

1 Title:

2 Gut Microbiome Functional and Taxonomic Diversity within an Amazonian semi-
3 nomadic hunter-gatherer group

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23 **Abstract**

24 **Background:** Human gut microbiome profiles have been associated with
 25 human health and disease. These profiles have been defined based on
 26 microbes' taxonomy and more recently, on their functionality. Human groups
 27 that still maintain traditional modes of subsistence (hunter-gatherers and rural
 28 agriculturalists) represent the groups non-impacted by urban-industrialized
 29 lifestyles, and therefore study them provide the basis for understanding the
 30 human microbiome evolution. The Yanomami is the largest semi-nomadic
 31 hunter-gatherer group of the Americas, exploring different niches of the Amazon
 32 rainforest in Brazil and Venezuela. In order to extend the analysis of this unique
 33 and diverse group, we focused on the gut microbiome of the Yanomami from
 34 Brazil and compared with those from Venezuela, and also with other traditional
 35 groups from the Amazon, considering taxonomic and functional profiles.

36 **Results:** A diversity of taxonomic biomarkers were identified to each South
 37 American traditional group studied, including the two Yanomami groups, despite
 38 their overall similarity in the taxonomic gut microbiome profiles. Broader levels
 39 of functional categories poorly discriminated traditional and urban-industrialized
 40 groups. Interestingly, a diversity was observed with the stratification of these
 41 categories, clearly segregating those groups. The Yanomami/Brazil gut
 42 microbiome presented unique functional features, such as a higher abundance
 43 of gene families involved in regulation/cell signaling, motility/chemotaxis, and
 44 virulence, contrasting with the microbiomes from the Yanomami/Venezuela and
 45 other groups.

46 **Conclusions:** Our study revealed biomarkers, taxonomic and functional
 47 differences between the gut microbiome of Yanomami/Brazil and

48 Yanomami/Venezuela individuals. This intra-Yanomami group diversity was
 49 accessed due to the increase number of individuals and group studied. These
 50 differences may reflect their semi-nomadic behavior, as well as, the local and
 51 seasonal diversity of the vast rainforest niche they explore, despite their shared
 52 cultural and genetic background. Overall, their microbiome profiles are shared
 53 with South American and African traditional groups, probably due to their
 54 lifestyle. The unique features identified within the Yanomami highlight the bias
 55 imposed by underrepresented sampling, and factors such as variations over
 56 space and time (seasonality) that impact, mainly, the hunter-gatherers.
 57 Therefore, to reach knowledge about human microbiome variations and their
 58 implications in human health, it is essential to enlarge data concerning the
 59 number of individuals, as well as the groups representing different lifestyles.

60

61 **Keywords**

62 Gut microbiome, hunter-gatherers, semi-nomadic, Yanomami, Amerindian,
 63 westernization, biomarkers, functionality, taxonomic

64

65 **Background**

66 The transition of the traditional modes of subsistence to the current western
 67 lifestyles that occurred with the advent of modern practices (urbanization and
 68 industrialization) brought wide differences in diet and environment, factors
 69 proposed to be the main determinants of the gut microbiome composition. In
 70 fact, cross-population studies have demonstrated distinct taxonomic and
 71 functional profiles between the gut microbiome of hunter-gatherers/rural

72 agriculturalists and urban-industrialized human groups. The main differences
 73 among the gut microbiome of westernized and non-westernized groups are that
 74 individuals from traditional communities harbor a more diverse gut microbiome,
 75 with higher levels of fiber-degrading bacteria, and unique taxa that are depleted
 76 in urban-industrialized populations [1-10]. The lifestyle aspects that
 77 characterize most of the westernized groups, concerning diet, environment,
 78 sedentary practices, among others, shape the gut microbiome, defining some
 79 taxonomic profiles that have been associated with an increased risk of
 80 metabolic and chronic disorders that affect modern populations [11]. Studies
 81 focusing on the differences between traditional and urban-industrialized groups
 82 may reveal diets and bacterial/archaeal taxa that can be helpful in the
 83 development of prebiotics and probiotics for modern disorders prevention and
 84 treatment. Considering that human groups that live in a non-western lifestyle
 85 are in decline, the study of the remaining traditional groups constitutes an
 86 extraordinary opportunity to explore and unravel the human gut microbiome
 87 before modernization.

88 The Amazon region is the largest tropical wilderness area in the world,
 89 covering ~ 7 million km². This region includes the most extensive and preserved
 90 rainforest in the world (the Amazon Rainforest), vast areas of scrub-savannah
 91 that dominate the headwaters of the Brazilian and Guyana shields, as well as
 92 the Andes highlands, which are characterized by tundra-like grassy tussocks
 93 called the Puna [12]. Moreover, this region presents elevations ranging from sea
 94 level at the river's mouth to an altitude of 6,500 meters in the Andes [13].
 95 Having such high variable geomorphology, climate and vegetation cover, and
 96 harboring estimated 400-500 indigenous Amazonian Indian groups

97 (Amerindians), this region offers a unique scenario for microbiome studies.
 98 These groups live in the same geographical region but explore distinct
 99 ecological niches, present distinct dietary habits, culture, language and degrees
 100 of isolation [14, 15]. This diversity may reflect in the gut microbiome composition
 101 and functionality, expressing the adaptation to evolutionary and ecological
 102 constraints of each site inhabited, despite being non-western populations.

