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Fecal microbiota dysbiosis in macaques and humans within a shared environment

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## 24 **Abstract**

25 Traditional zoonotic disease research focuses on detection of recognized pathogens and may  
26 miss opportunities to understand broader microbial transmission dynamics between humans,  
27 animals, and the environment. We studied human-macaque microbiome overlap in Kosum Phisai  
28 District, Maha Sarakham Province, Thailand, where a growing population of long-tailed  
29 macaques (*Macaca fascicularis*) in Kosumpee Forest Park interact with humans from an adjacent  
30 village. We surveyed workers in or near the park with elevated exposure to macaques to  
31 characterize tasks resulting in exposure to macaque feces in addition to dietary and lifestyle  
32 factors that influence gut microbiome composition. Fecal samples were collected from 12  
33 exposed workers and 6 controls without macaque exposure, as well as 8 macaques from  
34 Kosumpee Forest Park and 4 from an isolated forest patch with minimal human contact. The V4  
35 region of the 16S rRNA gene from fecal sample extracted DNA was amplified and sequenced  
36 using Illumina HiSeq to characterize the microbial community. A permuted betadisper test on the  
37 weighted UniFrac distances revealed significant differences in the dispersion patterns of gut  
38 microbiota from exposed and control macaques ( $p=0.03$ ). The high variance in gut microbiota  
39 composition of macaques in contact with humans has potential implications for gut microbiome  
40 stability and susceptibility to disease, described by the Anna Karenina principle (AKP). Human  
41 samples had homogenous variance in beta diversity but different spatial medians between groups  
42 ( $p=0.02$ ), indicating a shift in microbial composition that may be explained by fundamental  
43 lifestyle differences between the groups unrelated to exposure status. SourceTracker was used to  
44 estimate the percent of gut taxa in exposed humans that was contributed by macaques. While one  
45 worker showed evidence of elevated contribution, the overall trend was not significant. Task  
46 observations among workers revealed opportunities to employ protective measures or training to

47 reduce exposure to occupational hazards. These results suggest the potential for hygiene  
48 measures to mitigate negative aspects of contact between humans and macaques in order to  
49 optimize the health of both populations.

50

## 51 **Introduction**

## 52 **Background**

53 Habitat fragmentation and human encroachment results in a patchwork of isolated non-  
54 human primate populations across Thailand with potential for increased human-macaque contact  
55 [1]. Supplemental feeding for religious reasons or tourism contributes to a growing macaque  
56 population unconstrained by natural food resources. Amidst these changes, the high level of  
57 human-macaque conflict has led researchers to call for improved management plans and  
58 conservation strategies [1, 2]. The reasons for concern are two-fold: 1) human-macaque  
59 conflict—such as crop-raiding—can disrupt or damage livelihoods, resulting in negative  
60 perception of macaques and impairing conservation efforts and 2) the increased level of contact  
61 can provide opportunities for transmission of zoonotic diseases into either macaque or human  
62 populations.

63 Due to their genetic similarity, humans and macaques are susceptible to many of the same  
64 infectious diseases including tuberculosis and hepatitis [3]. Parasitic infections from soil-  
65 transmitted helminths capable of infecting humans and macaques, such as *Strongyloides*  
66 *fuelleborni* and *S. stercoralis* have also been documented in this region [4, 5]. These pathogens  
67 may (e.g. Ebola virus) or may not (e.g. Herpes B virus) cause overt disease in macaques,  
68 however cross-species pathogen transmission from wildlife reservoirs to human hosts is a  
69 recognized factor in the emergence of novel diseases [6]. These spillover events can readily

70 occur where natural and urban spaces meet. An example of such an interface is the Kosumpee  
71 Forest Park (KFP), a small fragmented forest in northeastern Thailand that is home to over 700  
72 long-tailed macaques (*Macaca fascicularis*) and lies adjacent to the Kosum Phisai community of  
73 4,235 persons [7, 8]. Unlike other popular tourist sites in Southeast Asia, macaques in KFP  
74 rarely climb onto people and feeding by the people often involves simply throwing food on the  
75 ground [9]. However, workers in and around the park regularly feed macaques and sweep  
76 macaque excrement from public spaces, elevating their risk of zoonotic disease transmission  
77 relative to other members of the Kosum Phisai community. While these workers represent a  
78 control point for broader spread of zoonoses, little is known about their knowledge, attitudes, and  
79 practices surrounding macaque exposure [10]. Based on task observations, it may be possible to  
80 identify intervention strategies to reduce exposure to macaque biological material. Such  
81 strategies could include the use of personal protective equipment (PPE), training in basic hand  
82 hygiene, or other measures to mitigate the risk of disease transmission. These measures would  
83 also promote responsible wildlife conservation by protecting macaques from pathogens that the  
84 workers could transmit through reverse zoonotic transmission.

85         The zoonotic spillover potential of certain pathogens, such as simian foamy virus, can be  
86 investigated through blood sample collection, however this method can be logistically  
87 challenging since it requires trapping and immobilization of wild macaques. Additionally,  
88 previous surveys among this worker population indicated that scratches or bites are infrequent,  
89 and ingestion of aerosolized fecal matter may be a more common route of exposure to zoonoses.  
90 Therefore, in this setting, we chose to analyze the microbial communities of fecal samples in  
91 humans and macaques with close contact. Advantages of analyzing the fecal microbiota include  
92 the fact that, compared to the skin microbiota, it is better characterized in literature, more

93 temporally stable, and yield higher read counts [11]. Recent studies have demonstrated that the  
94 community composition of human microbiota is influenced by our environment and the animals  
95 sharing that environment. The degree of contribution from these sources can be quantified using  
96 Bayesian approaches like SourceTracker [12, 13]. We performed a pilot study of fecal  
97 microbiota of workers and macaques in a shared environment to test the hypothesis that workers  
98 exposed to macaques will exhibit microbiota profiles that contain a greater percentage of  
99 microbes found in macaque feces compared to unexposed individuals. The goals of this study  
100 were to provide a baseline assessment of the risk of zoonotic disease transmission between  
101 macaques and workers and guide prevention recommendations.

102

## 103 **Materials and methods**

### 104 **Study design**

105 This pilot study was a cross-sectional sampling of humans and macaques, comparing  
106 humans with occupational contact with macaques (exposed humans) to humans without such  
107 contact (human controls), and macaques in close contact with humans (exposed macaques)  
108 compared to macaques without significant human contact (control macaques).

