), karina.montero@uni-
hamburg.de (B.K. Montero), bechet@tourduvalat.org (A. Béchet),
manuel.rendon@juntadeandalucia.es (M. Rendón-Martos), amat@ebd.csic.es (J.A. Amat),
enrico.dinelli@unibo.it (E. Dinelli), simone.sommer@uni-ulm.de (S. Sommer).

27

28 Keywords:

29 Environmental pollution, Trace elements, Heavy metals, Chick body condition Gut
30 microbiome, Feather bioaccumulation, Greater flamingos

31

32

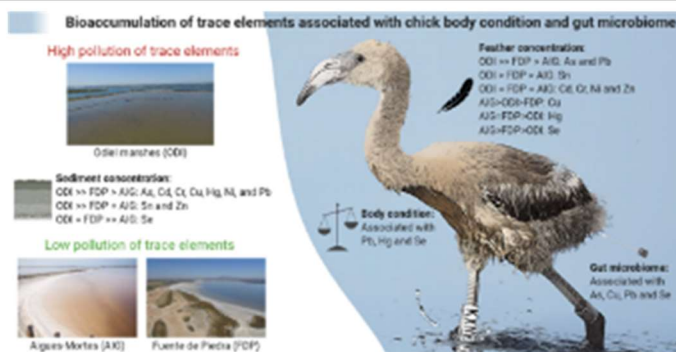
33 HIGHLIGHTS

- 34 • Sediment exposure results in bioaccu-mulation of As and Pb in flamingo
35 feathers.
- 36 • Feather bioaccumulation of Cu, Hg, Se, Sn and Zn likely reflects diet exposure.
- 37 • Feather bioaccumulation of Hg, Pb, and Se affects chick body condition.
- 38 • Feather bioaccumulation of As, Cu, Se and Pb affects microbiome composition.
- 39 • Pb and Se bioaccumulation is of particu-lar ecotoxicological concern.

40

41

42 GRAPHICAL ABSTRACT



43

44

45

46

47 **Abstract**

48 Elevated concentrations of trace elements represent a major concern to wetland
49 ecosystems, since river estuaries are geochemical endpoints that accumulate pollution.
50 Although the negative impact of environmental exposure of highly toxic elements such
51 as Pb and Hg has received substantial attention, we still lack a comprehensive
52 understanding of the effects that these and other common trace elements have on
53 natural populations. We used greater flamingos as a study system within three sites that
54 represent a gradient of pollution. Controlling for environmental sediment exposure, we
55 assessed if signatures of bioaccumulation in feathers for ten trace elements (As, Cd,
56 Cr, Cu, Hg, Ni, Pb, Se, Sn and Zn) are associated with two known proxies of health:
57 body condition and the gut bacterial microbiome. We found evidence of an adverse
58 effect of Se, Hg, and Pb bioaccumulation on body condition. Furthermore,
59 bioaccumulation of the elements As, Cu, Se, Pb and Zn influenced different aspects of
60 the gut microbiome. Bioaccumulation of Se led to a shift in the microbiome
61 composition, largely driven by an enrichment of *Bacteroides plebeius*, which is linked
62 to the breakdown of sulphated polysaccharides of algae. *Bacteroides plebeius* was
63 negatively associated with chick body condition, suggesting an adverse effect of a
64 microalgae diet rich in Se. Pb bioaccumulation was linked with a decrease in microbial
65 diversity (adjusted- $R^2 = 10.4\%$) and an increase in heterogeneity of the microbial
66 community (adjusted- $R^2 = 10.5\%$), an indication of impaired gut homeostasis. As, Cu
67 and Zn had more nuanced effects on gut microbiome heterogeneity according to
68 breeding site and bioaccumulation concentration. Our results therefore suggest that in
69 addition to well-studied elements, bioaccumulation of poorly studied elements also
70 adversely affect health of natural populations.

71

1. Introduction

Most trace elements, including metals, occur naturally in the environment and are essential for growth, development and physiology, to both micro- and macro-organisms. However, since the industrial revolution, anthropogenic activities have dramatically increased the levels of trace elements in the natural environment (Nriagu, 1989, 1996). As a result of limited microbial degradation on soil surfaces, anthropogenic sources of trace elements tend to accumulate and persist for long periods of time on the top layer of soil and sediment (Kirpichtchikova et al., 2006). In such conditions, toxic concentration levels are often prone to be taken up by organisms and bioaccumulated because they are normally not firmly bonded to inert materials (Bryan et al., 1979; Di Giuseppe et al., 2014; Migani et al., 2015). The detrimental effects of exposure to toxic levels of non-essential metals through the diet on organismal fitness are widely recognized (Järup, 2003; Nriagu, 1989, 1996). For instance, body condition, a key predictor of health and fitness (Peig and Green, 2009, 2010), has been found to be adversely associated with bioaccumulation of heavy metals, such as As, Cd, Cu, Hg, Pb and Zn in birds (e.g. Debacker et al., 2000; Janssens et al., 2003; Takekawa et al., 2002). However, less attention has been given to the potential adverse effects of constitutive and essential elements that can be toxic at the high exposure levels recorded at polluted sites, particularly for natural populations.

Aside from body condition, another key proxy of health and fitness in multicellular organisms is the gut microbiome community (Gould et al., 2018; Shin et al., 2011). The tight interaction between the microbial flora inhabiting the gastrointestinal tract and the host is crucial in facilitating nutrient intake, gut development, immune system maturation and resistance against enteric pathogens (Delsuc et al., 2014; Ley et al., 2008a; Ley et al., 2008b; Phillips et al., 2012; Shin et al., 2011; Stecher and Hardt, 2008). The gut microbiota may also play an important role in protecting the host against exposure to toxic levels of metals by regulating bioavailability in a number of ways. First, the microbiome may act as a physical barrier limiting absorption of trace elements via microbial-host interactions that involve epithelial junctions and physical

101 impediments of the mucous layer (Breton et al., 2013a; Ninkov et al., 2015). Second,
102 the microbiota may passively bind to, absorb and metabolize toxins and trace elements
103 (Halttunen et al., 2008; Morozzi et al., 1986). Finally, the metabolic interaction
104 between the microbiota and the host may regulate the pH, oxidative balance,
105 detoxification enzymes, and xenobiotic-metabolizing and transporting host proteins
106 within the gut (Claus et al., 2011), which may impact host absorption rates of trace
107 elements (Breton et al., 2013a; Ninkov et al., 2015).

108 Given the indiscriminate nature of high exposure to toxic levels of elements, which
109 will harm beneficial microbes in the gut, toxic exposure is likely to disrupt the
110 homeostasis of the gut microbiome (Assefa and Köhler, 2020; Claus et al., 2016;
111 Rosenfeld, 2017). Most of our current understanding of the impact of toxic trace
112 elements on the microbiome and gastrointestinal diseases comes from experiments
113 using laboratory rodents (e.g. Breton et al., 2013a, 2013b; Fazeli et al., 2011; Gao et
114 al., 2017; Liu et al., 2014; Ninkov et al., 2015; Ruan et al., 2018; Tinkov et al., 2018).
115 However, gut microbiomes of laboratory animals are rarely representative of natural
116 microbiota (Bolnick et al., 2014; Chandler et al., 2011) and few studies have
117 investigated the impact of toxic trace elements on the microbiome of natural
118 populations (but see: Coolon et al., 2010; Zhang et al., 2016 that compare gut
119 microbiomes between polluted and unpolluted sites).

120 Birds are regularly used as bioindicators for various pollutants, including metals
121 (Furness and Greenwood, 1993). If careful methodological techniques are applied to
122 avoid falling into known pitfalls, such as external contamination, feather analysis can
123 be an effective non-invasive method to assess bioaccumulation for some trace elements
124 (Borghesi et al., 2011, 2016, 2017; Burger, 1993; Jaspers et al., 2019; Smith et al.,
125 2003; Tsipoura et al., 2008). An important benefit of feathers relative to blood samples,
126 is that metal accumulation represents a longer-term contamination process rather than
127 a recent contamination directly associated with a feeding bout (Carvalho et al., 2013).
128 Waterbirds are of particular interest since they inhabit wetland and river estuaries
129 which are particularly vulnerable to pollution because they are geochemical endpoints

130 of large river systems that often flow near or through cities, roads, factories, industries,
131 cultivated lands, and/or mines.

132 Greater flamingos (*Phoenicopterus roseus*) regularly breed within Odiel marshes,
133 which is located at the mouth of the Odiel and Tinto rivers, both of which have been
134 strongly affected by acid mine drainage from long term mining activities (Guillén et
135 al., 2011; Nieto et al., 2007). The latter combined with natural mineralisation of
136 massive sulphurs and waste from industrial activities (fertiliser factories and cop-per
137 foundries) makes the Odiel marshes one of the most polluted sites in trace elements in
138 the world (Borrego et al., 2002; Guillén et al., 2011; Nieto et al., 2007). Nevertheless,
139 the Odiel marshes is known to be a feeding site of adult greater flamingos breeding at
140 a nearby colony (~200 km) at the Fuente Piedra lagoon, one of the largest breeding
141 colony in the western Mediterranean (Amat et al., 2005). An essential com-ponent of
142 the greater flamingo diet, brine shrimp (*Artemia* spp.), represents a main route through
143 which toxic trace element exposure can enter the food web in Odiel marshes
144 (Rodríguez-Estival et al., 2019). As filter feeders greater flamingos also regularly ingest
145 large quantities of sediment (Jenkin, 1957), which directly exposes them to polluted
146 sediment. Greater flamingo chicks may be exposed to pollutants via crop milk fed from
147 adults which, along with proteins, fat, carotenoids and blood cells, may contain
148 pollutants (Fisher, 1972; Lang, 1963; Rendón et al., 2011). Furthermore, chicks are
149 further exposed to local environmental elements when from the age of three weeks,
150 they leave the nest and form a large crèche within the wetland surrounding the breeding
151 island (Johnson and Cézilly, 2007). Greater flamingo chicks therefore provide an ideal
152 model to investigate trace element bioaccumulation (using feather samples).

153 Apart from Hg (Borghesi et al., 2011), to date, little is known about the relationship
154 between environmental trace element expo-sure and bioaccumulation in greater
155 flamingos (Borghesi et al., 2016) and its potential effect on health. To address this issue,
156 we first evaluated differences in environmental (sediment) exposure to 10 trace
157 elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sn and Zn) across three breeding colonies in
158 the Mediterranean which are thought to represent a gradient in environmental pollution.

159 Second, by taking advantage of a new method to assess trace element bioaccumulation
160 in feathers (Borghesi et al., 2017), we assessed the relationship between variation in
161 environmental exposure and feather bioaccumulation between elements across
162 breeding sites. Finally we investigated the relationship between feather
163 bioaccumulation and two proxies of health: chick body condition, a known predictor
164 of dispersal in greater flamingos (Barbraud et al., 2003), and the gut microbiome which
165 has recently been described in greater flamingos (Gillingham et al., 2019).

166

167 **2. Methods**

168 ***2.1. Sampling sites***

169 Greater flamingos colonially breed in a few relatively unstable breeding sites in the
170 Mediterranean. Colony breeding success depends on favorable climatic conditions and
171 local water levels (Béchet et al., 2009a, 2012; Béchet and Johnson, 2008; Cézilly et al.,
172 1995; Johnson and Cézilly, 2007). In 2014, a greater flamingo colony established itself
173 in the salt pan of Aigues-Mortes in southern France (AIG; N 43° 33', E 4° 11'; Fig. 1)
174 due to unfavourable conditions in their more habitual site in the Fangassier lagoon,
175 located in the Rhône river delta in the Camargue region (Gillingham et al., 2019). The
176 second colony sampled was at the Fuente de Piedra lagoon (FDP; 37° 06'N; 04° 45'W;
177 Fig. 1), the largest breeding colony in the western Mediterranean (Balkız et al., 2010;
178 Rendón et al., 2001). AIG and FDP are thought to be relatively unpolluted sites and
179 were thus considered control sites in this study (Borghesi et al., 2017). Finally the
180 heavily polluted site investigated in this study is Odiel marshes, where a colony settled
181 and successfully produced chicks from 2008 onwards (ODI, 37° 17' N; 06° 55' W; Fig.
182 1).

183

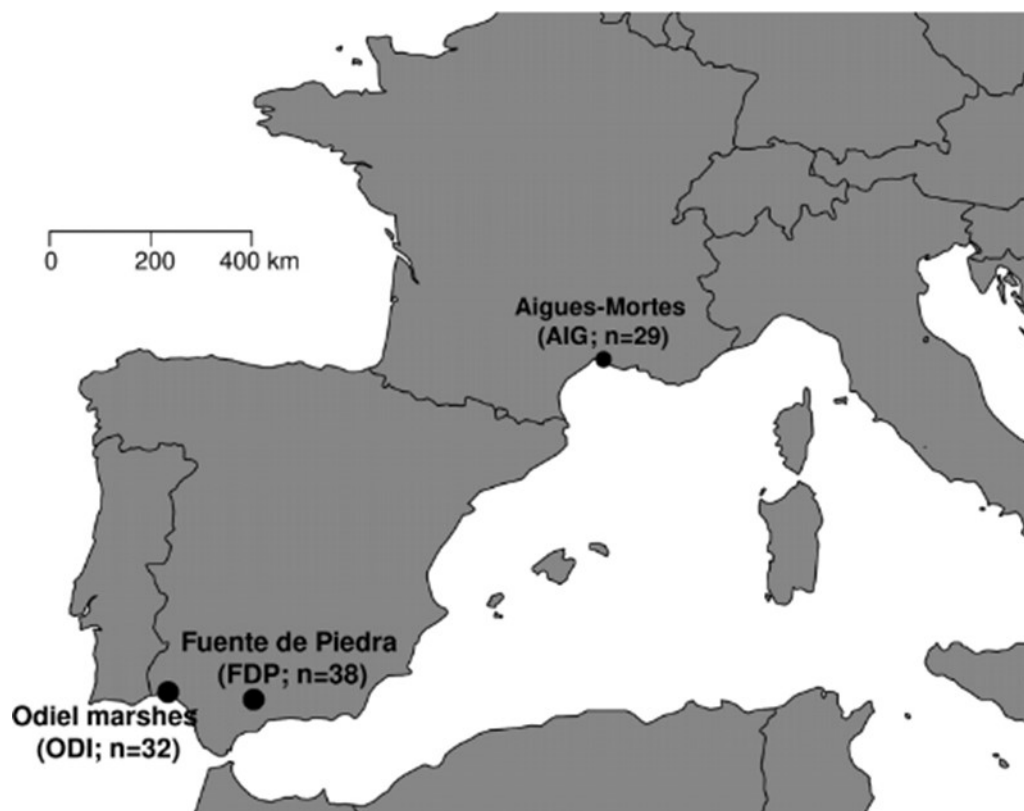


Fig. 1. Location of the sampling sites at the breeding colonies of the Greater flamingo. Sample size is indicated within

2.2. Sample collection

All samples (feather, sediment and swabs) were collected in 2014 during ringing operations (Borghesi et al., 2017; Gillingham et al., 2019; Johnson and Cézilly, 2007). Chicks were flightless and two to four months old at the time of sampling. Since greater flamingos are colonial breeders and chicks were forming a large crèche at the time of sampling, all chicks were captured and sampled at the same location within each breeding site.

We used trace element data (As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sn and Zn) of feathers and sediments that were collected and analysed following the new method to reliably interpret bioaccumulation in feathers by controlling for external environmental contamination (Borghesi et al., 2017). Thus, we used 119 chick feathers (AIG, n = 39; FDP, n = 40; ODI, n = 40) and 21 sediment samples (AIG, n = 7; FDP, n = 7; ODI, n =

200 7) that were collected in the three breeding colonies in the western Mediterranean (Fig.
201 1).

202 The gut microbiome dataset is a subset from our previous study which characterized
203 the gut microbiome of chicks in nine Mediterranean breeding sites (Gillingham et al.,
204 2019; but see below for molecular methods. We sexed birds using the CHD gene
205 sequence polymorphism as described by Balkız et al. (2007). For our analyses of the
206 effect of trace elements on chick body condition and gut microbiome, we used 99
207 samples for which we have trace element bioaccumulation data, gut microbiome, body
208 condition and sex data (Fig. 1; AIG, n = 29; FDP, n = 38; ODI, n = 32). We also included
209 the microbiome analysis of the 21 sediment samples, of which 20 (AIG, n = 6; FDP, n
210 = 7; ODI, n = 7) were retained after molecular and bioinformatic processing (see
211 molecular methods below), to investigate the relative contribution of the sediment
212 microbiome to the gut microbiome of chicks.

213

214 ***2.3. Trace element analyses for sediments and feather samples***

215 Methods for trace element analyses are identical to Borghesi et al. (2017). For each
216 individual, we collected ten feathers by cutting the distal part from random individuals
217 using stainless steel scissors. We selected the longest internal scapulars that were
218 protected from aerial deposition (Borghesi et al., 2011, 2016, 2017). We analysed 14
219 elements Al, As, Cd, Cr, Cu, Fe, Hg, K, La, Ni, Pb, Se, Sn and Zn. We removed the
220 vanes of the feathers from the rachides and we pooled five rachides per sample for
221 analysis. All 595 rachides from 119 samples were sequentially washed in three steps
222 using acetone, Triton XTM (Rohm and Haas, Philadelphia, PA, USA) detergent and
223 deionized water. During each step, washing sonication was performed for 20 min. After
224 washing, feathers were dried in a dry box at room temperature. All samples
225 (approximately 0.100 g from each sample) were digested and analysed at the Trace
226 Element Analysis Core Laboratory of Dartmouth College, Hanover, NH, USA.
227 Because Al, Fe, K and La concentration in feathers are almost exclusively due to

external contamination (Borghesi et al., 2017), only the remaining 10 bioaccumulated elements corrected for external contamination were analysed in this study.

To assess environmental exposure, we also collected seven sediment samples of 200–500 g at each sampling site within and on the shore of the water body where the breeding islet was situated. All sediment samples were kept in plastic containers in dry room temperature conditions prior to analysis. Digestion and trace analysis of sediment samples was carried out by ACME Labs, Vancouver (Canada).

