

1 **Impacts of radiation on the bacterial and fungal microbiome of small mammals in the**
2 **Chernobyl Exclusion Zone**

3

4 Rachael E. Antwis^{1#}, Nicholas A. Beresford^{1,2}, Joseph A. Jackson¹, Ross Fawkes¹,
5 Catherine L. Barnett², Elaine Potter², Lee Walker², Sergey Gaschak³, Michael D. Wood¹

6

7 1. School of Science, Engineering and Environment, University of Salford, UK
8 2. UK Centre for Ecology & Hydrology, Lancaster Environment Centre, UK
9 3. Chernobyl Center for Nuclear Safety, Radioactive Waste and Radioecology, International
10 Radioecology Laboratory, Slavutych, Ukraine
11 # Corresponding author: r.e.antwis@salford.ac.uk

12

13 **RUNNING TITLE:** Small mammal microbiomes in Chernobyl

14 **KEYWORDS:** 16S rRNA, ITS rRNA, ¹³⁷Cs, ⁹⁰Sr, mouse, vole, amplicon sequencing,
15 dosimetry, Red Forest

16

17

18

19

20

21

22

23 **ABSTRACT**

24 Environmental impacts of the 1986 Chernobyl Nuclear Power Plant accident are much
25 debated, but the effects of radiation on host microbiomes has received little attention to date.
26 We present the first analysis of small mammal gut microbiome from the Chernobyl Exclusion
27 Zone in relation to total absorbed dose rate and including caecum as well as faeces sample.
28 The associations between microbiome communities and radiation exposure varied between
29 host species. Associations between microbiome and radiation was different for analyses
30 based on ambient versus total weighted absorbed dose rates. We found considerable
31 variation between patterns for faecal and gut samples of bank voles, suggesting faecal
32 samples are not an accurate indicator of gut composition. For bank vole guts, associations
33 between radiation and bacterial community composition were robust against geographical
34 and habitat variation. We found limited associations between radiation and fungal
35 communities. Host physiological mechanisms or environmental factors may be driving these
36 patterns.

37 **INTRODUCTION**

38 Multicellular organisms host a complex community of microbes (the microbiome) that is
39 critical for host health and function^{1,2}. The gut microbiota has been shown to affect animal
40 development, immune response, food digestion and behaviour³. Microbiome composition
41 varies according to biological and environmental factors such as host species⁴, host age⁵,
42 diet⁶, season⁷, and contaminant-induced stress⁸, among others⁹. Less well understood is
43 the relationship between radiation exposure and microbiome composition, particularly in wild
44 animal systems.

45 Over the last decade there has been a growing interest in the effect of contaminants
46 on the composition of the gut microbiome, with some studies reporting changes in the two
47 most prevalent bacterial phyla within the gut^{10,11}, namely Firmicutes and Bacteroidetes¹².
48 Different chemical stressors have been found to affect Firmicutes: Bacteroidetes (F:B)
49 ratios, with As¹³, Cd¹⁴, chlorpyrifos¹⁵, permethrin¹⁶ and pentachlorophenol¹⁷ leading to
50 decreases in F:B ratios, whereas Pb¹¹ and carbendazim¹⁸ cause increased F:B ratios.
51 These changes in gut microbiota composition have also been linked to changes in host
52 immune responses¹².

53 High acute radiation exposure (> 1 Gy) has been shown to influence gut microbial
54 communities (e.g. Dubois & Walker 1989; Packey & Ciorba 2011), leading to the suggestion
55 that gut microbiota could be a potential biomarker of radiation exposure^{21,22}. Improvements
56 in the responses of both humans and model organisms to acute radiation exposure have
57 also been observed when bacterial probiotics (particularly *Lactobacillus* spp.) were
58 administered (e.g. Demers *et al.* 2014; Meng-Meng *et al.* 2017). Some studies suggest that
59 faecal microbiomes may be associated with lower radiation exposure in contaminated
60 environments, such as the Chernobyl Exclusion Zone (CEZ)^{25,26}. For example, Lavrinienko
61 *et al.*²⁵ report a reduction in the faecal F:B ratios of bank voles (*Myodes glareolus*) at their
62 most contaminated sites (mean ambient dose rate 30 µSv h⁻¹). Radiation-induced changes
63 in the microbiome of skin and feathers in organisms from Chernobyl have also been

64 investigated. Lavrinienko et al. ²⁶ found no effect of radiation on skin microbiome of bank
65 voles, but radiation-induced changes in feather bacterial communities have been suggested
66 ^{27,28}.

67 The extent to which radiation exposure is affecting wildlife in Chernobyl is highly
68 contested ^{29,30}. A fundamental problem with many of the studies undertaken to date is that
69 they use ambient dose rates from the air (often reported in units of absorbed radiation dose
70 rate for humans, $\mu\text{Sv/h}$), rather than estimating the total absorbed dose rate of study
71 organisms, taking account of both internal and external exposure ³¹. As such, it is not
72 possible to accurately determine dose-effect relationships, making interpretation of these
73 studies difficult. Here we present the first study of GI tract microbiome composition in CEZ
74 small mammals for which individual total absorbed dose rates have been estimated.
75 Previous studies in the CEZ have only considered the bacterial microbiome of one small
76 mammal species (bank vole) using faecal samples; here we report on the faecal microbiome
77 of four small mammal species using faecal samples, as well as the first direct analysis of gut
78 microbiome using caecum samples from bank voles. In addition, our microbiome analysis
79 includes both bacteria and fungi, extending our limited general knowledge on the fungal
80 component of animal microbiomes.

81

82 **METHODS**

83 *Field sampling in the Red Forest (2017)*

84 In August 2017, we sampled small mammals from the Red Forest, an area of c. 4-6 km²
85 over which pine trees were killed by radiation in 1986; subsequently there has been sparse
86 regrowth of deciduous trees and some understorey vegetation. In 2016, approximately 80%
87 of the Red Forest was damaged by fire. Our 2017 sampling sites (Figure 1) included a total
88 of eight sites across three burn categories, namely 'burnt with regrowth' (n = 2), 'burnt with
89 minimal regrowth' (n = 3) and 'unburnt' (n = 3). At each of these sampling sites, a 60 m x 60

90 m trapping grid was used, with traps positioned at 10 m intervals (each grid comprised a
91 total of 49 traps). To maximise trapping success, the trapping grids were established one
92 week prior to the beginning of the study and pre-baited with rolled oats and
93 carrots/cucumber. Trapping occurred over eight consecutive days; traps were baited and set
94 each evening and visited early in the morning to retrieve captured small mammals. The
95 small mammals were transferred to the Chernobyl field station where each animal was live
96 monitored to determine its ^{137}Cs whole body activity concentration using an unshielded 51
97 mm x 51 mm NaI (TI) detector (GMS 310 core gamma logger) supplied by John Caunt
98 Scientific Ltd. Additional regular background measurements were made each day. The
99 detector was calibrated using the results for small mammals ($n = 14$) that were live
100 monitored on the GMS 310 and subsequently analysed using a calibrated detector at the
101 Chernobyl Center's main laboratory ($R^2 = 0.98$). The limit of detection (LOD) was estimated
102 as three times the standard deviation of the background measurement. The sex of each
103 animal was determined and their live mass recorded.

104 Freshly excreted faecal samples were collected directly from animals for subsequent
105 microbiome analysis. We sampled striped field mice (*Apodemus agrarius*; $n = 29$), yellow-
106 necked mice (*Apodemus flavicollis*; $n = 58$), wood mice (*Apodemus sylvaticus*; $n = 27$) and
107 bank voles (*Myodes glareolus*; $n = 22$; Table S1). Faecal samples were immediately placed
108 into vials containing 100% ethanol and subsequently stored at -20°C. Samples were
109 transported under licence to the University of Salford (UK); sample integrity was maintained
110 during transit using dry ice and the samples were then stored at -20°C prior to DNA
111 extraction. We used fur clipping to mark each small mammal prior to release at the point of
112 capture, which allowed us to check whether each capture over the subsequent days was a
113 new animal. Only faeces from new animal captures were included in this study.

114

115 *Field sampling across the CEZ (2018)*

116 Small mammals were trapped in July/August 2018 over 10 consecutive days, with only bank
117 voles included in this study. Twelve transects of Sherman traps were established at sites
118 across a gradient of ambient dose rates (Figure 1). Each transect measured 290 m with a
119 trap interval of 10 m (30 traps per transect). The 2018 sampling adopted the same protocol
120 for baiting and collection of captured animals that was used in 2017. For some of the
121 analyses, bank voles from 2018 have been categorised by collection ‘site category’, defined
122 as inside or outside of the Red Forest.

123 Captured animals were transferred to the Chernobyl field station, where each animal
124 was live monitored to quantify the whole-body activity concentrations of both ^{137}Cs and ^{90}Sr .
125 This was done using a new field portable Radioanalysis of Small Samples (ROSS) detector
126 developed at the University of Salford ³². ROSS comprises a sample holding chamber with a
127 capacity of 170 x 60 x 50 mm. Two CsI gamma detectors (each measuring 70 x 40 x 25 mm)
128 were mounted on opposite sides of the sample holding chamber and two plastic scintillator
129 beta detectors were mounted one above (100 x 50 x 0.5 mm) and one below (100 x 60 x 0.5
130 mm) the chamber. The entire assembly was enclosed within a lead shield (>10 mm
131 thickness). ROSS was calibrated using ^{137}Cs and ^{90}Sr standards developed by Chornobyl
132 Center; standards ranged from 4 to 20 g to represent small mammals. We included Cs-only
133 standards, Sr-only standards and mixed standards. Counting of the Cs standards on the
134 beta detectors provided a correction for the influence of gamma emissions. Multiple
135 background counts were performed daily (at least nine per day) and the LOD was estimated
136 using the method described by Currie ³³.

137 Bank voles were killed by an overdose of anaesthetic (isofluorane) followed by
138 exsanguination (in accordance with Schedule 1 of the Animals (Scientific Procedures) Act
139 1986). The sex and mass of each animal was recorded. Gastrointestinal tracts (n = 142)
140 were dissected immediately and stored in laboratory vials containing 100% ethanol and
141 stored at -20°C. The frozen vials were transported to the University of Salford under licence
142 and stored as described for faeces.

143

144 *Dosimetry: Ambient dose rate*

145 All dose data are provided as supplementary data. At every trapping location in 2017 and
146 2018, ambient dose rate ($\mu\text{Sv h}^{-1}$) was measured using an MKS-01R meter at 5 cm above
147 the soil surface.