109

### 110 **Human participants**

111 Eligible workers (n=12) were defined as members of the community who contact  
112 macaques or macaque bodily fluids (blood, feces, urine) as a component of their paid work at  
113 least once per week. Workers were excluded if they had not worked at that site for a minimum of  
114 three months. Human controls (n=6) were recruited from a convenience sampling of adults at

115 Maharakham University and were eligible if they were over 18 years of age and reported no  
116 contact with macaques. Recruited participants were informed of study objectives and their rights  
117 as participants and offered 100 Thai baht as compensation for their time.

118

## 119 **Animal participants**

120 Exposed macaques (n=8) were sampled at Kosumpee Forest Park, with an effort to  
121 collect samples from macaques belonging to each of the social groups and age/sex distribution  
122 representative of the overall population. These macaques are individually identifiable by facial  
123 features or other unique characteristics by RCK. Control macaques (n=4) were sampled from a  
124 nearby forest in Phon Ngam in the same manner as exposed macaques, and age/sex were  
125 recorded.

126

## 127 **Environment**

128 Sites were selected based on the level of interaction between humans and macaques. The  
129 study site for exposed human participants was a village of approximately 4,235 individuals  
130 adjacent to Kosumpee Forest Park (KFP), Kosum Phisai District, Maha Sarakham Province in  
131 northeastern Thailand (16°15'19"N 103°04'06"E) [8]. The forest park is an isolated forest patch  
132 of approximately 0.2 km<sup>2</sup>, bordered on the east by the Chi River and to the south by the Kosum  
133 Phisai village. The park contains over 700 long-tailed macaques, divided into five social groups  
134 with largely overlapping ranges [7]. Control sites were Maharakham University for humans,  
135 approximately 24 km E of KFP, and a small forest tract in Phon Ngam (16°21'01"N  
136 102°56'54"E) for macaques, approximately 16 km NW of KFP, where there is minimal human-  
137 macaque interaction.

138

## 139 **Measurement**

140 Interviews, task observation, and sample collection was conducted from Sept 24 – Oct 7,  
141 2017. Survey data and sample metadata were collected and stored using the REDCap electronic  
142 database [14].

143 Macaque workers were surveyed regarding practices, training (e.g. macaque behavior,  
144 PPE use, wound care) and their knowledge of the principle that macaques and humans can share  
145 diseases. We piloted the occupational risk factor survey used in this study for eight park workers  
146 in October 2016 and revised it to address limitations that emerged during administration and  
147 analysis. Additions included a dietary questionnaire based on a modified food frequency  
148 questionnaire (FFQ). The full occupational questionnaire is available in supplemental materials.  
149 Task observations of workers were recorded using a GoPro HERO5 video recorder (GoPro, Inc.,  
150 San Mateo, CA, USA) in order to assess work activities and supplement characterization of  
151 exposure opportunities identified in the survey. Task observations were performed at the job title  
152 level (vendor, park worker, and janitor), not for each individual, for feasibility. The scoring  
153 criteria was devised by authors based on probable routes for fecal microbe transmission to  
154 humans. The video recordings were reviewed by two individuals to maintain consistency and  
155 discrepancies were addressed by reexamining the video segment. During review of recorded  
156 tasks, an exposure category was assigned by the reviewer at 5 minute intervals, based on  
157 proximity of macaques (high=direct contact or within 3m, low=beyond 3m or not visible) and  
158 behavior (aerosol generation or hand-to-mouth contact).

159 Fresh fecal samples were placed immediately into OMNIgene.GUT kits (DNA Genotek,  
160 Ontario, Canada) to stabilize and preserve microbial community composition and stabilize DNA

161 in the absence of a cold chain. Workers were provided with sterile collection kits and instructions  
162 in Issan Thai for proper specimen collection; macaque samples were similarly collected using a  
163 sterile spatula from the center of fresh excrement. Samples stored at ambient temperature as per  
164 OMNIgene.GUT kit instructions until they were processed at Khon Kaen University. QIAamp  
165 PowerFecal DNA Isolation kit (Qiagen, Hilden, Germany) was used to extract genomic DNA,  
166 following manufacturer protocols. DNA concentration was determined using a NanoDrop2000  
167 spectrophotometer (NanoDrop Technologies Inc., DE, USA) and the integrity of DNA was  
168 evaluated by running 5 ul of sample on a 0.8% agarose gel under 100 V for 30 min and assessing  
169 bands. Extracted DNA samples were shipped overnight on blue ice to Genewiz Laboratories in  
170 Suzhou, China. DNA quality was verified by Genewiz using NanoDrop, Qubit, and agarose  
171 electrophoresis. The V4 region of the bacterial 16S genes were amplified using the 515F-806R  
172 primers, based on the Earth Microbiome Project protocol [15]. Amplicons were sequenced on an  
173 Illumina HiSeq platform by Genewiz Laboratories. Raw FASTQ files and metadata can be  
174 accessed through the Qiita database (<https://qiita.ucsd.edu/>) (accession no. 11835) and the  
175 European Bioinformatics Institute, European Nucleotide Archive (accession no. ERP111664).  
176

## 177 **Analysis**

178 DNA sequences or reads in the form of FASTQ files were analyzed with QIIME2 version  
179 2017.12.0 pipeline [16]. DADA2 version 2017.12.1 was used for sequence quality control and  
180 feature table construction [17]. Forward reads were truncated to 280 bp and reverse reads to 260  
181 bp. Alpha diversity metrics (observed OTUs, Shannon's diversity index, Faith's Phylogenetic  
182 Diversity, and Pielou's Evenness) were calculated in QIIME2. In order to attain valid  
183 comparisons of abundance and diversity across samples, we normalized to the lowest sample



184 depth of 12,466 reads per sample [18]. Sequences were assigned taxonomy using the SILVA 132  
185 reference database [19]. Analysis of Composition of Microbiomes (ANCOM) was performed in  
186 QIIME2 between species and exposure groups with significantly different abundance values  
187 identified based on the W-statistic [20]. Principal Coordinate of Analysis (PCoA) plots and taxa  
188 bar plots were generated using the phyloseq package (version 1.22.3) in R [21]. PCoA plots were  
189 generated to visualize clustering patterns based on weighted UniFrac distance measures, which  
190 describes the degree of similarity between sample compositions by measuring the fraction of  
191 unique branch length from the phylogenetic tree of sample features and weights the distance by  
192 the relative abundance of that taxa within a sample. Profile clustering patterns from weighted  
193 UniFrac distance measures were analysed using adonis and betadisper tests from the vegan  
194 package (version 2.5.1) [22]. All tests were performed using 999 permutations based on the  
195 spatial median. To further characterize microbial sharing, SourceTracker [23] was applied to  
196 feature tables with macaques as source and humans as the sink under the default settings at a  
197 rarefaction depth of 1000 with 100 burn-ins and 10 re-starts.