Since external environmental contamination is mainly due to particles from the sediment, Borghesi et al.'s (2017) method enabled us to infer what the predicted concentration of feather elements would be if external contamination was 100% for each element. From this predicted concentration, we could then estimate the concentration of the trace element that is due to external contamination and corrected our estimates using simple ratios and a reference element known to not be bioaccumulated (Borghesi et al., 2017). Here, we chose Fe as the reference element for external contamination because it is well recovered by the methods applied in our study, it is reported to be only negligibly bioaccumulated in shafts of seabirds (Howell et al., 2012) and it represents a wide gamma of compounds in sediments (Borghesi et al., 2017).

2.4. Estimating chick body condition

Chick body condition was estimated using the scaled mass index (SMI) proposed by Peig and Green (2009):

$$M_i = M_i |T_0/T_i|^{bSMA}$$

where M_i and T_i are the mass and structural size indicator of individual i respectively, T_0 is the population mean of the structural size indicator and $bSMA$ is the slope of an standardized major axis (SMA) regression between the log of M_i and the log of T_i . Chick tarsus length was used as a proxy for body size since it is a good indicator of

255 structural body size in tall lengthy greater flamingos (Amat et al., 2007; Béchet and
256 Johnson, 2008; Cézilly et al., 1995; Gillingham et al., 2012, 2013; Yohannes et al.,
257 2014). The SMA slope between log transformed mass and log trans-formed tarsus
258 length was used as the scaling factor (SMA: p-value <0.001; n = 352; R² = 0.666; β
259 [95%CI] = 2.269 [2.135; 2.411]; we used the larger dataset from Gillingham et al.
260 (2019) to obtain more precise SMA estimates). The allometric relationship between
261 mass and tarsus did not differ significantly between breeding sites (SMA: p-value =
262 0.714; n = 352) and sexes (SMA: p-value = 0.454; n = 340), thus justifying the pooling
263 of data across sites and gender when estimating body condition.

264

265 ***2.5. DNA extraction of swabs, PCR amplification, library preparation, and high-*** 266 ***throughput sequencing***

267 Cloacal and sediment swabs were collected using forensic swabs (Sarstedt,
268 Nuembrecht, Germany) that were placed in 2 ml Eppendorf tubes with 600 μ l of
269 RNeasy®. All samples were stored at -20 °C within 48 h of sampling until DNA
270 extraction was performed. DNA extraction, PCR amplification, library preparation and
271 high through-put sequencing methods are identical to Gillingham et al. (2019). Briefly,
272 we amplified a 291-bp fragment of the hypervariable V4 region of the 16S rRNA gene
273 using the universal bacterial primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and
274 806R (5'-GGAC TACHVGGGTWTCTAAT-3') (Caporaso et al., 2011). Individual
275 PCR reactions were tagged with a 10-base pair identifier, using a standardized Fluidigm
276 protocol (Access Array™ System for Illumina Sequencing Systems, ©Fluidigm
277 Corporation). Amplifications were quantified by UV/VIS spectroscopy on the Xpose
278 (Trinean, Gentbrugge, Belgium) and samples were pooled to equimolar amounts of
279 DNA. The library was prepared as recommended by Illumina (Miseq System Denature
280 and Dilute Libraries Guide 15039740 v05) and was loaded at 7.5 pM on a MiSeq flow
281 cell with a 10% PhiX spike. Paired-end sequencing was performed over 2×251 cycles.
282 We ran our 814 cloacal swab samples (of which only 99 samples are used in this study),

283 21 sediment samples, 41 ex-traction blanks and 44 PCR blanks (a total of 85 extraction
284 and PCR controls) across five sequencing runs.

285

286

287 **2.6. Bioinformatics**

288 We used DADA2 (Callahan et al., 2016) with nQIIME2 ([https:// qiime2.org](https://qiime2.org); Bolyen
289 et al., 2018) to denoise our data from artifacts (including chimeras), merge paired-end
290 sequences and remove primer sequences. Any ASV with fewer than 20 sequences in
291 the entire dataset were filtered out of the dataset and we excluded samples with fewer
292 than 10,000 sequences. We assigned ASVs to taxonomy within QIIME 2 using the
293 Greengenes database (DeSantis et al., 2006). We excluded sequences that did not assign
294 to any known bacterial lineage at the phylum taxonomic level. We used FastTree 2.1.8
295 (Price et al., 2010) to build a tree with an archaea sequence (Accession number:
296 KU656649) used to root the tree. The branch and taxonomic name of the root archaea
297 sequence was removed prior to analysis. The R package “phyloseq” (McMurdie and
298 Holmes, 2013) was used to import and analyse the data into R version 3.6.3 (R Core
299 Team, 2020). As in Gillingham et al. (2019), sequences that were found with a relative
300 abundance of at least 1% across extraction and PCR blanks were considered to be
301 contaminant sequences and were excluded from the dataset.

302

303 **2.7. Statistics**

304 Statistical analyses aim at evaluating three main questions: first we tested for evidence
305 of bioaccumulation of trace elements across site, second we tested for the influence of
306 bioaccumulation on body condition and third we investigated the effect of
307 bioaccumulation on microbiome diversity, composition and stochasticity. For all
308 regression type analyses, we followed recommended model validation steps, plotted
309 residuals against fitted values and checked for violation of model assumptions (Zuur et

310 al., 2009; Zuur and Ieno, 2016). Validation plots for regression type analyses can be
311 found in the supplementary materials next to Tables S1–S193.

312 We first analysed whether concentrations in trace elements of the abiotic environment
313 in the soil and bioaccumulated in feathers varied between sites. We analysed
314 differences between sites for each trace element using linear model regression (Cu and
315 Zn), general least square (GLS) regression if controlling for heterogeneity between
316 sites was necessary (As, Hg, Ni, Pb, Se and Sn) with the R “nlme” package (Pinheiro
317 et al., 2020) and using zero-inflated general linear models (GLM) if controlling for
318 zero inflation was necessary (Cd and Cr) with the R “pscl” package (Zeileis et al., 2008)
319 (Fig. 2). Since trace element bioaccumulation can be age and gender specific (Scheirs
320 et al., 2006), we first analysed the effects of site, tarsus length (the best proxy of age in
321 lengthy greater flamingo chicks), sex and the interaction between all terms ($n = 105$;
322 AIG, $n = 31$; FDP, $n = 39$; ODI, $n = 35$). Since the effects of age and sex (and associated
323 interactions) were never supported statistically, we reanalysed the data on the full
324 dataset ($n = 119$; AIG, $n = 39$; FDP, $n = 40$; ODI, $n = 40$) and reported the effect of site
325 only (but see supplementary materials Tables S182–S191 for full analyses of all
326 parameters). Since chicks were 2–4 months old, our results suggest that a difference in
327 age of two months was too short to generate differences in trace element
328 bioaccumulation as a result of variable exposure time.

329 For all subsequent analyses, because there is a lack of independence between breeding
330 sites and the trace elements (see Results section), we analysed the effect of trace
331 elements on the microbiome and chick body condition both across and within breeding
332 sites. Because of collinearity between trace elements within sites and to avoid over-
333 parameterizing models, we modelled the effect of each trace element separately with
334 the exception of Hg and Se which were also modelled together since both elements are
335 known to be antagonists and bioaccumulation of Se has previously been shown to
336 mitigate the negative effects of Hg (El-Begearmi et al., 1977). In addition, unless
337 otherwise stated, we used the information-theoretic approach to infer model selection
338 (Burnham and Anderson, 2002), whereby all possible hypotheses are modelled and the

weight of support for each hypothesis is assessed using the model's Akaike Information Criterion for small sample sizes (AICc), AICc weight and adjusted-R². For all analyses, we checked for non-linear effects using General Additive Models (GAM) using the “mgcv” package within R (Wood, 2017) and proceeded with GAMs if a non-linear relationship was found. Effect sizes for linear continuous and categorical variables were estimated using partial-r and Cohen's D respectively and 95% confidence intervals were estimated by bootstrap (n = 10,000) (Nakagawa and Cuthill, 2007). Full model selection tables are presented in the supplementary materials (supplementary Tables S1–S181).

We evaluated the effect of trace element, sex, breeding site and the interaction between breeding site and trace element on chick body condition using linear models. We subsequently analysed the relationship between trace elements on microbiome alpha diversity, microbiome beta diversity and microbiome differential abundance. In addition, we investigated the relative contribution of sediment microbiome to the gut microbiome of chicks. Finally, we predicted that specific ASVs that were differentially abundant according to element bioaccumulation would also be associated with metabolism and thus with body condition. Therefore for those ASVs that were found to be differentially abundant according to bioaccumulation of an element, we tested if they were associated with body condition.

For alpha diversity, we chose to measure the number of ASVs (i.e. unweighted observed species diversity) and Shannon index (i.e. an alpha diversity metric that is weighted for species abundance). We modelled the effect of trace element, sex, breeding site, sequencing depth and the interaction between breeding site and trace element on the number of ASVs and Shannon index using GLMs. A GLM with a negative binomial error distribution and Gamma distribution with a log link was used to model the number of ASVs and Shannon index, respectively.

There is increasing recognition of the importance of treating microbiome data as compositional data during data analyses (Martino et al., 2019; Quinn et al., 2018,

2019). Therefore, for beta diversity analyses we applied the Aitchison's log-ratio approach (Aitchison, 1982), which consists in transforming the data into centered log ratio (clr) and applying euclidean distance matrix for ordination analyses. While unconstrained ordination analyses are frequently used methods to explore variability in beta diversity of the microbiome, these methods do not allow the direct assessment of how different environmental variables contribute to observed patterns (Anderson and Willis, 2003; Shankar et al., 2017). We therefore applied a redundancy analysis (RDA) with a Euclidean distance matrix to investigate the relationship between beta diversity and the explanatory variables breeding site, sex, trace element concentration and the interaction between breeding site and trace element. To deal with positively skewed data due to sparse taxa and high zero inflation which may bias beta diversity analyses (Martino et al., 2019), we applied a high filter threshold whereby only taxa with prevalence of 0.32 were kept in the dataset (which was also the same cutoff threshold we used for differential abundance analyses described below). Applying such a stringent filtering step meant that all RDA residuals were very close to normal distribution. P-values and adjusted-R² were estimated by permutation (n = 99,999).

To investigate the effect of trace elements bioaccumulation on microbiome stochasticity/heterogeneity, we used the betadisper function with the R “vegan” package (Oksanen et al., 2019) using a Euclidean distance and using the median distance to centroid within site. We used a linear model to explore the effect of breeding site, sex, trace element concentration and the interaction between breeding site and trace element concentration on microbiome stochasticity.

To investigate the relative contribution of sediment microbiome exposure to chick gut microbiome composition, we analysed the percentage of gut microbiome ASVs that could be traced to sediment microbiome using the Bayesian method from the R package “SourceTracker” (Knights et al., 2011), as has previously been done in a study on Red-necked stints, *Calidris ruficollis* (Risely et al., 2017). SourceTracker makes use of the relative abundances in both the host and sediment sample to estimate the probability that the source of each ASV found in the gut is the sediment. In so doing,

the output of SourceTracker is an estimate of the proportions of ASVs in the host that can be traced to the local sediment for each sample. Since sediment microbiome is site specific, SourceTracker analyses were carried out within site. We report the median percent-age and 95% range (i.e. the 2.5% and 97.5% percentile) of ASVs that were traced to local sediment samples.

To investigate the effect of trace elements bioaccumulation on differential relative abundance we used an analysis of composition of microbiomes (ANCOM) (Mandal et al., 2015). Because of high variability in ASV relative abundance between sites, we modified the original ANCOM function to apply GLS models to control for heterogeneity between sites. We also controlled for the additive effect of site. The same prevalence threshold of 0.32 was used as for beta analyses since it was the cutoff threshold value at which GLS models converged. Furthermore high prevalence values are recommended to avoid high zero inflation and false positives (Mandal et al., 2015). ANCOM generates W scores, which is the number of rejected null sub-hypotheses of the log ratios of ASV_i/ASV_j according to the predictor. Thus, the higher the W score the stronger the evidence that the relative abundance of a specific ASV is associated with a given predictor. For each element, we plotted vol-cano plots of the ANCOM W score in relation to the estimates of a GLS controlling for the additive effect of site and using the same clr trans-formed dataset used for beta diversity. We only considered the relative abundance of a specific ASV to be differentially abundant according to a predictor if the W was above a threshold of 0.6 (60% of sub-hypotheses are rejected) and if the 95% confidence interval of GLS estimates (here-after referred to as differential logs) did not overlap 0.

In total, ANCOM revealed three ASVs of interest (see Results section), which were assigned taxonomically as members of Tissierellaceae, Porphyromonas and Bacteroides plebeius. The effects of the Tissierellaceae and Porphyromonas ASVs on chick body condition were analysed using linear models, whilst the effect of the Bacteroides plebeius ASV was analysed using a GLS controlling for heterogeneity across sites. The full models additionally include the fixed effects of site and sex.

425

426 **3. Results**

427 ***3.1. Trace element concentrations from the abiotic environment of sampling sites***

428

429 A gradient ODI >> FDP > AIG was evident for: As, Cd, Cr, Cu, Hg, Ni, and Pb, while
430 FDP and AIG show similar levels of Sn and Zn (>> ODI) (Fig. 2). The sole exception
431 of the above trends was Se which was similar in FDP and ODI, with AIG showing very
432 low concentrations (Fig. 2). ODI sediments were heavily polluted for some of the most
433 important elements of environmental concern, which include As (more than 30-fold
434 higher than the other two sites), Cu (20-fold FDP, 60-folds AIG), Hg (8-fold FDP, 16
435 fold AIG), Pb (6-fold FDP, 12-fold AIG), Zn (28-fold). Sn in AIG was highly variable,
436 because the presence of a warning peak of 7.0 mg/kg.

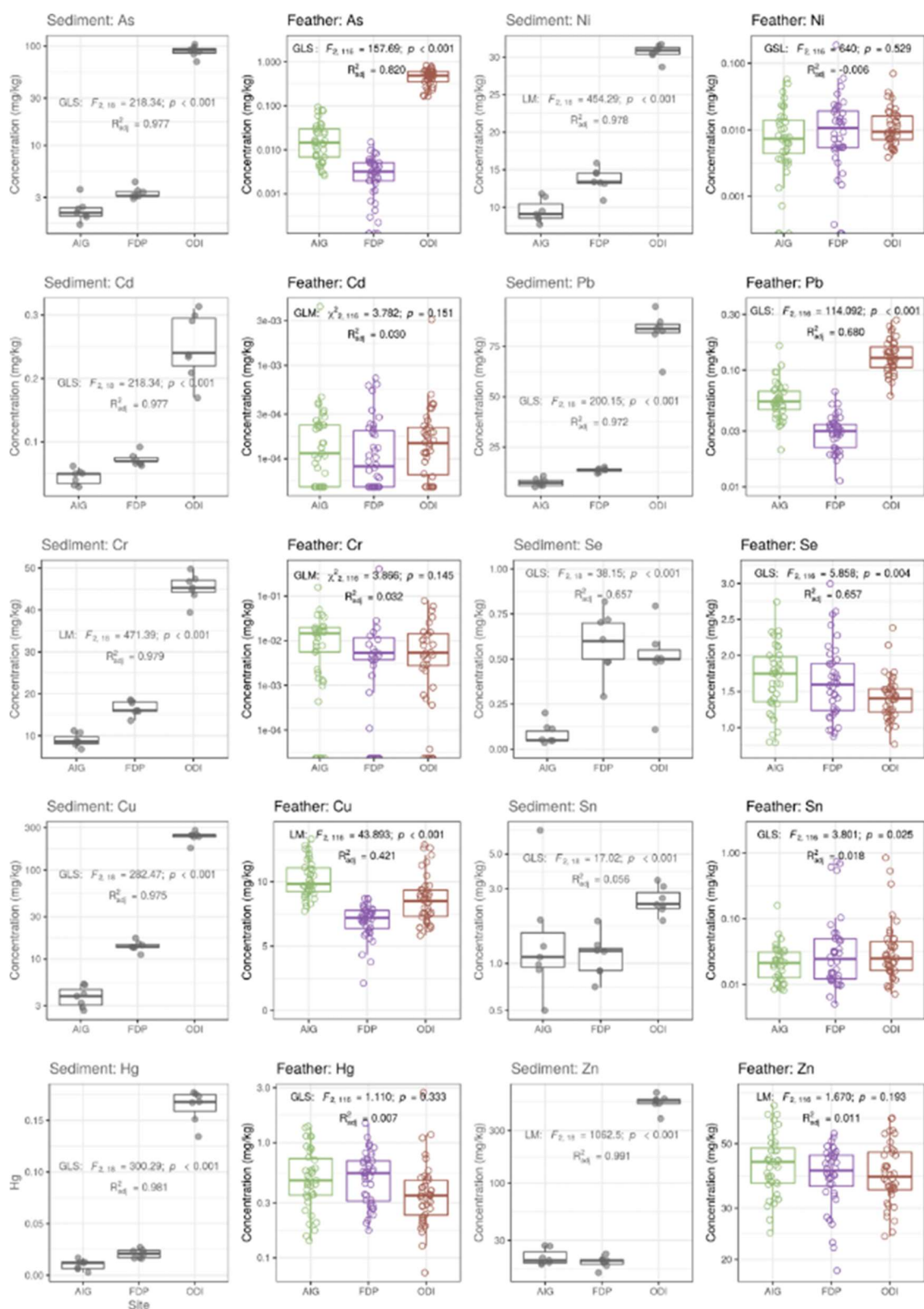
437

438 ***3.2. Variation in bioaccumulation of trace elements and between sites***

439 In feathers, there was a strong spike in concentration of As and Pb in ODI, which
440 mirrored abiotic environmental differences. In contrast to the abiotic environment, FDP
441 had lower concentrations for these elements than AIG (Fig. 2). In addition, there were
442 no significant differences for Cd, Cr, Hg, Ni and Zn between sites (Fig. 2). While
443 bioaccumulation varied significantly between sites for Cu, Se, and Sn, once again
444 differences did not mirror the patterns observed in the abiotic environment (Fig. 2).
445 The highest concentration of Cu was found in AIG, while the lowest was in FDP.
446 Selenium was significantly lower in ODI (Fig. 2). Although significant, differences
447 between sites in Sn concentration were weak with a gradient ODI > FDP > AIG (Fig.
448 2).