103 The Yanomami is the largest indigenous semi-isolated group in the Amazon
 104 to maintain a traditional system of production based on hunting, fishing,
 105 gathering, and swidden horticulture [16]. They inhabit an area of 192,000 km² in
 106 the Amazon region encompassing the Brazil and Venezuela border. Of the
 107 estimated 40,000 Yanomami, approximately 26,000 live in the 37,260 m²
 108 reserve in Brazil and another 16,000 in Venezuela. Even though they represent
 109 a single semi-nomadic ethnic group of hunter-gatherers, they speak four
 110 different languages of the same family and live in villages located at sea level
 111 as well as on high mountains in a huge area in the Amazon [17]. Their diet is
 112 low in fat and salt, and high in fruits, fiber, and sylvatic animals. Atherosclerosis
 113 and obesity are virtually unknown among semi-isolated Yanomami, having low
 114 blood pressure, with no apparent increase as they age [18].

115 A previous study with uncontacted Yanomami from Venezuela revealed
 116 some aspects from the gut microbiome of this group [4]. In order to go deeper
 117 into the characterization of gut microbiome among traditional subsistence
 118 groups, we studied semi-isolated hunter-gatherers Yanomami individuals from
 119 Brazil. For this, we generated and analyzed metagenomic data of Yanomami
 120 from Brazil, and performed comparative analyses with those from Venezuela,
 121 other traditional groups from the Amazon (the Matsigenka and the Tunapucos), as

well as an urban-industrialized group (Figure 1). The Matses and the Tunapuco inhabit the borders of Peru that comprise the Amazon Region, but their lifestyles and environment are strikingly different. The Matses are traditional hunter-gatherers living by the sea level, while the Tunapuco is a rural agriculturalist community situated in the Andes highlands [6].

We hypothesized that, since the previous Yanomami group studied was uncontacted and lived in a remote area in Venezuela [4], their gut microbiome would present unique features in comparison with the large semi-isolated Yanomami group living in the Brazilian Amazon. Even though they are hunter-gatherers, their diet varies depending on the niche explored, since they live in areas ranging from near rivers to frankly mountainous regions. Moreover, we explored the taxonomy and functionality of these microbiomes, contributing to the understanding of features that can affect the health outcomes observed in modern populations.

Results

Intra and Inter Individual Diversity of the Gut Microbiomes

To unravel the gut microbiome diversity of Yanomami from Brazil individuals (Yanomami/Brazil, $n = 15$), we performed alpha and beta diversity analyses based on the bacterial genera profile identified by Kraken. For these analyses, we also reanalyzed and compared gut microbiome data gathered from other South American traditional communities: the Yanomami from the Venezuelan Amazon (Yanomami/Venezuela, $n = 8$) [4], the Matses from the

147 Peruvian Amazon (n= 24) [6], the Tunapuco from the Andean highlands (n= 12)
148 [6]; and a representative group of urban individuals from United States (US, n=
149 44) [19,20].

150 There was no statistically difference between the Yanomami/Brazil and
151 Yanomami/Venezuela regarding intra and inter diversity (alpha- and beta-
152 diversity, respectively) of gut microbiome, although the group from Brazil had
153 the lowest alpha-diversity values. However, the Yanomami individuals showed
154 the lowest bacterial alpha-diversity among the traditional groups and all the
155 traditional human groups presented higher bacterial diversity compared to the
156 urban individuals (Figure 2a). Regarding the beta-diversity, the Yanomami/Brazil
157 presented the highest interpersonal variation, and the urbans presented the
158 lowest, although there was no significant difference with the
159 Yanomami/Venezuela group (Figure 2b). A clear segregation was observed
160 among the semi-isolated and westernized individuals (PERMANOVA, $P=0.001$)
161 based on Principal Coordinate Analysis (PcoA) generated with Bray-Curtis
162 distances. In addition, a higher dispersion of Yanomami/Brazil and
163 Yanomami/Venezuela samples was observed, stressing their higher
164 interpersonal variation (Figure 2c).

165

166 **Microbiomes Taxonomic Characterization**

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168 In order identify which bacterial and archaeal taxa differentiate the
169 traditional groups from the urban group, the microbiomes were compared at
170 both phylum and genus scales. Thirty-two bacterial phyla were identified, with
171 16 phyla having significant differences in the relative abundances among the

172 groups (Kruskal-Wallis test: $P < 0.0001$). Considering the traditional and urban
173 groups, a clear difference at the phylum level was observed, with the former
174 having a higher biodiversity characterized by *Firmicutes*, *Proteobacteria*,
175 *Bacteroidetes* and *Spirochaetes*, while the urban group is mainly characterized
176 by *Bacteroidetes* (Figure 3a).