148

149 *Dosimetry: Estimation of small mammal total absorbed dose rate for the 2017 study*

150 Soil samples (0 - 10 cm soil depth) were available from each of the trapping sites used in the
151 2017 study. These samples were analysed using laboratory detectors at Chornobyl Centre
152 to determine ^{137}Cs and ^{90}Sr activity concentrations within the soil (see ³⁴ for methodology).
153 For each small mammal species, an external dose conversion coefficient was calculated
154 using the ERICA Tool version 1.2 ³⁵. To define the geometry for each species, the length,
155 width and height were determined through literature review. Soil activity concentrations were
156 input into the ERICA Tool and external dose rates estimated using the derived external dose
157 conversion coefficients and appropriate occupancy factors (assuming 50% of time in soil and
158 50% on the soil surface for mice and 70% in soil and 30% on soil for bank voles).

159 The measured ^{137}Cs whole body activity concentrations were used to determine the
160 internal absorbed dose from ^{137}Cs . In 2017. The internal ^{90}Sr activity concentrations were not
161 directly measured; these were estimated using the species-specific transfer parameters
162 measured in the CEZ and presented by Beresford et al. ³⁴ and the soil ^{90}Sr activity
163 concentrations for the appropriate sampling site. For each small mammal species, an
164 internal dose conversion coefficient was calculated using the ERICA Tool and the same
165 assumed geometries as used for the external dose conversion coefficient derivation. The
166 ERICA Tool was then run using the default radiation weighting factors to calculate total
167 weighted absorbed dose rate. Whilst other radionuclides (e.g. Pu-isotopes and ^{241}Am) are
168 present in the CEZ, Beresford et al. ³⁴ demonstrated that the contribution of these isotopes

169 to small mammal total absorbed dose rate of small mammals within the Red Forest was low
170 (< 10 %).

171 For each individual animal, the total weighted absorbed dose rate (hereafter referred
172 to as the total absorbed dose rate) was calculated by summing the internal and external
173 dose rates for that individual.

174

175 *Dosimetry: Estimation of small mammal total absorbed dose rate for the 2018 study*

176 Soil activity concentrations were not available for all of the sites within the 2018 study.
177 However, Beresford et al. ^{34,36} reported relatively good agreement between estimated
178 external dose from ¹³⁷Cs and the external ambient dose field at sites in the CEZ. Based on
179 our small mammal dose rate data from 2017, the mean ratio of external dose from ¹³⁷Cs to
180 the external ambient dose field is 0.98. We used the dosimetry approach of the ERICA Tool,
181 which assumes a shielding effect from fur and skin for external beta exposure ³⁷ and
182 subsequently also adopted by the ICRP ³⁸. The estimated contribution of ⁹⁰Sr (a beta
183 emitter) to the external whole-body dose rate of small mammals is therefore negligible.
184 Therefore, the external dose rates measured at each trapping location were used to
185 estimate the external absorbed dose rate for each small mammal using the occupancy
186 factors defined above.

187 The ¹³⁷Cs and ⁹⁰Sr whole body activity concentrations measured using ROSS were
188 input into the ERICA Tool and the species-specific internal dose conversion coefficients
189 were used to estimate the internal absorbed dose rate for each animal. At the lowest
190 contamination sites in 2018, some of the whole-body activity concentrations for both ¹³⁷Cs
191 and ⁹⁰Sr were below the LOD. Using these LOD values to determine total absorbed internal
192 dose rate led to a maximum estimated dose of 0.6 μ Gy h⁻¹, introducing some uncertainty in
193 radiation exposure estimates at the lowest end of our total absorbed dose rate range.

194

195 *Dosimetry: Incorporation of estimated dose rates with subsequent analyses*

196 We assigned animals to total absorbed dose rate categories based on the suggested
197 derived consideration reference level for ICRPs Reference Rat³⁸, i.e. approximately 4-42
198 µGy h⁻¹. As such, animals estimated to receive total absorbed dose rates of <4 µGy h⁻¹ were
199 assigned ‘low’, those with estimated dose rates of 4-42 µGy h⁻¹ assigned ‘medium’, and
200 those >42 µGy h⁻¹ assigned to a ‘high’ category. The ‘high’ and ‘low’ total absorbed dose
201 rates are in-effect also a comparison of inside and outside the Red Forest (i.e. the ‘inside’
202 and ‘outside’ Red Forest site categories; Table S1).

203 We correlated ambient and total estimated absorbed dose rates using a Spearman’s
204 rank correlation. To quantify whether correlation coefficients varied based on radiation dose,
205 we also repeated the correlations for each total absorbed dose rate category separately, and
206 visualised these using a scatter plot in ggplot2³⁹.

207

208 *DNA extraction and molecular work*

209 For faecal samples, we extracted DNA from the full sample (~0.1 g) of the four host species.
210 For gut samples, we isolated ~25% of the distal end of the caecum of bank voles and
211 homogenised the contents by hand in sterile petri dishes, before weighing out ~0.1 g for
212 DNA extraction. We conducted all DNA extractions using the PureLink™ Microbiome DNA
213 Purification Kit (Invitrogen, UK) according to the manufacturer’s instructions.

214 To identify bacterial communities, we conducted 16S rRNA amplicon sequencing
215 according to Kozich et al.⁴⁰ and Griffiths et al.⁴¹. Briefly, we ran PCRs in duplicate using
216 Solis BioDyne 5x HOT FIREPol® Blend Master Mix, 2µM primers and 15ng of sample DNA
217 under thermocycling conditions of 95 °C for 10 min; 25 cycles of 95°C for 30s, 55°C for 20s,
218 and 72°C for 30s; and a final extension of 72 °C for 8 minutes. We included negative
219 (extraction blanks) and positive (mock community) controls. We combined PCR replicates
220 into a single PCR plate and cleaned these using HighPrep™ PCR clean up beads (MagBio,

221 USA) according to the manufacturers' instructions. We quality checked PCR products
222 throughout on an Agilent 2200 TapeStation. To quantify the number of sequencing reads per
223 sample, we constructed a library pool using 1ul of each sample. We conducted a titration
224 sequencing run using this pool with a v2 nano cartridge (2 x 150bp) on the Illumina MiSeq
225 platform. We calculated the volume of each sample required based on the percentage of
226 reads obtained and pooled these accordingly. We sequenced the final normalised library
227 using paired end (2 x 250bp) reads on a v2 cartridge on an Illumina MiSeq at the University
228 of Salford.

229 We identified fungal communities via the ITS1F-2 rRNA gene using a modified
230 protocol of Smith & Peay⁴² and Nguyen et al.⁴³, as in Griffiths et al.⁴⁴. We ran PCRs in
231 duplicate using thermocycling conditions of 95°C for 10 minutes, followed by 28 cycles of
232 95°C for 30s, 54°C for 45s, and 72°C for 60s; with a final extension at 72°C for 10 minutes.
233 We included extraction blanks and a mock community as negative and positive controls,
234 respectively. We quantified and normalised individual libraries as described above, before
235 conducting full paired-end sequencing using Illumina v2 (2 x 250bp) chemistry on an Illumina
236 MiSeq at the University of Salford.

237

238 *Pre-processing of amplicon sequencing data*

239 Unless otherwise stated, we conducted all data processing and analysis in RStudio
240 (v1.2.1335)⁴⁵ for R (v3.6.0)⁴⁶. A total of 13,371,018 raw sequence reads from 279 samples,
241 plus one mock community and ten negative controls, were generated during 16S rRNA gene
242 amplicon sequencing, which we processed in DADA2 v1.5.0⁴⁷. Modal contig length was
243 253bp once paired-end reads were merged. We removed sequence variants (SVs) with
244 length >260bp (26 SVs; 0.002% of total sequences) along with chimeras and five SVs found
245 in the negative controls. We assigned taxonomy using the SILVA v132 database^{48,49}.
246 DADA2 identified 20 unique sequence variants in the sequenced mock community sample

247 comprising 20 bacterial isolates. We stripped out mitochondria from samples along with SVs
248 with less than 0.0001% abundance across all samples. We removed three samples from
249 which poor sequence data were obtained (<1000 reads), leaving a median of 26,106 reads
250 (6,557 to 119,871) per sample. We exported the final SV table, taxonomy table and sample
251 metadata to the phyloseq package ⁵⁰.

252 We obtained a total of 2,778,887 raw sequence reads from 279 samples (plus mock
253 community and negative controls) during ITS rRNA gene sequencing. We trimmed
254 remaining adapters and primers from ITS rRNA sequencing data using cutadapt ⁵¹ in
255 RStudio. As with 16S rRNA sequence data, we pre-processed ITS rRNA amplicons in
256 DADA2 v1.5.0 ⁴⁷. Modal contig length was 219bp (167-457bp) once paired-end reads were
257 merged. We did not conduct additional trimming based on sequence length as the ITS
258 region is highly variable ⁵². We removed chimeras and one contaminant found in the
259 negative controls, and then assigned taxonomy using the UNITE v7.2 database ⁵³. DADA2
260 identified 12 unique sequence variants in the sequenced mock community sample
261 comprising 12 fungal isolates. We removed 54 samples from which poor sequence data
262 were obtained (<500 reads), leaving a median of 1863 reads (506 to 17,226) per sample. As
263 with 16S rRNA data, we exported the final SV table, taxonomy table and sample metadata to
264 the phyloseq package ⁵⁰ for subsequent analysis.

265

266 *Community analyses*

267 For both bacterial and fungal community data, we normalised the clean count data using
268 centred-log ratio (clr) transformations ⁵⁴ in phyloseq ⁵⁰, and visualised beta-diversity
269 (between species and between sample type, i.e. gut or faeces) using PCA plots with
270 Euclidean distances in ggplot2 ³⁹. We used PERMANOVAs to test for significant differences
271 in beta-diversity according to species, sex, sample type and total absorbed dose rate
272 category using the adonis function in the vegan package ⁵⁵. We agglomerated the data to

273 family level and visualised differences in clr-transformed data according to the five sampling
274 groups (faecal samples from the three mice species, plus faecal and gut samples from bank
275 voles) using jitter box plots in ggplot2³⁹. We tested for significant differences between
276 sampling groups in the clr-transformed values of these 24 families (12 per microbial
277 kingdom) using Kruskal-Wallis non-parametric tests with Dunn's pairwise tests and
278 Hochberg-adjusted p values in the dunn.test and FSA packages^{56,57}. We also converted the
279 raw SV counts to relative abundance and visualised the 12 most abundant families (for each
280 kingdom separately) as a stacked chart according to species and sample type.

281 We then split the clr-transformed data by sampling year and re-ran the
282 PERMANOVA analysis for the 2017 faecal samples with species, sex, total absorbed dose
283 rate category, grid line and burn category as predictor variables. We visualised the variation
284 in clr-transformed values for the 12 most abundant genera in faecal samples from yellow-
285 necked mice (as this was the only species with sufficient samples across all three burn
286 categories; Table S1) using PCA plots of beta-diversity and jitter plots for the clr values of
287 the 12 most abundant genera. We also re-ran the PERMANOVA analysis for the 2018 gut
288 data with total absorbed dose rate category, site category, sex and transect line as predictor
289 variables, and again visualised these using PCA plots of beta-diversity and jitter plots of the
290 clr values of the 12 most abundant genera for each microbial kingdom separately.