449 Across sites Pb and As were highly significantly correlated (Spearman rho = 0.82; p-
450 value < 0.001), however this correlation was mainly driven by collinearity between site
451 and element concentration since this correlation was no longer significant when

452 analysed within site (see supplementary material Figs. S1-S4). Arsenic was highly
453 correlated with Se within AIG (Spearman rho = 0.62; p-value < 0.001). Cd was highly
454 correlated with Ni and Sn within FDP (Spearman rho = 0.56; p-value < 0.001 and
455 Spearman rho = 0.56; p-value < 0.001, respectively) as was Ni with Sn within FDP
456 (Spearman rho = 0.55; p-value < 0.001). All other significant correlations between trace
457 elements within and across sites had a Spearman rho below 0.5 (see supplementary
458 materials Figs. S1-S4).



459

460 Fig. 2. Trace element concentrations of sediment and feathers between sites. Statistical
 461 differences between sites are shown within the figures of each element

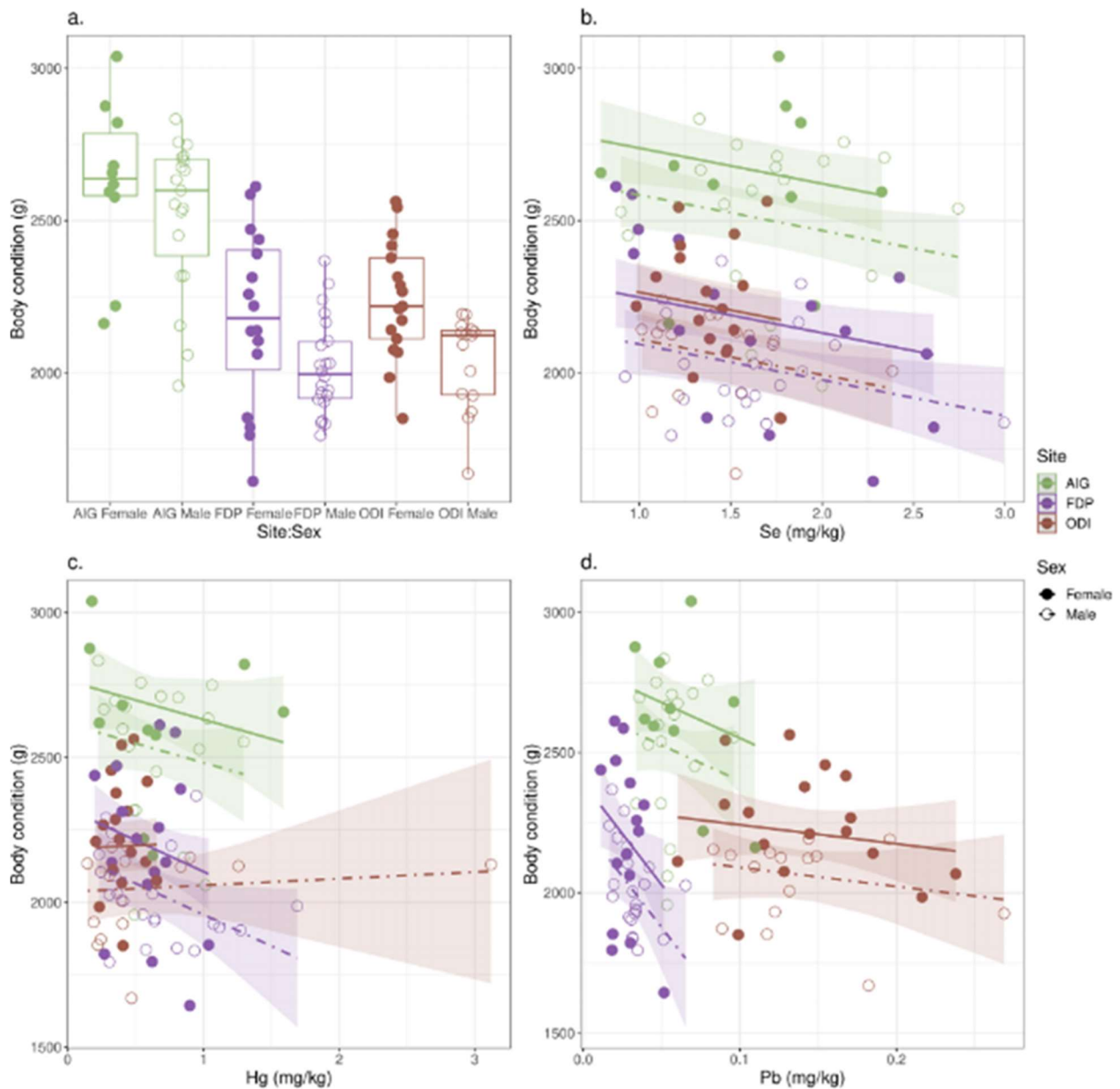
462

3.3. Association between chick body condition and bioaccumulation of trace elements

Females had a higher chick body condition than males ($\Delta\text{AICc} = 9.96$; Cohen's D $[\pm 95\% \text{CI}] = 0.730 [0.655; 1.192]$; adjusted- $R^2 = 6.5\%$; Fig. 3a.). In addition, chick body condition differed between breeding sites with a gradient of $\text{AIG} \gg \text{ODI} = \text{FDP}$ ($\Delta\text{AICc} = 60.96$; Cohen's D $\text{AIG-FDP} [\pm 95\% \text{CI}] = 1.706 [1.152; 2.311]$; Cohen's D $\text{AIG-ODI} [\pm 95\% \text{CI}] = 1.610 [1.053; 2.244]$; Cohen's D $\text{FDP-ODI} [\pm 95\% \text{CI}] = 0.162 [-0.258; 0.595]$; adjusted- $R^2 = 43.8\%$; Fig. 3a.). Se was the only trace element found to have a small negative effect on chick body condition ($\Delta\text{AICc} = 3.16$; partial- $r [\pm 95\% \text{CI}] = -0.231 [-0.444; -0.195]$; adjusted- $R^2 = 2.6\%$; Fig. 3b.). None of the other trace elements were supported by model selection (supplementary Tables S1–S181).

Because there is a lack of independence between breeding sites and the trace elements (see above), we reanalysed the effect of trace elements on chick body condition within each breeding site. The only site for which we found an association between trace elements and chick condition was in FDP, whereby there was a negative association with Se ($\Delta\text{AICc} = 6.35$; partial- $r [\pm 95\% \text{CI}] = -0.461 [-0.675; -0.149]$; adjusted- $R^2 = 12.8\%$; Fig. 3b.) and Pb ($\Delta\text{AICc} = 2.58$; partial- $r [\pm 95\% \text{CI}] = -0.358 [-0.660; -0.027]$; adjusted- $R^2 = 15.5\%$; Fig. 4d.).

When controlling for the effect of Se, we also found a strong negative association between body condition and Hg ($\Delta\text{AICc} = 5.00$; partial- $r [\pm 95\% \text{CI}] = -0.427 [-0.639; -0.046]$; adjusted- $R^2 = 12.8\%$; Fig. 3c.). The effect of Se was also slightly better supported and stronger when controlling for Hg ($\Delta\text{AICc} = 11.34$; partial- $r [\pm 95\% \text{CI}] = -0.567 [-0.757; -0.247]$; adjusted- $R^2 = 18.4\%$).



488

489 Fig. 3. The effect of breeding site, sex (a.), Se (b.), Hg (c.) and Pb (d.) on chick body
 490 condition. In b. to d. fitted lines from linear models are shown along with 95%
 491 confidence intervals.

492

493 3.4. Association between microbiome alpha diversity and bioaccumulation of trace 494 elements

495 An effect of site on Shannon index was not supported, although there was a tendency
 496 for ODI to have a lower Shannon index than AIG and FDP ($\Delta AIC_c = 1.52$; Cohen's D
 497 AIG-FDP [$\pm 95\%CI$] = 0.313 [-0.093 ; 0.670]; Cohen's D AIG-ODI [$\pm 95\%CI$] = -0.197
 498 [-0.644 ; 0.253]; Cohen's D FDP-ODI [$\pm 95\%CI$] = -0.602 [-1.035 ; -0.170]; adjusted-

499 $R^2 = 7.1\%$; Fig. 4a.). There was a strong support for an association between the number
500 of ASVs and breeding site, with higher ASV richness in FDP compared to the two other
501 sites and AIG tending to have a similar number of ASVs to ODI ($\Delta AICc = 35.76$;
502 Cohen's D AIG-FDP [$\pm 95\%CI$] = 1.150 [0.656; 1.625]; Cohen's D AIG-ODI [$\pm 95\%$
503 CI] = 0.007 [-0.426; 0.476]; Cohen's D FDP-ODI [$\pm 95\%CI$] = -1.218 [-1.692; 0.693];
504 adjusted- $R^2 = 23.2\%$; Fig. 4b.). There was no evidence of an association between alpha
505 diversity and sex (Fig. 4a. and b.).

506 When analysing across sites, a non-linear negative relationship between Pb and
507 Shannon index was supported by model selection ($\Delta AICc = 3.141$; adjusted- $R^2 =$
508 10.4% ; Fig. 4c.), where Pb bioaccumulation was negatively associated with Shannon
509 index when bio-accumulation was less than 0.1 mg/kg (partial-r [$\pm 95\%CI$] = -0.291
510 [-0.468; -0.044]). Whilst a similar tendency was found when analysing the number of
511 ASVs, it was not statistically supported following model selection due to the lack of
512 independence between breeding site and Pb, and insufficient power within site (Fig.
513 4d.). Model selection also retained an effect of Sn across sites ($\Delta AICc = 28.681$) and
514 within AIG ($\Delta AICc = 65.526$) on Shannon index, however this effect was driven by a
515 single outlier and was no longer supported once the outlier was removed. Model
516 selection did not retain an effect of any other trace element on Shannon index and the
517 number of ASVs across or within sites (supplementary Tables S1–S181).

518

519

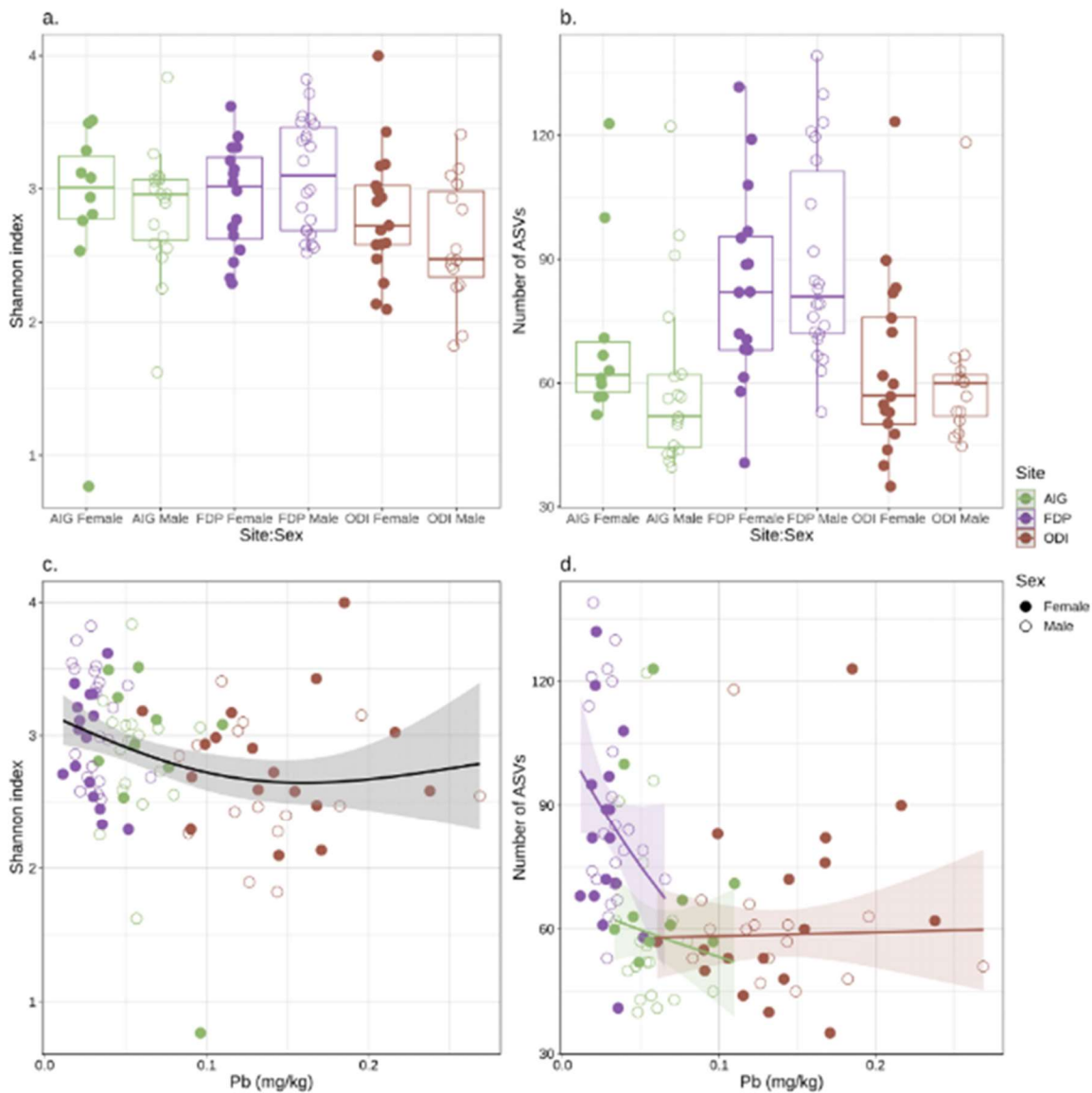


Fig. 4. The effect of breeding site and sex on Shannon index (a.) and number of ASVs (b.). The effect of Pb across breeding sites on Shannon index (c.) and within sites on number of ASVs (d.). Inc.tod.fitted lines from general linear models are shown along with 95% confidence intervals.

3.5. Association between microbiome beta diversity and bioaccumulation of trace elements

We found that breeding site was significantly associated with beta diversity based on RDA analysis ($F_{2,96} = 12.083$; $p\text{-value} < 0.001$, adjusted- $R^2 = 0.18$, Fig. 4a.). However SourceTracker analyses revealed that environmental exposure of sediment microbiome plays a minor role in explaining the strong site specific effects, since a median of only 0.013% (95% range: 0–0.568%), 0.155% (95% range: 0.004–7.336%) and 0.016%

533 (95% range: 0–4.4%) of gut microbiome ASVs in AIG, FDP and ODI respectively
534 could be traced to environmental sediment sources. The effect of sex was not
535 statistically supported ($F_{1,95} = 1.395$; p-value = 0.125).

536 When analysing across sites we found significant support for a small effect of Se on
537 beta diversity ($F_{1,95} = 1.926$; p-value = 0.031, adjusted- $R^2 = 0.01$), with the effect
538 mainly driven by an ASV assigned as *Bacteroides plebeius* (Fig. 5a). Within site, Se
539 was significantly supported within FDP ($F_{1,37} = 2.138$; p-value = 0.002, adjusted- $R^2 =$
540 0.03), but the effect of Se was not significant within AIG ($F_{1,27} = 1.308$; p-value = 0.150,
541 adjusted- $R^2 = 0.01$) or ODI ($F_{1,33} = 0.888$; p-value = 0.632, adjusted- $R^2 = 0$). We found
542 no significant effect of any other trace element, both across and within sites, or any
543 interaction between trace elements and breeding site on beta diversity (all p-values
544 >0.05).