177 The Yanomami/Brazil as well as the other traditionalists individuals follow a
178 trend in which they have higher *Firmicutes* and lower *Bacteroidetes* levels, while
179 the opposite was observed in the urban individuals (Figure 3b). Even though,
180 the *Firmicutes* in each traditional group was characterized by distinct genera, no
181 prevalent genus was consistently observed in the groups. In fact, all traditional
182 groups presented different genera from the *Firmicutes* phylum as biomarkers
183 (Figure 3c). Genera from *Bacteroidetes* phylum were demonstrated to be the
184 biomarkers of the urban group (Figure 3c).

185 Distinctly from the other groups, *Proteobacteria* was the most prevalent
186 phylum among the Yanomami individuals, despite their geographic origin (Brazil
187 and Venezuela). The most abundant genera of this phylum in the traditional
188 groups were *Escherichia* and *Klebsiella*, however, there is a contrasting higher
189 abundance of *Escherichia* and *Ralstonia* genera in the Yanomami/Brazil, and
190 therefore, they were defined as Yanomami/Brazil biomarkers. On the other
191 hand, *Neisseria* and *Desulfovibrio* were defined as Yanomami/Venezuela
192 biomarkers, while *Klebsiella* was the biomarker of the Matses group. It is
193 noteworthy that *Cutibacterium* from the Actinobacteria phylum and *Akkermansia*
194 from the Verrucomicrobia phylum were also deemed as the biomarkers of the
195 Yanomami/Brazil (Figure 3c). Besides that, the Yanomami/Brazil, similarly with
196 the other semi-isolated, present *Treponema* and *Brachyspira*, two genera from

197 the Spirochaetes that were not detected in the urban group.

198 With respect to Archaea, we observed that the most abundant genus in the
199 traditional groups was *Methanobrevibacter*, comprising ~70% of all archaea
200 classified reads, while in the urban population, there were a high abundance of
201 *Methanoculleus* and *Methanothermobacter*, all methane-producers
202 archaea (Figure 3d).

203

204 **Microbiomes Functional Characterization**

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206 For functional characterization, the metagenomic reads of all groups were
207 assigned to gene families from the SEED database, and were categorized for
208 their functional roles in subsystems with 3 levels of resolution, in which level 1
209 represent the broader category. We observed a segregation between the
210 traditional and urban groups regarding the abundance of functions at the level 3
211 subsystems. Interestingly, among the traditional groups, the Matsigenka and
212 Yanomami/Brazil individuals exhibits a clear segregation, however the
213 Yanomami/Brazil present a more disperse pattern, indicating a quite diverse
214 functional characteristic concerning functions at the level 3 subsystem (Figure
215 4a).

216 The most abundant metabolic functions present in the microbiome of all
217 groups at the level 1 were the metabolism of carbohydrates and proteins, while
218 the membrane transport was also a main function in the urban group, but was
219 depleted in the traditional groups (Figure 4b). We observed differences in the
220 carbohydrate metabolic functions between the traditional and the urban groups.
221 At level 2, the main carbohydrate metabolic functions in the traditional groups

222 belonged to the central carbohydrate metabolism, with functions as pyruvate
 223 metabolism and glycolysis/gluconeogenesis being the most predominant; while
 224 in the urbans, the major functions were the monosaccharides and di-
 225 /oligosaccharides metabolism (Figure 4b). In monosaccharides metabolism,
 226 there was also differences between the groups: D-galacturonate/D-glucuronate
 227 and xylose use were the most abundant functions in the traditional groups,
 228 while mannose metabolism was the most abundant in the urban group. The di-
 229 /oligosaccharides microbiome metabolism of US individuals is mainly driven by
 230 functions associated with lactose utilization, which was depleted in the
 231 microbiome of traditional groups. Regarding protein metabolism, there was no
 232 difference among the groups' microbiome, with the most abundant functions
 233 being those associated with protein biosynthesis and degradation. We also
 234 observed differences in metabolic pathways related to cofactors, vitamins,
 235 prosthetic groups and pigments among the groups, the major gene families
 236 found in the microbiome of the Yanomami/Brazil and Yanomami/Venezuela
 237 were associated with folate/pterines, in the Matsigenka and Tunapucos was
 238 riboflavin, and in the US group was tetrapyrroles (Figure 4b).

239 Interestingly, at level 1 subsystems, the microbiome of the Yanomami/Brazil
 240 was distinct from the other groups due to its significant higher abundance of
 241 gene families involved in regulation/cell signaling, motility/chemotaxis, and
 242 virulence (Figure 4b). The regulation/cell signaling functions in the
 243 Yanomami/Brazil is driven by abundance of programmed cell death and toxin-
 244 antitoxin systems, while the motility/chemotaxis function is driven by the
 245 presence of genes involved in flagellar motility in prokaryotes. The most
 246 abundant virulence function at subsystems 3 in the Yanomami/Brazil is cobalt,

247 zinc and cadmium resistance.

