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1 **Gut Microbiota and Parasite Dynamics in an Amazonian Community Undergoing**
2 **Urbanization in Colombia**
3
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Abstract

Studies on human gut microbiota have recently highlighted a significant decline in bacterial diversity associated with urbanization, driven by shifts toward processed diets, increased antibiotic usage, and improved sanitation practices. This phenomenon has been largely overlooked in the Colombian Amazon, despite rapid urbanization in the region. In this study, we investigate the composition of gut bacterial microbiota and intestinal protozoa and soil-transmitted helminths (STHs) in both urban and rural areas of Leticia, which is located in the southern Colombian Amazon. Despite their geographic proximity, the urban population is predominantly non-indigenous, while indigenous communities mostly inhabit the rural area, resulting in notable lifestyle differences between the two settings. Our analyses reveal a reduction in bacterial families linked to non-processed diets, such as *Lachnospiraceae, Spirochaetaceae*, and *Succinivibrionaceae*, in the urban environment compared to their rural counterparts. Interestingly, *Prevotellaceae*, typically associated with non-processed food consumption, shows a significantly higher abundance in urban Leticia. STH infections were primarily detected in rural Leticia, while intestinal protozoa were ubiquitous in both rural and urban areas. Both types of parasites were associated with higher gut bacterial richness and diversity. Additionally, microbial metabolic prediction analysis indicated differences in pathways related to unsaturated fatty acid production and aerobic respiration between rural and urban bacterial microbiomes. This finding suggests a tendency towards dysbiosis in the urban microbiota, possibly increasing susceptibility to non-communicable chronic diseases. These findings provide new insights into the impact of urbanization on gut microbiota dynamics in the Amazonian context and underscore the need for further research to elucidate any associated health outcomes.

Relevance

Changes in the diversity and composition of gut microbiota in urban populations have been linked to the rise of non-communicable chronic diseases, such as autoimmune conditions, diabetes, and cancer. As developing countries undergo a demographic shift towards increased urbanization, accompanied by changes in diet, housing, and medication use, there is a concerning loss of microbial diversity. Therefore, it is essential to investigate microbiota changes in overlooked populations, such as indigenous communities in the Colombian Amazon basin. A better understanding of local and generalizable changes in gut microbial composition through urbanization may facilitate the development of targeted programs aimed at promoting lifestyle and diet changes, to prevent diseases that healthcare systems may be ill-equipped to effectively address.

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Introduction

The taxonomic composition of the human gut microbiota is dynamic and shaped by factors such as diet, medication, sanitation, and occupational exposures [1]. Surveys on microbiota composition during population rural-to-urban transition have consistently observed a reduction in microbial diversity, particularly of bacterial groups associated with traditional lifestyles. These are known as VANISH (volatile and/or negatively associated with industrialized human societies) taxa. Examples include *Prevotellaceae, Spirochaetaceae*, and *Succinivibrionaceae [2–5]*, whose decline is attributed to dietary shifts towards processed foods, widespread antibiotic usage, and decreased physical and outdoor activity. This is accompanied by a corresponding expansion of BloSSUM (bloom or selected in societies of urbanization/modernization) taxa, such as *Bacteroidaceae* and *Verrucomicrobia*, which are linked to an increased incidence of chronic disease [6,7]. These trends are expected to increase with the global rise of urbanization, with over half of the world's population currently residing in urban areas [8].

Gut bacteria also interact with commensal and parasitic eukaryotes in the human host, including protists and soil-transmitted helminths (STHs) [9,10]. These single-celled and multicellular organisms interact with the bacterial community and are associated with increased richness and diversity, or changes in the abundance of specific bacterial taxa [11–17]. For example, abundance of the *Prevotella* genus of gut bacteria is positively associated with *Blastocystis spp*. and *Endolimax nana* protozoa [12], but negatively associated with the presence of *Entamoeba* [18]. These interactions can also be clinically relevant, such as the negative correlation between the *Megasphaera* genus of bacteria and diarrheal symptoms during *Cryptosporidium* infection [19]. Helminth parasites also alter host-microbiota interactions [20], with several well-known immunomodulatory effects of helminth infection requiring host microbiota [21,22]. Infection with *Trichuris muris* and *Heligmosomoides polygyrus* roundworms inhibits proinflammatory bacterial taxa while promoting colonization with protective *Clostridiales* species, and this relationship can be reversed with deworming [23]. The relative abundance of *Clostridiales* also changes in individuals infected with hookworm (*Necator americanus*) following anthelmintic treatment [24]. Both protozoa and helminth parasites also stimulate intestinal tuft cells [25,26]. These cells promote type II immunity and alter intestinal microbiota composition [27,28] while also secreting signals that directly modulate parasite biology [29].

Demographic and cultural change in the Amazonian region is characterized by an urban expansion of existing riverside towns and the establishment of new peri-urban settlements driven by agriculture, extractive industries, and infrastructure development [30]. However, gut microbiota studies have predominantly focused on rural horticulturists and hunter-gatherers, with limited attention given to urban populations [31–36]. Leticia, situated in the southern Colombian Amazon, serves as the capital city of the Departamento del Amazonas and forms an urban complex straddling the borders of Brazil, Colombia, and Peru. The city's population is estimated at 100,000 inhabitants, with distinct demographic characteristics observed between urban and peri-urban rural areas [37,38]. Mixed-race populations predominantly inhabit the urban area, while indigenous groups primarily populate rural communities settled in response to extractive booms (e.g. rubber and coca) and assimilation efforts such as missionary campaigns [39].

