Effects of host species identity and diet on biodiversity of oral and rectal microbiomes of Puerto Rican bats

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Abstract

Microbiomes perform vital functions for their mammalian hosts, making them potential drivers of host evolution. Understanding effects of environmental factors and host characteristics on the composition and biodiversity of microbiomes may provide novel insights into the origin and maintenance of these symbiotic relationships. Our goals were to (1) characterize biodiversity of oral and rectal microbiomes of bats in Puerto Rico; and (2) determine the effects of geographic location and host characteristics on that biodiversity. We collected bats and their microbiomes from 3 sites, and used 4 metrics (species richness, Shannon diversity, Camargo evenness, Berger-Parker dominance) to characterize biodiversity. We evaluated the relative importance of site, host sex, host species identity, and host foraging guild on microbiome biodiversity. Microbiome biodiversity was highly variable among conspecifics. Geographical location exhibited consistent effects, whereas host sex did not do so. Within each host guild, host species exhibited consistent differences in oral and rectal microbiome biodiversity. Oral microbiome biodiversity was indistinguishable between guilds, whereas rectal microbiome biodiversity was significantly greater in carnivores than in herbivores. The high intraspecific and spatial variation in microbiome biodiversity necessitate a large number of samples to isolate the effects of environmental or host characteristics on microbiomes. Species-specific biodiversity of oral microbiomes suggests these communities are structured by direct interactions with the host immune system via epithelial receptors. In contrast, the number of microbial taxa that a host gut supports may be contingent on the number and kinds of functions a host requires of its microbiome.

Keywords

Chiroptera, Mormoopidae, Phyllostomidae, spatial variation, Vespertilionidae

INTRODUCTION

Microbiomes perform vital functions for their mammalian hosts, including nutrient acquisition, pathogen defense, and immune development [1–3]. This suggests that microbiomes may be important drivers of host evolution, affecting their physiology, immunocompetence, diet, and ultimately fitness [4]. Moreover, aspects of mammalian physiology, anatomy, behavior, diet, and niche affect which microbes encounter particular host habitats (e.g. skin, oral cavity, gastrointestinal tract). Consequently, these symbiotic associations likely represent co-evolutionary relationships [5, 6].

Understanding effects of environmental factors and host characteristics on the composition and biodiversity of microbiomes may provide novel insights into the origin and maintenance of symbiotic relationships. Indeed, host phylogeny, host diet, and environmental characteristics are the primary candidates likely to affect variation in microbiome composition or biodiversity [7–11]. Host phylogeny is a particularly attractive explanation as it forms the basis for coevolutionary dynamics. Because organisms evolve via descent with modification, phylogenetic inertia gives rise to a priori expectations that more closely related species will be more similar (from genetic, functional, behavioral, anatomical, and physiological perspectives), and that more distantly related species will be less similar [12]. Consequently, host phylogeny may be an effective proxy for combinations of host characteristics that affect the composition

and biodiversity of microbiomes rather than an explanatory mechanism for variation among hosts in aspects of their microbiome per se.

Host diet has been a focal point for understanding microbiome composition and biodiversity, especially for gastrointestinal microbiomes, which facilitate digestive processes and are directly exposed to the ingested food. Consequently, intra- or inter-specific differences in host diet may result in differences in gastrointestinal microbiomes due to exposure (i.e. animals with similar diets may consume similar microbiota) or due to the digestive functions provided by the microbiota [9, 13]. In addition, hosts that live in similar environments may be exposed to similar microbiota, resulting in similar microbiome composition or biodiversity [13]. Important aspects of the environment that may affect microbiome composition and biodiversity include host abundance and community composition, as well as ambient environmental characteristic s (e.g. roost type, habitat type, and abiotic factors).

Studies typically consider samples from intestinal contents, intestinal linings, or feces to represent the same microbial communities [9, 14, 15]. Nonetheless, microbiomes isolated from the mucosal layer of the intestines are distinct from those isolated from feces or intestinal contents [14]. Moreover, variation among microbiomes from the intestinal mucosa are closely associated with host evolutionary relationships, whereas variation in fecal microbiomes are closely associated with dietary variation among hosts [14].

Bats as microbiome hosts

Bats are an ideal model taxon for the study of variation in microbiome biodiversity [14]. They compose the 2nd most species-rich order of mammals, and are nearly cosmopolitan, occurring everywhere but the Arctic, Antarctica, and a few small oceanic islands [16]. In addition, bats are

97 locally abundant, live in close proximity to humans, travel long distances to forage or between
98 winter and summer ranges, and are functionally diverse [17, 18]. Moreover, bats are important
99 agents of pollination, seed dispersal, and pest control [19, 20], and exhibit specializations to
100 forage on nectar, fruit, insects, fish, small vertebrates, and blood [17, 18, 21, 22]. As with other
101 mammals, functional traits and behaviors are evolutionarily conserved in bats, often confounding
102 the ability to evaluate independent effects of diet or phylogeny on ecological patterns [23].
103 Understanding bat microbiome composition and diversity may be especially important
104 because bats often live in proximity to humans [24] and are reservoirs or vectors for many well-
105 known zoonoses [25–29]. Their presence in settlements affects infection rates of diseases in
106 humans [30]. Bats use many human-dominated habitats: they feed on fruits in orchards, forage
107 for insects around streetlights, and use buildings for maternity colonies, roosts, and hibernacula
108 [24]. In addition, bats are highly vagile and capable of traveling long distances in a single night.
109 This creates opportunities for exposure to novel microbes and to enhance dispersal [31]. In
110 addition, microbiomes may drive host evolution, physiology, and fitness [2]. For example, the
111 successful evolution of new dietary strategies within a clade (e.g. the diversification of
112 herbivorous strategies within the Phyllostomidae) may be contingent of the functional diversity
113 of their associated microbiomes. Finally, the digestive physiology of flying vertebrates (bats and
114 birds) differ from that of other vertebrates [32], including reliance on paracellular glucose
115 absorption, resulting in different mechanisms structuring the microbiomes of bats and birds than
116 in terrestrial vertebrate groups.
117 Thirteen species of bat occur on Puerto Rico [33], including 7 insectivores (*Eptesicus*
118 *fuscus*, *Lasiurus borealis*, *Molossus molossus*, *Mormoops blainvillii*, *Pteronotus quadridens*, *P.*
119 *parnellii*, *Tadarida brasiliensis*), a piscivore (*Noctilio leporinus*), a nectarivore (*Monophyllus*