248

249 **Discussion**

250

251 The gut microbiome is a diverse ecosystem with multiple metabolic and
252 immune functions associated with the diet and lifestyle of the host [11,21].
253 Therefore, considering the current variety of lifestyles and diets in human
254 society, many aspects concerning the gut microbiome composition and
255 functionality are yet to be accessed and explored to understand the influence of
256 these factors in the gut microbiome. The study of human groups that still
257 maintain traditional modes of subsistence (hunter-gatherers and rural
258 agriculturalists) provides valuable information regarding the ancestral
259 microbiome that existed before the urbanization and industrialization impacted
260 human diet and lifestyle. So far, few studies explored worldwide traditional
261 groups gut microbiome, and it is essential to enlarge data concerning the
262 number of individuals, as well as different groups.

263 Therefore, in the present study, we characterized the gut microbiome of 15
264 semi-isolated Yanomami individuals from Brazil, and compared with other South
265 American traditional groups (uncontacted Yanomami from Venezuela, the
266 Matsigenka and the Tunapucos) as well as an urban-industrialized group (US)
267 [4,6,19,20], enlarging the number of Yanomami individuals analyzed, as well as
268 of hunter-gatherers. These traditional groups explore different Amazonian
269 niches and contrast with the US group, which lives in a densely populated
270 urbanized and industrialized society with access to medical care and high
271 hygiene standards.

272 Consistent with previous studies [1-10, 22], our analysis point to a higher
 273 bacterial diversity in the traditional groups, with diverse taxonomic and
 274 functional features that distinguish them from urban-industrialized individuals.
 275 Microbiomes harboring a high diversity showed a positive association with
 276 health, as consequence of the presence of a higher global metabolic potential,
 277 providing the host with a wide range of health-relevant metabolites [23, 24].
 278 Besides that, the microbiomes of the traditional South American groups share
 279 features with traditional African groups (Western, Central and Eastern Africa):
 280 they are also more diverse than the urbans, are enriched in *Proteobacteria*, with
 281 the presence of some *Spirochaetes* that are depleted in industrialized
 282 populations (*Treponema* and *Brachyspira*) [1-10, 22]. Despite the South
 283 American and the African groups being in different continents and having
 284 distinct genetic origin, they maintain a traditional mode of subsistence and do
 285 not have access to processed and refined food in their daily diet [21]. This
 286 corroborates that population lifestyle and diet are the major determinants of the
 287 gut microbiome composition and diversity, overruling genetic backgrounds and
 288 geographic origin.

289 The taxonomic analysis of the South-American traditional groups
 290 demonstrated a common profile at bacterial genera level, even though each
 291 group presented a specific set of biomarkers. Interestingly, some biomarkers
 292 converge in their functional profile, e.g. *Roseburia*, *Anaerostipes*, *Eubacterium*,
 293 *Flavonifactor*, biomarkers of the Yanomami/Brazil, Yanomami/Venezuela,
 294 Matses and Tunapuco, respectively, are butyrate-producing bacteria. Butyrate is
 295 an anti-inflammatory short chain fatty acid (SCFA) that induces mucin synthesis,
 296 contributing to colon health and gut integrity [25, 26]. In contrast, the urban-

297 industrialized biomarkers produce SCFAs other than butyrate, such as
 298 propionate, acetate, and succinate, which, in high proportions, may increase gut
 299 permeability, leading to a further unhealthy status [27]. Other biomarker of the
 300 Yanomami/Brazil group is Akkermansia, a mucin degrader, which has been
 301 associated with healthier metabolic status and better clinical outcomes [28,29].

302 Broader levels of functional categories poorly discriminated traditional and
 303 urban-industrialized groups. Interestingly, the stratification of these categories
 304 clearly segregated those groups. Differences were identified at level 3 of
 305 monosaccharides metabolism, where the main functions in the traditionalists
 306 and urbans were xylose and mannose metabolism, respectively. Xylans and
 307 Mannans, polysaccharides of xylose and mannose, are the two major classes of
 308 hemicelluloses that accumulate in plant secondary walls [30]. Interestingly,
 309 recent studies with mouse models revealed that mannose increased the
 310 Bacteroidetes to Firmicutes ratio in the gut, a characteristic observed in urban-
 311 industrialized groups [31]. On the other hand, Treponema, a prevalent genus in
 312 traditional populations that consume polysaccharide-rich diets, is a key xylan-
 313 degrader [32]. Another difference observed in the US versus traditional groups
 314 was the lactose utilization, which is enriched in the former and depleted in the
 315 latter group. This difference may be related to the lack of intake of dairy in the
 316 traditional groups [6]. Within the traditional groups, there was differences at
 317 level 3 of biosynthesis of vitamins: both Yanomami groups presented an
 318 enrichment in folate biosynthesis while the Matses and Tunapuco presented an
 319 enrichment in riboflavin biosynthesis, as well as in the US group. Riboflavin is
 320 the most commonly synthesized vitamin in the gut [33], and has been
 321 associated with the immune response through the activation of T-cells [34].