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107 This study provides novel insights into the urban and rural gut microbiota and parasite
108 communities of a Colombian Amazonian population. We aim to understand how social
109 factors, medical history, and current infection with parasitic protozoa and STHs interact with
110 the bacterial microbiota composition. Additionally, using metabolic pathways predictions, we
111 explore the potential health implications of the bacterial taxonomic differences between
112 urban and rural areas.
113
114 **Materials and methods**
115
116 Ethics and Human Subjects
117 All work conducted in this study received approval from the Research Ethics Committee of
118 Universidad Nacional de Colombia (protocol number CEMED-060-19), the University of
119 Wisconsin-Madison Health Sciences Institutional Review Board (protocol number
120 2020-0214), and local indigenous community leaders. Healthy volunteers were recruited in
121 March 2021 from two field sites: (a) the peri-urban rural multi-ethnic indigenous community
122 of Nimaira Naimeki Ibiri Kilómetro 11 (referred to as Km11) (n = 80); and (b) within the urban
123 city limits of Leticia (referred to as Leticia) (n = 20). Colombian Army and Air Force support
124 facilitated operations at the Km11 field site. Before enrollment, consent was obtained from all
125 participants. For individuals under 18 years old, formal written consent was provided by their
126 parents or legal guardians. Before sampling, each participant completed a survey
127 addressing socioeconomic and health status.
128
129 Donor sampling
130 Enrolled participants were provided with a specialized kit for fecal specimen collection, along
131 with verbal instructions for proper sample acquisition. Upon collection, fecal specimens were
132 promptly stored in pack ice for transportation to the State Public Health Laboratory. Aliquots
133 of 1 g of the specimens were preserved in 2 ml of DNA/RNA Shield (Zymo Research) and
134 stored at -80°C. Preserved specimens were transferred to the One Health Genomic
135 Laboratory (OHGL) at the Universidad Nacional Sede Medellín and shipped to the University
136 of Wisconsin-Madison for DNA extraction and sequencing. In addition to fecal specimens,
137 serum samples and nasal swabs were collected for SARS-CoV-2 testing. Recent infections
138 were assessed using the Abbott Architect SARS-CoV-2 IgG antibody assay in serum (Abbott
139 Park, IL). Active infections were determined through genomic DNA extraction from nasal
140 swabs using Gene E RT-PCR, as previously described [40].
141
142 DNA Extraction
143 DNA extraction from fecal specimens was conducted using the QIAamp PowerFecal Pro
144 DNA kit (Qiagen). The extraction process followed the manufacturer's protocol, with a
145 modification at Step 1, where the input material consisted of 50 μl of fecal slurry in DNA/RNA
146 Shield, 500 μl of CD1 buffer, and 300 μl of ATL buffer (Qiagen; not included in the kit). Bead
147 beating was performed using a TissueLyser II (Qiagen) for two cycles, each lasting 5
148 minutes at 25 Hz. Between cycles, adaptors containing the specimens were repositioned so
149 that samples that were closer to the machine body were further away in the second cycle.
150 Finally, samples were eluted in a final volume of 50 μl RNase-free water and stored at -80°C.
151
152 16s rRNA metabarcoding sequencing and analysis
153 Qiagen Genomic Services conducted 16S rRNA microbiome profiling using the QIAseq
154 16S/ITS Screening Panel for library preparation. First, starting with 1 ng of DNA, target

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155 regions were selected and amplified through 12 cycles of PCR. Samples underwent cleanup
156 using QIAseq Beads (Qiagen), followed by the addition of sequencing adapters and
157 enrichment in a second PCR of 12 cycles. After a second bead cleanup, the libraries
158 underwent quality control assessment using capillary electrophoresis (Tape D1000).
159 High-quality libraries were then pooled in equimolar concentrations, determined by the
160 Bioanalyzer automated electrophoresis system (Agilent Technologies). The library pool(s)
161 were quantified using qPCR, and the optimal concentration was used to generate clusters on
162 the surface of a flow cell before sequencing on a MiSeq (Illumina Inc.) instrument (2x276).
163 Sequencing data were deposited in the NCBI SRA database (bioproject accession number
164 PRJNA1246579).
165
166 Raw V4-V5 16S rRNA fragment reads were processed using a QIIME2 pipeline [41]. The
167 DADA2 plugin [42] was utilized for trimming reads, removing sequences with ambiguous
168 nucleotides and chimeras, and discarding singletons. The remaining sequences, with a
169 length of approximately 370 bp, were clustered into Operational Taxonomic Units (OTUs) at
170 a 99% identity level. Taxonomic classification was performed using the q2-feature-classifier
171 plugin with the Bayes machine-learning classifier method [43] trained with the Greengenes
172 515F/806R database v.13.8 [44] with OTUs identified as Mitochondria, Chloroplast, or
173 Archaea discarded. After removing low abundance OTUs (≤ 0.01% total sampling) and
174 samples (≤ 1000 sequences), two individuals from Km11 were discarded, resulting in a final
175 sample size of 98 individuals.
176
177 Alpha and beta diversity analyses were conducted in R in the phyloseq package [45]. To
178 assess the overall influence of donor location, a permutational multivariate analysis of
179 variance (PERMANOVA) with 999 permutations was performed on weighted UniFrac
180 distances using the vegan package [46]. To identify microbial composition differences
181 between the locations, two approaches were employed: (a) identify OTUs with differential
182 abundance using the Wald significance test implemented in DESeq2 [47], with Km11 as the
183 treatment group and Leticia as the control (threshold cutoff, $\alpha = 0.01$); (b) evaluate the
184 differential abundance of selected BloSSUM and VANISH taxa by using Wilcoxon paired
185 tests. For BloSSUM taxa, *Bacteroidaceae*, *Verrucomicrobiaceae*, and *Rikenellaceae*, were
186 chosen. For for the VANISH taxa, *Prevotellaceae* plus *Paraprevotellaceae* (referred as
187 *Prevotellaceae*), *Succinivibrionaceae*, *Spirochaetaceae*, and the clostridiales
188 *Lachnospiraceae* with *Ruminococcaceae* as one group (referred as *Lachnospiraceae*) were
189 chosen. For microbiota functional analysis, PICRUSt 2.0 [48] was utilized to predict
190 biological pathways. Results were classified with the MetaCyc database [49] and differential
191 abundances between Km11 and Leticia microbial pathways were evaluated using a Wald
192 significance test within the ggpicrust2 package [50].
193
194 To describe the Leticia bacterial microbiota structure in a regional context, we performed a
195 comparative analysis of our dataset against previously reported Amazonian and
196 non-Amazonian Colombian microbiotas. We included datasets that meet two criteria: used
197 the V4 region of the 16S rRNA sequence and have reads with >100 bp in length. The final
198 analysis included nine datasets, divided into six Amazonian populations: (a) the urban and
199 rural Leticia sampling as one group; (b) the urban Belém and rural indigenous (c) Suruí, (d)
200 Tupaiú, and (e) Xikrin [4] communities in Brazil; and the (f) rural indigenous Tsimané
201 community in Bolivia [34]; and three non-Amazonian datasets, two urban Colombian (g)
202 Bogotá and (h) Medellín) [45]; and (i) an urban American cohort from Ohio, subsampled from