291 To determine whether microbiome beta-diversity correlated with total absorbed dose
292 rate independently to geographic location for bank vole gut data, we conducted partial
293 Mantel tests using the vegan package⁵⁵ on the gut data from bank voles. We constructed
294 microbiome distance matrices in phyloseq⁵⁰ between samples for bacteria and fungi
295 separately using Euclidean distances of clr-transformed data. We generated Euclidean
296 distance matrices from the total absorbed dose rate data for each individual using the proxy
297 package⁵⁸. Finally, we constructed a geographic distance matrix between radiation distance
298 and samples using longitude and latitude coordinates in Microsoft Excel. We then ran partial
299 Mantel tests with Spearman's rank correlation between total absorbed dose rate distance

300 and microbiome distance (for bacteria and fungi separately), with the geographic distance
301 matrix as a covariate.

302 For bank vole gut data, we calculated alpha diversity (SV richness, and community
303 evenness using the inverse Simpson index) of bacterial and fungal communities by
304 subsampling the raw SV count table to a standardised number of reads (equal to the sample
305 with the lowest number of reads) using an iterative approach (100 times), and averaging the
306 diversity estimates across these iterations. We correlated these alpha-diversity measures
307 with total absorbed dose rate for each bank vole sample using Spearman's correlations.

308 We used Spearman's rank correlation (with Benjamini-Hochberg corrected p values
309 and False Discovery Rate adjustment) in the associate function of the microbiome package
310⁵⁹ to identify relationships between the two radiation dose measures (ambient and total) and
311 clr-transformed 16S and ITS rRNA sequence data, agglomerated to genus level. These
312 analyses were conducted separately for the gut and faecal samples, according to host
313 species. We then visualised the resultant correlation coefficients using heatmaps in ggplot2
314³⁹.

315 We calculated Firmicutes: Bacteriodetes (F:B) ratios in vole guts using both clr-
316 transformed data, and data converted to relative abundance (as in²⁵). We also calculated
317 F:B ratios in faecal samples of all four mammal species using relative abundance data. We
318 visualised these ratios according to total absorbed dose rate category using jitter plots. We
319 tested for significant differences between total absorbed dose rate categories within a set of
320 data using Kruskal-Wallis non-parametric tests, with Dunn's pairwise tests and Hochberg-
321 adjusted p values where necessary.

322

323 **RESULTS**

324 *How do ambient dose rates compare to total absorbed dose rates?*

325 There was a significant positive correlation between ambient and total absorbed dose rates
326 across all animals captured during both 2017 and 2018 ($r = 0.529$, $p < 0.001$), although there
327 was considerable variation around the data (Figure 2). When data were split into the three
328 different total absorbed dose rate categories, all relationships remained statistically
329 significant (low ($< 4 \mu\text{Gy h}^{-1}$): $r = 0.898$, $p < 0.001$; medium ($4-42 \mu\text{Gy h}^{-1}$): $r = 0.879$, $p <$
330 0.001 ; high ($>42 \mu\text{Gy h}^{-1}$): $r = 0.236$, $p < 0.001$), however the variation in the ‘high’ dose data
331 was particularly evident (although note the greater sample size; Figure 2). The estimated
332 total absorbed dose rate gives a better estimation than ambient dose rate of each
333 individual’s radiation exposure, and hence we used total absorbed dose rates for the
334 majority of our analyses. In this study we have used the ERICA dosimetry approach, which
335 assumes shielding by fur and skin of beta radiation and consequently the external dose
336 rates from ^{90}Sr are estimated to be negligible. We acknowledge that estimates using
337 different modelling approaches may lead to a higher estimated external dose rate from ^{90}Sr
338 (e.g. ^{60}Co). We have estimated the external dose contributions using an alternative model
339 (<https://wiki.ceh.ac.uk/x/9wHbBg>) which does not consider fur or skin shielding^{61,62}. Using
340 this model, we find that the maximum difference in total dose rate estimate would be
341 approximately 30%, with an average difference of about 10%.

342

343 *How does microbiome beta-diversity vary according to host factors and total absorbed dose
344 rate?*

345 A PERMANOVA analysis demonstrated sample type ($F_{1,260} = 49.408$, $R^2 = 0.143$, $p = 0.001$),
346 species ($F_{3,260} = 9.846$, $R^2 = 0.085$, $p = 0.001$) and total absorbed dose rate category ($F_{2,260}$
347 = 2.631, $R^2 = 0.015$, $p = 0.001$) all significantly predicted bacterial community beta-diversity,
348 whereas sex did not ($F_{1,268} = 1.206$, $R^2 = 0.003$, $p = 0.138$). Similarly, fungal community beta-
349 diversity was significantly predicted by sample type ($F_{1,212} = 12.574$, $R^2 = 0.052$, $p = 0.001$),
350 species ($F_{3,212} = 2.183$, $R^2 = 0.027$, $p = 0.001$), and total absorbed dose rate category ($F_{2,212}$
351 = 2.024, $R^2 = 0.016$, $p = 0.001$), but not sex ($F_{2,212} = 1.108$, $R^2 = 0.004$, $p = 0.206$). As such,

352 sample type was the biggest driver of variation in both bacterial and fungal community
353 composition. Differences between host species were much more evident for bacterial
354 community composition than for fungal community composition (Figures 3a, b), for which
355 8.5% and 2.7% of the variation was explained by host species, respectively. A full
356 description (with statistical testing) of the differences in community composition based on
357 host species and sample type can be found in Supplementary Material.

358

359 *How does beta-diversity of faecal samples vary according to host species, burn category*
360 *and total absorbed dose rate?*

361 When using faecal samples (i.e. 2017 data) only, the PERMANOVA analysis indicated that
362 host species ($F_{3,128} = 11.944$, $R^2 = 0.217$, $p = 0.001$; Figure S3), burn category ($F_{2,128} =$
363 1.632 , $R^2 = 0.020$, $p = 0.004$; Figure S3) and grid line ($F_{5,128} = 1.562$, $R^2 = 0.047$, $p = 0.001$)
364 had a significant effect on beta-diversity of faecal bacterial communities, but that total
365 absorbed dose rate category ($F_{1,128} = 0.912$, $R^2 = 0.006$, $p = 0.616$) and sex ($F_{1,128} = 1.103$,
366 $R^2 = 0.007$, $p = 0.228$) did not. There were only sufficient samples for yellow-necked mice to
367 visualise differences in microbiome composition across all three burn categories (Table S1).
368 Although the differences were relatively small in the 12 most abundant bacterial genera,
369 some showed directional changes based on burn category (Figure S4), for instance,
370 Bacteroides were most abundant at ‘burnt (minimal regrowth)’ sites and least abundant at
371 ‘unburnt’ sites, whereas Ruminococcaceae_UCG-003 showed the inverse.

372 As with bacterial communities, host species ($F_{3,111} = 1.945$, $R^2 = 0.048$, $p = 0.001$),
373 burn category ($F_{2,111} = 1.969$, $R^2 = 0.033$, $p = 0.001$) and grid line ($F_{5,111} = 1.726$, $R^2 = 0.072$,
374 $p = 0.001$) had a significant effect on beta-diversity of faecal fungal communities, but total
375 absorbed dose rate category ($F_{1,111} = 1.157$, $R^2 = 0.010$, $p = 0.153$) and sex ($F_{1,111} = 0.823$,
376 $R^2 = 0.007$, $p = 0.911$) did not. Burn category effects on faecal community composition were
377 clearer from the fungal community PCA plot (Figure S5) than for the bacterial community

378 PCA (Figure S3); individuals captured in the burnt areas with minimal regrowth tended to
379 appear in the lower left-hand side of the plot (Figure S5). When looking at the samples from
380 yellow-necked mice, differences in clr values for the 12 most abundant fungal genera based
381 on burn category were more pronounced than for bacterial genera (Figure S6). For example,
382 yellow-necked mice trapped in unburnt areas had faecal communities characterised by low
383 *Gelatoporia*, *Pyrenophaetopsis* and *Wickerhamomyces* relative to burnt areas, as well as
384 high *Tritirachium* (Figure S6).

385

386 *How do alpha-diversity and beta-diversity of bank vole gut samples vary according to site*
387 *category and total absorbed dose rate?*

388 Converse to faecal samples, the PERMANOVA analysis showed total absorbed dose rate
389 category had a significant effect on bacterial community beta-diversity of bank vole guts
390 ($F_{2,138} = 2.706$, $R^2 = 0.036$, $p = 0.001$; Figure S7), as did sex ($F_{1,138} = 1.296$, $R^2 = 0.009$, $p =$
391 0.028) and transect line ($F_{14,138} = 1.558$, $R^2 = 0.146$, $p = 0.001$), whereas site category (i.e.
392 inside or outside the Red Forest) did not ($F_{1,138} = 1.221$, $R^2 = 0.008$, $p = 0.091$). There were
393 subtle differences evident in the abundance of the 12 most abundant genera across the
394 three total absorbed dose rate categories, of note are the increases in Lachnospiraceae
395 NK4A136, Roseburia, Ruminiclostridium 9, and UBA1819 as radiation dose increased and
396 the decrease in unidentified SVs from the Muribaculaceae family (Figure S8).

397 The same results were also found for fungal communities of bank vole gut samples,
398 whereby total absorbed dose rate category ($F_{2,107} = 2.428$, $R^2 = 0.044$, $p = 0.001$; Figure S9)
399 and transect line ($F_{13,107} = 1.335$, $R^2 = 0.151$, $p = 0.001$) had a significant effect on
400 community beta-diversity, whereas site category did not ($F_{1,107} = 1.066$, $R^2 = 0.009$, $p =$
401 0.384). However unlike with bacterial community beta-diversity, sex did not have a
402 significant effect on fungal community beta-diversity ($F_{2,107} = 1.174$, $R^2 = 0.010$, $p = 0.142$).
403 Again, subtle differences between total absorbed dose rate categories were evident in the

404 12 most abundant fungal genera, including steady increases in *Arthrobotrys* and *Aspergillus*,
405 and steady decreases in *Candida* and *Wickerhamomyces*, as total absorbed dose rate
406 category increased from low to high (Figure S10).

407 The partial Mantel test for association between microbial community beta diversity
408 and total absorbed dose rate distance (weighted by geographic distance) indicated a
409 significant relationship for bacterial communities of bank vole guts ($r = 0.078$, $p = 0.047$), but
410 the same pattern was not found for fungal communities ($r = -0.095$, $p = 0.866$).