545 Beta diversity divergence was higher in the polluted site of ODI than in AIG and FDP,
546 with the latter two having similar beta diversity divergence ($\Delta AICc = 5.262$; Cohen's
547 D AIG-FDP [$\pm 95\%CI$] = 0.131 [−0.422; 0.716]; Cohen's D AIG-ODI [$\pm 95\%CI$] =
548 0.645 [0.064; 1.218]; Cohen's D FDP-ODI [$\pm 95\%CI$] = 0.747 [0.271; 1.212]; adjusted-
549 $R^2 = 9.2\%$; Fig. 5b.) but no effect of sex was supported by model selection. We found
550 support for an effect of the interaction between As and breeding site on beta diversity
551 divergence ($\Delta AICc = 2.449$; adjusted- $R^2 = 8.1\%$; Fig. 5c). The latter was driven by a
552 negative association within AIG which had low to intermediate bioaccumulation of As
553 ($\Delta AICc = 2.908$; partial-r [$\pm 95\%CI$] = −0.412 [−0.583; −0.158]; adjusted- $R^2 = 17\%$;
554 Fig. 5c), but no association was found either within FDP which had low
555 bioaccumulation nor within ODI which had high As bio-accumulation. We also found
556 strong support for an interaction between Cu and breeding site on beta diversity
557 divergence ($\Delta AICc = 3.661$; Fig. 5d), with a negative association supported within FDP
558 ($\Delta AICc = 4.463$; partial-r [$\pm 95\%CI$] = −0.405 [−0.613; −0.045]; Fig. 4d) and a positive
559 association supported within ODI ($\Delta AICc = 3.019$; partial-r [$\pm 95\%CI$] = 0.396
560 [−0.035; 0.714]; Fig. 5d). We found strong support of a positive association between
561 Pb and beta diversity divergence across sites ($\Delta AICc = 3.700$; partial-r [$\pm 95\%CI$] =

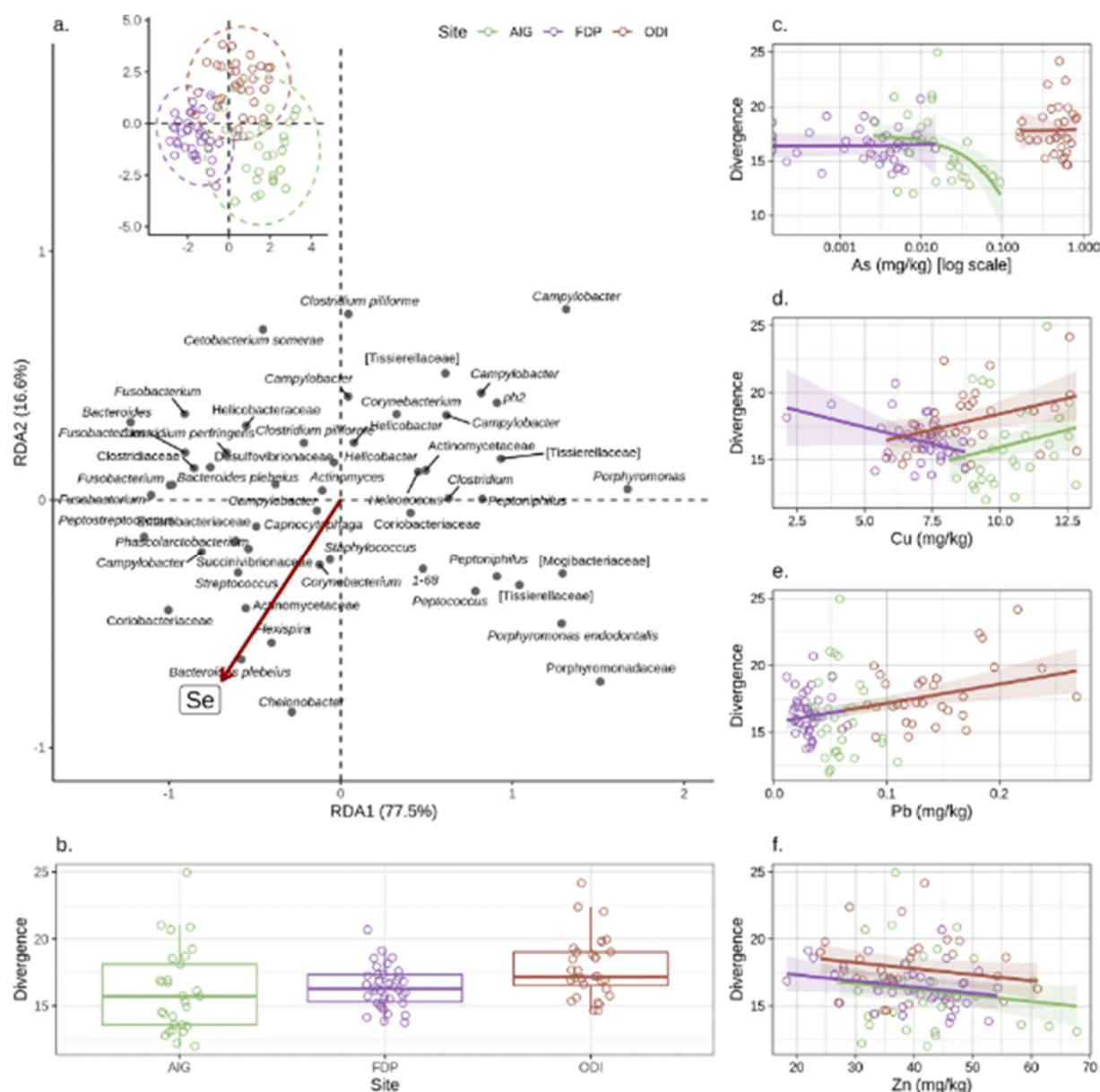
0.326 [0.145; 0.518]; Fig. 5e). When modelling the effect of Pb on beta diversity divergence, the effect of breeding site was no longer supported suggesting that most of the variation in beta diversity divergence between sites was driven by variation in Pb bioaccumulation. Finally, there was support for a negative association between beta diversity divergence and Zn across sites ($\Delta AICc = 1.021$; partial-r [$\pm 95\%CI$] = -0.179 [-0.341 ; -0.007]; Fig. 5e).

None of the other trace elements, both across and within sites, or any other interaction between trace elements and breeding site on beta diversity divergence was supported by model selection (supplementary Tables S1–S181).

3.6. Association between differential relative abundance of specific ASVs and bioaccumulation of trace elements

Mirroring results from beta diversity we found evidence of an association between relative differential abundance of specific ASVs and four elements: As, Cu, Pb and Se (Fig. 6a). For both As and Pb, ANCOM identified six ASVs whose relative abundance was differentially expressed according to trace element concentrations, but parameter estimates of differential logs overlapped 0 for each of these ASVs (Fig. 6a). The latter result further supports that stochastic processes are driving differences in microbiome assemblage according to the elements, rather than a systemic shift of specific ASVs. In contrast for Cu, two out of 12 ASVs above the ANCOMW threshold of 0.6 had differential log estimates whose 95% confidence intervals did not overlap 0. The two ASVs were assigned to Tissierellaceae and Porphyromonas and both were negatively associated with Cu concentrations (Fig. 6b and c, respectively; partial-r [$\pm 95\%CI$] = -0.325 [-0.719 ; -0.002] and partial-r [$\pm 95\%CI$] = -0.220 [-0.641 ; 0.036], respectively; adjusted- $R^2 = 4.39\%$ and adjusted- $R^2 = 2.47\%$, respectively). ANCOM found support for a single ASV, assigned as *Bacteroides plebeius*, to be significantly enriched along Se concentrations (Fig. 6 d; partial-r [$\pm 95\%CI$] = 0.239 [0.046 ; 0.433]; adjusted- $R^2 = 3.54\%$). In turn, *Bacteroides plebeius* was negatively associated with

body condition ($\Delta\text{AICc} = 2.852$; partial-r [$\pm 95\%\text{CI}$] = -0.230 [-0.455 ; 0.019];
adjusted- $R^2 = 4.46\%$; Fig. 7). In contrast, we found no support for an effect of
Tissierellaceae and Porphyromonas on body condition (supplementary Tables S1–
S181).



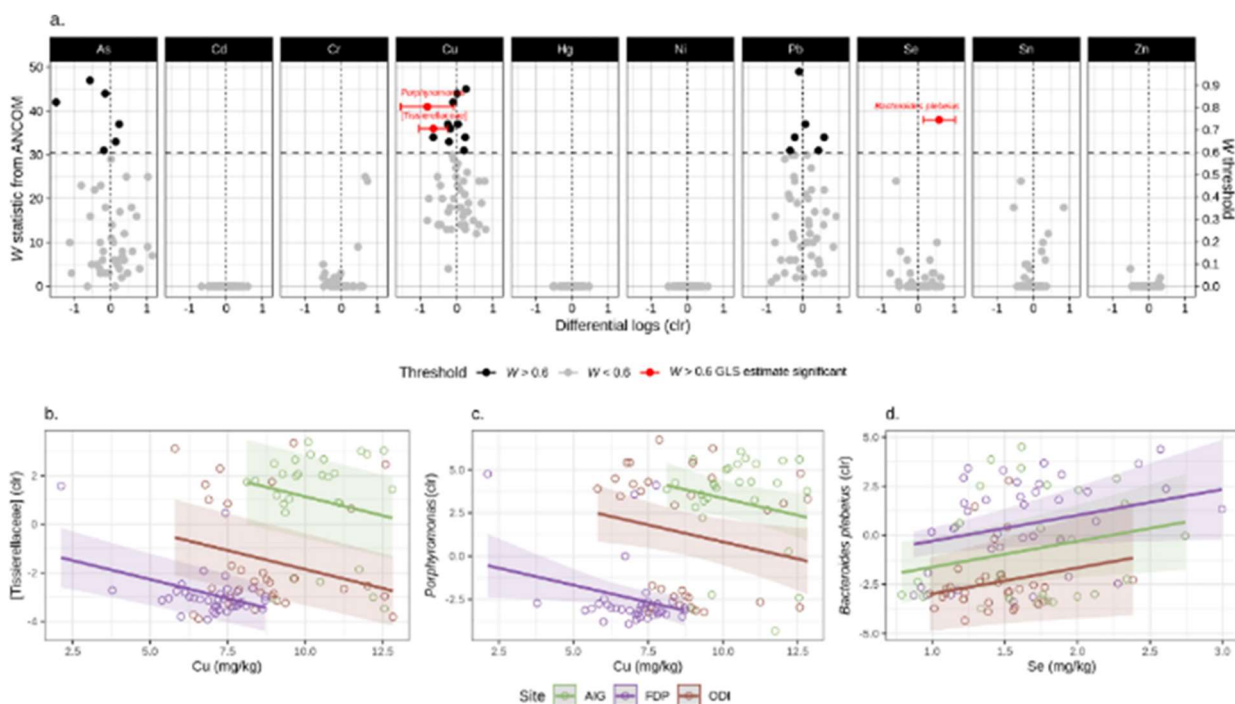


Fig. 6. Volcano plots show the results of analysis of composition of microbiomes (ANCOM) according to differential log estimates of centred logged ratios (clr) from general least square (GLS) regressions across the ten trace elements investigated in this study (a.). Both ANCOM and GLS analyses control for the fixed effect of breeding sites and the effect of heterogeneity within breeding sites. Dots represent individual ASVs and are colored grey if below the ANCOM W threshold of 0.6. If the W threshold was above 0.6 dots were colored red if the 95% confidence intervals of the GLS parameter estimate overlapped 0 and black if confidence intervals did not overlap 0. Taxonomic assignment and confidence intervals are shown only for ASVs in red. Relative differential abundance (clr) of an ASV assigned as Tisseriellaceae (b.) and Porphyromonas (c.) according to bioaccumulation of trace elements Cu are fitted with GLS regressions controlling the fixed effect of breeding site and breeding site heterogeneity. An identical model structure was used to investigate the relationship between Bacteroides plebeius and Se bioaccumulation (d.). In b. to d. fitted lines are shown along with 95% confidence intervals

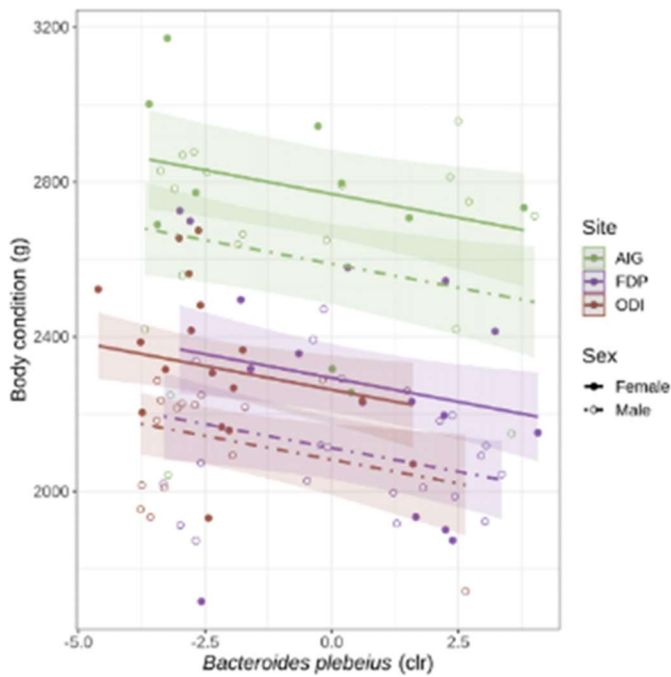


Fig. 7. The effect of *Bacteroides plebeius* relative abundance on chick body condition controlling for the effect of site and sex. Fitted lines from general least squares models are shown along with 95% confidence intervals.

4. Discussion

Our results demonstrate that high environmental exposure of trace elements from the sediment only equated to high feather bioaccumulation for the harmful elements As, Pb and perhaps Sn but not for the remaining trace elements. Importantly, we found that body condition was adversely associated with bioaccumulation of two antagonist trace elements, Se and Hg, and with Pb. Among these trace elements, Se and Pb bioaccumulation influenced gut microbiome composition, while we also found subtler effects of As, Cu and Zn.

4.1. Variation in sediment element concentration is not mirrored in feather bioaccumulation of trace elements

Our results are consistent with the extensive literature which demonstrates that ODI is one of the most polluted estuarine areas in the world (Achterberg et al., 2003; Barba-Brioso et al., 2010; Guillén et al., 2011; Morillo et al., 2008; Ruiz et al., 2008), since

641 ODI sediment element concentration was much higher for 9 out of 10 elements (the
642 exception was Se) compared to AIG and FDP. In contrast, our results confirmed that
643 both AIG and FDP can be considered relatively unpolluted sites for the trace elements
644 investigated in this study. FDP tended to be slightly richer in trace elements than AIG,
645 which is likely driven by the fact that FDP sediment is characterized by more fine
646 particles (clays and organic matter) which are more prone to accumulate undesirable
647 toxic metals and metalloids (Davis, 1984; Lukman et al., 2013).

648 Despite evidence of high environmental pollution in ODI, we found that variation in
649 bioaccumulation in feathers between sites did not mirror the pattern observed in
650 sediment, whereby higher concentration of trace elements in ODI was only observed
651 in feathers for the harmful elements As, Pb and to a lesser extent Sn. Overall two
652 alternative, but non-mutually exclusive, interpretations can explain our results: (i)
653 feathers are a good indicator of trace element bioaccumulation in greater flamingos for
654 As and Pb in highly polluted sites but not for the remaining trace elements or (ii) the
655 relative role of local sediment and diet exposure on trace element bioaccumulation
656 varies according to the trace element and site investigated.

657 Both explanations are likely for Cd, Cr and Ni for which bioaccumulation was rather
658 low and variable within site, despite high exposure from the abiotic environment in
659 ODI. In contrast, several lines of evidence makes the former an unsatisfactory
660 explanation for the remaining elements. First, metals have a strong affinity to the
661 sulfhydryl groups in the keratin of feathers (Burger and Gochfeld, 2002). In theory, all
662 trace elements circulating in the blood at the time of feather growth, including the
663 essential elements Cu and Zn required for feather formation (Lai et al., 2010; Leeson,
664 2009; Sunde, 1972; Supplee et al., 1958; Vohra and Kratzer, 1957), may enter the
665 structure of the feather and may be a good bioindicator of exposure (Burger, 1993).
666 Indeed trace element concentrations in feathers have been shown to be correlated with
667 blood concentrations of chicks for As, Hg, Se, and Pb (Burger and Gochfeld, 1990;
668 Dolan et al., 2017; Monteiro and Furness, 2001). Furthermore, a previous study on fish
669 in Tinto and Odiel rivers revealed that Cu and Zn are more bioavailable than Pb

(Vicente-Martorell et al., 2009) and high concentration levels across sites indicate that both elements clearly bioaccumulated in feathers in our study. Second, the bioaccumulation of the toxic elements Hg, Se and, to a lesser extent, Sn followed a similar pattern to supposedly constitutive and essential elements (Cu and Zn) and were considerably more concentrated relative to the basal level in the environment than the other toxic bioaccumulated elements (As, Cd, Cr, Ni, and Pb). For instance, in FDP, the ratio Pb:Hg is about 655 in sediments but only 0.08 in feathers; the ratio Pb:Se is 23 in sediments and about 0.02 in feathers. For Sn, the ratio of Pb:Sn is lower by a factor of 2.3–9.2 in sediment compared to feathers depending on the site and the high bioaccumulation in feathers observed in this study is consistent with previous results in chickens (Sun et al., 2014). Therefore, our results are a strong indication that feathers are incorporating the toxic elements Hg, Se and Sn more efficiently than Pb. Third, there is extensive evidence demonstrating that feathers play a primary role as an excretion pathway for methyl-Hg in seabirds (Becker et al., 1994; Lewis and Furness, 1991) and low levels of Hg in feathers in ODI relative to sediment exposure is consistent with previous studies in water birds (Borghesi et al., 2011, 2016; Rubio et al., 2016). Therefore, overall our results suggest that, in addition to As and Pb, there is clear bioaccumulation of Cu, Hg, Se, Sn and Zn in feathers. However, unlike As and Pb in ODI, the main source of exposure for these elements is likely to be the greater flamingo's diet rather than local sediment. Indeed, diet exposure has been shown to result in Cd, Cu, Hg, Ni, Pb, Se, Sn and Zn feather bioaccumulation (Cd: Pilastro et al., 1993; Cu: Kim and Kil, 2015; Kim et al., 2016; Hg: Monteiro et al., 1998; Falkowska et al., 2013; Keller et al., 2014; Thompson et al., 1998; Ni: Eastin and O'Shea, 1981; Pb: Markowski et al., 2013; Se: Ševčíková et al., 2006; Couloigner et al., 2015; Sn: Sun et al., 2014; Cu and Zn: Vohra et al., 1968) and indirect evidence also points to diet resulting in As feather bioaccumulation (Tasneem et al., 2020; Sánchez-Virosta et al., 2015; Dauwe et al., 2004).

In greater flamingo chicks diet exposure comes from crop milk fed by the adults and hence reflects adult foraging behaviour. While foraging behaviour by breeding greater

699 flamingos shows extensive geo-graphic variation, individuals are known to cover large
700 distances. Resighting data from dye-marked breeding birds at the Etang du Fangassier
701 site in the Camargue, south of France (which is 34 km from AIG and the preferred
702 breeding site in the south of France when environmental conditions are favorable)
703 indicate that around a third of birds forage within 10 km of the breeding site but some
704 individuals were recorded to forage up to 70 km away (Béchet et al., 2009b). In AIG,
705 Cu was significantly higher than in other sites and AIG is surrounded by vineyards
706 (Sable-de-camargue IGP) where Cu has been heavily dispersed since the late 19th
707 century to fight and control fungal diseases (Ballabio et al., 2018), including organic
708 farms who continue to preferentially use Cu as a fungicide (Ballabio et al., 2018;
709 Provenzano et al., 2010). Therefore the higher concentrations of Cu feather
710 bioaccumulation in AIG may be explained by agricultural contamination of adult
711 feeding sites. The baseline level of Cu in flamingos should be assessed in future studies
712 in order to properly use Cu in feathers as a bioindicator.