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203 the American Gut Project [46] **(Table S1)**. Given that some datasets only sampled adult
204 donors, infant samples (<15 years old) were removed before the analysis. Raw 16S rRNA
205 reads were trimmed to 130 bp and preprocessed using the QIIME2 pipeline. Comparative
206 analysis of alpha and beta diversity and selected BIoSSUM and VANISH taxon abundance
207 were performed in R.
208
209 <u>18s rRNA metabarcoding sequencing and analysis</u>
210 For the same DNA samples used for 16s rRNA analysis, we carried out eukaryotic analysis
211 using the VESPA (Vertebrate Eukaryotic Endo-Symbiont and Parasite Analysis)
212 metabarcoding protocol [51] targeting the 18S rRNA gene V4 region. Library pools were
213 sequenced using MiSeq (Illumina Inc.) instrument with a 300×300 cycle chemistry.
214 Sequencing data were deposited in the NCBI SRA database (bioproject accession number
215 PRJNA1246579). Raw reads were processed using a QIIME2 pipeline with OTUs at a 99%
216 identity level classified using the PR2 reference sequence database v.5.0 [52]. OTUs with
217 unassigned or incomplete taxonomy using the PR2 database were manually classified using
218 the full NCBI nucleotide database. Three Km11 samples with ≤ 1000 sequences were
219 discarded from the final dataset. PERMANOVA tests were used to evaluate the influence of
220 parasitic protists and nematodes in bacterial community structure.
221
222 **Results**
223
224 We evaluate the impact of urbanization on local microbiota structure in the gut microbiota of
225 Leticia by comparing the peri-urban rural indigenous community of Kilometro 11 (Km11) with
226 the non-indigenous urban population (Leticia). Participants completed surveys that provided
227 insights into the conditions of the community (**Table 1**). The cohort showed a representative
228 sex distribution (Leticia, 60% female, 40% male; Km11, 58% female, 42% male) with donors
229 ranging from 5 to 82 years (Leticia, median age 36.5 years old; Km11 38.0 years old). As
230 expected, ethnic identification varied significantly by location, with 95% of the Km11
231 community identifying as indigenous (primarily Wuitoto, Ticuna, and Murui). In contrast, only
232 one donor from Leticia identified as indigenous, with most self-describing as *mestizos*
233 (mixed-race). Most Km11 participants typically spend the day around their residence with
234 high contact with livestock (82%), raising and sacrificing mostly poultry (chickens and
235 ducks). Conversely, companion animal contact showed no significant difference between
236 sites. Finally, over 90% of the donors stored water at home for domestic use.
237
238 Both groups reported low frequencies of historical diagnoses of vector-borne and chronic
239 diseases (18-35%, with cardiovascular disease and hypertension being the most prevalent).
240 Over 50% of donors at both locations had recent SARS-CoV-2 infection indicated by an IgG
241 antibody assay, and less than 10% had an active positive infection based on RT-PCR (**Table**
242 **1**) although they were asymptomatic at the time of sampling. Medication use reported during
243 the preceding month was high, particularly in Km11, especially for analgesics like
244 paracetamol and ibuprofen.
245
246 To describe the bacterial microbiota compositions of the two groups, we sequenced the
247 V4-V5 16S rRNA region. After preprocessing the reads, our final metabarcoding dataset
248 comprised an average of 28,798 ± 15,564 sequences per sample, distributed across 466
249 OTUs. Most samples exhibited good taxonomic coverage, reaching the maximum number of
250 OTUs with a subsampling of 10,000 sequences **(Fig. S1)**. Both richness (number of OTUs, p

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251 value = 0.0449) and diversity (Shannon index, p value = 0.001179) were statistically
252 significantly reduced in the Leticia group **(Fig. 1A).** At the family level, *Prevotellaceae* was
253 the most abundant group in the sampling, with >38% of the overall abundance for both
254 locations **(Fig. 1B)**, followed by *Ruminococcaceae* (~15%). We evaluated the influence of
255 the surveyed variables on microbiota structure using a PERMANOVA analysis. The results
256 revealed a statistically significant influence of location (F = 7.904, R^2 = 0.08; p = 0.002), with
257 the Leticia samples clustering in the PCA plot (**Fig. 1C**). Other variables also differed
258 between locations, such as ethnic identification, livestock contact, mosquito net use,
259 healthcare coverage, and educational level, and these were significantly associated with the
260 gut microbiota community structure (**Table 1**).
261
262 Next, to identify the taxa driving differences between Leticia and Km11, we analyzed
263 bacterial differential abundance using two approaches. For the Wald significance test, 135
264 out of 466 total OTUs showed a statistically significant log fold change (**Table S2**), with
265 increased abundance in Km11 compared to Leticia (**Fig. 2A**). Most of these OTUs (55%)
266 belong to the clostridial families *Ruminococcaceae* and *Lachnospiraceae*, and 22% were
267 absent in Leticia samples (**Table S2**). The selected family abundance analysis (**Fig. 2B**)
268 showed significant reductions of VANISH taxa in Leticia, with *Spirochaetaceae* locally extinct
269 and only *Prevotellaceae* increased in the urban setting. No significant difference was
270 observed for BloSSUM groups, being in low abundance for most donors.
271
272 In order to infer physiological implications of these bacterial microbial repertoires, we
273 performed a predictive functional analysis finding a total of 331 predicted metabolic
274 pathways. Using a Wald significance test, we identified 30 pathways with significant
275 differences in relative abundance between the two locations (**Fig. 3** and **Table S3**).
276 Compared to Leticia, 14 pathways were increased in Km11, with half of these belonging to
277 fatty acid and lipid biosynthesis pathways. A total of 16 pathways were increased in Leticia,
278 including pathways associated with aerobic respiration like cofactors biosynthesis pathways.
279
280 To evaluate the bacterial community compositions in a regional context, we performed a
281 comparative gut bacteria microbiota analysis comprising nine datasets, including six
282 Amazonian datasets (two urban groups including our sampling, and four rural groups) and
283 three urban non-Amazonian datasets, for a total of 489 samples (**Table S1; Fig. S2**). For the
284 alpha diversity (**Fig. 4A**), rural Amazonian groups showed higher richness than urban
285 communities, where Leticia has similar values to the other Colombian urban microbiotas. We
286 performed a PERMANOVA analysis to evaluate how much the sampled location can explain
287 the microbiota structure (F = 37.94, R^2 = 38.09; p = 0.001). This was visualized in the PCA,
288 where Ohio samples formed a separated cluster from three South American subgroups,
289 Colombian urban Medellín-Bogotá, one transitional group for the Amazonian rural Tsimané,
290 and the Leticia samples indistinct from Brazilian rural and urban populations (**Fig. 4B**). The
291 clustering of Leticia and Brazilian samples is explained in the selected family analysis (**Fig.**
292 **4C**), where VANISH taxa *Prevotellaceae* and *Succinivibrionaceae* are increased compared
293 to the other populations, while BloSSUM groups dominated in the urban populations
294 (*Bacteroidaceae* and *Rikenellaceae* in Ohio, and *Verrucomicrobiacea* in Bogotá and
295 Medellín) are reduced. Belém, the other Amazonian city evaluated, is distinguished from
296 Leticia for the higher abundance of *Bacteroidaceae,* even with some samples clustering with
297 the Ohio samples.
298