411 There were no significant effects of total absorbed radiation dose rate on alpha-
412 diversity of microbial communities (all $p > 0.05$; Table S3).

413

414 *How do different microbial taxa correlate with the two radiation dose measures?*

415 The association analysis identified seven bacterial families in bank vole gut samples that
416 significantly correlated with total absorbed dose rate (Table S2). All of these had a negative
417 relationship with dose rate with the exception of Lachnospiraceae (Table S2). Two fungal
418 families in bank vole gut samples were significantly correlated with total absorbed dose rate,
419 with Steccherinaceae negatively correlated, and Strophariaceae positively correlated (Table
420 S2). Faecal and gut samples of bank voles showed considerably different fungal and
421 bacterial association patterns (Figures 4 and S11) (note that faecal samples were collected
422 in 2017 and guts, from different animals, in 2018). Fungal and bacterial association patterns
423 between faecal samples from the four small mammal species were also markedly different to
424 one another (Figures 2b and S11b).

425

426 *How do Firmicutes: Bacteroidetes ratios vary according to total absorbed dose rate
427 category?*

428 When using clr-transformed data, F:B ratios were less than 0 in vole guts (Figure S12a).
429 When using relative abundance data, voles in the ‘high’ total absorbed dose rate category
430 had slightly higher F:B ratios than those in the ‘low’ and ‘medium’ categories (Figure S12b).
431 The Kruskal-Wallis model indicated a significant effect of total absorbed dose rate category
432 ($X^2 = 7.489$, d.f. = 2, p = 0.024), with bank voles in the ‘high’ category exhibiting significantly
433 higher F:B ratios than those in the ‘medium’ category (p = 0.019), but ‘low’ was not
434 significantly different to ‘high’ or ‘medium’ (p > 0.05; Figure S12b). For the faecal sample
435 data, only striped field mice and yellow-necked mice had data for animals in more than one
436 absorbed dose rate category, i.e. medium and high for both. The Kruskal-Wallis analysis
437 was not statistically significant for either striped field mice ($X^2 = 0.012$, d.f. = 1, p = 0.911) or
438 yellow-necked mice ($X^2 = 0.019$, d.f. = 1, p = 0.896), meaning there were no differences in
439 F:B ratios between the ‘medium’ and ‘high’ categories for these two species (Figure S12c).

440

441 **DISCUSSION**

442 In this study we present the first analyses of small mammal faecal and gut microbial
443 communities from the Chernobyl Exclusion Zone for which individual total absorbed dose
444 rates have been estimated. This study also presents the first data from Chernobyl on the
445 fungal component of the gut microbiome, and considers a wider range of species than has
446 previously been studied (previous studies being limited to bank voles^{25,26}). Previous papers
447 have used faecal samples to characterise the gut microbiome^{25,26}, whereas our study
448 provides the first data on the true gut microbiome of Chernobyl bank voles using samples
449 from the distal section of the caecum.

450 We provide novel evidence that radiation has a small ($R^2 < 0.05$), but statistically
451 significant, association with changes in microbial communities of small mammals. We also
452 identified a limited number of taxa with a significant association (Table S2; Figures 4 and
453 S11) with total absorbed dose rate of the host animals. For subsequent discussion of these

454 findings, the relevance of the total absorbed dose rate and its association with specific
455 geographic locations in the CEZ needs to be considered. The total absorbed dose rate is
456 that of the host organism; we have not estimated the radiation exposure of gut microbiota
457 directly. All animals in the 'high' total absorbed dose rates ($>42 \mu\text{Gy h}^{-1}$) except two bank
458 voles from 2018 were collected from within the Red Forest. Other studies of radiation effects
459 in CEZ wildlife, including the microbiome studies of Lavrinienko et al.^{25,26}, also have their
460 most contaminated sampling sites within the Red Forest. The Red Forest is an area of
461 naturally poor habitat quality where soil and water conditions do not favour high biological
462 diversity. The forest was also severely damaged in 1986 as a consequence of the accident
463 at the Chernobyl Nuclear Power Plant and has not fully recovered. Furthermore, some of our
464 2017 Red Forest sampling sites were showing signs of fire damage from a large fire in July
465 2016. Any study that uses the Red Forest as a location for radiation effects studies on
466 wildlife needs to consider the historical impacts of radiation and other stressors (e.g.
467 wildfires) on this area of the CEZ⁶³.

468 Geographical location is known to affect bacterial community composition^{41,64}, and
469 here we find the same, whereby grid/transect line are significant predictors of bacterial beta-
470 diversity. We also provide novel evidence that geography affects fungal community
471 composition. The Mantel test shows that bacterial community composition of bank vole guts
472 varied predictably with total absorbed dose rate when taking geographical variation into
473 account (although the same was not true for fungal communities). Furthermore, the
474 PERMANOVA analysis of bank vole gut microbiome indicated that total absorbed dose rate
475 category was a significant predictor of both bacterial and fungal beta-diversity, whereas site
476 category (inside or outside the Red Forest) was not. Together, these results indicate that
477 differences in microbiome composition exhibited in bank vole guts were a result of radiation
478 exposure, rather than the confounding effects of geography or habitat type. However, given
479 that microbes are actually highly resilient to radiation exposure^{65,66}, environmental radiation
480 exposure at sites such as Chernobyl is unlikely to affect the microbiome directly. It is more

481 likely that co-correlating factors are driving observed relationships between radiation and gut
482 microbiome⁶⁷. For example, radiation exposure that causes changes in habitat quality, food
483 availability or a host physiological response may all influence the gut microbiome^{68–70}.

484 The gut communities of bank voles showed similar changes in composition in
485 response to both ambient and total dose radiation measures, although relationships were
486 generally less strong for ambient dose compared with total dose (Figures 4 and S11). This
487 may be due to differences in the way that individual dose rates are assigned; every
488 individual from a site is assigned the same dose rate whereas total absorbed dose rate is
489 calculated on an individual basis. The bacterial genera identified here (Figure 4; Table S2)
490 may serve as useful bioindicators for radiation exposure in mammals, although more work is
491 required to determine if these patterns are consistent across different host species. Our data
492 from faecal samples indicate that the relationship between the small mammal microbiomes
493 and total absorbed dose rate of the host may vary from species to species (Figures 4 and
494 S11), although there were relatively few significant relationships (identified by * on Figure 4)
495 between radiation dose and clr values for individual genera. However, these heatmaps
496 indicate that faecal sample communities exhibited considerably different results for the two
497 dose measures, suggesting that faecal samples and/or ambient dose measures are not a
498 reliable method of characterising microbiome changes in response to radiation exposure.

499 The relationships between microbial families and radiation exposure were
500 considerably different between gut and faecal samples for bank voles. This may be because
501 different taxa are being excreted to those that are retained in the gut. It has previously been
502 shown that a number of host species have significantly different communities associated
503 with the gut and faeces^{71,72}. As such, faecal samples may not directly reflect responses of
504 gut communities to radiation exposure or any other stressor. However, it is worth noting that
505 bank vole gut samples were collected in 2018 from across the CEZ, whereas the faeces
506 samples collected in 2017 were all from inside the Red Forest (including from a number of

507 sites that had been recently burnt), which may also be influencing the observed differences
508 between the gut and faecal samples.

509 Firmicutes and Bacteroidetes are the most abundant phyla within the microbiome of
510 small mammals; Firmicutes have been linked to processes such as the generation of
511 metabolites, fat storage, angiogenesis and immune system maturation¹⁰. Lavrinenko et al.
512²⁵ found a two-fold increase in F:B ratios in bank vole faeces from areas of elevated
513 radionuclide contamination in the CEZ (these sites would span our ‘medium’ and ‘high’ total
514 absorbed dose rate categories) compared with areas of lower contamination in the CEZ and
515 sites close to Kiev. The authors attribute the two-fold increase in F:B ratios to potential
516 changes in diet arising from reduced arthropod densities in their higher contamination areas
517 of the Chernobyl Exclusion Zone (referring to earlier work of Møller & Mousseau⁷³, the
518 findings of which have been contested^{30,74,75}) and/or an active increase in the consumption
519 of plant based foods. Indeed, F:B ratios in faeces have previously been used as a marker of
520 changes in diet⁷⁶. However, the authors also state that the bank vole diet is normally
521 dominated by plant material, with only occasional consumption of invertebrates.
522 Consequently, the effect of any reduction in arthropod consumption on the bank vole faecal
523 microbiome F:B ratios would likely be minimal. In the present study, we found no evidence of
524 altered F:B ratios in bank vole gut samples based on total absorbed radiation dose rate
525 category suggesting similar bank vole diets across our study locations, including inside and
526 outside the Red Forest.

527 Our results suggest that bacterial communities are more influenced by total absorbed
528 dose rate than fungal communities. For bank vole guts, the partial Mantel tests were not
529 significant for fungi ($p > 0.80$) but were for bacteria ($p < 0.05$). Fewer fungal families than
530 bacterial families were significantly associated with total absorbed dose rate (Table S2). In
531 addition, fungi and bacteria appeared to display opposing responses to radiation. For
532 example, the heatmaps suggest that family-level associations with total absorbed dose rate
533 in bank vole guts were mostly positive for fungi (Figure S11), but mostly negative for bacteria

534 (Figure 4). Together, our results suggest that changes in host-associated fungal
535 communities may be less associated with radiation exposure than changes in bacterial
536 communities (Table S2).

537 To our knowledge, we present here the first demonstration that host species is a
538 significant predictor of fungal community composition in ground-dwelling small mammal
539 populations; fungal community compositions are an under-explored aspect of host-
540 associated microbiomes in general^{9,77}. In agreement with previous studies on a range of
541 species^{78–80}, including small mammals⁴, we also find host species to be a significant
542 predictor of bacterial community composition. We found no effect of sex on bacterial or
543 fungal communities of faecal samples from any host species, or on fungal communities of
544 bank vole guts. However, we did find that sex had a significant effect on bacterial community
545 composition of bank vole guts. Previous studies have found mixed effects of sex on
546 microbiome composition of small mammals^{4,10,25}.

547

548 **Conclusions**

549 Using a range of statistical analyses, we identify a number of significant associations
550 between total absorbed radiation dose and changes in microbiome, particularly for the
551 bacterial component. For bank vole gut data, these results were robust against confounding
552 factors including geographic variation and habitat type. However, the overall evidence for a
553 significant impact of radiation on the fungal component of the microbiome was limited.
554 Furthermore, contrary to the findings of the only previous published study of small mammal
555 microbiome in the CEZ^{25,26}, we did not see any significant effect on the F:B ratio with
556 absorbed dose rate. We also provide evidence that faecal samples are not reliable for
557 examining microbiome changes in response to radiation, and that total absorbed radiation
558 doses provide considerably more accurate results than ambient dose measures.