713 Unlike AIG and ODI, the lagoon at the FDP breeding site dries out and the lack of local
714 foraging sites forces adults to forage in distant sites around 130–400 km away,
715 including the heavily polluted sites of the Tinto-Odiel estuary (~200 km from FDP)
716 (Amat et al., 2005; Rendón et al., 2011, 2014). We therefore speculated that FDP adults
717 may be ex-posing their chicks to toxic levels of trace elements through their crop milk,
718 leading to high bioaccumulation in chick feathers. However this is not supported by
719 our results since FDP had lower bioaccumulation concentrations for As and Pb than in
720 AIG, despite higher abiotic expo-sure in FDP. In fact, for all trace elements investigated
721 in this study, FDP had either similar levels of trace element bioaccumulation to AIG or
722 lower concentrations. Our results therefore suggest that ODI may not be a major
723 feeding site of adults (for both birds breeding in ODI and FDP). The latter is supported
724 by data of sightings of individually banded birds, when during the chick provisioning
725 period, adults show a strong fidelity to the foraging sites and about 80% of adults
726 breeding in FDP were observed to forage in a fish farm in Doñana (130 km from FDP),

about 10% in ODI, about 5% in Cádiz Bay saltpans, and the remain-ing in other areas (J. A. Amat, A. Garrido, M.A. Rendón, M. Rendón-Martos, unpubl. data).

Selenium bioaccumulation was also significantly different across breeding sites with a gradient of $AIG > FDP > ODI$. Se pollution is known to quickly bioaccumulate in aquatic environments through diet exposure and has only been recognized as an important pollutant in the 1990s (Lemly, 2004). Sources of Se pollution ranges from mining (coal, gold, silver, and nickel), agricultural practices (e.g. subsurface irrigation drainage) and industrial activities (e.g. coal power combustion) and water is the primary driver of anthropogenic sourced Se in aquatic environments (Lemly, 2004; Maher et al., 2010). Despite the fact that elevated Se pollution can cause devastating effects in waterbirds, resulting in high embryonic abnormalities and mortalities (Presser and Ohlendorf, 1987), there is scarce data of Se contamination and bioaccumulation in our study sites (Alvárez et al., 2013; Amiard-Triquet et al., 1991; Cosson et al., 1988; Guan and Martin, 1991). Our results suggest variation in dietary Se exposure across sites in greater flamingos and the effects of Se contamination in Mediterranean wetlands merits further research.

Overall our results are consistent with diet exposure playing a greater role in determining trace element exposure than local sediment exposure for AIG and FDP, as well as for all trace elements in ODI except for Pb, As and perhaps Sn. Only the sampling of crop milk can elucidate further the interplay between diet, environmental exposure and chick bioaccumulation, which merits further investigation in the future.

4.2. Site specific effects indicate that environmental and ecological factors affect body condition and gut microbiome

According to studies on both neutral and adaptive genetic markers, greater flamingo breeding colonies form a single genetic population with high gene flow at the scale of the Mediterranean (Geraci et al., 2012; Gillingham et al., 2017). Therefore differences in parental genotypes cannot explain site specific effects of chick phenotypes,

755 suggesting strong local environmental and ecological effects on chick body condition
756 and gut microbiome, as has previously been reported (body condition: Amat et al.,
757 2007; Béchet and Johnson, 2008; Cézilly et al., 1995; Yohannes et al., 2014; gut
758 microbiome: Gillingham et al., 2019). Variation in chick body condition is known to
759 be negatively associated with the number of breeding pairs in a colony, presumably
760 due to density-dependent effects (Béchet and Johnson, 2008). In the Bouche-du-Rhône
761 estuary, which includes the AIG site, chick body condition is also negatively associated
762 with river discharge levels of the Rhône. Béchet and Johnson (2008) speculated that
763 higher water discharge may increase the release of metal and sewage contaminants
764 suspended in solids in the Bouche-du-Rhône estuary and have a detrimental effect on
765 invertebrate productivity resulting in lower food availability, negatively affecting chick
766 body condition. In addition, evidence from feather and blood isotopes suggests that
767 chicks fed a diet richer in food from temporary marshes than permanent salt pans had
768 higher body condition (Yohannes et al., 2014). Food availability and diet, which will
769 vary across and within sites, is also known to be an important predictor of gut
770 microbiome composition in many taxa (Muegge et al., 2011), parameters that we could
771 not directly investigate in this study but merits further future investigation in the future.

772 Another potential source of site specific effects in microbiome gut composition is
773 variation in microbial exposure from the environment and in particular sediment.
774 However, sediment microbiome explained a small amount of variation of gut
775 microbiome composition (0.013–0.155% of gut microbiome ASVs could be traced to
776 sediment microbiome), as previously reported in the Red-necked stint, *Calidris*
777 *ruficollis* (Risely et al., 2017). Chick age is also not a significant parameter in
778 explaining chick gut microbiome composition in our study system (see Gillingham et
779 al., 2019). Therefore, chick gut microbiome at 2–4 months of age has already
780 undergone strong host selection (Gillingham et al., 2019) and is resilient to site specific
781 environmental microbial exposure.

782

783 ***4.3. Trace element bioaccumulation is associated with two proxies of health: body***
784 ***condition and the gut microbiome***

785 Our study reveals that exposure to environmental pollution of trace elements, including
786 heavy metals, is an important predictor of site specific variation in body condition and
787 microbiome composition. Toxic levels of heavy metal bioaccumulation can play an
788 ambivalent role on body condition depending on the element and the tissue sample
789 used to estimate bioaccumulation (e.g. Debacker et al., 2000; Esselink et al., 1995;
790 Janssens et al., 2003; Takekawa et al., 2002). These ambivalent results can be explained
791 by the fact that the liver and kidney can incorporate some trace elements at a higher
792 rate than feathers (Cosson et al., 1988) and that body condition estimates are an indirect
793 measure of true condition (Peig and Green, 2009, 2010). In this study, we found no
794 evidence of an effect of bioaccumulation As, Cd, Cr, Cu, Ni, Sn and Zn in feathers on
795 chick body condition but the aforementioned limitations do not allow us to exclude the
796 possibility that these elements do not affect physiological health. Indeed, we found
797 evidence that feather bioaccumulation of As, Cu and Zn did affect gut microbiome
798 divergence from the population median, an indicator of impairment in gut homeo-stasis
799 (Ma, 2020; Zaneveld et al., 2017).

800 Whilst As is toxic and has been shown to disrupt microbiome com-position in
801 experimental rodents and in humans (Assefa and Köhler, 2020; Brabec et al., 2020; Lu
802 Kun et al., 2014; McDermott et al., 2020), trace quantities are thought to be essential
803 to both eukaryotes and prokaryotes (Stolz et al., 2006). In our study, we found that
804 divergence from the median decreased with increasing As bioaccumulation within the
805 site with intermediate bioaccumulation for this element (AIG; range: 0.003–0.09
806 mg/kg), suggesting an optimal concentration in feathers of As at around 0.1 mg/kg (Fig.
807 5c).

808 Copper is an essential element to all living organisms but high exposure is toxic to
809 animals and whilst copper ions are naturally antimicrobial, few studies have
810 investigated the relationship between Cu and the intestinal flora (Dempsey et al., 2019).

811 Interestingly in birds it has been found that both low and high intake of Cu lead to gut
812 dysbiosis and a shift in gut microbiome composition (Song et al., 2017). We found a
813 negative association between Cu bioaccumulation and microbiome di-vergence in the
814 site with low to intermediate bioaccumulation values (FDP; range: 2.12–8.72 mg/kg)
815 and a positive association in the site with intermediate to high bioaccumulation values
816 (ODI; range: 5.80–12.84 mg/kg), suggesting an optimal Cu concentration in feathers
817 of around 7.5 mg/kg (Fig. 5 d.). Cu was also associated negatively with two ASVs
818 assigned to Tissierellaceae, of the Firmicutes phylum and Porphyromonas of the
819 Bacteroidetes phylum. The family Tissierellaceae and the genus Porphyromonas have
820 previously been described in host-associated microbiota, and although their functions
821 remain unknown, some opportunistic pathogens have been identified (Bemis et al.,
822 2011; Capellari et al., 2016; Kawamura et al., 2015; Sakamoto et al., 2015; Summanen
823 et al., 2009; Wagener et al., 2014). Therefore our re-sults are in broad agreement with
824 experimental work in birds which suggest that an optimal intake of dietary Cu is
825 associated with gut homeostasis (Song et al., 2017).

826 Zinc is also essential to both eukaryotes and prokaryotes and is only toxic at very
827 elevated levels (Berg and Shi, 1996; Blencowe and Morby, 2003; Gaither and Eide,
828 2001). Healthy dietary intake of Zn is known to be key to gut homeostasis (Højberg et
829 al., 2005; Pieper et al., 2012; Smith et al., 1972; Starke et al., 2014; Vahjen et al., 2010)
830 and a deficiency in Zn has been associated with decreased homeostasis of the gut in
831 chick microbiota of birds (Reed et al., 2015). Therefore, the negative association
832 between Zn bioaccumulation and microbiome divergence from the population median
833 in our study adds to the growing body of evidence of the central beneficial role of this
834 element in gut homeostasis in birds.

835 Our results indicate that there is a threshold effect of Pb bioaccumulation on chick body
836 condition. We found a strong negative association with chick body condition in the site
837 with low Pb bioaccumulation (FDP: range 0.011–0.065 mg/kg), but Pb
838 bioaccumulation was no longer associated with chick body condition in breeding sites
839 with intermediate bioaccumulation (AIG: range 0.033–0.110 mg/kg) and high

840 bioaccumulation (ODI: range 0.060–0.269 mg/kg). Pb also affected gut microbiome
841 richness (alpha diversity). A negative log-linear association between Pb and alpha
842 diversity is in broad agreement with previous work showing lower alpha diversity in a
843 mine affected polluted site than in an unpolluted site in the Mongolian toad
844 (*Strauchbufo raddei*) (Zhang et al., 2016) and with experimental work in mice
845 reporting decreased microbial diversity with high levels of exposure to Pb (Gao et al.,
846 2017).

847 We found that microbiome divergence from the population median was strongly
848 associated with Pb bioaccumulation (adjusted- $R^2 = 10.6\%$). Indeed, the higher
849 divergence in the polluted site of ODI was largely explained by Pb bioaccumulation.
850 Our results are consistent with a number of studies in experimental mice which have
851 found that Pb exposure disrupts the homeostasis of the gut microbiome (Breton et al.,
852 2013a, 2013b; Gao et al., 2017). Overall our results suggest a strong negative effect of
853 Pb bioaccumulation on chick greater flamingo health. Whilst Pb is well known to be
854 detrimental to wildlife health, our study highlights that the adverse effects of Pb on
855 both body condition and gut microbiome in a natural population was only detected be-
856 cause both polluted and unpolluted sites were sampled, capturing a broad range of
857 exposure for this harmful element.

858 We also found a negative association between Se bioaccumulation in feathers and body
859 condition in greater flamingo chicks. Despite being an essential element, exposure of
860 Se above beneficial doses is known to have detrimental effects in birds (Ackerman and
861 Eagles-Smith, 2009; Beijer and Jernelöv, 1978; Heinz and Fitzgerald, 1993; Heinz and
862 Hoffman, 1998; Takekawa et al., 2002). For instance, feeding of mallard ducks, *Anas*
863 platyrhynchos, with ad libitum diets enriched with 20 µg/g or more of selenium
864 significantly caused weight loss and mortality in an experimental study (Heinz and
865 Fitzgerald, 1993). In addition, Se is known to be an antagonist to Hg and can reverse
866 the negative effects of Hg and increase survival of birds exposed to toxic levels of Hg
867 (El-Begearmi et al., 1977). However, elevated levels of Se can cause increased
868 retention of Hg which counteract the positive effect of Se de-creased intoxication

869 (Beijer and Jernelöv, 1978; Heinz and Hoffman, 1998). An experimental study in
870 mallards, has found that the additive adverse effects of Se and Hg are worse than when
871 birds are exposed to elevated levels of only one of the two elements (Heinz and
872 Hoffman, 1998). In our study, we found that Hg and Se bioaccumulation had an
873 additive negative effect on body condition in FDP (the site with the highest median Hg
874 bioaccumulation). Thus, our study further suggests that both Hg and elevated Se
875 bioaccumulation is additively and negatively affecting host health in birds.

876 Selenium but not Hg, was also associated with a shift in microbiome composition,
877 mostly driven by an enrichment of *Bacteroides plebeius*. *B. plebeius* has previously
878 been associated with diets rich in red seaweed in humans (Hehemann et al., 2012). *B.*
879 *plebeius* is involved in the production of carbohydrate active enzymes, or CAZymes,
880 in the gut and plays an important function in the breakdown of sulphated
881 polysaccharides that are found in marine algae (Hehemann et al., 2012), a key part of
882 flamingo species diet (Johnson, 1997). Interestingly, microalgae is known to be
883 enriched in Se in many species for which it is required for growth and metabolic
884 functions via selenoproteins (Araie and Shiraiwa, 2009; Obata and Shiraiwa, 2005;
885 Schiavon et al., 2017). There-fore, *B. plebeius* enrichment may reflect higher intake of
886 selenium rich microalgae which requires the metabolization of the associated carbo-
887 hydrates. In addition, *B. plebeius* enrichment in chicks was negatively as-sociated with
888 chick body condition in our study. Our results therefore suggest that a Se rich diet of
889 microalgae may be associated with high Se bioaccumulation which in turn adversely
890 affected body condition.

891

892 **5. Conclusion**

893 We found that only three elements out of 10 were significantly higher in feathers of
894 greater flamingo chicks at the highly polluted breeding site of Odiel: As, Pb and to a
895 lesser extent Sn. We can therefore conclude that high local sediment exposure of As
896 and Pb pollutants and perhaps Sn resulted in high bioaccumulation in the polluted site.

897 In contrast, bioaccumulation in feathers did not reflect local sediment expo-sure for all
898 other elements investigated in this study (Cd, Cr, Cu, Hg, Ni, Se and Zn) and exposure
899 is likely driven by parental transfer of diet. Se, Hg and Pb bioaccumulation affected
900 chick body condition, whilst gut microbiome composition was affected by As, Cu, Pb
901 and Zn bioaccumulation. Unsurprisingly, Pb emerged as an element of particular
902 concern, affecting chick body composition, microbial diversity and composition. Our
903 study also highlights the concern of Se, which impacted chick body condition,
904 microbiome composition and the interplay between both of these parameters across all
905 sites. Our results therefore suggest that in addition to well-studied elements such as Pb
906 and Hg, bioaccumulation of poorly studied elements, such as Se, are adversely
907 affecting the health of natural populations. More studies are greatly needed on the
908 metabolic processes driving trace element accumulation of contaminants into feathers
909 and how they relate to direct measures of fitness such as survival and reproductive
910 success in order to further understand their potential use as bioindicators.

911

912 **CRedit authorship contribution statement**

913 MG, FB, ED and SS instigated the study. MG and SS acquired the funding. MG
914 collected the samples during field work and did the lab work of the 16S dataset. FB
915 and FM prepared the samples for trace ele-ment analyses and analysed the initial raw
916 data. MG did the bioinfor-matics and all statistical analyses with input from BKM. FB
917 and BKM provided data interpretation input. MG wrote the first draft of the man-
918 uscript with input from FB and BKM. AB participated in acquiring the funding and
919 organized the field work in France. MR-M and JA organized the field work in Spain
920 and helped to collect the samples. SS heads the lab where the 16S lab work was carried
921 out and ED heads the lab where trace elements were analysed. All authors provided
922 input to-wards the final draft of the manuscript.

923

924 Declaration of competing interest

925

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

928

929 Acknowledgements

930

931 Mark Gillingham was supported by a DFG grant (DFG Gi 1065/2-1). This study was
932 also supported by the TOTAL foundation. We are deeply grateful to Luc Hoffmann and
933 the late Alan Johnson for the instigation of the long-term study on the Greater flamingo
934 and we warmly thank the many volunteers who participated in collecting feather
935 samples of greater flamingos including: Sebastian Menke, Matthias Meier, Alexandre
936 Courtiol, Araceli Garrido Aguilera, Christophe Germain and Antoine Arnaud. We
937 acknowledge the Salt company Salins for granting access to the Aigues-Mortes site and
938 providing support to the ringing operation. We also thank our companions through
939 thick and thin: Jim Knopf and Kleine Tante.

940

941 Data accessibility

942 The individual gut bacterial 16S rRNA gene raw sequences are available in the NCBI
943 Sequence Read Archive (SRA; accession number: SAMN10576470-SAMN10577345)
944 under BioProject PRJNA485732. All trace element data are available in the public
945 database Dryad: [https:// doi.org/10.5061/dryad.27056](https://doi.org/10.5061/dryad.27056). The R phyloseq subsetted data
946 used in this manuscript can be found in the public database [https://doi.org/10.](https://doi.org/10.6084/m9.figshare.13110974.v1)
947 [6084/m9.figshare.13110974.v1](https://doi.org/10.6084/m9.figshare.13110974.v1).