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299 To describe the parasitic protists and nematodes associated with the Leticia gut microbiota,
300 we performed an 18s rRNA analysis using the VESPA (Vertebrate Eukaryotic endoSymbiont
301 and Parasite Analysis) protocol for eukaryotic endosymbiont metabarcoding [51]. Amplicons
302 were generated targeting the 18S rRNA gene V4 region, sequenced, and recovered reads
303 were classified into seven taxonomic categories using the PR2 reference sequence
304 database. Parasitic protist sequences were the most common group, followed by human
305 host. Parasitic nematodes were recovered in a low abundance (<1%) (**Fig. 5A**). Most
306 individuals in both locations were infected with two or more parasitic protist taxa (**Fig. 5B**).
307 Nematode infections were mostly detected in rural Km11 (**Fig. 5B**). Only two nematode taxa,
308 *Enterobius* and *Necator*, were detected in urban Leticia, while seven genera were found in
309 Km11 at rates ranging from 1 to 17% (**Fig. 5C**). Five protist taxa were detected in both
310 locations, with *Blastocytis* being the most common, detected in >85% of samples from both
311 locations (**Fig. 5C**).
312
313 Given the extensive literature indicating that parasite infection influences the host microbial
314 environment, we tested whether there was an association with protist or helminth infection
315 and either bacterial diversity and abundance. PERMANOVA tests were used to evaluate
316 alpha diversity metrics for the number of observed OTUs (richness), Shannon index
317 (diversity), and abundance of *Prevotellaceae* and *Lachnospiraceae* family of bacteria. The
318 Leticia and Km11 datasets were combined into one group, and comparisons were made
319 between positive and negative infection status with any STH taxa or infection with any of the
320 five most prevalent protists (*Blastocystis*, *Dientamoeba*, *Endolimax*, *Entamoeba*, and
321 *Enteromonas*). Parasite infection was significantly associated with increased richness of
322 bacterial tax for *Endolimax* ($F = 12.137$, $R^2 = 0.115$, $p = 0.001$) and *Entamoeba* ($F = 19.834$,
323 $R^2 = 0.176$, $p = 0.001$). Infection with the following protist and nematode parasites were
324 associated with increased diversity; *Endolimax* ($F = 12.618$, $R^2 = 0.119$, $p = 0.001$),
325 *Entamoeba* ($F = 12.625$, $R^2 = 0.119$, $p = 0.003$), and STH infection ($F = 6.919$, $R^2 = 0.069$, p
326 $= 0.007$). For the bacterial taxonomic abundance, STH infection influenced both
327 *Prevotellaceae* ($F = 6.722$, $R^2 = 0.068$, $p = 0.001$) and *Lachnospiraceae* ($F = 4.753$, $R^2 =$
328 0.048, $p = 0.018$). All the results were confirmed with Wilcoxon paired tests comparing
329 between positive and negative infection samples (**Fig. 6**).
330

331 ## Discussion

332
333 This study sheds new light on the gut bacterial microbiota of urban and rural populations in
334 the Colombian Amazonian. In urban Leticia, we found lower bacterial diversity than in Km11
335 communities, reflecting reduced abundance of families related to non-processed foods
336 (VANISH taxa), such as *Lachnospiraceae*, *Ruminococcaceae*, *Succinivibrio*, and
337 *Spirochaetaceae*. The latter includes spirochetes of the genus *Treponema*, which are
338 strongly associated with traditional rural populations of non-“Western” lifestyles [35]. Similar
339 reductions in microbiota diversity have also been reported in other locations undergoing
340 demographic and cultural transitions [53,54]. However, a noteworthy difference for Leticia is
341 the increase of *Prevotella* instead of taxa associated with processed foods diets (BloSSUM
342 taxa) in the urban setting.
343
344 Decreased microbial diversity in samples from the urban, mostly non-indigenous Leticia
345 population compared to the rural, mostly indigenous Km11 population may be explained by
346 lifestyle differences. Most of the Km11 donors practice activities such as small poultry

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347 farming for economic sustenance [55]. Outdoor activity [56] and livestock raising [57,58]
348 have been shown to enrich microbiota diversity. Also, the diet in urban Leticia has changed
349 significantly in recent decades compared to rural areas of the Amazonia, with reduced
350 consumption of traditional non-industrialized foods like fish broth, wild animals,
351 cassava-derived products like casabe and farinha, and fruit-based juices, and increased
352 consumption of products such as packaged chicken, eggs, rice, canned foods, and
353 powdered drink mixes of coffee, cocoa, or fruit [55].
354
355 Our comparative analyses show that the gut microbiota of people in and around Leticia is
356 similar to that described within the Brazilian Amazonian urban and rural communities [4,6],
357 with a high *Prevotellaceae* abundance and in general low presence of BloSSUM taxa. This
358 pattern may be explained by similarities in the diet of these riverine communities along the
359 Amazonian basin, which is still mostly composed of fish and polysaccharide-rich foods like
360 cassava [4]. This differs from the lowland forest Tsimané community in Bolivia [34], the other
361 Amazonian group evaluated, which has a diet rich in plant foraging and wild animals [59].
362 The taxonomic differences with Bogotá and Medellín, the other Colombian urban
363 microbiotas, where BloSSUM taxa are more abundant, seem to reinforce the concept of a
364 "tropical urban" category to describe microbiota of habitats of urban areas in tropical regions
365 that are in different stages of microbiota "westernization" compared to non-tropical
366 populations [4], although the different levels of urban transition, exemplified in the taxonomic
367 differences between Leticia and Belém, indicate the necessity to further sample more
368 Amazonian urban locations.
369
370 We also described the Leticia gut eukaryotic parasite community using a recently developed
371 18s rRNA metabarcoding protocol. We found a high abundance of intestinal parasites in
372 both urban and rural samples, with *Endolimax* and *Entamoeba* presence having a positive
373 effect on microbial richness and diversity. Infection with protists is already known to influence
374 microbiota structure, being associated with higher microbial richness and abundance of
375 VANISH taxa like *Prevotellaceae* and *Ruminococcaceae* [12,13]. We found that STH
376 parasites are associated with higher bacterial microbial diversity and VANISH taxa
377 abundance. A similar result, increased bacterial species diversity and *Prevotellaceae*
378 abundance, was reported in a cohort of Colombian harboring mixed STH infections [60].
379 These results indicate potential parasite-microbiome interactions that could influence human
380 health. This phenomenon has been extensively studied for both protists and helminths,
381 which have been proposed to be beneficial by protecting against allergic and metabolic
382 diseases [61–63].
383
384 Finally, predictive metabolic analysis indicates several significant results that may be
385 relevant to health outcomes of the microbiota structure in the study area. Urban samples had
386 relative depletion of fatty acid biosynthesis pathways. This finding may be explained by
387 reduced abundance of *Lachnospiraceae*, which are key producers of butyrate and other
388 short-chain fatty acids and decrease in diets high in saturated fatty acids [64]. Interestingly,
389 depletion in short chain fatty acid producers was also observed in *Trichuris* infected
390 individuals in a large study spanning Côte d'Ivoire, Laos, and Tanzania [65]. There was also
391 an increase in the aerobic respiration pathways in urban samples, which may indicate
392 increased saturated fatty acid consumption. These changes can increase the epithelial
393 oxygenation in the colon, triggering a microbiome dysbiosis, characterized by an elevated
394 abundance of facultatively aerobic bacteria compared to the healthy microbiota composition

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395 dominated by anaerobic bacteria [66]. Finally, although *Prevotellaceae* has been associated
396 with a healthy microbiota, the high prevalence found in Leticia should be carefully
397 interpreted, as this group has also been linked with inflammatory autoimmune diseases like
398 rheumatoid arthritis [67].
399
400 Differences in the taxonomic composition and predicted physiology of the urban and rural
401 Leticia microbiota documented in this study are likely associated with cultural and health
402 transitions and intestinal protozoa and STH infections. These findings have relevance to
403 public health, as such changes may underlie increases in chronic non-communicable
404 diseases in the region, highlighting the need for further investigations into microbiota
405 dynamics among urban and rural populations across the Colombian Amazon. This expanded
406 analysis will be important for enhancing our understanding of the local health transitions and
407 implementing proactive measures to improve public health outcomes and healthcare system
408 preparedness.
409
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411 Institute Pilot Grant (A.J.E.), NIH-NIAID funding R01AI151171 (M.Z.) and R21AI163592
412 (T.L.G.). S.D. was supported by a UW-Madison Global Health Institute Postdoctoral
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414 (T32AI007414).