322 Folate is associated with high-fiber and low-fat diets [35], which agrees with
323 Yanomami diet from the present study.

324 The microbiome of the Yanomami/Brazil is unique concerning the presence
325 of higher levels of functions associated with virulence, driven by the cobalt, zinc
326 and cadmium resistance. Cobalt is commonly distributed in nature and has a
327 biological role as metal constituent of the vitamin B12, however, excessive
328 exposure induces adverse health effects [36]. Zinc is an essential nutrient and
329 play a role in gene expression, biomolecular activity and structural DNA
330 stabilization [37]. Cadmium is a non-essential element, representing an
331 environmental hazard to human health when contaminates the food chain,
332 causing cumulative toxic effects in diverse human organs [38]. Cadmium and
333 Zinc are present in mine discharges, which disperses into air, water and soils,
334 contaminating areas nearby mines [39]. However, in Yanomami/Brazil area,
335 Cadmium contamination may occur as consequence of the continuous
336 discharge of batteries anywhere by the Yanomami along decades.

337

338 **Conclusions**

339

340 Exploring the gut microbiome of traditional groups is challenging, mainly
341 due the difficult to access them. These groups are important, since they
342 represent living representatives of ancestral behaviors/dietary long lost for a
343 long time in the westernized groups. Our study revealed that even within very
344 close and related traditional groups (as Yanomami/Brazil and
345 Yanomami/Venezuela), there are taxonomic differences that distinguish their gut
346 microbiome. These variations may reflect their nomadic behavior, as well as,

347 the local and seasonal diversity of the vast rainforest niche they explore, despite
348 their shared cultural and genetic background. Overall, their microbiome profiles
349 are shared with South American and African traditional groups, probably due to
350 their diet and lifestyle. This highlight the need to characterize larger sampling of
351 human microbiomes, considering not only distinct lifestyle but also a broad
352 population representing a particular lifestyle. Thus, we expect novel insights into
353 the diverse factors that are associated with microbiome composition and human
354 health.

355

356 **Methods**

357

358 **Study Participants and Sample Collection**

359

360 The protocol of this study was reviewed and approved by Oswaldo Cruz
361 Foundation's Ethics Research Committee N ° 638/11 and by the National Ethics
362 the Committee in Research – CONEP N° 16907. Before participating in the
363 study, a bilingual interpreter (a Yanomami native who spoke Portuguese)
364 explained the leaders and/or Indigenous representatives, the purpose and
365 importance of the study, the procedures to be carried out and finally requested
366 permission by fingerprint consent of each participant. Participants were
367 requested to provide a morning faecal sample and a labelled screw-capped
368 plastic container was provided. A single stool sample was collected from each
369 subject on the following day and samples were stored in separate sterile feces
370 containers. At the time of the collection, age and sex information of the
371 individuals were also acquired. These details are summarized in Table S1.

372 **DNA extraction, library preparation, and sequencing**

373

374 Total DNA was extracted from 15 stool samples with FastDNA® SPIN Kit
375 (MP Biomedicals), following the manufacturer's instructions. DNA concentration
376 were evaluated using Qubit® 2.0 Fluorometer (Life Technologies). Metagenomic
377 libraries were constructed with TruSeq DNA Sample Preparation v2 Kit following
378 the standard protocols. Purified libraries were sequenced on a HiSeq® 2500
379 sequencer (Oswaldo Cruz Foundation High-throughput sequencing Platform) in
380 two batches, producing a total of ~ 219 million reads, with an average of ~ 14
381 million reads per sample.

382

383 **Bioinformatic Processing**

384

385 Raw reads were trimmed and filtered (phred quality < 20 , length < 30)
386 using Trimmomatic [40]. The remaining reads (~ 206 million reads) were
387 mapped to a human reference genome (Hg38) using Bowtie2 [41]. Non-host
388 reads (~ 198 million reads) were used in further analysis. Besides the
389 metagenomes generated in this study, we also analyzed shotgun metagenomic
390 data from previously published studies: two hunter-gatherer communities
391 (Yanomami from Venezuela, n=8 [4] ; Matses, n=24 [6]), a rural agricultural
392 community (Tunapuco, n=12 [6]) and urban populations (USA, n=44 [19,20]).
393 These datasets were sequenced on Illumina platforms and bioinformatic
394 processing was performed in parallel with the data generated in this study.

395 Taxonomic classification was performed by Kraken [42], using a database
396 of whole genomes of bacteria and archaea from NCBI. Functional classification

397 were classified by SUPER-FOCUS [43] based on the genes families from the
398 SEED database. Linear discriminant analysis (LDAs) were performed using
399 LEfSe [44] to detect bacterial genera that characterize the differences between
400 the groups (LDA score of > 4.0).