948

949 Appendix A. Supplementary data

950

951 **References**

- 952 Achterberg, E.P., Herzl, V.M.C., Braungardt, C.B., Millward, G.E., 2003. Metal behaviour in an
953 estuary polluted by acid mine drainage: the role of particulate matter. *Environ. Pollut.* 121, 283–
954 292. [https://doi.org/10.1016/S0269-7491\(02\)00216-6](https://doi.org/10.1016/S0269-7491(02)00216-6).
- 955 Ackerman, J.T., Eagles-Smith, C.A., 2009. Selenium bioaccumulation and body condition in
956 shorebirds and terns breeding in San Francisco Bay, California. USA. *Environ. Toxicol. Chem.* 28,
957 2134–2141. <https://doi.org/10.1897/09-101.1>.
- 958 Aitchison, J., 1982. The statistical analysis of compositional data. *J. R. Stat. Soc. Ser. B Methodol.*
959 44, 139–160. <https://doi.org/10.1111/j.2517-6161.1982.tb01195.x>.
- 960 Álvarez, C.R., Moreno, M.J., Alonso, L.L., Gómara, B., Bernardo, F.J.G., Martín-Doimeadios,
961 R.C.R., González, M.J., 2013. Mercury, methylmercury, and selenium in blood of bird species
962 from Doñana National Park (Southwestern Spain) after a mining accident. *Environ. Sci. Pollut.*
963 Res. 20, 5361–5372. <https://doi.org/10.1007/s11356-013-1540-1>.
- 964 Amat, J.A., Rendón, M.A., Rendón-Martos, M., Garrido, A., Ramírez, J.M., 2005. Ranging behaviour
965 of greater flamingos during the breeding and post-breeding periods: linking connectivity to
966 biological processes. *Biol. Conserv.* 125, 183–192. <https://doi.org/10.1016/j.biocon.2005.02.018>.
- 968 Amat, J.A., Hortas, F., Arroyo, G.M., Rendón, M.A., Ramírez, J.M., Rendón-Martos, M., Pérez-
969 Hurtado, A., Garrido, A., 2007. Interannual variations in feeding frequencies and food quality of
970 greater flamingo chicks (*Phoenicopterus roseus*): Evidence from plasma chemistry and effects
971 on body condition. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 147, 569–576 Issue
972 includes papers from a presentation at a Memorial Symposium in honor of Dr. Peter Lutz held
973 at Florida Atlantic University on September 23rd, 2005.
974 <https://doi.org/10.1016/j.cbpa.2007.02.006>.
- 975 Amiard-Triquet, C., Pain, D., Delves, H.T., 1991. Exposure to trace elements of flamingos living in a
976 biosphere reserve, the Camargue (France). *Environ. Pollut.* 69, 193–201.
977 [https://doi.org/10.1016/0269-7491\(91\)90143-K](https://doi.org/10.1016/0269-7491(91)90143-K).
- 978 Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of
979 constrained ordination for ecology. *Ecology* 84, 511–525. [https://doi.org/10.1890/0012-9658\(2003\)084\[0511:CAOPCA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2).
- 981 Araie, H., Shiraiwa, Y., 2009. Selenium utilization strategy by microalgae. *Molecules* 14, 4880–4891.
982 <https://doi.org/10.3390/molecules14124880>.
- 983 Assefa, S., Köhler, G., 2020. Intestinal microbiome and metal toxicity. *Curr. Opin. Toxicol.*
984 Mechanistic Toxicology 19, 21–27. <https://doi.org/10.1016/j.cotox.2019.09.009>.
- 985 Balkız, Ö., Dano, S., Barbraud, C., Tekin, S., Özesmi, U., Dündar, M., Béchet, A., 2007. Sexing
986 greater flamingo chicks from feather bulb DNA. *Waterbirds* 30, 450–453. [https://doi.org/10.1675/1524-4695\(2007\)030\[0450:SGFCFF\]2.0.CO;2](https://doi.org/10.1675/1524-4695(2007)030[0450:SGFCFF]2.0.CO;2).
- 988 Balkız, Ö., Béchet, A., Rouan, L., Choquet, R., Germain, C., Amat, J.A., Rendón-Martos, M.,
989 Baccetti, N., Nissardi, S., Özesmi, U., Pradel, R., 2010. Experience-dependent natal philopatry
990 of breeding greater flamingos. *J. Anim. Ecol.* 79, 1045–1056. <https://doi.org/10.1111/j.1365-2656.2010.01721.x>.
- 992 Ballabio, C., Panagos, P., Lugato, E., Huang, J.-H., Orgiazzi, A., Jones, A., Fernández-Ugalde, O.,
993 Borrelli, P., Montanarella, L., 2018. Copper distribution in European topsoils: an assessment

- 994 based on LUCAS soil survey. *Sci. Total Environ.* 636, 282–298. <https://doi.org/10.1016/j.scitotenv.2018.04.268>.
- 995
- 996 Barba-Brioso, C., Fernández-Caliani, J.C., Miras, A., Cornejo, J., Galán, E., 2010. Multi-source water
997 pollution in a highly anthropized wetland system associated with the estuary of Huelva (SW
998 Spain). *Mar. Pollut. Bull.* 60, 1259–1269.
- 999 Barbraud, C., Johnson, A.R., Bertault, G., 2003. Phenotypic correlates of post-fledging dispersal in a
1000 population of greater flamingos: the importance of body condition. *J. Anim. Ecol.* 72, 246–257.
1001 <https://doi.org/10.1046/j.1365-2656.2003.00695.x>.
- 1002 Béchet, A., Johnson, A.R., 2008. Anthropogenic and environmental determinants of greater flamingo
1003 *Phoenicopterus roseus* breeding numbers and productivity in the Camargue (Rhône delta,
1004 southern France). *Ibis* 150, 69–79. <https://doi.org/10.1111/j.1474-919X.2007.00740.x>.
- 1005 Béchet, A., Germain, C., Johnson, A., 2009a. Greater Flamingos stop breeding in the Camargue
1006 (southern France) in 2007, for the first time in 38 years; the beginning of a new era? *Flamingo* 1,
1007 26.
- 1008 Béchet, A., Germain, C., Sandoz, A., Hirons, G.J.M., Green, R.E., Walmsley, J.G., Johnson, A.R.,
1009 2009b. Assessment of the impacts of hydrological fluctuations and salt pans abandonment on
1010 Greater flamingos in the Camargue, South of France. *Biodivers. Conserv.* 18, 1575–1588.
1011 <https://doi.org/10.1007/s10531-008-9544-8>.
- 1012 Béchet, A., Rendón-Martos, M., Rendón, M.Á., Amat, J.A., Johnson, A.R., Gauthier-Clerc, M., 2012.
1013 Global economy interacts with climate change to jeopardize species conservation: the case of the
1014 greater flamingo in the Mediterranean and West Africa. *Environ. Conserv.* 39, 1–3.
1015 <https://doi.org/10.1017/S0376892911000488>.
- 1016 Becker, P.H., Henning, D., Furness, R.W., 1994. Differences in mercury contamination and
1017 elimination during feather development in gull and tern broods. *Arch. Environ. Contam. Toxicol.*
1018 27, 162–167. <https://doi.org/10.1007/BF00214258>.
- 1019 Beijer, K., Jernelöv, A., 1978. Ecological aspects of mercury-selenium interactions in the marine
1020 environment. *Environ. Health Perspect.* 25, 43–45. <https://doi.org/10.2307/3428707>.
- 1021 Bemis, D.A., Greenacre, C.B., Bryant, M.J., Jones, R.D., Kania, S.A., 2011. Isolation of a variant
1022 *Porphyromonas* sp. from polymicrobial infections in central bearded dragons (*Pogona vitticeps*).
1023 *J. Vet. Diagn. Investig. Off. Publ. Am. Assoc. Vet. Lab. Diagn. Inc* 23, 99–104.
1024 <https://doi.org/10.1177/104063871102300116>.
- 1025 Berg, J.M., Shi, Y., 1996. The galvanization of biology: a growing appreciation for the roles of zinc.
1026 *Science* 271, 1081–1085. <https://doi.org/10.1126/science.271.5252.1081>. Blencowe, D.K.,
1027 Morby, A.P., 2003. Zn(II) metabolism in prokaryotes. *FEMS Microbiol. Rev.* 27, 291–311.
1028 [https://doi.org/10.1016/S0168-6445\(03\)00041-X](https://doi.org/10.1016/S0168-6445(03)00041-X).
- 1029 Bolnick, D.I., Snowberg, L.K., Hirsch, P.E., Lauber, C.L., Knight, R., Caporaso, J.G., Svanbäck, R.,
1030 2014. Individuals' diet diversity influences gut microbial diversity in two freshwater fish
1031 (threespine stickleback and Eurasian perch). *Ecol. Lett.* 17, 979–987. <https://doi.org/10.1111/ele.12301>.
- 1032
- 1033 Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H.,
1034 Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn,
1035 C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E., Silva, R.D.,
1036 Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki,
1037 M., Fouquier, J., Gauglitz, J.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B.,
1038 Holmes, S., Holste, H., Huttenhower, C., Huttley, G., Janssen, S., Jarmusch, A.K., Jiang, L.,

1039 Kaehler, B., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciulek,
1040 T., Kreps, J., Langille, M.G., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M.,
1041 Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C.,
1042 Morton, J., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T.,
1043 Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Michael, S.,
1044 Robeson, I.I., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R.,
1045 Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan,
1046 S., van der Hooft, J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters,
1047 W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H., Willis, A.D., Xu, Z.Z.,
1048 Zaneveld, J.R., Zhang, Y., Knight, R., Caporaso, J.G., 2018. QIIME 2: reproducible, interactive,
1049 scalable, and extensible microbiome data science (No. e27295v1). PeerJ Inc.
1050 <https://doi.org/10.7287/peerj.preprints.27295v1>.

1051 Borghesi, F., Andreotti, A., Baccetti, N., Bianchi, N., Birke, M., Migani, F., Dinelli, E., 2011.
1052 Flamingo feathers to monitor metal contamination of coastal wetlands: methods and initial results
1053 concerning the presence of mercury at six Mediterranean sites. *Chem. Ecol.* 27, 137–151.
1054 <https://doi.org/10.1080/02757540.2011.625942>.

1055 Borghesi, F., Migani, F., Andreotti, A., Baccetti, N., Bianchi, N., Birke, M., Dinelli, E., 2016. Metals
1056 and trace elements in feathers: a geochemical approach to avoid misinterpretation of analytical
1057 responses. *Sci. Total Environ.* 544, 476–494.

1058 Borghesi, F., Dinelli, E., Migani, F., Béchet, A., Rendón-Martos, M., Amat, J.A., Sommer, S.,
1059 Gillingham, M.A.F., 2017. Assessing environmental pollution in birds: a new methodological
1060 approach for interpreting bioaccumulation of trace elements in feather shafts using geochemical
1061 sediment data. *Methods Ecol. Evol.* 8, 96–108. <https://doi.org/10.1111/2041-210X.12644>.

1062 Borrego, J., Morales, J., de la Torre, M., Grande, J., 2002. Geochemical characteristics of heavy metal
1063 pollution in surface sediments of the Tinto and Odiel river estuary (southwestern Spain). *Environ.*
1064 *Geol.* 41, 785–796. <https://doi.org/10.1007/s00254-001-0445-3>.

1065 Brabec, J.L., Wright, J., Ly, T., Wong, H.T., McClimans, C.J., Tokarev, V., Lamendella, R.,
1066 Sherchand, S., Shrestha, D., Uprety, S., Dangol, B., Tandukar, S., Sherchand, J.B., Sherchan, S.P.,
1067 2020. Arsenic disturbs the gut microbiome of individuals in a disadvantaged community in
1068 Nepal. *Heliyon* 6, e03313. <https://doi.org/10.1016/j.heliyon.2020.e03313>.

1069 Breton, J., Daniel, C., Dewulf, J., Pothion, S., Froux, N., Sauty, M., Thomas, P., Pot, B., Foligné, B.,
1070 2013a. Gut microbiota limits heavy metals burden caused by chronic oral expo-sure. *Toxicol.*
1071 *Lett.* 222, 132–138. <https://doi.org/10.1016/j.toxlet.2013.07.021>.

1072 Breton, J., Massart, S., Vandamme, P., De Brandt, E., Pot, B., Foligné, B., 2013b. Ecotoxicology
1073 inside the gut: impact of heavy metals on the mouse microbiome. *BMC Pharmacol. Toxicol.* 14,
1074 62. <https://doi.org/10.1186/2050-6511-14-62>.

1075 Bryan, G.W., Waldichuk, M., Pentreath, R.J., Darracott, A., 1979. Bioaccumulation of marine
1076 pollutants [and discussion]. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 286, 483–505.
1077 <https://doi.org/10.1098/rstb.1979.0042>.

1078 Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. *Rev Env. Toxicol*
1079 5, 203–311.

1080 Burger, J., Gochfeld, M., 1990. Tissue levels of lead in experimentally exposed herring gull (*Larus*
1081 *argentatus*) chicks. *J. Toxicol. Environ. Health* 29, 219–233. <https://doi.org/10.1080/15287399009531385>.

1083 Burger, J., Gochfeld, M., 2002. Effects of Chemicals and Pollution on Seabirds. CRC Press, Boca
1084 Raton, FL, Biology of marine birds.

1085 Burnham, K., Anderson, D., 2002. Model Selection and Multi-Model Inference - A Practical
1086 Information-Theoretic Approach. Springer, New York.

1087 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016.
1088 DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.
1089 <https://doi.org/10.1038/nmeth.3869>.

1090 Capellari, G., Caimmi, F., Bruggi, M., Mariani, S., 2016. A multiscale approach to the smart
1091 deployment of micro-sensors over flexible plates. Proceedings 1, 40. [https://doi.org/](https://doi.org/10.3390/ecsa-3-D005)
1092 10.3390/ecsa-3-D005.

1093 Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer,
1094 N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences
1095 per sample. Proc. Natl. Acad. Sci. 108, 4516–4522. [https://doi.org/ 10.1073/pnas.1000080107](https://doi.org/10.1073/pnas.1000080107).

1096 Carvalho, P.C., Bugoni, L., McGill, R.A.R., Bianchini, A., 2013. Metal and selenium concentrations
1097 in blood and feathers of petrels of the genus procellaria. Environ. Toxicol. Chem. 32, 1641–1648.
1098 <https://doi.org/10.1002/etc.2204>.

1099 Cézilly, F., Boy, V., Green, R.E., Hirons, G.J.M., Johnson, A.R., 1995. Interannual variation in greater
1100 flamingo breeding success in relation to water levels. Ecology 76, 20–26.
1101 <https://doi.org/10.2307/1940628>.

1102 Chandler, J.A., Morgan Lang, J., Bhatnagar, S., Eisen, J.A., Kopp, A., 2011. Bacterial communities
1103 of diverse Drosophila species: ecological context of a host–microbe model sys-tem. PLoS Genet.
1104 7, e1002272. <https://doi.org/10.1371/journal.pgen.1002272>.

1105 Claus, S.P., Ellero, S.L., Berger, B., Krause, L., Bruttin, A., Molina, J., Paris, A., Want, E.J., de
1106 Waziers, I., Cloarec, O., Richards, S.E., Wang, Y., Dumas, M.-E., Ross, A., Rezzi, S., Kochhar,
1107 S., Bladeren, P.V., Lindon, J.C., Holmes, E., Nicholson, J.K., 2011. Colonization-induced host-
1108 gut microbial metabolic interaction. mBio 2, e00271-10. <https://doi.org/10.1128/mBio.00271-10>.

1109 Claus, S.P., Guillou, H., Ellero-Simatos, S., 2016. The gut microbiota: a major player in the toxicity
1110 of environmental pollutants? Npj Biofilms Microbiomes 2, 1–11. [https://doi.](https://doi.org/10.1038/npjbiofilms.2016.3)
1111 [org/10.1038/npjbiofilms.2016.3](https://doi.org/10.1038/npjbiofilms.2016.3).

1112 Coolon, J.D., Jones, K.L., Narayanan, S., Wisely, S.M., 2010. Microbial ecological response of the
1113 intestinal flora of Peromyscus maniculatus and P. leucopus to heavy metal con-tamination. Mol.
1114 Ecol. 19, 67–80. <https://doi.org/10.1111/j.1365-294X.2009.04485.x>.

1115 Cosson, R.P., Amiard, J.-C., Amiard-Triquet, C., 1988. Trace elements in little egrets and flamingos
1116 of Camargue. France. Ecotoxicol. Environ. Saf. 15, 107–116. [https://doi.org/ 10.1016/0147-](https://doi.org/10.1016/0147-6513(88)90047-4)
1117 [6513\(88\)90047-4](https://doi.org/10.1016/0147-6513(88)90047-4).

1118 Couloigner, F., Jlali, M., Briens, M., Rouffineau, F., Geraert, P.-A., Mercier, Y., 2015. Selenium
1119 deposition kinetics of different selenium sources in muscle and feathers of broilers. Poult. Sci.
1120 94, 2708–2714. <https://doi.org/10.3382/ps/pev282>.

1121 Dauwe, T., Janssens, E., Bervoets, L., Blust, R., Eens, M., 2004. Relationships between metal
1122 concentrations in great tit nestlings and their environment and food. Environ. Pollut. 131, 373–
1123 380. <https://doi.org/10.1016/j.envpol.2004.03.009>.

1124 Davis, J.A., 1984. Complexation of trace metals by adsorbed natural organic matter. Geochim.
1125 Cosmochim. Acta 48, 679–691. [https://doi.org/10.1016/0016-7037\(84\)90095-4](https://doi.org/10.1016/0016-7037(84)90095-4).