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415 **Figure and Table Legends**

416

417 **Table 1.** Socio-economic and health parameters for Leticia and Km11 groups. PERMANOVA
418 results for each parameter with p-value indicating statically significant influence on
419 microbiota structure. [1]Housing social strata based in the Colombian official strata divisions,
420 rural and urban one to six levels. [2]Livestock contact includes raising and/or sacrificing
421 domestic animals. [3]Three samples from Km11 were excluded because of the lack of
422 biological material for testing.

423

424 **Fig 1**. Leticia gut bacteria microbiota alpha and beta diversity analysis. **(A)** Alpha diversity
425 estimators, (a) Number of OTUs (richness) and (b) Shannon Index (diversity). Difference
426 between locations evaluated using Wilcoxon Paired-tests. *p = 0.05–0.005, **p =
427 0.0049–0.0005, ***p < 0.00049. **(B)** Relative bacterial family abundance for locations.
428 Families with < 1% abundance were merged into one group. **(C)** PCA plot of bacterial
429 community structure based on the weighted UniFrac distances.

430

431 **Fig 2.** Differential abundance analysis between Leticia and Km11. **(A)** Significant OTUs
432 according to the Wald significance test. Km11 as the treatment and Leticia as the control
433 group, with an α = 0.01 threshold cutoff. **(B)** Differential abundance of VANISH and
434 BloSSUM taxa. Difference between locations evaluated using Wilcoxon paired-tests. *p =
435 0.05–0.005, **p = 0.0049–0.0005, ***p < 0.00049.

436

437 **Fig 3.** Predictive bacterial metabolic pathways analysis. **(Left)** Relative abundance of
438 pathways with differential abundance between locations. **(Right)** Logarithmic fold change
439 between pathways based on Wald Significance Test. Km11 as the treatment and Leticia as
440 the control group, with an α = 0.01 threshold cutoff.

441

442 **Fig 4.** Amazonian and non-Amazonian gut bacteria microbiota datasets alpha and beta
443 diversity comparative analysis. **(A)** Alpha diversity estimators of number of OTUs (richness)
444 and Shannon Index (diversity). Letters reflect grouping and differences between datasets
445 evaluated using Tukey HSD test. **(B)** PCA plot of bacterial community structure based on the
446 weighted UniFrac distances. **(C)** Abundance of VANISH and BloSSUM taxa across bacteria
447 microbiota datasets.

448

449 **Fig 5.** Leticia eukaryotic parasite metabarcoding description. **(A)** Relative sequence
450 abundance from the 18s rRNA metabarcoding for each taxonomic category. **(B)** Overall
451 detection rate of parasite nematodes and protists in Km11 and Leticia samples and **(C)**
452 breakdown of taxa detected.

453

454 **Fig 6.** Differential analysis between parasite positive and negative Leticia (both urban and
455 rural) samples. **(A)** Richness (observed number of OTUs) for *Endolimax* and *Entamoeba*
456 infection. **(B)** Diversity (Shannon index) for STH, *Endolimax*, and *Entamoeba* infection. **(C)**
457 *Prevotellaceae* and *Lachnospiraceae* abundance for STH infection. Difference between
458 infection status evaluated using Wilcoxon paired-tests. *p = 0.05–0.005, **p =
459 0.0049–0.0005, ***p < 0.00049.

460

461

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462 **Table S1.** Sample list for Amazonian and non-Amazonian datasets used for the comparative
463 gut bacterial microbiota analysis.
464
465 **Table S2.** Overall abundance and logarithmic fold change value for OTUs with a differential
466 abundance between Leticia and Km11 based on the Wald Significance Test.
467
468 **Table S3**. Metacyc database classification of the metabolic pathways with a differential
469 abundance between Leticia and Km11 based on the Wald Significance Test.
470
471 **Fig S1.** Rarefaction curve for Leticia and Km11 samples.
472
473 **Fig S2.** Relative bacterial family abundance for Amazonian and non-Amazonian datasets
474 with each bar summarizing the total of samples per dataset. Families with < 1% abundance
475 were merged into one group.
476
477

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478 **Table 1**

Socio-economic and health parameters	Permanova result	Parameter levels	Leticia (%)	Kilómetro 11 (%)
Sex	F = 0.56, R^2 = 0.01 , p = 0.62	Female	60	59
		Male	40	41
Age group	F = 2.03, R^2 = 0.04 , p = 0.08	1-15 yo	5	10
		16-30 yo	25	22
		31-85 yo	70	68
Ethnic Identification	F = 10.18, R^2 = 0.10, p = 1.0E-3 ***	Indigenous	5	91
		Non-indigenous	95	9
Scholarity level	F = 1.97, R^2 = 0.10 p = 0.02 *	None	0	3
		Elementary school	20	27
		High school	25	60
		Technical school	40	6
		Undergraduate education	10	3
		Graduate education	5	1
Ocupation	F = 1.12, R^2 = 0.05, p = 0.33	Unemployed	0	17
		Employee	65	14
		Independent	15	13
		Housewife	10	38
		Student	10	18
Housing social strata[1]	F = 1.58, R^2 = 0.05, p = 0.14	Rural	0	76
		Level one	20	21
		Level two	50	4
		Level three	30	0
Number of house inhabitants	F = 0.90, R^2 = 0.02, p = 0.45	One	10	5
		Two to four	35	28
		Five or more	55	67
Place where stay most of the day	F = 0.86, R^2 = 0.02, p = 0.52	Home	30	78
		Work/Chagra	70	21
		School	0	1
Livestock contact[2]	F = 8.01, R^2 = 0.08, p = 1.0E-3 ***	Yes	5	82
		No	95	18
Companion animal contact	F = 1.29 , R^2 = 0.01, p = 0.21	Yes	55	22
		No	45	78
Insecticide use	F = 1.43, R^2 = 0.08, p = 0.19	Yes	95	73
		No	5	27
Mosquito net use	F = 6.44, R^2 = 0.06, p = 2.0E-3 **	Yes	25	94
		No	75	6
Water storing at home	F = 1.01, R^2 = 0.01, p = 0.35	Yes	90	94
		No	10	6
Healthcare coverage	F = 3.37, R^2 = 0.06, p = 0.01 **	No coverage	5	3
		Subsidized coverage	15	86
		Private coverage	80	12
Hospitalization in the last year	F = 0.39, R^2 = 4.6E-3, p = 0.79	Yes	0	12
		No	100	88
Chronic diseases history	F = 1.49, R^2 = 0.01, p = 0.18	Yes	35	22
		No	65	78
Vector-borne diseases history	F = 1.12, R^2 = 0.01, p = 0.28	Yes	25	18
		No	75	82
Chemiluminescence assay IgG SARS-CoV-2[3]	F = 1.06, R^2 = 0.02, p = 0.32	Yes	55	58
		No	45	38
		Not measured	0	4
Gen E RT-PCR SARS-CoV-2	F = 0.81, R^2 = 8.4E-3, p = 0.42	Yes	5	9
		No	95	91
Medication use in the last month	F = 2.39 , R^2 = 0.02, p = 0.08	Yes	70	45
		No	30	55
Smoking history	F = 1.94, R^2 = 0.02, p = 0.14	Yes	5	6
		No	95	94