401 For general data manipulation and statistical analysis we employed the
402 vegan [45] and phyloseq [46] packages in R. Shannon index of alpha-diversity
403 was estimated for each metagenome, with pairwise Wilcoxon test being used
404 for statistical difference evaluation. Beta diversity was estimated using Bray–
405 Curtis dissimilarity and permutational multivariate analysis of variance
406 (PERMANOVA) were performed with 999 permutations to estimate a P-value for
407 differences among traditional and westernized groups.

408

409 **List of abbreviations**

410

411 **LDA:** Linear discriminant analysis

412 **LefSe:** Linear discriminant analysis effect size

413 **PcoA:** Principal coordinates analysis

414 **PERMANOVA:** Permutational multivariate analysis of variance

415 **SCFA:** Short chain fatty acid

416

417

418 **Declarations**

419 **Ethics approval and consent to participate**

420 The protocol of this study was reviewed and approved by Oswaldo Cruz
421 Foundation's Ethics Research Committee N ° 638/11 and by the National Ethics
422 the Committee in Research – CONEP N° 16907.

423

424 **Consent for publication**

425 Not applicable.

426

427 **Availability of data and materials**

428 The quality-filtered metagenomic sequences are available on the NCBI under
429 the BioProject PRJNA527208.

430

431 **Competing interests**

432 The authors declare that they have no competing interests.

433

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438

439 **Authors' contributions**

440 JO collected the samples. LCC processed the samples and analyzed the data.
441 LCC and ACPV interpreted the data and drafted the manuscript. All authors
442 revised it. All authors approve the final version to be published and agree to be
443 accountable for the work.