- 1126 Debacker, V., Jauniaux, T., Coignoul, F., Bouqueneau, J.-M., 2000. Heavy metals contamination and
1127 body condition of wintering guillemots (*Uria aalge*) at the Belgian coast from 1993 to 1998.
1128 *Environ. Res.* 84, 310–317. <https://doi.org/10.1006/enrs.2000.4087>.
- 1129 Delsuc, F., Metcalf, J.L., Wegener Parfrey, L., Song, S.J., González, A., Knight, R., 2014.
1130 Convergence of gut microbiomes in myrmecophagous mammals. *Mol. Ecol.* 23, 1301–1317.
1131 <https://doi.org/10.1111/mec.12501>.
- 1132 Dempsey, J.L., Little, M., Cui, J.Y., 2019. Gut microbiome: an intermediary to neurotoxicity.
1133 *NeuroToxicology* 75, 41–69. <https://doi.org/10.1016/j.neuro.2019.08.005>.
- 1134 DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D.,
1135 Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene data-base and
1136 workbench compatible with ARB. *Appl. Environ. Microbiol.* 72, 5069–5072.
1137 <https://doi.org/10.1128/AEM.03006-05>.
- 1138 Di Giuseppe, D., Vittori Antisari, L., Ferronato, C., Bianchini, G., 2014. New insights on mobility
1139 and bioavailability of heavy metals in soils of the Padanian alluvial plain (Ferra Province,
1140 northern Italy). *Chem. Erde - Geochem.* 74, 615–623. <https://doi.org/10.1016/j.chemer.2014.02.004>.
- 1142 Dolan, K.J., Ciesielski, T.M., Lierhagen, S., Eulaers, I., Nygård, T., Johnsen, T.V., Gómez-Ramírez,
1143 P., García-Fernández, A.J., Bustnes, J.O., Ortiz-Santaliestra, M.E., Jaspers, V.L.B., 2017. Trace
1144 element concentrations in feathers and blood of Northern goshawk 60313.
- 1145 Esselink, H., van der Geld, F.M., Jager, L.P., Posthuma-Trumpie, G.A., Zoun, P.E.F., Baars, A.J.,
1146 1995. Biomonitoring heavy metals using the barn owl (*Tyto alba guttata*): sources of
1147 variation especially relating to body condition. *Arch. Environ. Contam. Toxicol.* 28, 471–486.
1148 <https://doi.org/10.1007/BF00211630>.
- 1149 Falkowska, L., Szumiło, E., Hajdryh, J., Grajewska, A., Beldowska, M., Krause, I., 2013. Effect of
1150 diet on the capacity to remove mercury from the body of a penguin (*Spheniscus demersus*) living
1151 in the ZOO. *E3S Web Conf.* 1, p. 12002. <https://doi.org/10.1051/e3sconf/20130112002>.
- 1152 Fazeli, M., Hassanzadeh, P., Alaei, S., 2011. Cadmium chloride exhibits a profound toxic effect on
1153 bacterial microflora of the mice gastrointestinal tract. *Hum. Exp. Toxicol.* 30, 152–159.
1154 <https://doi.org/10.1177/0960327110369821>.
- 1155 Fisher, H., 1972. Chapter 7 - the nutrition of birds A2 - king. In: Donald, S., Farnerjames, R. (Eds.),
1156 *Avian Biology*. Academic Press, Amsterdam, pp. 431–469.
- 1157 Furness, R.W., Greenwood, J.J.D. (Eds.), 1993. *Birds as Monitors of Environmental Change*. Springer
1158 Netherlands, Dordrecht.
- 1159 Gaither, L.A., Eide, D.J., 2001. Eukaryotic zinc transporters and their regulation. *Biometals* 14, 251–
1160 270. <https://doi.org/10.1023/A:1012988914300>.
- 1161 Gao, B., Chi, L., Mahbub, R., Bian, X., Tu, P., Ru, H., Lu, K., 2017. Multi-omics reveals that lead exposure
1162 disturbs gut microbiome development, key metabolites, and metabolic pathways. *Chem. Res.*
1163 *Toxicol.* 30, 996–1005. <https://doi.org/10.1021/acs.chemrestox.6b00401>.
- 1164 Geraci, J., Béchet, A., Cézilly, F., Ficheux, S., Baccetti, N., Samraoui, B., Wattier, R., 2012. Greater
1165 flamingo colonies around the Mediterranean form a single interbreeding population and share a
1166 common history. *J. Avian Biol.* 43, 341–354. <https://doi.org/10.1111/j.1600-048X.2012.05549.x>.
- 1167 Gillingham, M.A.F., Béchet, A., Geraci, J., Wattier, R., Dubreuil, C., Cézilly, F., 2012. Genetic
1168 polymorphism in dopamine receptor D4 is associated with early body condition in a large
1169 population of greater flamingos, *Phoenicopterus roseus*. *Mol. Ecol.* 21, 4024–4037.
1170 <https://doi.org/10.1111/j.1365-294X.2012.05669.x>.

1171 Gillingham, M.A.F., Cézilly, F., Wattier, R., Béchet, A., 2013. Evidence for an association between
1172 post-fledging dispersal and microsatellite multilocus heterozygosity in a large population of
1173 greater flamingos. *PLoS One* 8, e81118. <https://doi.org/10.1371/journal.pone.0081118>.

1174 Gillingham, M.A.F., Béchet, A., Courtiol, A., Rendón-Martos, M., Amat, J.A., Samraoui, B., Onmuş,
1175 O., Sommer, S., Cézilly, F., 2017. Very high MHC Class IIB diversity without spatial
1176 differentiation in the mediterranean population of greater Flamingos. *BMC Evol. Biol.* 17, 56.
1177 <https://doi.org/10.1186/s12862-017-0905-3>.

1178 Gillingham, M.A.F., Béchet, A., Cézily, F., Wilhelm, K., Rendón-Martos, M., Borghesi, F., Nissardi,
1179 S., Baccetti, N., Azafzaf, H., Menke, S., Kayser, Y., Sommer, S., 2019. Offspring microbiomes
1180 differ across breeding sites in a panmictic species. *Front. Microbiol.* 10.
1181 <https://doi.org/10.3389/fmicb.2019.00035>.

1182 Gould, A.L., Zhang, V., Lamberti, L., Jones, E.W., Obadia, B., Korasidis, N., Gavryushkin, A.,
1183 Carlson, J.M., Beerenwinkel, N., Ludington, W.B., 2018. Microbiome interactions shape host
1184 fitness. *Proc. Natl. Acad. Sci.* 201809349. <https://doi.org/10.1073/pnas.1809349115>.

1185 Guan, D.M., Martin, J.-M., 1991. Selenium distribution in the Rhône delta and the Gulf of lions. *Mar.*
1186 *Chem. Reactivity of Chemical Species in Aquatic Environments* 36, 303–316.
1187 [https://doi.org/10.1016/S0304-4203\(09\)90068-8](https://doi.org/10.1016/S0304-4203(09)90068-8).

1188 Guillén, M.T., Delgado, J., Albanese, S., Nieto, J.M., Lima, A., De Vivo, B., 2011. Environmental
1189 geochemical mapping of Huelva municipality soils (SW Spain) as a tool to deter-mine
1190 background and baseline values. *J. Geochem. Explor. Pedogeochemical mapping of potentially*
1191 *toxic elements International Conference of the European Geosciences Union Session SSS32* 109,
1192 59–69. <https://doi.org/10.1016/j.gexplo.2011.03.003>.

1193 Halttunen, T., Collado, M.C., El-Nezami, H., Meriluoto, J., Salminen, S., 2008. Combining strains of
1194 lactic acid bacteria may reduce their toxin and heavy metal removal efficiency from aqueous
1195 solution. *Lett. Appl. Microbiol.* 46, 160–165. [https://doi.org/10.1111/j.1472-](https://doi.org/10.1111/j.1472-765X.2007.02276.x)
1196 [765X.2007.02276.x](https://doi.org/10.1111/j.1472-765X.2007.02276.x).

1197 Hehemann, J.-H., Kelly, A.G., Pudlo, N.A., Martens, E.C., Boraston, A.B., 2012. Bacteria of the
1198 human gut microbiome catabolize red seaweed glycans with carbohydrate-active en-zyme
1199 updates from extrinsic microbes. *Proc. Natl. Acad. Sci. U. S. A.* 109, 19786–19791.
1200 <https://doi.org/10.1073/pnas.1211002109>.

1201 Heinz, G.H., Fitzgerald, M.A., 1993. Overwinter survival of mallards fed selenium. *Arch. Environ.*
1202 *Contam. Toxicol.* 25, 90–94. <https://doi.org/10.1007/BF00230717>.

1203 Heinz, G.H., Hoffman, D.J., 1998. Methylmercury chloride and selenomethionine interactions on
1204 health and reproduction in mallards. *Environ. Toxicol. Chem.* 17, 139–145.
1205 <https://doi.org/10.1002/etc.5620170202>.

1206 Højberg, O., Canibe, N., Poulsen, H.D., Hedemann, M.S., Jensen, B.B., 2005. Influence of dietary
1207 zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. *Appl.*
1208 *Environ. Microbiol.* 71, 2267–2277. [https://doi.org/10.1128/ AEM.71.5.2267-2277.2005](https://doi.org/10.1128/AEM.71.5.2267-2277.2005).

1209 Howell, N., Lavers, J., Paterson, D., Garrett, R., Banati, R., 2012. Trace metal distribution in feathers
1210 from migratory, pelagic birds using high-resolution synchrotron X-ray fluorescence microscopy.
1211 *Res. Sel.* 2012, 41.

1212 Janssens, E., Dauwe, T., Pinxten, R., Bervoets, L., Blust, R., Eens, M., 2003. Effects of heavy metal
1213 exposure on the condition and health of nestlings of the great tit (*Parus major*), a small songbird
1214 species. *Environ. Pollut.* 126, 267–274. [https://doi.org/10.1016/S0269-7491\(03\)00185-4](https://doi.org/10.1016/S0269-7491(03)00185-4).

1215 Järup, L., 2003. Hazards of heavy metal contamination. *Br. Med. Bull.* 68, 167–182.

- 1216 Jaspers, V.L.B., Covaci, A., Herzke, D., Eulaers, I., Eens, M., 2019. Bird feathers as a biomonitor for
1217 environmental pollutants: prospects and pitfalls. *TrAC Trends Anal. Chem.* 118, 223–226.
1218 <https://doi.org/10.1016/j.trac.2019.05.019>.
- 1219 Jenkin, P.M., 1957. The filter-feeding and food of flamingoes (*Phoenicopteri*). *Philos. Trans. R. Soc.*
1220 *Lond. Ser. B Biol. Sci.* 401–493.
- 1221 Johnson, A.R., 1997. *Phoenicopterus ruber* greater flamingo. *BWP Update* 1, 15–23. Johnson, A.,
1222 Cézilly, F.C., 2007. *The Greater Flamingo* (Poyser).
- 1223 Kawamura, Y., Kuwabara, S., Kania, S.A., Kato, H., Hamagishi, M., Fujiwara, N., Sato, T., Tomida,
1224 J., Tanaka, K., Bemis, D.A., 2015. *Porphyromonas pogonae* sp. nov., an anaerobic but low
1225 concentration oxygen adapted coccobacillus isolated from lizards (*Pogona vitticeps*) or human
1226 clinical specimens, and emended description of the genus *Porphyromonas* Shah and Collins
1227 1988. *Syst. Appl. Microbiol.* 38, 104–109. <https://doi.org/10.1016/j.syapm.2014.11.004>.
- 1228 Keller, R.H., Xie, L., Buchwalter, D.B., Franzreb, K.E., Simons, T.R., 2014. Mercury
1229 bioaccumulation in Southern Appalachian birds, assessed through feather concentrations.
1230 *Ecotoxicology* 23, 304–316. <https://doi.org/10.1007/s10646-013-1174-6>.
- 1231 Kim, J.W., Kil, D.Y., 2015. Determination of relative bioavailability of copper in tribasic copper
1232 chloride to copper in copper sulfate for broiler chickens based on liver and feather copper
1233 concentrations. *Anim. Feed Sci. Technol.* 210, 138–143. <https://doi.org/10.1016/j.anifeedsci.2015.09.022>.
- 1235 Kim, J.W., Kim, J.H., Shin, J.E., Kil, D.Y., 2016. Relative bioavailability of copper in tribasic copper
1236 chloride to copper in copper sulfate for laying hens based on egg yolk and feather copper
1237 concentrations. *Poult. Sci.* 95, 1591–1597. <https://doi.org/10.3382/ps/pew049>.
- 1238 Kirpichtchikova, T.A., Manceau, A., Spadini, L., Panfili, F., Marcus, M.A., Jacquet, T., 2006.
1239 Speciation and solubility of heavy metals in contaminated soil using X-ray microfluorescence,
1240 EXAFS spectroscopy, chemical extraction, and thermodynamic modeling. *Geochim.*
1241 *Cosmochim. Acta* 70, 2163–2190.
- 1242 Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D.,
1243 Knight, R., Kelley, S.T., 2011. Bayesian community-wide culture-independent microbial source
1244 tracking. *Nat. Methods* 8, 761–763. <https://doi.org/10.1038/nmeth.1650>.
- 1245 Lai, P.W., Liang, J.B., Hsia, L.C., Loh, T.C., Ho, Y.W., 2010. Effects of varying dietary zinc levels
1246 and environmental temperatures on the growth performance, feathering score and feather mineral
1247 concentrations of broiler chicks. *Asian-Australas. J. Anim. Sci.* 23, 937–945
1248 (<https://doi.org/2010.23.7.937>).
- 1249 Lang, E.M., 1963. Flamingoes raise their young on a liquid containing blood. *Experientia* 19, 532–
1250 533.
- 1251 Leeson, S., 2009. Copper metabolism and dietary needs. *Worlds Poult. Sci. J.* 65, 353–366.
1252 <https://doi.org/10.1017/S0043933909000269>.
- 1253 Lemly, A.D., 2004. Aquatic selenium pollution is a global environmental safety issue. *Ecotoxicol.*
1254 *Environ. Saf.* 59, 44–56. [https://doi.org/10.1016/S0147-6513\(03\)00095-2](https://doi.org/10.1016/S0147-6513(03)00095-2).
- 1255 Lewis, S.A., Furness, R.W., 1991. Mercury accumulation and excretion in laboratory reared black-
1256 headed gull *Larus ridibundus* chicks. *Arch. Environ. Contam. Toxicol.* 21, 316–320.
- 1257 Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L.,
1258 Tucker, T.A., Schrenzel, M.D., Knight, R., Gordon, J.I., 2008a. Evolution of mammals and their
1259 gut microbes. *Science* 320, 1647–1651. <https://doi.org/10.1126/science.1155725>.

1260 Ley, R.E., Lozupone, C.A., Hamady, M., Knight, R., Gordon, J.I., 2008b. Worlds within worlds:
1261 evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* 6, 776–788.
1262 <https://doi.org/10.1038/nrmicro1978>.

1263 Liu, Y., Li, Y., Liu, K., Shen, J., 2014. Exposing to cadmium stress cause profound toxic effect on
1264 microbiota of the mice intestinal tract. *PLoS One* 9, e85323. [https://doi.org/](https://doi.org/10.1371/journal.pone.0085323)
1265 [10.1371/journal.pone.0085323](https://doi.org/10.1371/journal.pone.0085323).

1266 Lukman, S., Essa, M.H., Mu'azu, N.D., Bukhari, A., Basheer, C., 2013. Adsorption and desorption
1267 of heavy metals onto natural clay material: influence of initial pH. *J. Environ. Sci. Technol.* 6, 1.

1268 Lu Kun, Phillip, Abo Ryan, Ann, Schlieper Katherine, Graffam, Michelle E., Stuart, Levine, Wishnok,
1269 John S., Swenberg, James A., Tannenbaum, Steven R., Fox, James G., 2014. Arsenic exposure
1270 perturbs the gut microbiome and its metabolic profile in mice: an integrated metagenomics and
1271 metabolomics analysis. *Environ. Health Perspect.* 122, 284–291.
1272 <https://doi.org/10.1289/ehp.1307429>.

1273 Ma, Z. (Sam), 2020. Testing the Anna Karenina principle in human microbiome-associated diseases.
1274 *iScience* 23, 101007. <https://doi.org/10.1016/j.isci.2020.101007>.

1275 Maher, B., Roach, T., Doblin, M., Fan, T., Foster, S., Garrett, R., Moller, G., Oram, L., Wallschlager,
1276 D., 2010. Environmental sources, speciation and partitioning of selenium. *Ecol. Assess. Selenium*
1277 *Aquat. Environ.* 47–92.

1278 Mandal, S., Treuren, W.V., White, R.A., Eggesbø, M., Knight, R., Peddada, S.D., 2015. Analysis of
1279 composition of microbiomes: a novel method for studying microbial composition. *Microb. Ecol.*
1280 *Health Dis.* 26, 27663. <https://doi.org/10.3402/mehd.v26.27663>.

1281 Markowski, M., Kaliński, A., Skwarska, J., Wawrzyniak, J., Bańbura, M., Markowski, J., Zieliński,
1282 P., Bańbura, J., 2013. Avian feathers as bioindicators of the exposure to heavy metal
1283 contamination of food. *Bull. Environ. Contam. Toxicol.* 91, 302–305.
1284 <https://doi.org/10.1007/s00128-013-1065-9>.

1285 Martino, C., Morton, J.T., Marotz, C.A., Thompson, L.R., Tripathi, A., Knight, R., Zengler, K., 2019.
1286 A novel sparse compositional technique reveals microbial perturbations. *mSystems*, 4
1287 <https://doi.org/10.1128/mSystems.00016-19>.

1288 McDermott, T.R., Stolz, J.F., Oremland, R.S., 2020. Arsenic and the gastrointestinal tract
1289 microbiome. *Environ. Microbiol. Rep.* 12, 136–159. <https://doi.org/10.1111/1758-2229.12814>.

1290 McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and
1291 graphics of microbiome census data. *PLOS ONE* 8, e61217. [https://doi.org/](https://doi.org/10.1371/journal.pone.0061217)
1292 [10.1371/journal.pone.0061217](https://doi.org/10.1371/journal.pone.0061217).

1293 Migani, F., Borghesi, F., Dinelli, E., 2015. Geochemical characterization of surface sediments from
1294 the northern Adriatic wetlands around the Po river delta. Part I: Bulk composition and relation to
1295 local background. *J. Geochem. Explor.* 156, 72–88.

1296 Monteiro, L.R., Furness, R.W., 2001. Kinetics, dose–response, and excretion of methyl-mercury in
1297 free-living adult Cory's shearwaters. *Environ. Sci. Technol.* 35, 739–746.
1298 <https://doi.org/10.1021/es000114a>.

1299 Monteiro, L.R., Granadeiro, J.P., Furness, R.W., 1998. Relationship between mercury levels and diet
1300 in Azores seabirds. *Mar. Ecol. Prog. Ser.* 166, 259–265. <https://doi.org/10.3354/meps166259>.

1301 Morillo, J., Usero, J., Rojas, R., 2008. Fractionation of metals and As in sediments from a biosphere
1302 reserve (Odiel salt marshes) affected by acidic mine drainage. *Environ. Monit. Assess.* 139, 329–
1303 337.

1304 Morozzi, G., Cenci, G., Scardazza, F., Pitzurra, M., 1986. Cadmium uptake by growing cells of gram-
1305 positive and gram-negative bacteria. *Microbios* 48, 27–35.

1306 Muegge, B.D., Kuczynski, J., Knights, D., Clemente, J.C., González, A., Fontana, L., Henrissat, B.,
1307 Knight, R., Gordon, J.I., 2011. Diet drives convergence in gut microbiome functions across
1308 mammalian phylogeny and within humans. *Science* 332, 970–974. <https://doi.org/10.1126/science.1198719>.

1310 Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a
1311 practical guide for biologists. *Biol. Rev.* 82, 591–605. <https://doi.org/10.1111/j.1469-185X.2007.00027.x>.