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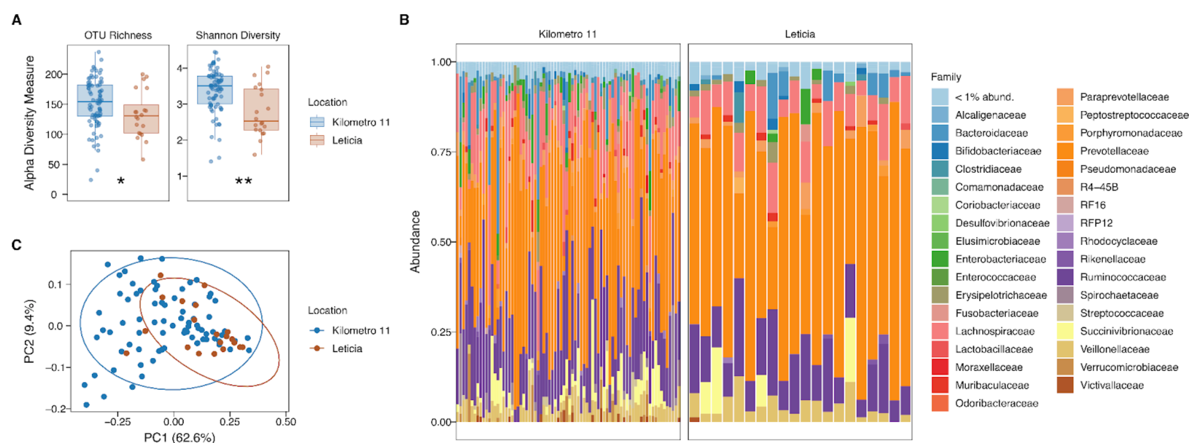
481 **Figure 1**

482



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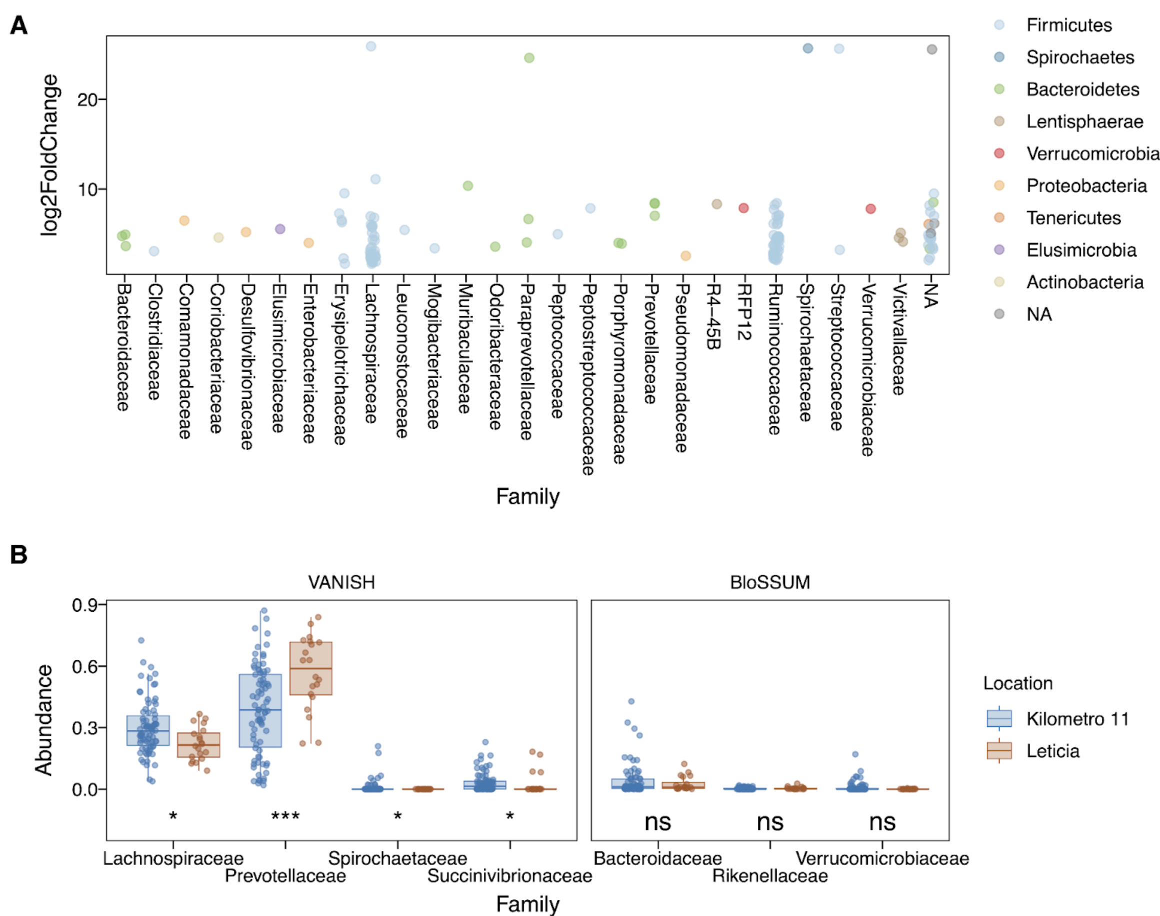
484 **Figure 2**



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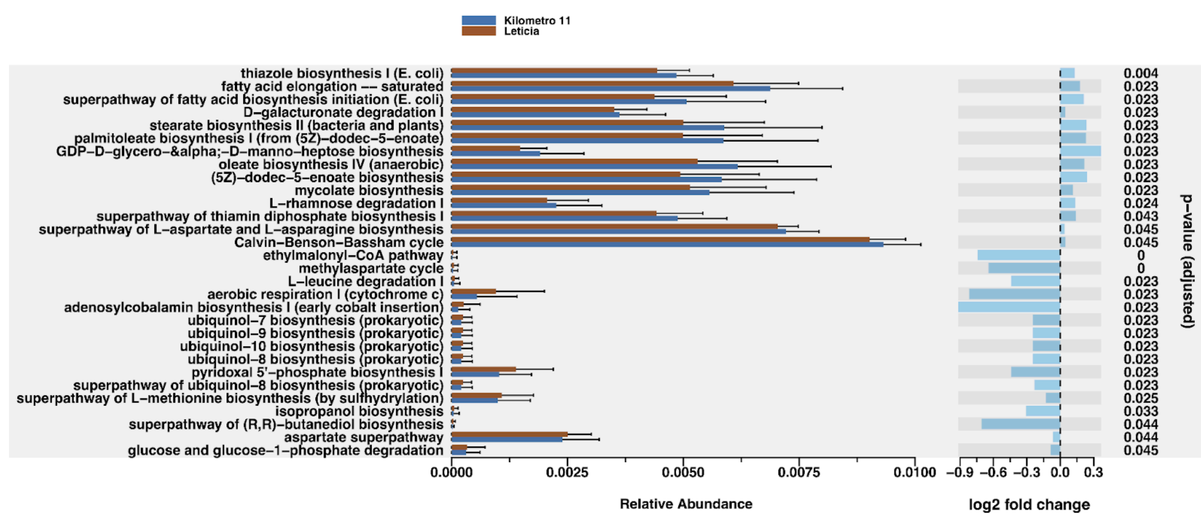
486 **Figure 3**