198

## 199 **Study team**

200 The COHERE guidelines for reporting of One Health studies were followed in the  
201 preparation of this manuscript [24]. Study members represented the following areas of expertise:  
202 primatology (RCK, PK), human health (PR), anthropological medicine (VR), microbial ecology  
203 (EG), molecular biology (PP, RD), computational biology (PT), and environment/resource  
204 management (TT).

205

## 206 **Ethics statement**

207           The research in this study was approved through the University of Washington  
208 Institutional Review Board (IRB) for human subjects research and Institutional Animal Care and  
209 Use Committee (IACUC) for animal research (#51546 and #3143-04, respectively). The study  
210 also received approval through Mahasarakham University for human and animal subjects  
211 research (protocol numbers 037/2016 and 0009/2016, respectively). Written informed consent  
212 was obtained from all human participants and they were informed that participation was  
213 voluntary, they could withdraw at any time, and questionnaire responses, individual microbiota  
214 results, and task observation videos would be kept confidential and de-identified. Macaque  
215 samples were obtained from fresh defecations, therefore no direct macaque handling occurred as  
216 part of this study. This study was part of a larger project approved by the National Research  
217 Council of Thailand (NRCT project approval to RCK - Project ID: 2016/048; “Healthy  
218 Coexistence between Human and Non-human Primates: A One Health Approach”).

219

## 220 **Results**

### 221 **Questionnaire**

222           Exposed workers included government employees of Kosumpee Forest Park (n=8),  
223 janitors at a nearby school (n=3), and a vendor stationed near the park entrance (n=1). All study  
224 participants were born in Thailand and lived in the Maha Sarakham province for over a year.  
225 Demographic factors are summarized in Table 1.

226

227 **Table 1. Human metadata.**

Factor	Exposed (n=12)	Control (n=6)
Age, years (mean $\pm$ SD)	47.17 $\pm$ 11.36	27.5 $\pm$ 9.44
Sex		
Male	75% (9)	50% (3)
Female	25% (3)	50% (3)
Education, years (mean $\pm$ SD)	9.0 $\pm$ 3.05	16.8 $\pm$ 5.76 <sup>1</sup>
Household size		
1-3	25% (3)	67% (4)
4-6	58% (7)	33% (2)
7-9	17% (2)	0
Self-rated general health		
Fair	77% (8)	0
Good	33% (4)	83% (5)
Excellent	0	17% (1)
Smoker	75% (9)	0
Health problems in past year		
Fever	92% (11)	67% (4) <sup>1</sup>
Respiratory problems	58% (7)	67% (4) <sup>1</sup>
Gastrointestinal problems	33% (4)	67% (4)
Skin problems	25% (3)	0
Infectious diseases in lifetime		
Tuberculosis	8% (1)	0
Malaria	8% (1)	0
Dengue	17% (2)	0
Other parasites, hookworm	58% (7) <sup>1</sup>	0 <sup>1</sup>
Vaginal birth method	77% (8)	50% (3)
Breast-fed as infant	92% (11)	33% (2)
BMI	25.5 $\pm$ 5.8	23.8 $\pm$ 3.5
Antibiotic use in past month	17% (2) <sup>4</sup>	33% (2) <sup>2</sup>

228 Demographic, early life history, dietary, and other health factors for exposed and control  
229 humans, which may influence gut microbiota or may be related to macaque exposure.  
230 Superscripted numbers reflect the number of missing datapoints.

231  
232 Occupational factors related to microbial transmission are presented in Table 2. More  
233 than half of workers regularly wash hands without soap. All participants reported handwashing  
234 before and after eating (not listed in table), however task observation footage suggested this was  
235 not the case for at least four participants. PPE use as reported in the survey was low, which was  
236 further confirmed by the video recorded task observations. Respondents did not report receiving  
237 training relevant to safe animal handling or disease prevention before working around macaques.  
238 In an assessment of zoonotic disease knowledge, one-third of workers thought a diseased animal  
239 could transmit that agent to a human. Only one worker thought a human could make an animal  
240 sick and remarked that this would be with a high degree of contact. Workers typically only have  
241 direct physical contact with carcasses, but occasionally trap live macaques to move them from  
242 private properties to the forest park or when helping researchers. In one instance, a janitor had to  
243 remove a macaque from a classroom using a stick and grabbing it by hand. When around animals  
244 that appear sick, workers' primary form of precaution was to avoid contact.

245

246 **Table 2. Occupational risk factors.**

Factor	Response
Years at current job (mean $\pm$ SD)	18.40 $\pm$ 11.79
Hrs/wk around macaques or their feces (mean $\pm$ SD)	45.08 $\pm$ 8.694
Handwashing	
Water only	58% (7)

Soap and water	50% (6)
Alcohol-based sanitizer	8% (1)
PPE	
Disposable gloves	8% (1)
Paper or cloth dust masks	17% (2)
Rubber boots	25% (3)
Received animal/disease safety training <sup>a</sup>	0% (0)
Change in macaque behavior	42% (5)
Knowledge of animal to human transmission	33% (4)
Concerned about diseases from animals at work	33% (4)
Knowledge of human to animal transmission	8% (1)
Take precautions around animals that look sick	83% (10)

247 Occupational risk factors related to macaque exposure among park workers, janitors and vendors  
248 based on a questionnaire.

249 <sup>a</sup> Training topics included animal behavior, animal capture/restraint, infectious disease  
250 prevention, PPE use, or wound care.

251

252 Since starting their current job, workers noted that macaques seem “naughtier”, wait for  
253 provisioning or do not look for natural food, and eat more human food (e.g. chicken, meatballs,  
254 soda). All workers reported finding macaques that looked sick or had died. Carcasses were  
255 typically buried or burned. One janitor remarked that, “Last month 3 monkeys die, pick them up  
256 by broom into plastic bag and then threw them into the forest.”