559 We suggest that, given the importance of the microbiome to host health and the
560 limited studies on microbiome (especially fungal microbiome and gut microbiome) in wild
561 animals, further studies of the microbiome response to radiation and other factors within the
562 CEZ should be undertaken. In particular, there are outstanding questions around whether
563 host microbiomes are directly affected by radiation exposure, or rather mediated by some
564 mechanism of host physiology. For this, it is important to establish directionality; i.e. whether
565 the host microbiome alters host physiology in response to radiation exposure, or vice versa.
566 Furthermore, changes in diet, resulting from impacts of radiation on the ecosystem (e.g. in
567 an area such as the Red Forest) rather than the host, may also be expected to affect the gut
568 microbiome. More work is required to understand the mechanisms that are driving changes
569 in host microbiomes of wildlife in general, and the implications of this for host function and
570 fitness.

571

572 **ACKNOWLEDGEMENTS**

573 The work described in this paper was conducted within the TREE (<https://tree.ceh.ac.uk/>)
574 and RED FIRE (<https://www.ceh.ac.uk/redfire>) projects. TREE was funded by the Natural
575 Environment Research Council (NERC), Radioactive Waste Management Ltd. and the
576 Environment Agency as part of the RATE Programme; RED FIRE was a NERC Urgency
577 Grant. The study was undertaken in line with ethical approval obtained from the University of
578 Salford. For the 2017 study, the GMS 310 core gamma logger was kindly loaned by John
579 Caunt Scientific Ltd.

580

581 **STATEMENT OF AUTHORSHIP**

582 MDW, NAB & SG designed the study and undertook sample collection (along with RF, JAJ,
583 CLB, EP & LW); SG characterised sites; NAB and RF live-monitored small mammals; REA

584 conducted the DNA extraction, molecular work and statistical analysis; REA, NAB and MDW
585 wrote the paper; all authors revised and approved the final manuscript.

586

587 **DATA ACCESSIBILITY STATEMENT**

588 Sequence data are available from the NCBI SRA database under project numbers
589 PRJNA594002 and PRJNA592322.

590

591 **REFERENCES**

- 592 1. Mckenney, E. A., Koelle, K., Dunn, R. R. & Yoder, A. D. The ecosystem services of
593 animal microbiomes. *Mol. Ecol.* 0–1 (2018). doi:10.1111/mec.14532
- 594 2. McFall-Ngai, M. *et al.* Animals in a bacterial world, a new imperative for the life
595 sciences. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 3229–3236 (2013).
- 596 3. Viney, M. The gut microbiota of wild rodents: Challenges and opportunities. *Lab.*
597 *Anim.* **53**, 252–258 (2019).
- 598 4. Knowles, S. C. L., Eccles, R. M. & Baltrūnaitė, L. Species identity dominates over
599 environment in shaping the microbiota of small mammals. *Ecol. Lett.* **22**, 826–837
600 (2019).
- 601 5. McKenney, E. A., Rodrigo, A. & Yoder, A. D. Patterns of gut bacterial colonization in
602 three primate species. *PLoS One* **10**, 1–18 (2015).
- 603 6. McKenney, E. A., O'Connell, T. M., Rodrigo, A. & Yoder, A. D. Feeding strategy
604 shapes gut metagenomic enrichment and functional specialization in captive lemurs.
605 *Gut Microbes* **9**, 202–217 (2018).
- 606 7. Maurice, C. F. *et al.* Marked seasonal variation in the wild mouse gut microbiota.
607 *ISME J.* **9**, 2423–2434 (2015).

- 608 8. Zhang, L. *et al.* Perfluorooctane Sulfonate Alters Gut Microbiota-Host Metabolic
609 Homeostasis in Mice Limin. *Toxicology* **431**, 152365 (2020).
- 610 9. Antwis, R. E., Cox, M. & Harrison, X. A. *Host Microbiomes of Soils, Plants and*
611 *Animals: An Integrated Approach*. (Cambridge University Press, 2020).
- 612 10. Weldon, L. *et al.* The gut microbiota of wild mice. *PLoS One* **10**, 1–15 (2015).
- 613 11. Wu, J. *et al.* Perinatal lead exposure alters gut microbiota composition and results in
614 sex-specific bodyweight increases in adult mice. *Toxicol. Sci.* **151**, 324–333 (2016).
- 615 12. Jin, Y., Wu, S., Zeng, Z. & Fu, Z. Effects of environmental pollutants on gut
616 microbiota. *Environ. Pollut.* **222**, 1–9 (2017).
- 617 13. Lu, K. *et al.* Arsenic exposure perturbs the gut microbiome and its metabolic profile in
618 mice: An integrated metagenomics and metabolomics analysis. *Environ. Health*
619 *Perspect.* **122**, 284–291 (2014).
- 620 14. Zhang, S., Jin, Y., Zeng, Z., Liu, Z. & Fu, Z. Subchronic Exposure of Mice to Cadmium
621 Perturbs Their Hepatic Energy Metabolism and Gut Microbiome. *Chem. Res. Toxicol.*
622 **28**, 2000–2009 (2015).
- 623 15. Joly Condette, C., Bach, V., Mayeur, C., Gay-Quéheillard, J. & Khorsi-Cauet, H.
624 Chlorpyrifos Exposure during Perinatal Period Affects Intestinal Microbiota Associated
625 with Delay of Maturation of Digestive Tract in Rats. *J. Pediatr. Gastroenterol. Nutr.* **61**,
626 30–40 (2015).
- 627 16. Nasuti, C. *et al.* Changes on fecal microbiota in rats exposed to permethrin during
628 postnatal development. *Environ. Sci. Pollut. Res.* **23**, 10930–10937 (2016).
- 629 17. Kan, H., Zhao, F., Zhang, X. X., Ren, H. & Gao, S. Correlations of Gut Microbial
630 Community Shift with Hepatic Damage and Growth Inhibition of Carassius auratus
631 Induced by Pentachlorophenol Exposure. *Environ. Sci. Technol.* **49**, 11894–11902
632 (2015).

- 633 18. Jin, Y., Zeng, Z., Wu, Y., Zhang, S. & Fu, Z. Oral exposure of mice to carbendazim
634 induces hepatic lipid metabolism disorder and gut microbiota dysbiosis. *Toxicol. Sci.*
635 **147**, 116–126 (2015).
- 636 19. Packey, C. D. & Ciorba, M. A. Microbial influences on the small intestinal response to
637 radiation injury. *Curr. Opin. Gastroenterol.* **46**, 564–574 (2011).
- 638 20. Dubois, A. & Walker, R. I. Gastrointestinal Injury Associated With the Acute Radiation
639 Syndrome. *Gastroenterology* **95**, 500–507 (1988).
- 640 21. Zhang, A. & Steen, T. Y. Gut microbiomics—a solution to unloose the Gordian knot of
641 biological effects of ionizing radiation. *J. Hered.* **109**, 212–221 (2018).
- 642 22. Goudarzi, M. *et al.* An Integrated Multi-Omic Approach to Assess Radiation Injury on
643 the Host-Microbiome Axis. *Radiat. Res.* **186**, 219 (2016).
- 644 23. Demers, M., Dagnault, A. & Desjardins, J. A randomized double-blind controlled trial:
645 Impact of probiotics on diarrhea in patients treated with pelvic radiation. *Clin. Nutr.* **33**,
646 761–767 (2014).
- 647 24. Meng-Meng, L., Shu-Ting, L., Yan, S. & He-Qin, Z. Probiotics for prevention of
648 radiation-induced diarrhea: A meta-analysis of randomized controlled trials. *PLoS One*
649 **12**, 1–15 (2017).
- 650 25. Lavrinienko, A. *et al.* Environmental radiation alters the gut microbiome of the bank
651 vole *Myodes glareolus*. *ISME J.* **12**, 2801–2806 (2018).
- 652 26. Lavrinienko, A., Tukalenko, E., Mappes, T. & Watts, P. C. Skin and gut microbiomes
653 of a wild mammal respond to different environmental cues. *Microbiome* **6**, 1–16
654 (2018).
- 655 27. Ruiz-González, M. X. *et al.* Resistance of Feather-Associated Bacteria to Intermediate
656 Levels of Ionizing Radiation near Chernobyl. *Sci. Rep.* **6**, 1–7 (2016).
- 657 28. Czirják, G. Á., Möller, A. P., Mousseau, T. A. & Heeb, P. Microorganisms Associated

- 658 with Feathers of Barn Swallows in Radioactively Contaminated Areas Around
659 Chernobyl. *Microb. Ecol.* **60**, 373–380 (2010).
- 660 29. Mousseau, T. A. & Moller, A. P. Landscape portrait: A look at the impacts of
661 radioactive contaminants on Chernobyl's wildlife. *Bull. At. Sci.* **67**, 38–46 (2011).
- 662 30. Beresford, N. A., Scott, E. M. & Copplestone, D. Field effects studies in the Chernobyl
663 Exclusion Zone: Lessons to be learnt. *J. Environ. Radioact.* **211**, (2020).
- 664 31. Beaugelin-Seiller, K., Garnier-Laplace, J. & Beresford, N. A. Estimating radiological
665 exposure of wildlife in the field. *J. Environ. Radioact.* **211**, (2020).
- 666 32. Fawkes, R., Wood, M. D., Beresford, N. A. & Burgess, P. H. An innovative portable
667 detector for the live-monitoring of radionuclides in small terrestrial animals. (2018).
- 668 33. Currie, L. Limits for qualitative detection and quantitative determination. Application to
669 radiochemistry. *Anal. Chem.* **40**, 586–93 (1968).
- 670 34. Beresford, N. A. *et al.* Radionuclide transfer to wildlife at a 'Reference site' in the
671 Chernobyl Exclusion Zone and resultant radiation exposures. *J. Environ. Radioact.*
672 **211**, 105661 (2020).
- 673 35. Brown, J. E. *et al.* A new version of the ERICA tool to facilitate impact assessments of
674 radioactivity on wild plants and animals. *J. Environ. Radioact.* **153**, 141–148 (2016).
- 675 36. Beresford, N. A. *et al.* Estimating the exposure of small mammals at three sites within
676 the Chernobyl exclusion zone - a test application of the ERICA Tool. *J. Environ.*
677 *Radioact.* **99**, 1496–1502 (2008).
- 678 37. Ulanovskya, A., Pröhla, G. & Gómez-Rosb, J. M. Methods for calculating dose
679 conversion coefficients for terrestrial and aquatic biota. *J. Environ. Radioact.* **99**,
680 1440–1448 (2008).
- 681 38. ICRP. *Environmental protection: the concept and use of reference animals and plants*,
682 ICRP publication 108, Ann. ICRP 38, 4-6, 2008. (2008).