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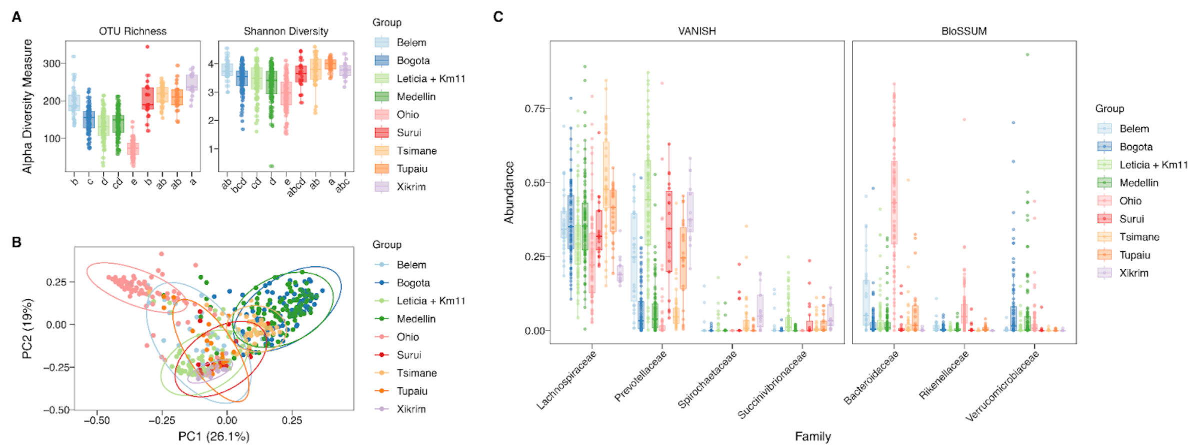
488 **Figure 4**



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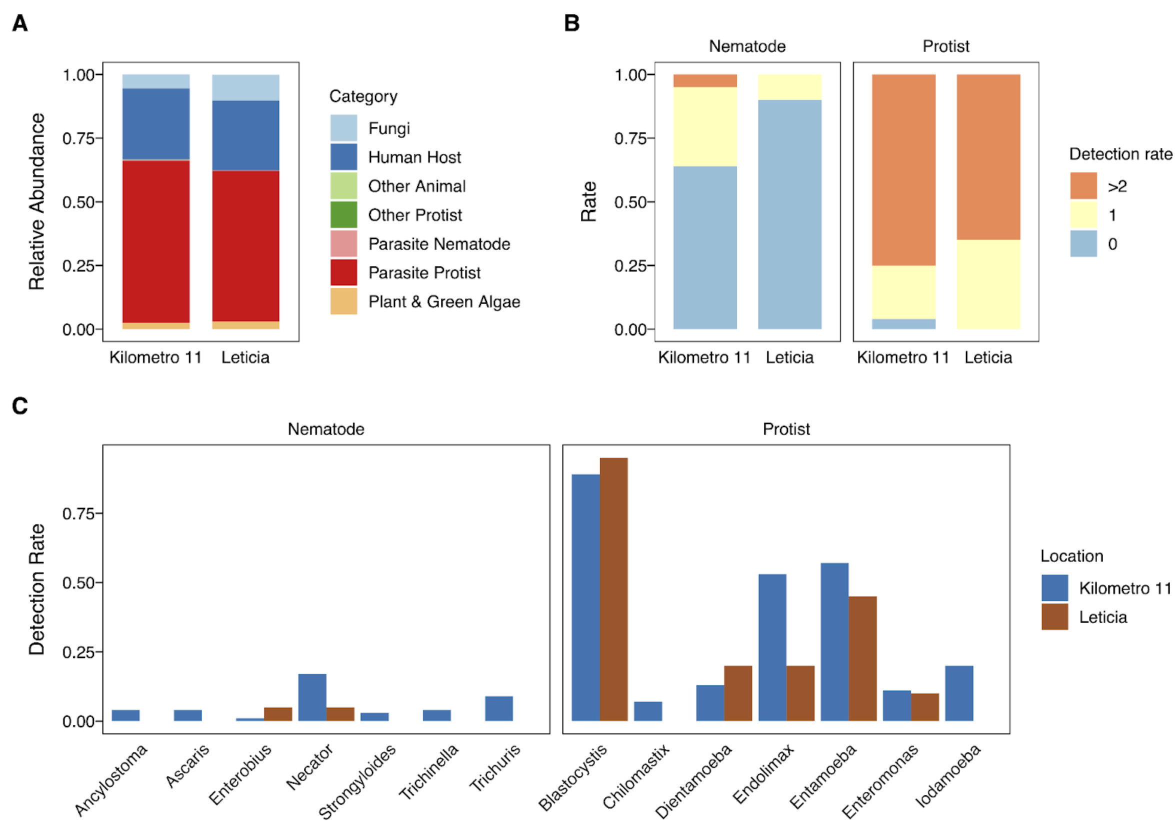
490 **Figure 5**