257 Workers were asked what diseases they were primarily concerned about getting in  
258 general, not necessarily from macaques. Responses included leptospirosis (n=3), cancer (n=2),  
259 the common cold (n=2), cirrhosis (n=1), allergies (n=1), and an airborne infectious disease (n=1)  
260 (Table 1). One worker was concerned about a “disease that come with monkey poo because I

261 have to sweep it every day.” In contrast, non-communicable diseases like high blood pressure  
262 (n=3), cancer (n=1), diabetes (n=1) and hemorrhoids or constipation (n=1) were the primary  
263 disease concerns among controls.

264 An abbreviated food frequency questionnaire revealed dietary differences in the type of  
265 animal protein consumed. Control group members consumed more pork (p=0.04) and snail  
266 (p=0.03), whereas exposed workers typically ate more frog (p=0.04). There were no significant  
267 differences found in other dietary categories, including raw meat consumption. All respondents  
268 reported that they pass normal formed stool (Type 3/4 on Bristol stool scale), except one, from  
269 the exposed group, who reported Type 1/2.

270

## 271 **Task observation of workers**

272 Park workers engaged in the highest exposure activities based on recorded task  
273 observations, followed by individuals working as school janitors, then vendors (Table 3). Using  
274 the number of exposure events divided time observed to calculate relative risk (RR), a park  
275 worker is 1.78 times more likely than a school janitor and 2.84 times more likely than a vendor  
276 to work within 3m of macaques or engage in risk elevating activities (e.g. aerosol generation,  
277 hand-mouth contact) during the task observation.

278

279 **Table 3. Potential exposure to macaque feces based on video-recorded task observation**

Occupation	Proximity to macaques or macaque feces				Risk elevation <sup>c</sup>	Time observed	RR (95% CI)
	Not visible <sup>a</sup>	Beyond 3m <sup>a</sup>	Within 3m <sup>b</sup>	Contact <sup>b</sup>			
Vendor	0	16	3	0	4	95 min	Ref
Janitor	4	7	2	0	11	65 min	1.78 (1.67-1.90)

Park worker	0	3	6	4	9	65 min	2.84 (2.67-3.02)
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280 Task observation assessment for the three worker groups (vendor, janitor, and park worker)  
281 reveals variation in proximity to macaques and behaviors that elevate risk of potential exposure.  
282 Risk of a potential exposure event during the task observation is presented relative to the lowest  
283 exposure occupation (vendor).

284 <sup>a</sup> No exposure

285 <sup>b</sup> Exposure

286 <sup>c</sup> Exposure, defined as engagement in activities that generate aerosols and/or eating, drinking, or  
287 smoking without prior handwashing

288

## 289 **Macaque demographics**

290 Macaques were age and sex identified according to Table 4. Among exposed macaques,  
291 members of four of the five social groups within Kosumpee Forest Park are represented in this  
292 study. Two individuals were sampled from each group (i.e. Red Dot, Stump Tail, Droop Lip and  
293 Hare Lip). At the control site, all four members were from the same social group.

294

295 **Table 4. Macaque metadata.**

Macaques	Exposed (n=8)	Control (n=4)
Age		
Juvenile	0	75% (3)
Subadult	37.5% (3)	0
Adult	62.5% (5)	25% (1)
Sex		
Male	50% (4)	50% (2) <sup>1</sup>
Female	50% (4)	25% (1) <sup>1</sup>

296 Age and sex of sampled macaques. Superscripted numbers reflect the number of missing  
297 datapoints where age or sex could not be determined.

298

## 299 **Fecal microbiota analysis**

### 300 **Phylum-level abundance**

301 A total of 3,307 amplicon sequence variants (ASVs) were generated from 628,623 total  
302 read counts. There was an average of 20,954 reads per sample (range: 12,466-35,318). Fig. 1  
303 shows the relative abundance of bacterial phyla in each sample, after rarefaction to minimum  
304 sample size. All sample profiles were dominated by Bacteroidetes, Firmicutes, and  
305 Proteobacteria.

306

307 **Figure 1. Phylum-level abundance bar plot.** Relative abundance of bacterial phyla in macaque  
308 control (n=4), macaque exposed (n=8), human exposed (n=12), and human control (n=6)  
309 samples following rarefaction to the minimum library size. Samples are labelled with their record  
310 ID, group (HC, Human Control; HE, Human Exposed; ME, Macaque Exposed; MC, Macaque  
311 Control), and, if applicable, level of exposure (L, Low; M, Medium; H, High) and social group  
312 (R, S, D, H).

313 Using ANCOM analysis, no taxonomic features were significantly different in abundance  
314 between exposed and unexposed humans. Statistically significant differences in abundance  
315 between humans and macaques are listed in Table 5.

316

### 317 **Table 5. ANCOM analysis of differential abundance in humans and macaques**

Feature taxonomy	W	Enriched in
------------------	---	-------------



Cyanobacteria>Melainabacteria	25	Macaques
Kiritimatiellaeota>Kiritimatiellae>WCHB1-41	45	Macaques
Bacteroidetes> Bacteroidia>Bacteroidales>Marinifilaceae	85	Macaques
Fusobacteria>Fusobacteriia>Fusobacteriales>Fusobacteriaceae	86	Humans
Proteobacteria>Gammaproteobacteria>Enterobacteriales>Enterobacteriaceae	90	Macaques
Bacteroidetes>Bacteroidia>Bacteroidales>Bacteroidaceae>Bacteroides	237	Macaques
Bacteroidetes> Bacteroidia>Bacteroidales>Rikenellaceae>Rikenellaceae RC9 gut group	249	Macaques

318 Features (ASVs) that were differentially abundant in humans or macaques are reported at the  
319 most resolved taxonomic level. W-statistic indicated is the number of other items from which a  
320 single item is found to be significantly different using the default  $\alpha=0.05$ .

321

## 322 **Alpha and beta diversity**

323 Fig. 2 displays the 1) total number of observed features in each sample, 2) Shannon's  
324 index, which accounts for abundance and evenness of the taxa present using a natural logarithm,  
325 and 3) Simpson's index, which measures the relative abundance of the different species making  
326 up the sample richness. For both humans and macaques, alpha diversity was consistently lower  
327 in the exposed groups (Fig 2), though this difference was only statistically significant among  
328 exposed humans compared to non-exposed controls.