444

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450

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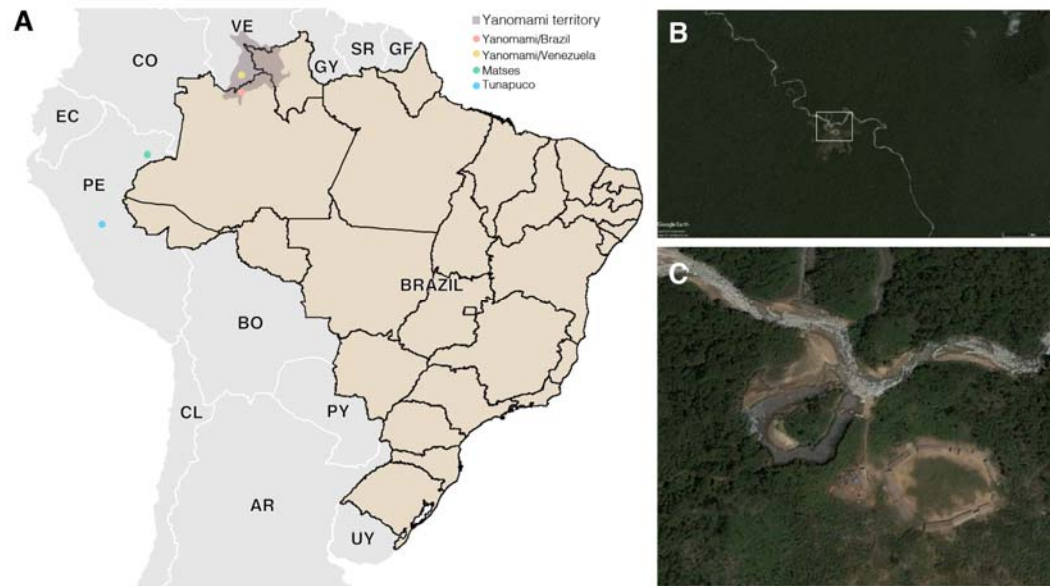
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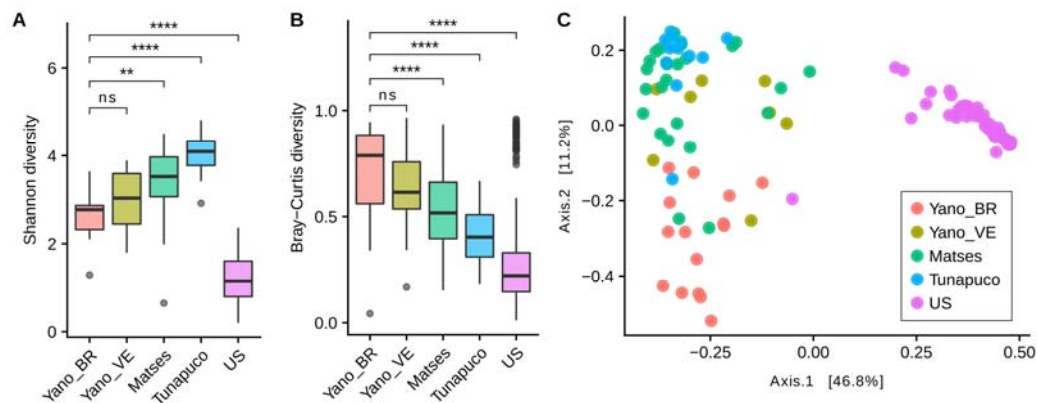
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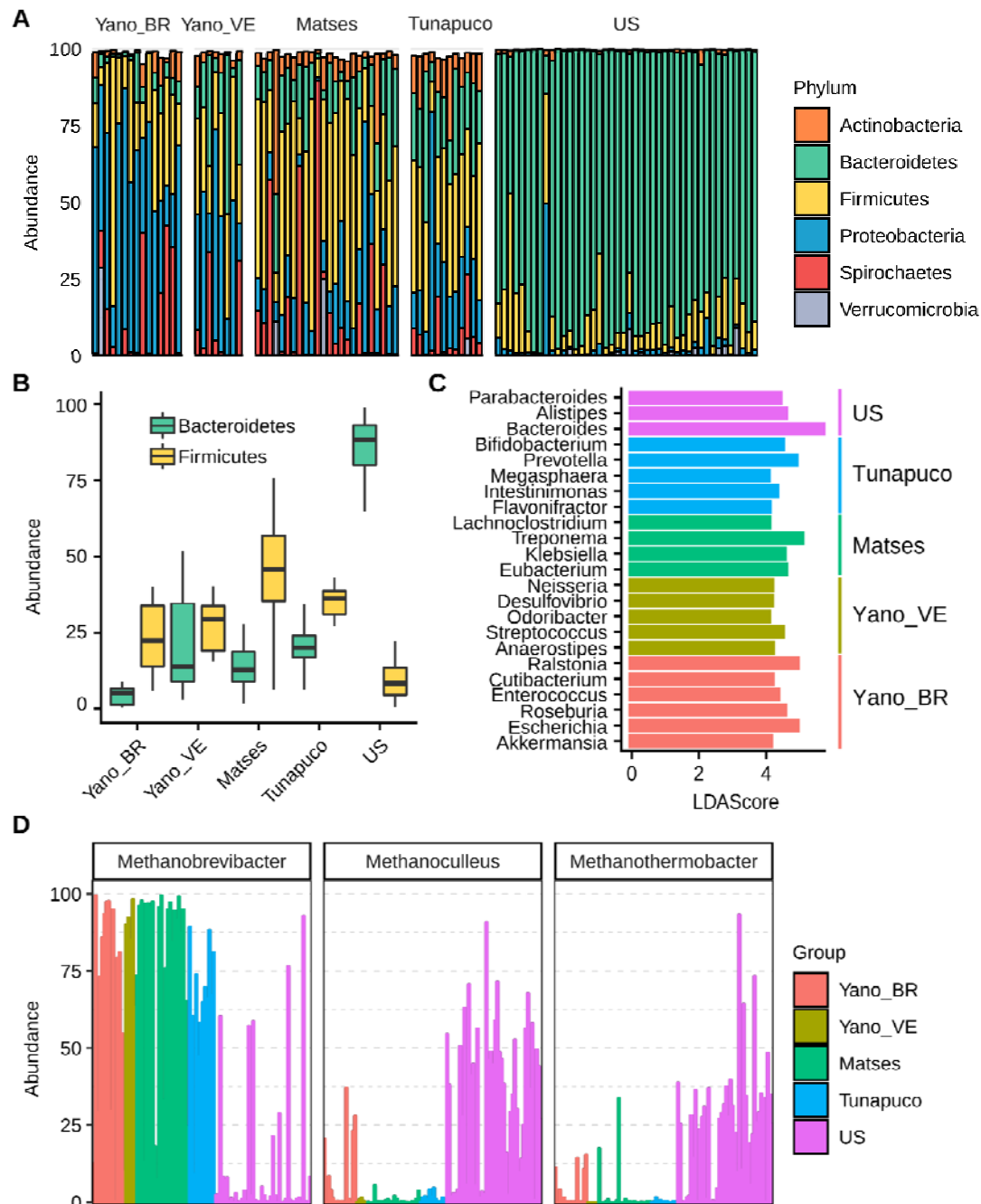
611 **Figure 1:** (A) Geographic locations of the South American traditional groups (B and C)
612 Satellite image of a Yanomami village in the Brazilian Amazon. Source: Google Earth
613 and Instituto Socioambiental (<https://acervo.socioambiental.org/>)