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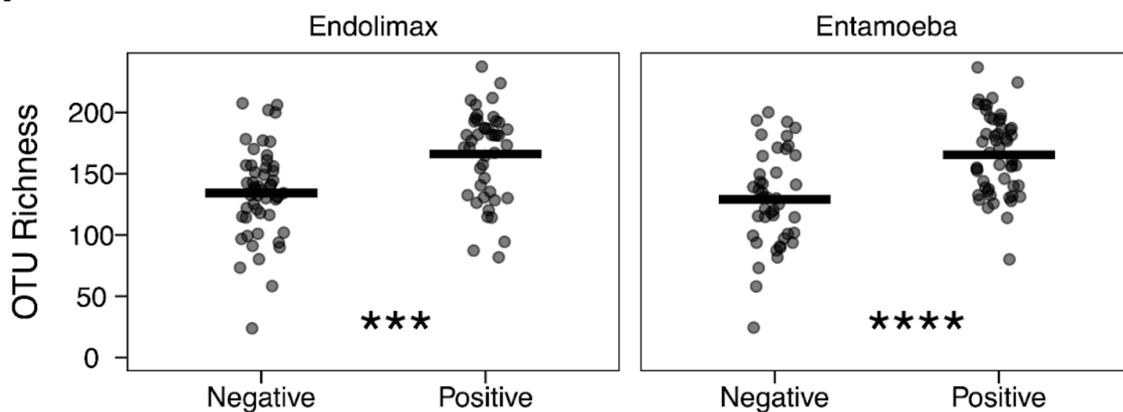
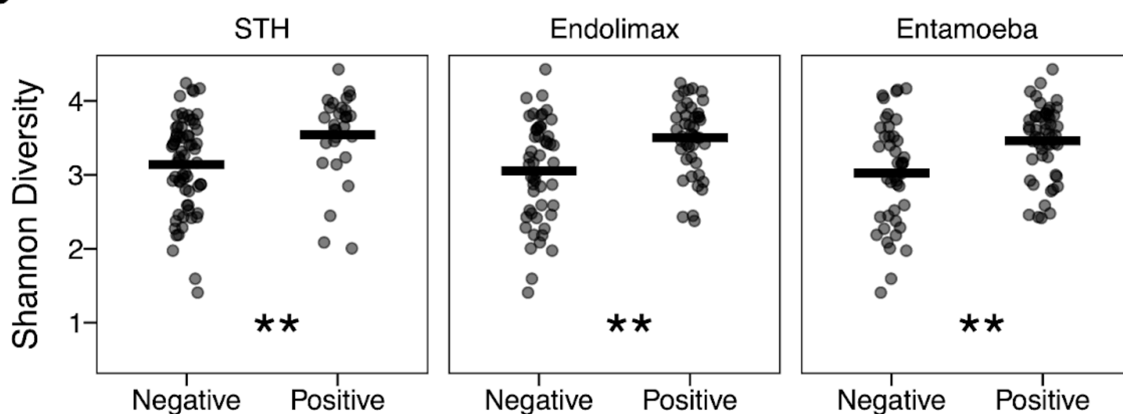
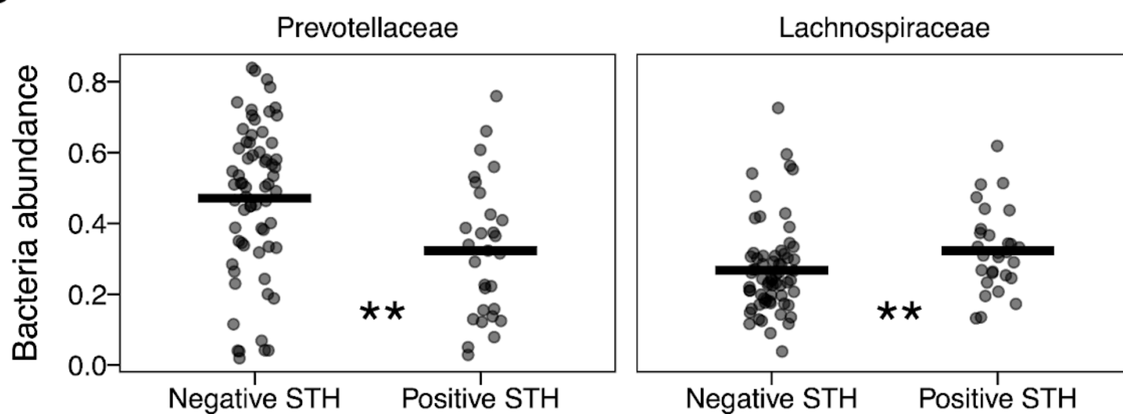
492 **Figure 6**







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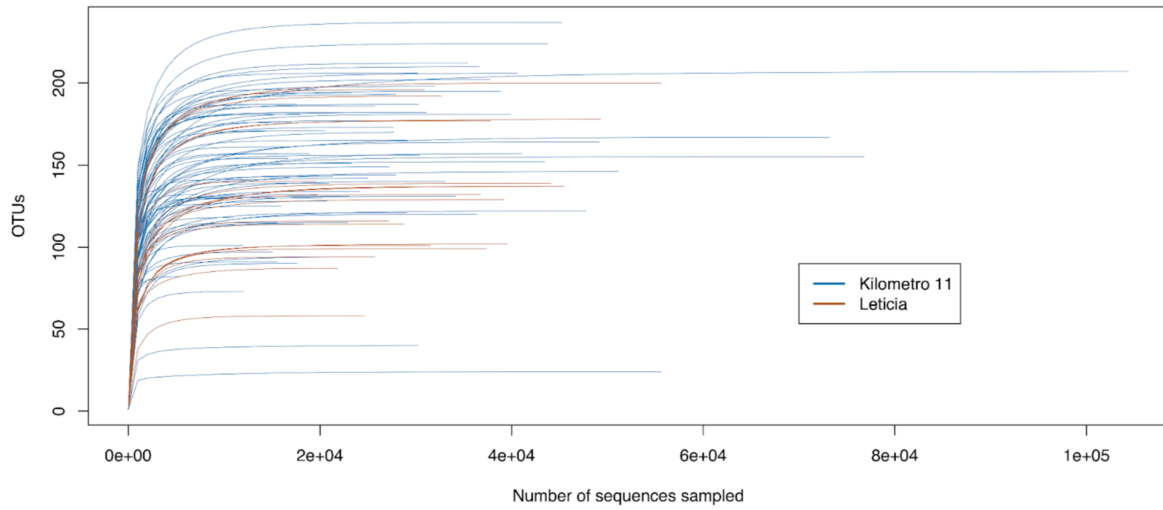
494 **Figure S1**



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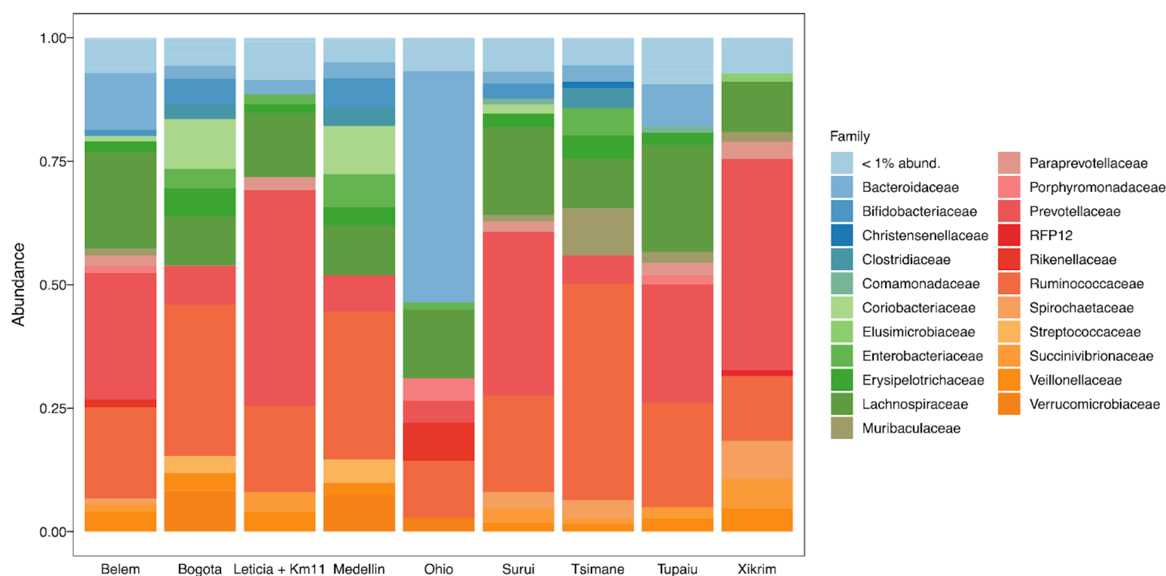
496 **Figure S2**



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