329

330 **Figure 2. Alpha diversity.** Alpha diversity was significantly lower among human exposed (HE)  
331 relative to human controls (HC) based on the observed features ( $p=0.04$ ), Shannon's index  
332 ( $p=0.02$ ), and Simpson's index ( $p=0.04$ ). While there was a trend toward lower alpha diversity in  
333 exposed macaques, this was not statistically significant.

334

335 PCoA plots for human and macaque gut microbial communities are shown in Fig. 3.  
336 Dispersion using the betadisper test was significant for macaques ( $p=0.03$ ), but not humans  
337 ( $p=0.66$ ). Findings among humans were unchanged after excluding people who reported taking  
338 antibiotics in the past month (2 participants from control and 2 from exposed). Adonis [22] was  
339 used to test for location shift of the spatial median based on exposure status, and this statistic was  
340 significant for macaques and humans ( $p=0.04$  and  $0.02$ , respectively). Dispersion and location  
341 tests were also performed for unweighted UniFrac, Bray-Curtis, and Jaccard distance measures,  
342 with the same conclusions.

343

344 **Figure 3. PCoA plot on weighted UniFrac distances.** 2D PCoA plot based on weighted  
345 UniFrac distances demonstrate clustering and dispersion patterns for exposed human/macaque  
346 and control human/macaque samples.

347

### 348 **SourceTracker analysis**

349 SourceTracker analysis (Fig. 4) revealed a higher percentage of microbes potentially  
350 sourced by macaque microbiota in the exposed human samples (mean=3.37%) compared to the  
351 controls (mean=1.84%). However, this difference was not significant by Mann-Whitney test  
352 ( $p=0.95$ ) and was driven by higher proportions for one individual. Similarly, the reverse analysis,  
353 with humans as the source and macaques as the sink, showed a difference in proportions  
354 attributed to human samples for exposed and control macaques (mean=4.21% and 3.98%,  
355 respectively) that was not significant ( $p=0.83$ ).

356

357 **Figure 4. SourceTracker analysis.** (A) Percent of each human sample attributed to macaque  
358 exposed or macaque control source, remainder is an unknown source. (B) Percent of each  
359 macaque sample attributed to human exposed or human control source, remainder is an unknown  
360 source.

361

## 362 **Discussion**

363 Our study of gut microbiota in humans and macaques in close contact found that the  
364 degree of sharing between was not statistically significant. The gut microbiota of the exposed  
365 workers was significantly different from the control humans, although demographic differences  
366 could explain the shift. Exposed macaques in close contact with humans, compared to a less  
367 exposed population, exhibited beta-diversity dispersion effects that may reflect a dysbiotic,  
368 unstable gut microbiota composition, which may be tied human contact in an urban environment.

369 SourceTracker analysis revealed no significant difference in microbial sharing between  
370 humans and macaques. However, one exposed worker had a greater proportion of their  
371 microbiota sourced from macaques than the other workers, suggesting that microbial sharing  
372 could be occurring and could depend largely on individual factors or behavior.

373 It is also worth noting that a common diet may play a role in the detected similarities,  
374 instead of or in addition to a shared environment. A study among urban Saudi and Bedouin  
375 populations compared to local baboons found that the shared environment and dietary overlap  
376 between Bedouins and local baboons resulted in more similar gut microbiome compositions  
377 relative to urban populations [25]. However, the relative importance of environment or diet was  
378 not characterized in that study. It is not clear to what degree the diet of macaques and humans  
379 overlap in our study setting. In addition to bananas, local residents and tourists bring a variety of

380 foods to the macaques. During observations conducted between Sept-Dec 2016 [7] much of the  
381 provisioning consisted of fruits and vegetables, but also included chips, breads, and other foods  
382 not traditionally found in a macaque diet. Some macaques routinely foraged in trash or  
383 consumed more atypical food than others, so individual level dietary differences should be better  
384 characterized in the future.

385         Our analysis revealed that workers exhibited a different composition of fecal microbial  
386 communities than controls, as evidenced by significantly different spatial medians. This finding  
387 may be due to a number of other exposure factors that warrant further investigation to determine  
388 the consequences of this location effect, including differences in age, SES, smoking status,  
389 delivery mode, and history of infectious diseases. Early life factors are believed to play an  
390 important role in shaping the adult microbiome, and there were differences in delivery method  
391 and infant diet between exposed and control groups. While there is a considerable difference in  
392 age, all subjects were adults, so this factor alone is not expected to greatly influence results as  
393 gut microbiota, which tends to be well-established in healthy adults. Healthy adults' gut  
394 microbiomes are usually less sensitive to perturbations than infants, whose microbiota are  
395 developing and have not reached a stable state and elderly (>75 years old), who tend to have  
396 lower total bacterial levels [26]. However, the difference age may be related to other factors  
397 (e.g., infectious disease history), which could shift their microbial composition. We also  
398 emphasize the need to exercise caution when excluding participants based on antibiotics use.  
399 Some respondents listed paracetamol or anthelmintic medication when asked about antibiotics  
400 use, or indicated they are unsure whether a drug they took was an antibiotic. Future studies  
401 should ask participants about antibiotic use by referencing specific drugs based on locally used  
402 names and example pills or obtain packaging from the medications used, if possible. A sub-

403 analysis excluding participants who reported antibiotic use did not alter the general conclusions  
404 of this study.

405         The macaques in the park have a high level of gut microbiota dispersion relative to the  
406 macaques with minimal human contact. Dispersion essentially reflects variation of microbiota  
407 composition, that is the taxa present and their abundance differs from sample to sample among  
408 exposed macaques, whereas the control macaques are composed of similar taxa at a similar  
409 abundance, and therefore cluster tightly together, with minimal dispersion. This significant  
410 dispersion pattern on exposed macaques is suggestive of the “Anna Karenina principle,” a  
411 signature of dysbiosis characterized by increased variation in profiles of individuals in a disease  
412 state [27]. This dysbiosis may be due to environmental stressors or diseases that perturb a stable  
413 state in an unpredictable manner. We cannot definitively determine whether AKP effects are  
414 occurring without longitudinal sampling, however the initial findings are suggestive of these  
415 effects. In the KFP population, this dysbiosis could be a result of increased stress and  
416 competition among macaques, an increased disease burden, or may be attributable to their  
417 atypical diet. When asked if they noticed any changes in macaque behavior, workers reported  
418 that the macaques drank more Coca-Cola and ate more chicken than they used to. While most of  
419 the provisioned food appears to be fruits and vegetables, according to author RCK, who has  
420 observed this population extensively, the more extreme dietary changes like foraging in trash,  
421 might explain the high variation in composition among macaques at KFP. The population density  
422 of the macaques in KFP also is approximately 3,670 individuals/km<sup>2</sup> which is considerably  
423 higher than found in more natural settings [7]. This likely results in elevated stress and  
424 aggression among macaques, which may ultimately facilitate pathogen spread. Since their  
425 microbiota appear to be in a dysbiotic state relative to macaques with low levels of human

426 contact, a condition that may predispose them to gut-related diseases, they might be expected to  
427 present a greater health threat to humans than wild macaques with typical gut flora [28, 29].