- 683 39. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*. (Springer-Verlag New York,
684 2009).
- 685 40. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D.
686 Development of a dual-index sequencing strategy and curation pipeline for analyzing
687 amplicon sequence data on the miseq illumina sequencing platform. *Appl. Environ.*
688 *Microbiol.* **79**, 5112–5120 (2013).
- 689 41. Griffiths, S. M. *et al.* Genetic variability and ontogeny predict microbiome structure in a
690 disease-challenged montane amphibian. *ISME J.* **1** (2018). doi:10.1038/s41396-018-
691 0167-0
- 692 42. Smith, D. P. & Peay, K. G. Sequence Depth, Not PCR Replication, Improves
693 Ecological Inference from Next Generation DNA Sequencing. *PLoS One* **9**, e90234
694 (2014).
- 695 43. Nguyen, N. H., Smith, D., Peay, K. & Kennedy, P. Parsing ecological signal from
696 noise in next generation amplicon sequencing. *New Phytol.* **205**, 1389–1393 (2015).
- 697 44. Griffiths, S. M. *et al.* Complex associations between cross-kingdom microbial
698 endophytes and host genotype in ash dieback disease dynamics. *J. Ecol.* **1–19**
699 (2019). doi:10.1111/1365-2745.13302
- 700 45. RStudio Team. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA
701 URL <http://www.rstudio.c> (2016).
- 702 46. R Core Team. R: A language and environment for statistical computing. R Foundation
703 for Statistical Computing. Vienna, Austria. URL <https://www.R-project.org/>. (2017).
- 704 47. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon
705 data. *Nat. Methods* **13**, 581–583 (2016).
- 706 48. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data
707 processing and web-based tools. *Nucleic Acids Res.* **41**, 590–596 (2013).

- 708 49. Yilmaz, P. *et al.* The SILVA and ‘all-species Living Tree Project (LTP)’ taxonomic
709 frameworks. *Nucleic Acids Res.* **42**, 643–648 (2014).
- 710 50. McMurdie, P. J. & Holmes, S. Phyloseq: An R Package for Reproducible Interactive
711 Analysis and Graphics of Microbiome Census Data. *PLoS One* **8**, e61217 (2013).
- 712 51. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing
713 reads. *EMBnet.journal*, 17:10-12. (2011).
- 714 52. Schoch, C. L. *et al.* Nuclear ribosomal internal transcribed spacer (ITS) region as a
715 universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci.* **109**, 6241–6246
716 (2012).
- 717 53. UNITE. UNITE general FASTA release. Version 01.12.2017. (2017).
- 718 54. Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V. & Egoscue, J. J. Microbiome
719 datasets are compositional: And this is not optional. *Front. Microbiol.* **8**, 1–6 (2017).
- 720 55. Oksanen, J. *et al.* vegan: Community Ecology Package. (2018).
- 721 56. Dinno, A. *dunn.test*: Dunn’s Test of Multiple Comparisons Using Rank Sums. R
722 package version 1.3.5. <https://CRAN.R-project.org/package=dunn.test>. (2017).
- 723 57. Ogle, D. H., Wheeler, P. & Dinno, A. *FSA*: Fisheries Stock Analysis. R package
724 version 0.8.25. <https://github.com/droglenc/FSA>. (2019).
- 725 58. Meyer, D. & Buchta, C. *proxy*: Distance and Similarity Measures. R package version
726 0.4-23. <https://CRAN.R-project.org/package=proxy>. (2019).
- 727 59. Lahti, L. & Shetty, S. Tools for microbiome analysis in R. Microbiome package version
728 1.1.10013. <http://microbiome.github.com/microbiome>. (2017).
- 729 60. Gaschak, S. P. *et al.* Radiation ecology issues associated with murine rodents and
730 shrews in the Chernobyl Exclusion Zone. *Health Phys.* **101**, 416–430 (2011).
- 731 61. Copplestone, D. *et al.* *Impact assessment of ionising radiation on wildlife. R&D*

- 732 *Publication 128, Environment Agency and English Nature, Bristol.* (2001).
- 733 62. Vives I Batlle, J. *et al.* Inter-comparison of absorbed dose rates for non-human biota.
- 734 *Radiat. Environ. Biophys.* **46**, 349–373 (2007).
- 735 63. Beresford, N. *et al.* Towards solving a scientific controversy – The effects of ionising
- 736 radiation on the environment. *J. Environ. Radioact.* **211**, 106033 (2020).
- 737 64. Antwis, R. E., Lea, J. M. D., Unwin, B. & Shultz, S. Gut microbiome composition is
- 738 associated with spatial structuring and social interactions in semi-feral Welsh
- 739 Mountain ponies. *Microbiome* **6**, 207 (2018).
- 740 65. Whicker, F. W. & Schultz, V. *Radioecology: Nuclear Energy and the Environment.*
- 741 Volume 2. (CRC Press, Boca Raton., 1982).
- 742 66. McNamara, N. P., Black, H. I. J., Beresford, N. A. & Parekh, N. R. Effects of acute
- 743 gamma irradiation on chemical, physical and biological properties of soils. *Appl. Soil*
- 744 *Ecol.* **24**, 117–132 (2003).
- 745 67. Newbold, L. K. *et al.* Genetic, epigenetic and microbiome characterisation of an
- 746 earthworm species (*Octolasion lacteum*) along a radiation exposure gradient at
- 747 Chernobyl. *Environ. Pollut.* **255**, 113238 (2019).
- 748 68. Joyce, S. A. *et al.* Regulation of host weight gain and lipid metabolism by bacterial bile
- 749 acid modification in the gut. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 7421–7426 (2014).
- 750 69. Schirmer, M. *et al.* Linking the Human Gut Microbiome to Inflammatory Cytokine
- 751 Production Capacity. *Cell* **167**, 1125-1136.e8 (2016).
- 752 70. Friberg, I. M., Taylor, J. D. & Jackson, J. A. Diet in the driving seat: natural diet-
- 753 immunity-microbiome interactions in wild fish. *Front. Immunol.* **10**, 243 (2019).
- 754 71. Ingala, M. R. *et al.* Comparing microbiome sampling methods in a wild mammal:
- 755 Fecal and intestinal samples record different signals of host ecology, evolution. *Front.*
- 756 *Microbiol.* **9**, 1–13 (2018).

- 757 72. Leite, G. *et al.* Sa1911 – Analysis of the Small Intestinal Microbiome Reveals Marked
758 Differences from Stool Microbiome in a Large Scale Human Cohort: Redefining the
759 “Gut Microbiome”. *Gastroenterology* **156**, S-449-S-450 (2019).
- 760 73. Møller, A. P. & Mousseau, T. A. Reduced abundance of insects and spiders linked to
761 radiation at Chernobyl 20 years after the accident. *Biol. Lett.* **5**, 356–359 (2009).
- 762 74. Garnier-Laplace, J. *et al.* Are radiosensitivity data derived from natural field conditions
763 consistent with data from controlled exposures? A case study of Chernobyl wildlife
764 chronically exposed to low dose rates. *J. Environ. Radioact.* **121**, 12–21 (2013).
- 765 75. Smith, J. Field evidence of significant effects of radiation on wildlife at chronic low
766 dose rates is weak and often misleading. A comment on “Is non-human species
767 radiosensitivity in the lab a good indicator of that in the field? Making the comparison
768 more robust”. *J. Environ. Radioact.* **211**, 105895 (2020).
- 769 76. Carmody, R. N. *et al.* Diet dominates host genotype in shaping the murine gut
770 microbiota. *Cell Host Microbe* **17**, 72–84 (2015).
- 771 77. Rowan-Nash, A. D., Korry, B. J., Mylonakis, E. & Belenky, P. Cross-Domain and Viral
772 Interactions in the Microbiome. *Microbiol. Mol. Biol. Rev.* **83**, 1–63 (2019).
- 773 78. Davenport, E. R. *et al.* The human microbiome in evolution. *BMC Biol.* **15**, 1–12
774 (2017).
- 775 79. Mazel, F. *et al.* Is Host Filtering the Main Driver of Phylosymbiosis across the Tree of
776 Life? *mSystems* **3**, 1–15 (2018).
- 777 80. Youngblut, N. D. *et al.* Host diet and evolutionary history explain different aspects of
778 gut microbiome diversity among vertebrate clades. *Nat. Commun.* **10**, 1–15 (2019).
- 779 81. Shestopalov, V. *Atlas of Chernobyl exclusion zone*. Kiev: Ukrainian Academy of
780 Science. (1996).
- 781

782 **FIGURE LEGENDS**

783 **Figure 1**

784 Location of the study sites in the CEZ where small mammals were trapped in 2017 and
785 2018; the approximate location of the Red Forest is indicated by the black rectangle. The
786 underlying ^{137}Cs soil data shown (decay corrected to summer 2017) are from Shestopalov
787 (1996).

788

789 **Figure 2**

790 Relationship between ambient and total absorbed dose rates associated with animals
791 sampled in the study, split into total absorbed dose rate categories (low <4 microGy/h;
792 medium = 4-42 microGy/h; high > 42 microGy/h).