1313 Nieto, J.M., Sarmiento, A.M., Olías, M., Canovas, C.R., Riba, I., Kalman, J., Delvalls, T.A., 2007.
1314 Acid mine drainage pollution in the Tinto and Odiel rivers (Iberian Pyrite Belt, SW Spain) and
1315 bioavailability of the transported metals to the Huelva Estuary. *Environ. Int.* 33, 445–455.

1316 Ninkov, M., Popov Aleksandrov, A., Demenesku, J., Mirkov, I., Mileusnic, D., Petrovic, A., Grigorov,
1317 I., Zolotarevski, L., Tolinacki, M., Kataranovski, D., Brceski, I., Kataranovski, M., 2015. Toxicity
1318 of oral cadmium intake: impact on gut immunity. *Toxicol. Lett.* 237, 89–99.
1319 <https://doi.org/10.1016/j.toxlet.2015.06.002>.

1320 Nriagu, J.O., 1989. A global assessment of natural sources of atmospheric trace metals. *Nature* 338,
1321 47–49.

1322 Nriagu, J.O., 1996. A history of global metal pollution. *Science* 272, 223.
1323 <https://doi.org/10.1126/science.272.5259.223>.

1324 Obata, T., Shiraiwa, Y., 2005. A novel eukaryotic selenoprotein in the haptophyte alga *Emiliania*
1325 *huxleyi*. *J. Biol. Chem.* 280, 18462–18468. <https://doi.org/10.1074/jbc.M501517200>.

1326 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R.,
1327 O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019.
1328 *vegan: Community Ecology Package*.

1329 Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/ length data:
1330 the scaled mass index as an alternative method. *Oikos* 118, 1883–1891.
1331 <https://doi.org/10.1111/j.1600-0706.2009.17643.x>.

1332 Peig, J., Green, A.J., 2010. The paradigm of body condition: a critical reappraisal of current methods
1333 based on mass and length. *Funct. Ecol.* 24, 1323–1332. <https://doi.org/10.1111/j.1365-2435.2010.01751.x>.

1335 Phillips, C.D., Phelan, G., Dowd, S.E., McDonough, M.M., Ferguson, A.W., Delton Hanson, J., Siles,
1336 L., Ordóñez-Garza, N., San Francisco, M., Baker, R.J., 2012. Microbiome analysis among bats
1337 describes influences of host phylogeny, life history, physiology and geography. *Mol. Ecol.* 21,
1338 2617–2627. <https://doi.org/10.1111/j.1365-294X.2012.05568.x>.

1339 Pieper, R., Vahjen, W., Neumann, K., Kessel, A.G.V., Zentek, J., 2012. Dose-dependent effects of
1340 dietary zinc oxide on bacterial communities and metabolic profiles in the ileum of weaned pigs.
1341 *J. Anim. Physiol. Anim. Nutr.* 96, 825–833. <https://doi.org/10.1111/j.1439-0396.2011.01231.x>.

1342 Pilastro, A., Congiu, L., Tallandini, L., Turchetto, M., 1993. The use of bird feathers for the
1343 monitoring of cadmium pollution. *Arch. Environ. Contam. Toxicol.* 24, 355–358.
1344 <https://doi.org/10.1007/BF01128733>.

1345 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2020. *sigma*, S.H. (Author fixed, R-core). *nlme: Linear*
1346 *and Nonlinear Mixed Effects Models*.

- Presser, T.S., Ohlendorf, H.M., 1987. Biogeochemical cycling of selenium in the San Joaquin Valley, California. USA. *Environ. Manag.* 11, 805–821. <https://doi.org/10.1007/BF01867247>.
- Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS One* 5, e9490. <https://doi.org/10.1371/journal.pone.0009490>.
- Provenzano, M.R., El Bilali, H., Simeone, V., Baser, N., Mondelli, D., Cesari, G., 2010. Copper contents in grapes and wines from a Mediterranean organic vineyard. *Food Chem.* 122, 1338–1343. <https://doi.org/10.1016/j.foodchem.2010.03.103>.
- Quinn, T.P., Erb, I., Richardson, M.F., Crowley, T.M., 2018. Understanding sequencing data as compositions: an outlook and review. *Bioinformatics* 34, 2870–2878. <https://doi.org/10.1093/bioinformatics/bty175>.
- Quinn, T.P., Erb, I., Gloor, G., Notredame, C., Richardson, M.F., Crowley, T.M., 2019. A field guide for the compositional analysis of any-omics data. *GigaScience* 8. <https://doi.org/10.1093/gigascience/giz107>.
- R Core Team, 2020. R Core Team (2020). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL. <https://www.R-project.org/>.
- Reed, S., Neuman, H., Moscovich, S., Glahn, R.P., Koren, O., Tako, E., 2015. Chronic zinc deficiency alters chick gut microbiota composition and function. *Nutrients* 7, 9768–9784. <https://doi.org/10.3390/nu7125497>.
- Rendón, M.A., Garrido, A., Ramírez, J.M., Rendón-Martos, M., Amat, J.A., 2001. Despotism establishment of breeding colonies of greater flamingos, *Phoenicopterus ruber*, in southern Spain. *Behav. Ecol. Sociobiol.* 50, 55–60. <https://doi.org/10.1007/s002650100326>.
- Rendón, M.A., Rendón-Martos, M., Garrido, A., Amat, J.A., 2011. Greater flamingos *Phoenicopterus roseus* are partial capital breeders. *J. Avian Biol.* 42, 210–213. <https://doi.org/10.1111/j.1600-048X.2011.05236.x>.
- Rendón, M.A., Garrido, A., Rendón-Martos, M., Ramírez, J.M., Amat, J.A., 2014. Assessing sex-related chick provisioning in greater flamingo *Phoenicopterus roseus* parents using capture–recapture models. *J. Anim. Ecol.* 83, 479–490. <https://doi.org/10.1111/1365-2656.12138>.
- Risely, A., Waite, D., Ujvari, B., Klaassen, M., Hoyer, B., 2017. Gut microbiota of a long-distance migrant demonstrates resistance against environmental microbe incursions. *Mol. Ecol.* 26, 5842–5854. <https://doi.org/10.1111/mec.14326>.
- Rodríguez-Estival, J., Sánchez, M.I., Ramo, C., Varo, N., Amat, J.A., Garrido-Fernández, J., Hornero-Méndez, D., Ortiz-Santaliestra, M.E., Taggart, M.A., Martínez-Haro, M., Green, A.J., Mateo, R., 2019. Exposure of black-necked grebes (*Podiceps nigricollis*) to metal pollution during the moulting period in the Odiel Marshes, Southwest Spain. *Chemosphere* 216, 774–784. <https://doi.org/10.1016/j.chemosphere.2018.10.145>.
- Rosenfeld, C.S., 2017. Gut dysbiosis in animals due to environmental chemical exposures. *Front. Cell. Infect. Microbiol.* 7. <https://doi.org/10.3389/fcimb.2017.00396>.
- Ruan, Y., Wu, C., Guo, X., Xu, Z., Xing, C., Cao, H., Zhang, C., Hu, G., Liu, P., 2018. High doses of copper and mercury changed cecal microbiota in female mice. *Biol. Trace Elem. Res.* 189, 134–144. <https://doi.org/10.1007/s12011-018-1456-1>.
- Rubio, I., Martínez-Madrid, M., Méndez-Fernández, L., Galarza, A., Rodríguez, P., 2016. Heavy metal concentration in feathers of Little Egret (*Egretta garzetta*) nestlings in three coastal breeding colonies in Spain. *Ecotoxicology* 25, 30–40.

1390 Ruiz, F., Borrego, J., González-Regalado, M.L., González, N.L., Carro, B., Abad, M., 2008. Impact
1391 of millennial mining activities on sediments and microfauna of the Tinto River estuary (SW
1392 Spain). *Mar. Pollut. Bull.* 56, 1258–1264.

1393 Sakamoto, M., Li, D., Shibata, Y., Takeshita, T., Yamashita, Y., Ohkuma, M., 2015. *Porphyromonas*
1394 *pasteri* sp. nov., isolated from human saliva. *Int. J. Syst. Evol. Microbiol.* 65, 2511–2515.
1395 <https://doi.org/10.1099/ijls.0.000294>.

1396 Sánchez-Virosta, P., Espín, S., García-Fernández, A.J., Eeva, T., 2015. A review on exposure and
1397 effects of arsenic in passerine birds. *Sci. Total Environ.* 512–513, 506–525.
1398 <https://doi.org/10.1016/j.scitotenv.2015.01.069>.

1399 Scheirs, J., de Coen, A., Covaci, A., Beernaert, J., Kayawe, V.M., Caturla, M., de Wolf, H., Baert, P.,
1400 Oostveldt, P.V., Verhagen, R., Blust, R., de Coen, W., 2006. Genotoxicity in wood mice
1401 (*Apodemus sylvaticus*) along a pollution gradient: exposure-, age-, and gender-related effects.
1402 *Environ. Toxicol. Chem.* 25, 2154–2162. <https://doi.org/10.1897/05-419R.1>.

1403 Schiavon, M., Ertani, A., Parrasia, S., Vecchia, F.D., 2017. Selenium accumulation and metabolism
1404 in algae. *Aquat. Toxicol.* 189, 1–8. <https://doi.org/10.1016/j.aquatox.2017.05.011>.

1405 Ševčíková, S., Skřivan, M., Dlouhá, G., Koucký, M., 2006. The effect of selenium source on the
1406 performance and meat quality of broiler chickens. *Czech J. Anim. Sci.* 51, 449–457.

1407 Shankar, V., Agans, R., Paliy, O., 2017. Advantages of phylogenetic distance based constrained
1408 ordination analyses for the examination of microbial communities. *Sci. Rep.* 7.
1409 <https://doi.org/10.1038/s41598-017-06693-z>.

1410 Shin, S.C., Kim, S.-H., You, H., Kim, B., Kim, A.C., Lee, K.-A., Yoon, J.-H., Ryu, J.-H., Lee, W.-J.,
1411 2011. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin
1412 signaling. *Science* 334, 670–674. <https://doi.org/10.1126/science.1212782>.

1413 Smith, J.C., McDaniel, E.G., McBean, L.D., Doft, F.S., Halsted, J.A., 1972. Effect of microorganisms
1414 upon zinc metabolism using germfree and conventional rats. *J. Nutr.* 102, 711–719.
1415 <https://doi.org/10.1093/jn/102.6.711>.

1416 Smith, T.B., Marra, P.P., Webster, M.S., Lovette, I., Gibbs, H.L., Holmes, R.T., Hobson, K.A.,
1417 Rohwer, S., Prum, R., 2003. A call for feather sampling. *Auk* 120, 218–221.

1418 Song, M., Li, X., Zhang, X., Shi, H., Vos, M.B., Wei, X., Wang, Y., Gao, H., Rouchka, E.C., Yin, X.,
1419 Zhou, Z., Prough, R.A., Cave, M.C., McClain, C.J., 2017. Dietary copper-fructose interactions
1420 alter gut microbial activity in male rats. *Am. J. Physiol.-Gastrointest. Liver Physiol.* 314, G119–
1421 G130. <https://doi.org/10.1152/ajpgi.00378.2016>.

1422 Starke, I.C., Pieper, R., Neumann, K., Zentek, J., Vahjen, W., 2014. The impact of high dietary zinc
1423 oxide on the development of the intestinal microbiota in weaned piglets. *FEMS Microbiol. Ecol.*
1424 87, 416–427. <https://doi.org/10.1111/1574-6941.12233>.

1425 Stecher, B., Hardt, W.-D., 2008. The role of microbiota in infectious disease. *Trends Microbiol.* 16,
1426 107–114. <https://doi.org/10.1016/j.tim.2007.12.008>.

1427 Stolz, J.F., Basu, P., Santini, J.M., Oremland, R.S., 2006. Arsenic and selenium in microbial
1428 metabolism. *Annu. Rev. Microbiol.* 60, 107–130. <https://doi.org/10.1146/annurev.micro.60.080805.142053>.

1430 Summanen, P.H., Lawson, P.A., Finegold, S.M., 2009. *Porphyromonas bennonis* sp. nov., isolated
1431 from human clinical specimens. *Int. J. Syst. Evol. Microbiol.* 59, 1727–1732.
1432 <https://doi.org/10.1099/ijls.0.001909-0>.

1433 Sun, L.-H., Zhang, N.-Y., Zhai, Q.-H., Gao, X., Li, C., Zheng, Q., Krumm, C.S., Qi, D., 2014. Effects
 1434 of dietary tin on growth performance, hematology, serum biochemistry, antioxidant status, and
 1435 tin retention in broilers. *Biol. Trace Elem. Res.* 162, 302–308. [https://doi.org/10.1007/s12011-](https://doi.org/10.1007/s12011-014-0129-y)
 1436 014-0129-y.

1437 Sunde, M.L., 1972. Zinc requirement for Normal feathering of commercial Leghorn-type pullets.
 1438 *Poult. Sci.* 51, 1316–1322. <https://doi.org/10.3382/ps.0511316>.

1439 Supplee, W.C., Combs, G.F., Blamberg, D.L., 1958. Zinc and potassium effects on bone formation,
 1440 feathering and growth of Poults¹. *Poult. Sci.* 37, 63–67. <https://doi.org/10.3382/ps.0370063>.

1441 Takekawa, J.Y., Wainwright-De La Cruz, S.E., Hothem, R.L., Yee, J., 2002. Relating body condition
 1442 to inorganic contaminant concentrations of diving ducks wintering in coastal California. *Arch.*
 1443 *Environ. Contam. Toxicol.* 42, 60–70. <https://doi.org/10.1007/s002440010292>.

1444 Tasneem, F., Abbasi, N.A., Iqbal Chaudhry, M.J., Mashiatullah, A., Ahmad, S.R., Qadir, A., Malik,
 1445 R.N., 2020. Dietary proxies ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) as signature of metals and arsenic exposure in birds
 1446 from aquatic and terrestrial food chains. *Environ. Res.* 183, 109191.
 1447 <https://doi.org/10.1016/j.envres.2020.109191>.

1448 Thompson, D.R., Furness, R.W., Monteiro, L.R., 1998. Seabirds as biomonitors of mercury inputs to
 1449 epipelagic and mesopelagic marine food chains. *Sci. Total Environ.* 213, 299–305.
 1450 [https://doi.org/10.1016/S0048-9697\(98\)00103-X](https://doi.org/10.1016/S0048-9697(98)00103-X).

1451 Tinkov, A.A., Gritsenko, V.A., Skalnaya, M.G., Cherkasov, S.V., Aaseth, J., Skalny, A.V., 2018. Gut
 1452 as a target for cadmium toxicity. *Environ. Pollut.* 235, 429–434.
 1453 <https://doi.org/10.1016/j.envpol.2017.12.114>.

1454 Tsipoura, N., Burger, J., Feltes, R., Yacabucci, J., Mizrahi, D., Jeitner, C., Gochfeld, M., 2008. Metal
 1455 concentrations in three species of passerine birds breeding in the Hackensack meadowlands of
 1456 New Jersey. *Environ. Res.* 107, 218–228.

1457 Vahjen, W., Pieper, R., Zentek, J., 2010. Bar-coded pyrosequencing of 16S rRNA gene amplicons
 1458 reveals changes in ileal porcine bacterial communities due to high dietary zinc intake. *Appl.*
 1459 *Environ. Microbiol.* 76, 6689–6691. <https://doi.org/10.1128/AEM.03075-09>.

1460 Vicente-Martorell, J.J., Galindo-Riaño, M.D., García-Vargas, M., Granado-Castro, M.D., 2009.
 1461 Bioavailability of heavy metals monitoring water, sediments and fish species from a polluted
 1462 estuary. *J. Hazard. Mater.* 162, 823–836.

1463 Vohra, P., Kratzer, F.H., 1957. The effect of dietary copper and molybdenum on Turkey poults. *Poult.*
 1464 *Sci.* 36, 1096–1098. <https://doi.org/10.3382/ps.0361096>.

1465 Vohra, P., Gottfredson, G.D., Kratzer, F.H., 1968. The effects of high levels of dietary EDTA, zinc or
 1466 copper on the mineral contents of some tissue of Turkey Poults¹. *Poult. Sci.* 47, 1334–1343.
 1467 <https://doi.org/10.3382/ps.0471334>.

1468 Wagener, K., Drillich, M., Baumgardt, S., Kämpfer, P., Busse, H.-J., Ehling-Schulz, M., 2014.
 1469 *Falsiporphyrromonas endometrii* gen. nov., sp. nov., isolated from the post-partum bovine uterus,
 1470 and emended description of the genus *Porphyromonas* Shah and Collins 1988. *Int. J. Syst. Evol.*
 1471 *Microbiol.* 64, 642–649. <https://doi.org/10.1099/ij.s.0.057307-0>.

1472 Wood, S.N., 2017. *Generalized Additive Models: An Introduction With R*. CRC Press, Second
 1473 Edition.

1474 Yohannes, E., Arnaud, A., Béchet, A., 2014. Tracking variations in wetland use by breeding flamingos
 1475 using stable isotope signatures of feather and blood. *Estuar. Coast. Shelf Sci.* 136, 11–18.
 1476 <https://doi.org/10.1016/j.ecss.2013.11.010>.

1477 Zaneveld, J.R., McMinds, R., Vega Thurber, R., 2017. Stress and stability: applying the Anna
1478 Karenina principle to animal microbiomes. *Nat. Microbiol.* 2, 1–8.
1479 <https://doi.org/10.1038/nmicrobiol.2017.121>.

1480 Zeileis, A., Kleiber, C., Jackman, S., 2008. Regression models for count data in R. *J. Stat. Softw.* 27,
1481 1–25. <https://doi.org/10.18637/jss.v027.i08>.

1482 Zhang, W., Guo, R., Yang, Y., Ding, J., Zhang, Y., 2016. Long-term effect of heavy-metal pollution
1483 on diversity of gastrointestinal microbial community of *Bufo raddei*. *Toxicol. Lett.* 258, 192–
1484 197. <https://doi.org/10.1016/j.toxlet.2016.07.003>.

1485 Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type
1486 analyses. *Methods Ecol. Evol.* 7, 636–645. <https://doi.org/10.1111/2041-210X.12577>.

1487 Zuur, A.F., Ieno, E., Walker, N., 2009. *Mixed Effects Models and Extensions in Ecology With R.*
1488 Springer.

1489