428         Given that AKP effects are associated with growths of opportunistic pathogens, we  
429 expect to find lower evenness among the exposed macaques. While evenness based on Shannon  
430 index, Pielou evenness, and Simpson evenness was marginally lower among exposed macaques,  
431 this difference was not statistically significant. Our small sample size limited power and  
432 increased the risk of beta error, in which a study may fail to reject the null hypothesis due to  
433 insufficient power. This is a limitation that should be addressed in any future studies. By further  
434 characterizing changes in susceptibility to pathogens related to gut dysbiosis, we can improve  
435 understanding of the consequences of human activities such as diet supplementation or habitat  
436 encroachment on wild macaque populations.

437         We also found a location shift in the spatial medians of control and exposed macaque  
438 sample. It should be noted that, since the assumption of equal group variances is violated among  
439 macaques, the test used is not technically valid, however, since the group larger sample size is  
440 the same that exhibits greater dispersion, the test is liable to be too conservative, therefore the  
441 detected shift in spatial medians likely represents a statistically significant finding [30].

442         Another limitation of the study was the choice of human controls, who differed in many  
443 aspects from the exposed human population. As a result, microbiota differences between the  
444 groups could be due to demographic differences rather than factors related to macaque contact or  
445 occupation.

446         Further research should 1) investigate temporal trends and the stability of the dysbiosis  
447 described in this study, 2) recruit well matched controls (e.g. matched age, SES, gender) in  
448 Kosum Phisai to minimize the number of confounding factors in microbiota comparisons, and 3)

449 incorporate testing for GI parasitism since many members of this community take anti-  
450 helminthic medication prophylactically and both factors have been shown to alter gut microbiota.  
451 The cross-sectional study design employed is practical as a baseline assessment that could be  
452 repeated in the future for continued, longitudinal surveillance of high risk worker populations  
453 and matched controls.

454         While the threat of acquiring an infectious disease shed through macaque feces from their  
455 work tasks appears low, we recommend that basic PPE be used, such as closed-toe shoes, to  
456 reduce the risk of acquiring environmentally transmitted parasites shed in macaque feces, which  
457 can enter through the skin. The high number of hand-to-mouth activities and work without  
458 respiratory protection represents a pathway for transmission of microbes that may be present in  
459 aerosolized macaque feces and offers insight into possible risk mitigating interventions. Due to  
460 the small sample size, we elected not to use scores from task observation videos in microbial  
461 composition analysis, instead treating all workers as exposed, however it is worth noting that the  
462 degree of exposure does indeed vary within this group. One worker noted that they experienced  
463 respiratory issues, which they attributed to the sweeping of macaque feces. Even if there were no  
464 microbial hazards from this exposure, the dust particles or endotoxin from Gram negative  
465 bacteria can cause irritation to the lungs. Therefore, future studies could assess lung function in  
466 the workers to help determine whether this workplace exposure contributes to decreased lung  
467 function or increased inflammation, respectively. Use of a mask during such tasks or misting of  
468 the ground prior to sweeping may reduce exposure to aerosolized macaque feces and protect  
469 worker health.

470         The use of a One Health approach, involving researchers from a range of disciplines,  
471 allowed us to compare the microbial status for both humans and the macaques of Kosumpee

472 Forest Park. A traditional approach concerning only the human health impacts of macaque  
473 exposure may not have yielded an overtly apparent risk. Our finding of dysbiosis in the gut flora  
474 of macaques with close human contact may indicate increased susceptibility to pathogens. While  
475 further investigation is needed to determine the implications of this finding, a microbiome-based  
476 approach considering human and animal health in parallel may provide a more complete picture  
477 of health in an ecosystem.

478

## 479 **Conclusions**

480 This study draws on a One Health approach to reduce human-animal conflict in a setting  
481 modified by habitat encroachment and fragmentation. Characterizing shifts in gut microbial  
482 communities allows for improved understanding of whether health changes are occurring due to  
483 increased human-macaque contact in a shared environment. Based on our analysis, workers' gut  
484 microbiota may under certain circumstances be influenced by their exposure to macaques, but  
485 this would need to be confirmed in larger studies. The dispersion effects seen in macaques of  
486 Kosumpee Forest Park suggest that their altered diet and/or interaction with an urban  
487 environment may contribute to gut dysbiosis with unknown health consequences. Given the risk  
488 of transmission or respiratory irritation from ingesting or inhaling fecal microbes, exposed  
489 individuals and their employers should consider greater use of basic PPE and infection  
490 prevention methods, particularly proper hand hygiene. Targeted health protection and disease  
491 awareness promotion among forest park workers could limit opportunities for disease spillover  
492 from macaque populations into the broader community.