793

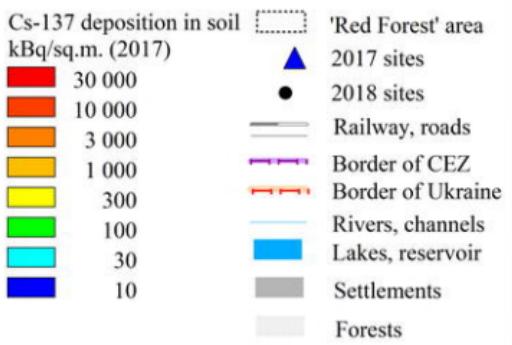
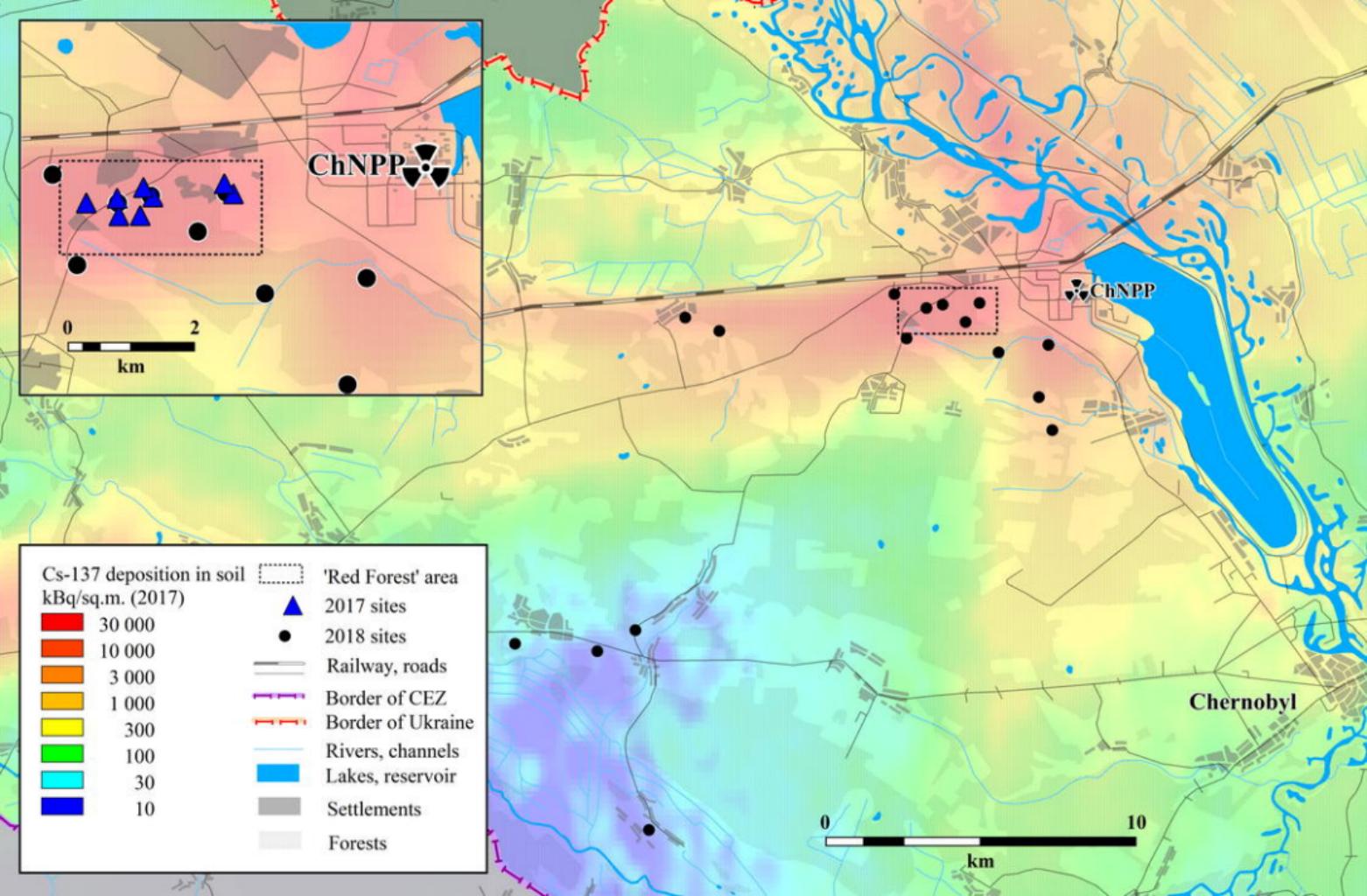
794 **Figure 3**

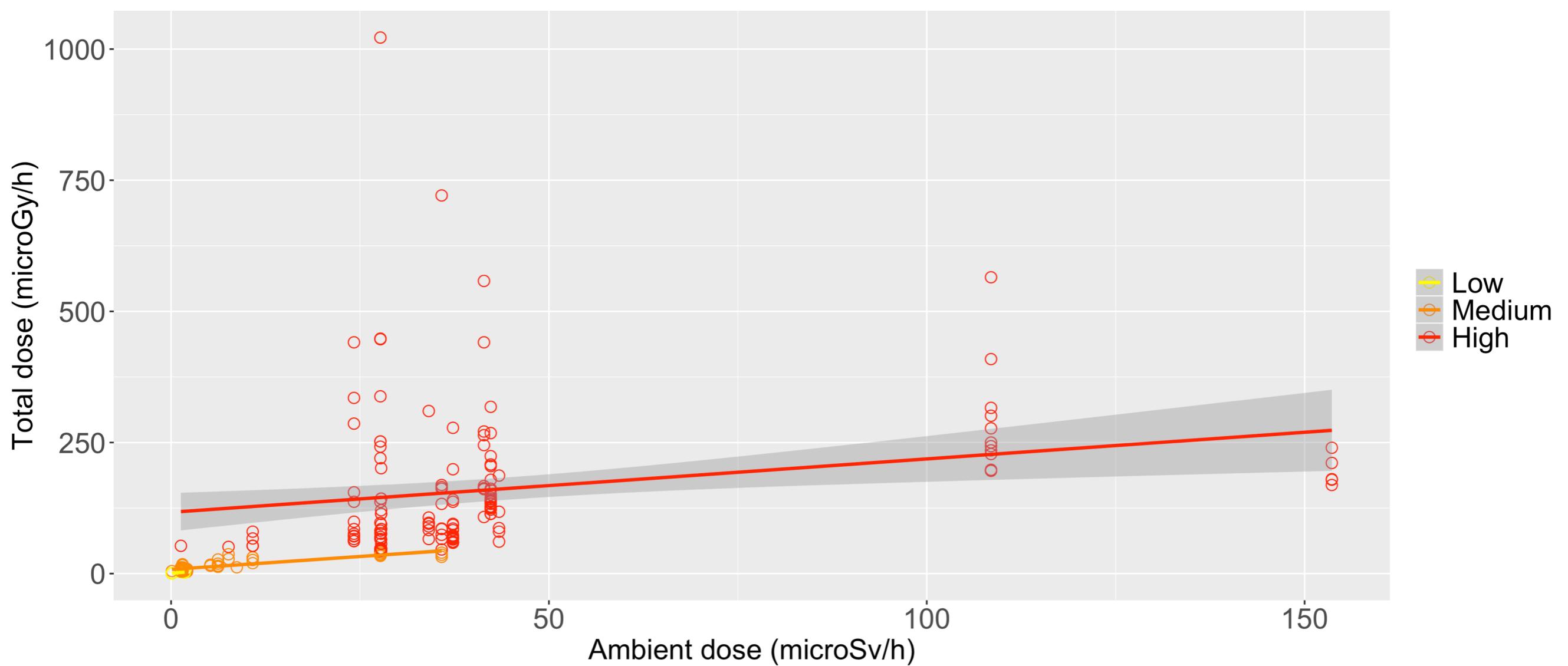
795 PCA plots showing Euclidean distances of clr-transformed bacterial (a) and fungal (b)
796 communities associated with faecal and gut samples from four small mammal species in the
797 Chernobyl Exclusion Zone. Jitter plots displaying the clr values of the 12 most abundant
798 bacterial (c) and fungal (d) families across the five sampling groups (faecal samples for the
799 three mice species along with faecal and gut samples for the bank voles).