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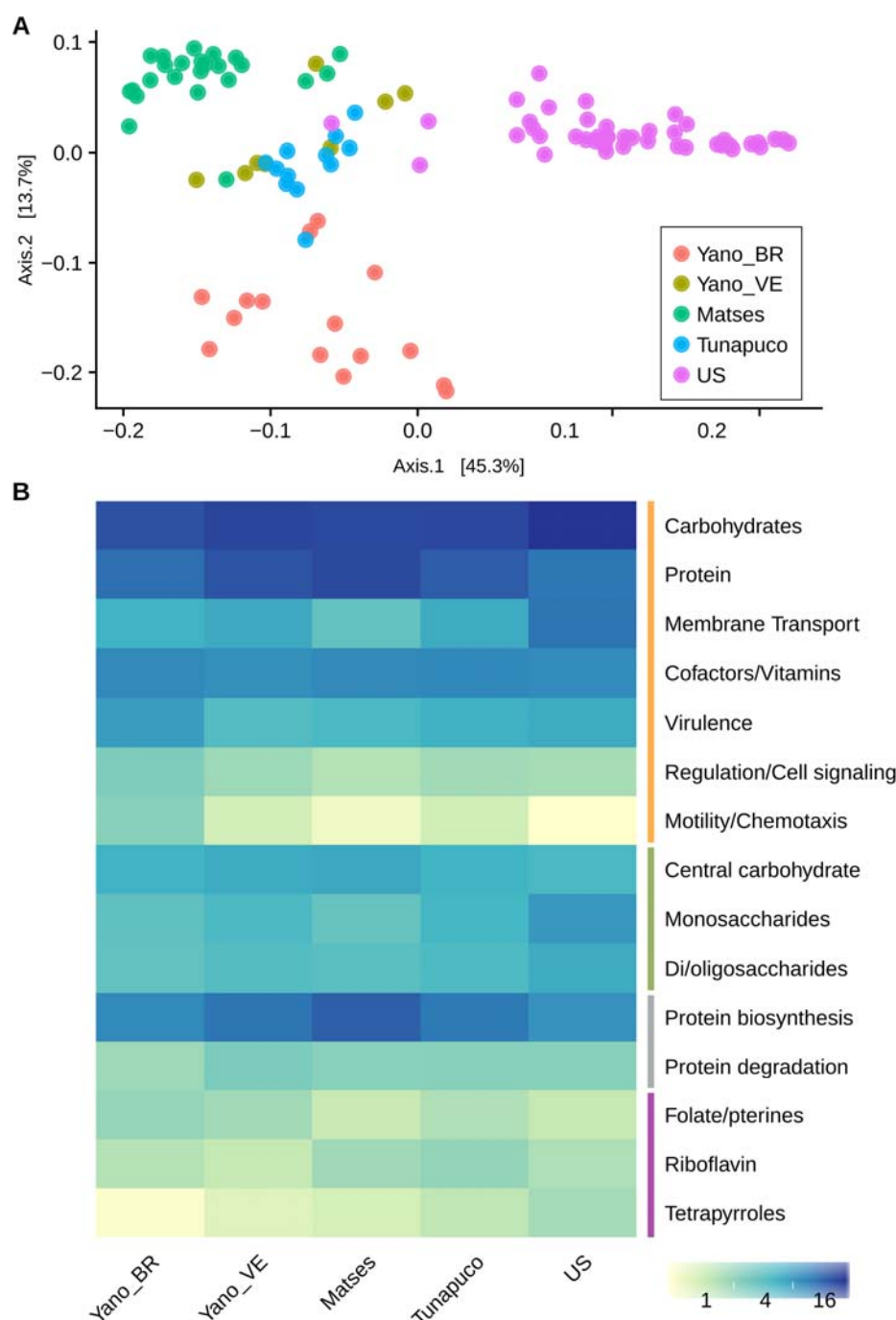
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616 **Figure 2:** Alpha and beta-diversity comparisons of the gut microbiomes of each group.
617 Analyses were performed on genus-level taxa tables. ns = not significant, ** $P < 0.01$,
618 **** $P < 0.0001$ (Wilcoxon test) (A) Boxplot of the Shannon diversity of each group (B)
619 Bray-Curtis distances within each group (C) Principal coordinate analysis of Bray-Curtis
620 distances. The colors of the boxplots and dots represent the different groups analyzed
621 according to the legend. Yano_BR, Yanomami/Brazil; Yano_VE, Yanomami/Venezuela;
622 US, US individuals.



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624 **Figure 3:** Bacterial and archaeal taxa differences among traditional and urban groups.
625 (A) Barplot representing the relative abundance (percentage) of the most frequent
626 phyla (B) Boxplots showing the *Bacteroidetes* and *Firmicutes* abundance (percentage)
627 in each group (C) Bar chart showing the LDA scores > 4 of bacterial genera found to be
628 significantly associated with each group (D) Relative abundance of the most prevalent
629 archaeas identified in the groups. Yano_BR, Yanomami/Brazil; Yano_VE,
630 Yanomami/Venezuela; US, US individuals.



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632 **Figure 4:** Functional metabolic characteristics of the traditional and urbanized
633 microbiomes. (A) Principal coordinate analysis of Bray-Curtis distances based on
634 functions at the level 3 subsystems. (B) Heatmap showing the main functions at level 1
635 (orange bar) and level 2 regarding carbohydrates metabolism (green bar), protein
636 metabolism (gray bar) and cofactors, vitamins, prosthetic groups and pigments (purple
637 bar). Yano_BR, Yanomami/Brazil; Yano_VE, Yanomami/Venezuela; US, US individuals.