493



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500

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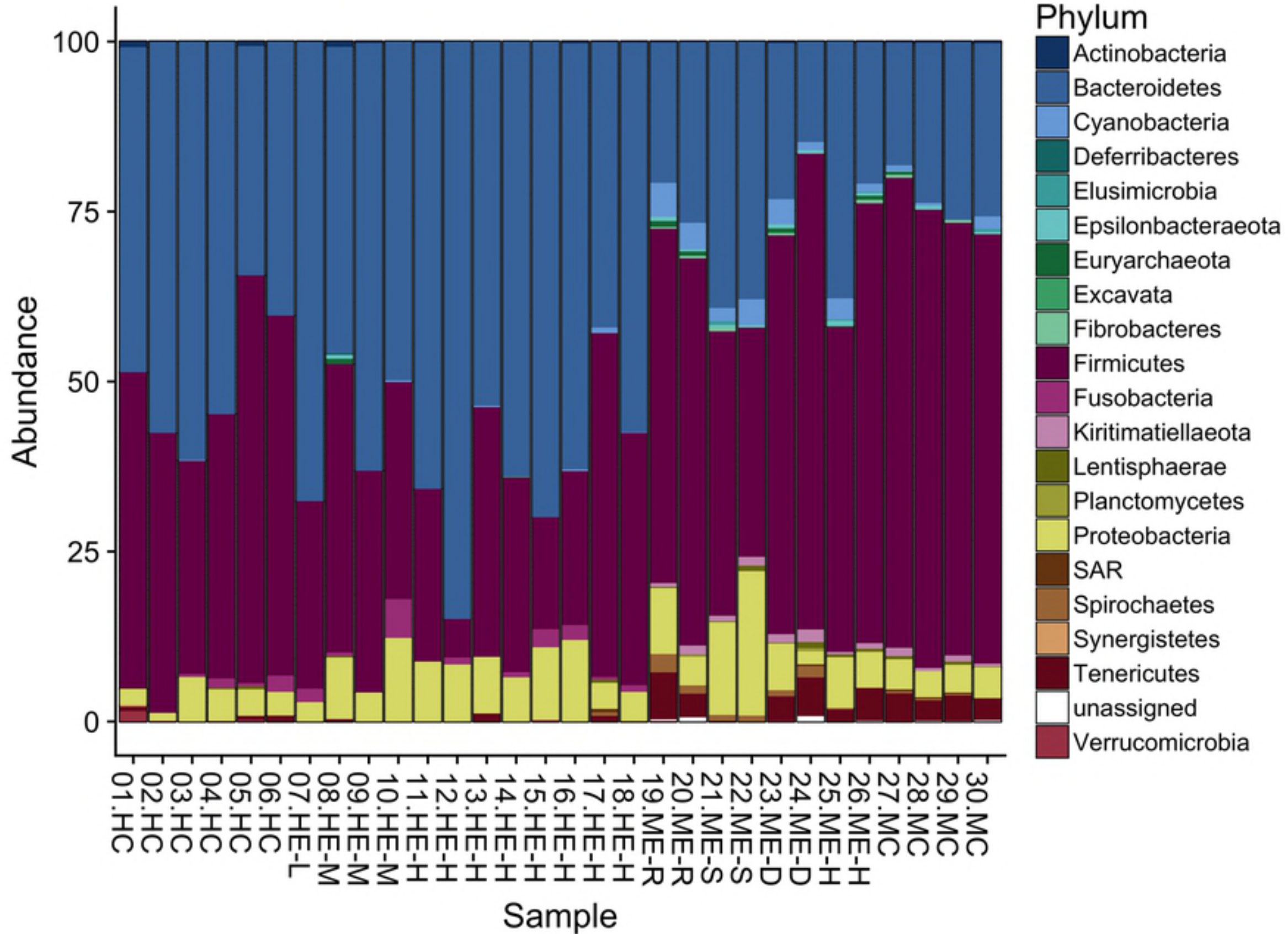
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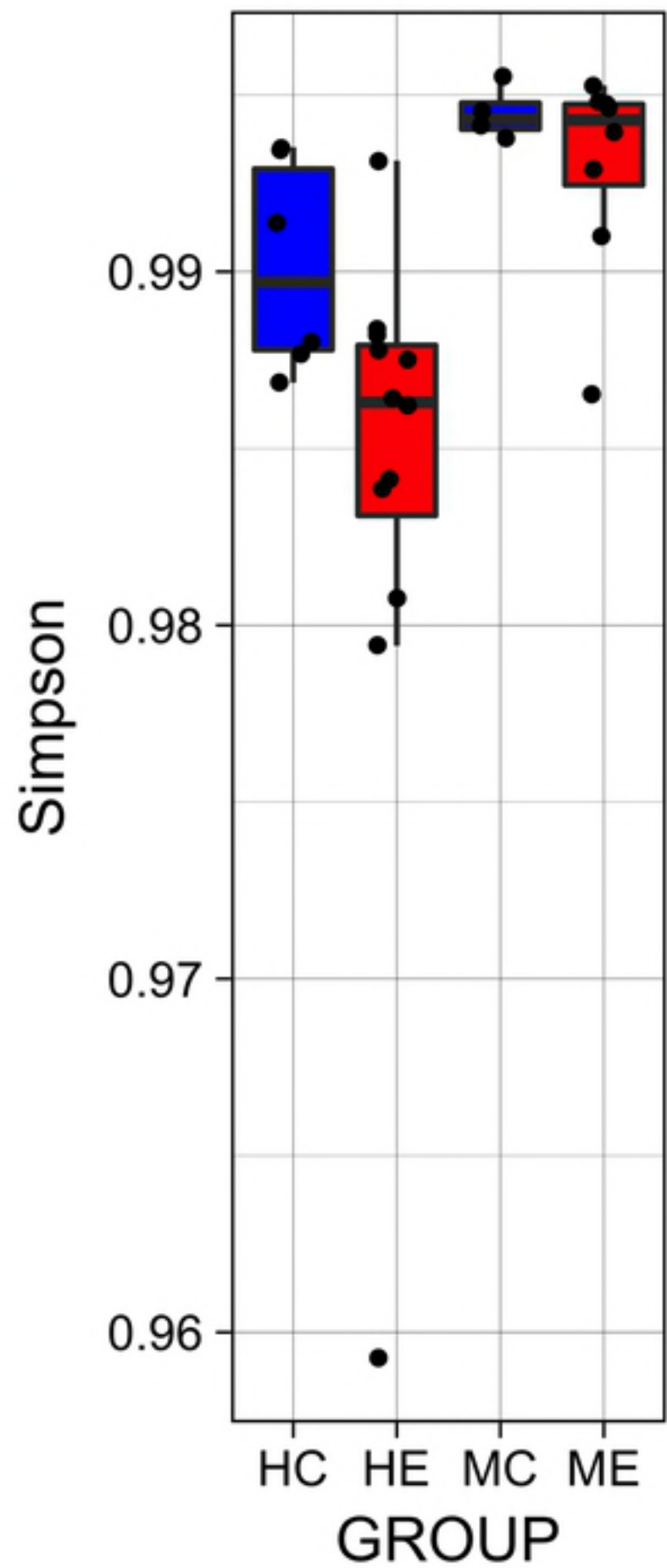
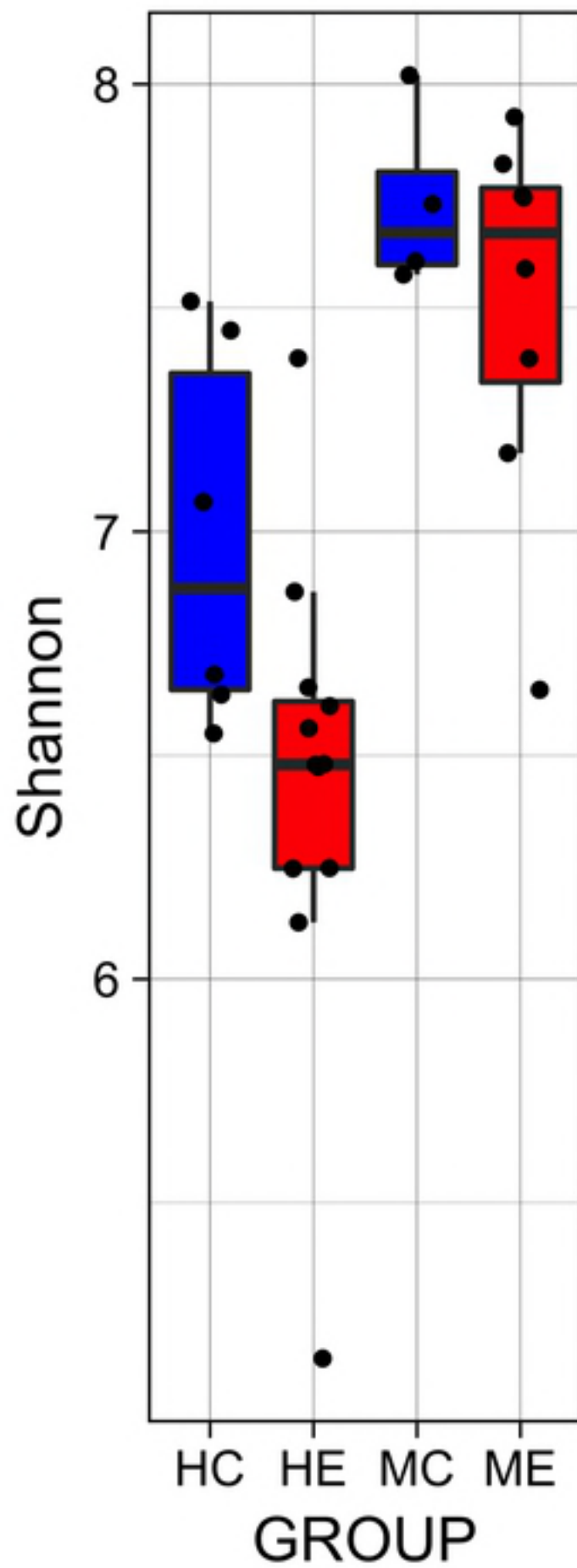
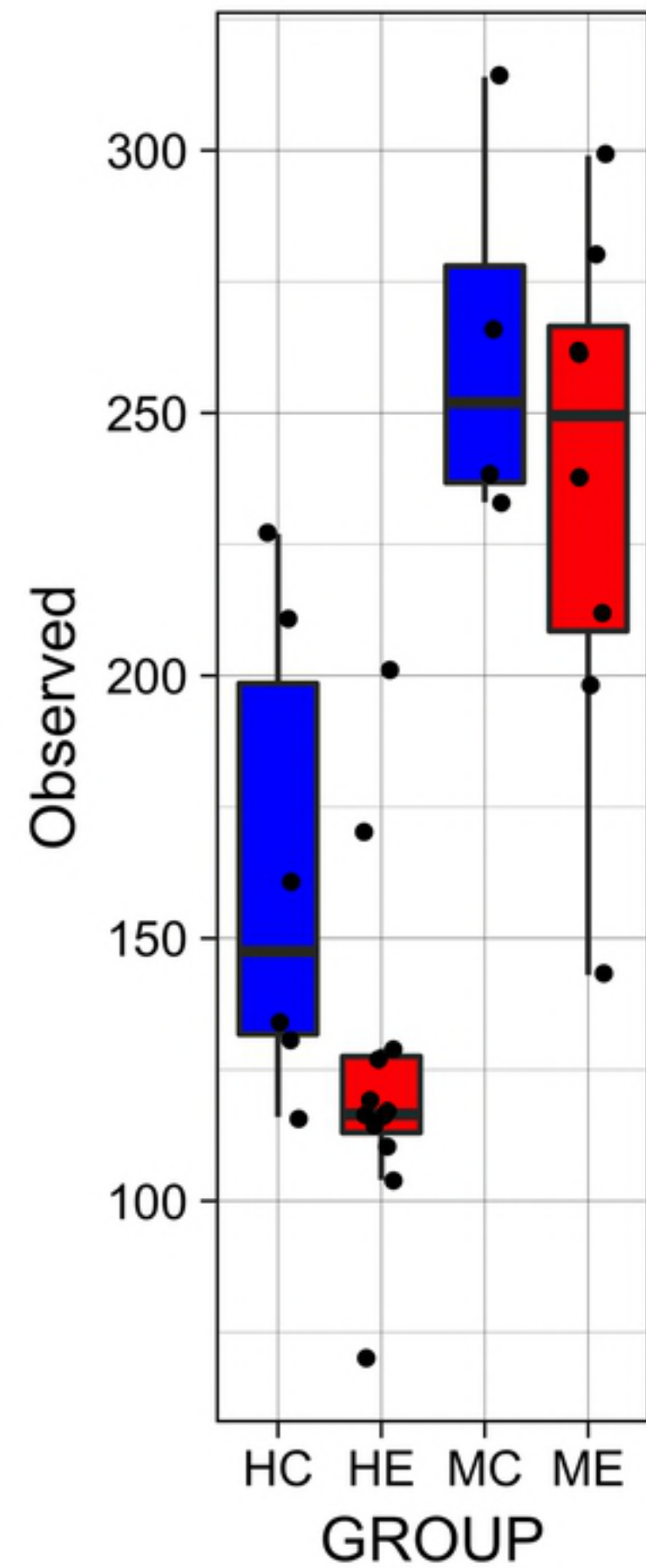
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591

## 592 **Supporting information**

593 **S1 File. Occupational exposure to macaques survey.** Questionnaire administered to workers to  
594 assess demographic, life history, diet, and general health, in addition to knowledge, attitudes and  
595 practices surrounding macaque exposure and zoonoses. Control surveys contained demographic,  
596 life history, diet, and general health sections only.





# PCoA on Weighted UniFrac Distance