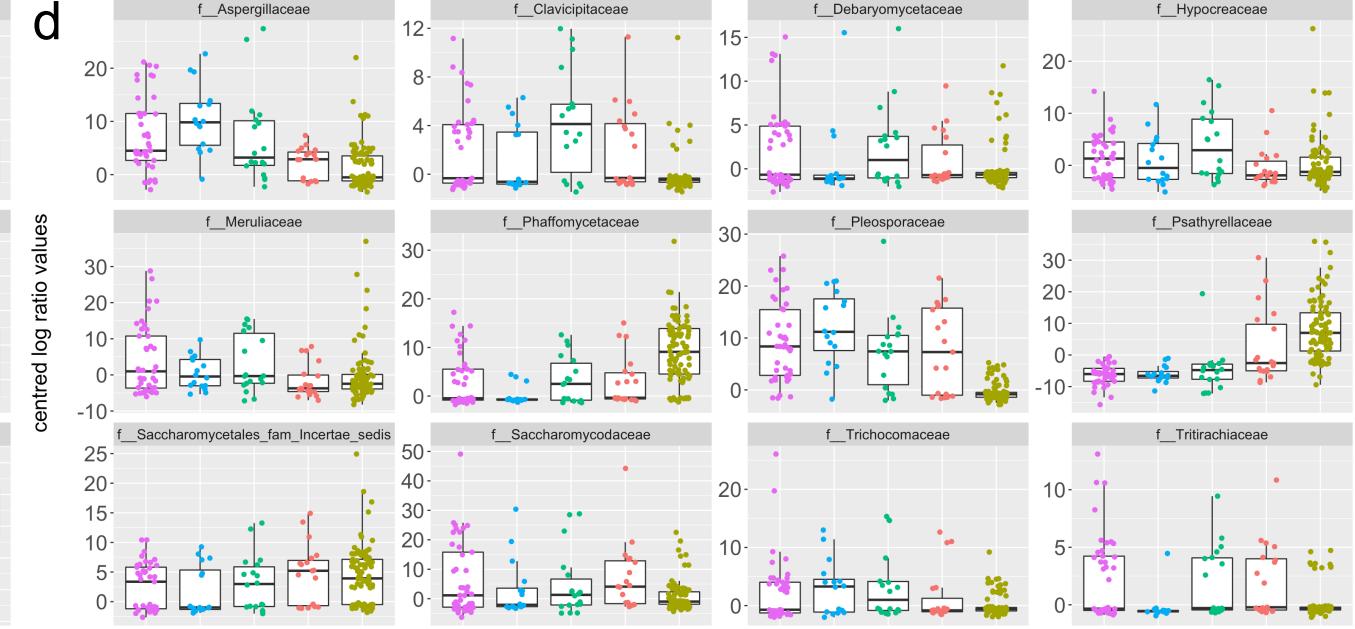
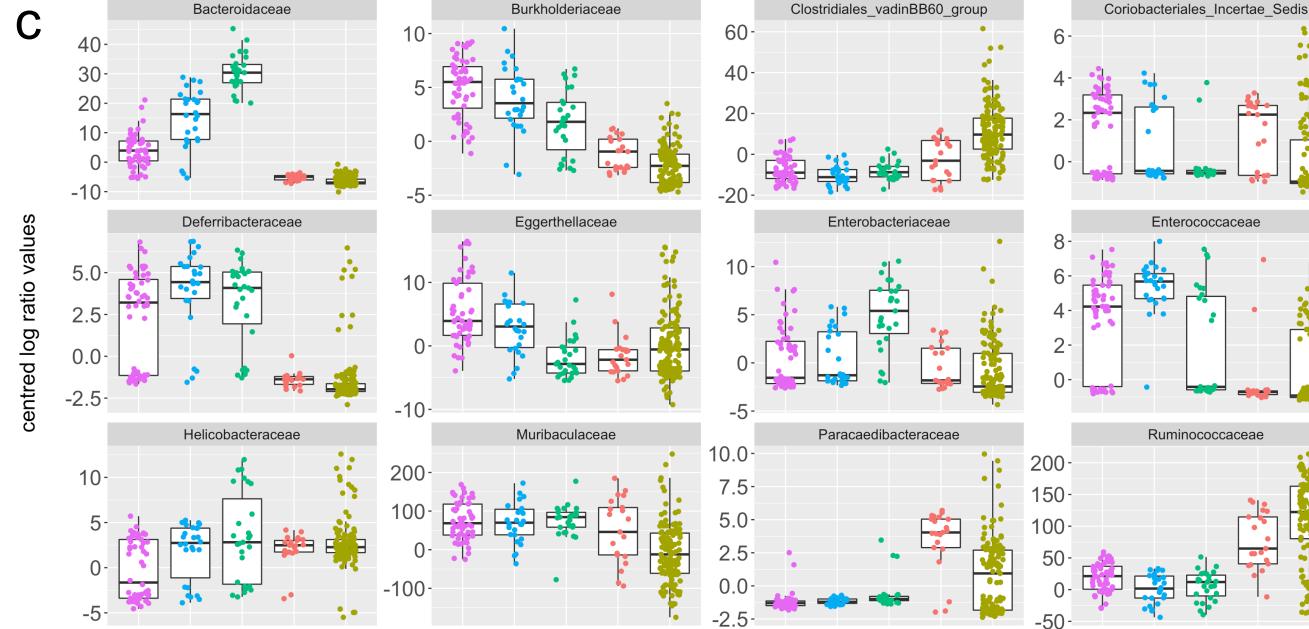
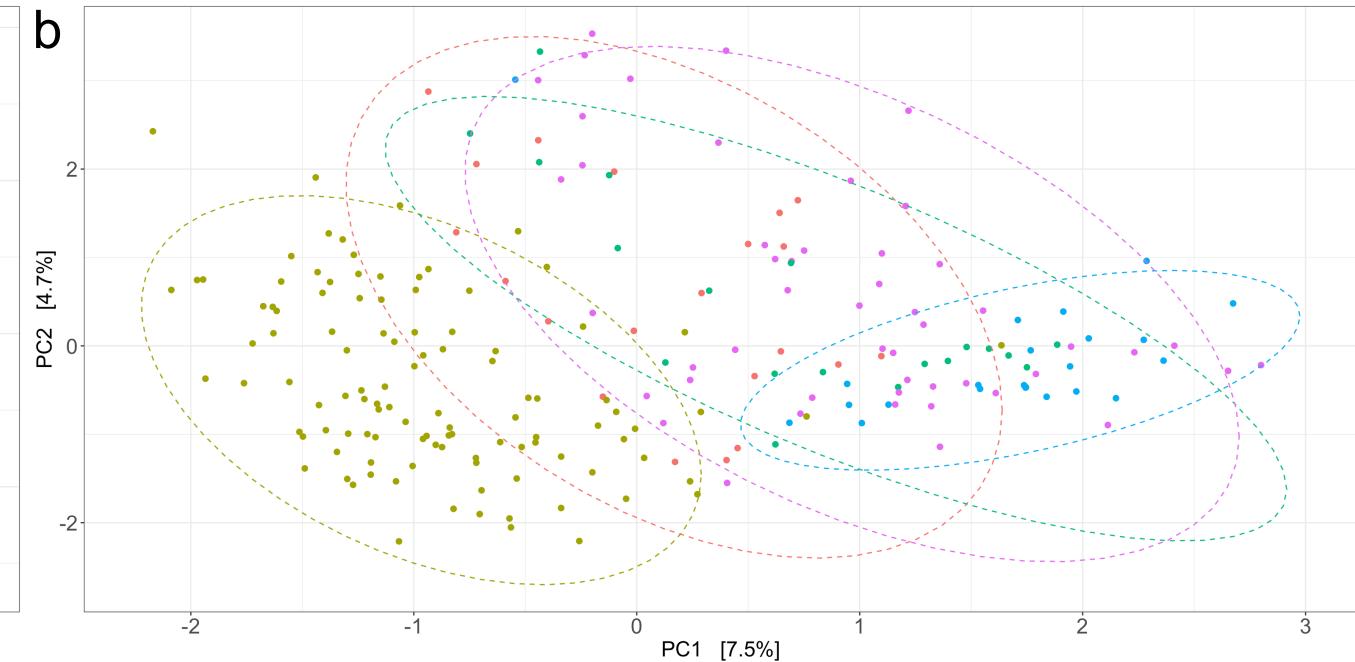
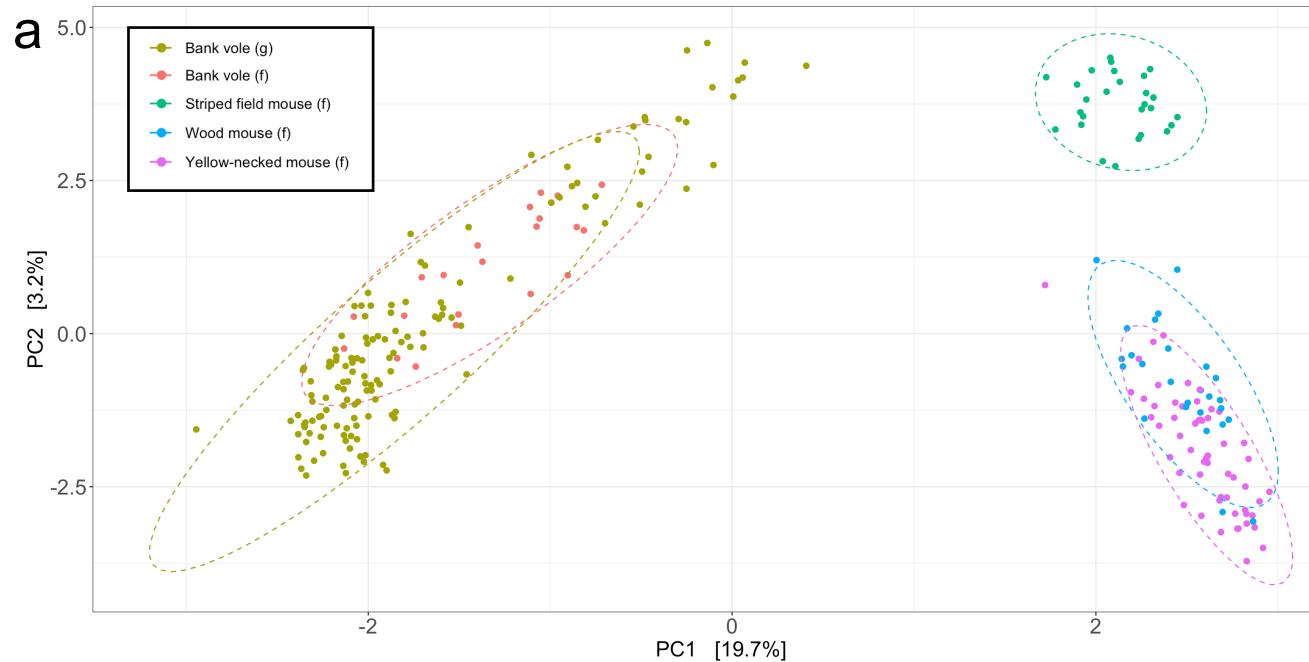
800

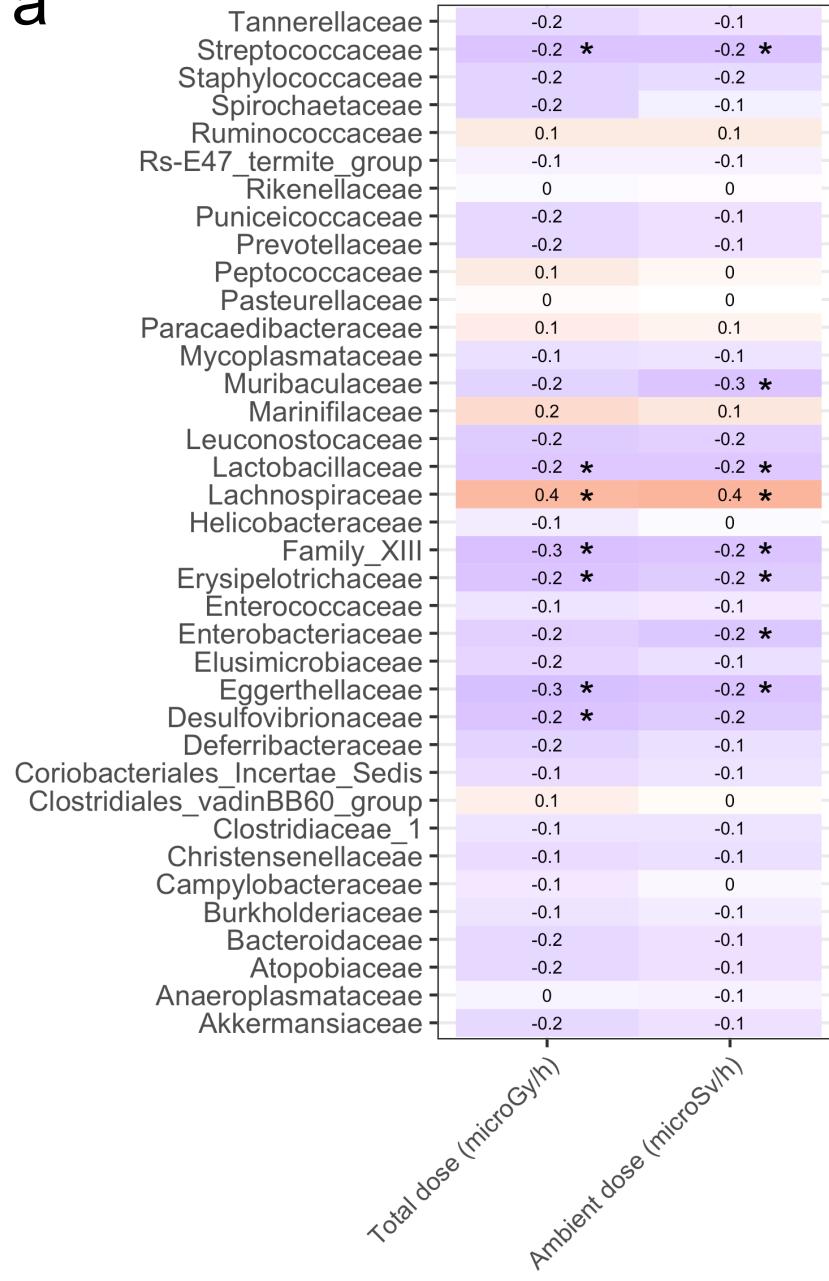
801 **Figure 4**

802 Heatmaps showing correlations between the two radiation dose rate measures (total and
803 ambient) and (a) clr-values of bacterial genera in vole guts and (b) clr-ratios of bacterial
804 genera in faecal samples from four small mammal species. Statistically significant
805 relationships are denoted by *.







a**b**