120 *redmani*), 2 frugivores (*Artibeus jamaicensis*, *Stenoderma rufum*), and 2 generalist herbivores
121 (*Brachyphylla cavernarum*, *Erophylla sezekorni*). Bats that consume fruit, nectar, flowers, or
122 pollen typically have diverse herbivorous diets that differ in preferred dietary items, with *M.*
123 *redmani* being primarily nectarivorous, *A. jamaicensis* and *S. rufum* being primarily frugivorous,
124 and *E. sezekorni* and *B. cavernarum* being generalist herbivores [33]. The insectivores belong to
125 3 families (Vespertilionidae, Molossidae, and Mormoopidae); the piscivore is a noctilionid; and
126 phyllostomids consume fruit, flowers, pollen, or nectar. The Noctilionidae, Mormoopidae, and
127 Phyllostomidae are members of the Noctilionoidea superfamily, whereas the Vespertilionidae
128 and Molossidae are members of the Vespertilionoidea superfamily [34]. These systematic
129 relationships decouple insectivory from phylogeny and may help disentangle the relative effects
130 of evolutionary history and ecological function as drivers of microbiome composition and
131 diversity. We grouped bats into broad foraging guilds (carnivores and herbivores) to evaluate
132 effects of general diet on microbiome biodiversity.
133
134 **Host environments**
135 Oral microbiomes provide benefits to the host, including prevention of infection by exogenous
136 microorganisms, regulation of immune responses, and the conversion of dietary nitrates into
137 nitrites that improve vascular health and stimulate gastric mucus production [35, 36]. The oral
138 environment (e.g. pH, saliva, temperature, nutrient sources, aerobic conditions) determines
139 which microbes colonize and become minor or major components of the oral microbiome [37].
140 In addition, the microbiome can modify the environment, facilitating or preventing establishment
141 by other microbes. Despite the importance of oral microbiomes to their hosts, they have rarely

142 been studied in wild animals (but see [15]) even though such studies are critical for advancing
143 evolutionary ecology in general [38].

144 Studies rarely have sufficient sample sizes from multiple locations, species, or foraging
145 guilds to powerfully and simultaneously address multiple factors that affect microbiome
146 composition or biodiversity in bats (but see [15]). Moreover, studies are lacking that
147 simultaneous consider effects of environmental and host factors on microbiomes from multiple
148 sources (e.g. oral cavity). To address these issues, we collected oral and rectal samples from bats
149 captured at three locations (hereafter called "sites") in Puerto Rico. We evaluated the relative
150 importance of site, host sex, host species identity, and host foraging guild on microbiome
151 biodiversity from oral and rectal samples. We used a hierarchical analytical design to evaluate
152 these factors (Fig. 1). First, for each host species with sufficient sample sizes from multiple
153 caves, we evaluated effects of site (i.e. host population) and host sex on microbiome biodiversity.
154 Second, we evaluated the effect of host species identity on microbiome biodiversity separately
155 for bats within each of two broadly defined foraging guilds. Finally, we evaluated the effect of
156 host foraging guild (carnivores versus herbivores) on microbiome biodiversity.

157 We expected factors that mold patterns in oral microbiomes to be different from those
158 that mold such patterns in rectal microbiomes. More specifically, we expected dietary guild to
159 have a larger impact on the biodiversity of rectal microbiomes than on that of oral microbiomes
160 because sources of nutrients and energy (fats, carbohydrates, proteins) have a dominant effect on
161 the composition and diversity of microbiomes associated with the digestive tract [8]. In contrast,
162 we expected biodiversity of the oral microbiome, but not that of the rectal microbiome, to
163 respond to host species identity and geographical site because oral microbiomes are affected

164 primarily by the interactions with the epithelia and exposure to local habitats (e.g. roost
165 locations, animals that share a roost, hot cave versus cold cave).
166
167 **METHODS**
168 **Study area and sample collection**
169 Field work was conducted at three sites (Mata de Plátano, Río Encantado, and Aguas Buenas) in
170 Puerto Rico (Fig. 2), Each is in an area characterized by limestone formations (karst region), in
171 which weathering has produced ridges, towers, fissures, sinkholes, and caves throughout the
172 landscape. Although bats captured in a location may not be roosting in a single cave, all are
173 using the same habitats and resources, meeting the criteria for a population.
174 The majority of sampling was conducted on the Mata de Plátano Nature Reserve
175 (operated by InterAmerican University, Bayamon, Puerto Rico) in north-central Puerto Rico (18°
176 24.87' N, 66° 43.53' W). Mata de Plátano harbors two adjacent, well-studied caves (Culebrones
177 and Larva). Culebrones is a structurally complex hot cave, with temperatures reaching 40 °C and
178 relative humidity at 100%. It is home to about 300,000 bats representing six species [39]: three
179 carnivores (*P quadridens*, *P. parnellii*, *M. blainvillii*) and three herbivores (*M. redmani*, *E.*
180 *sezekorni*, *B. cavernarum*). Bats were sampled at Culebrones for 28 nights from June to August
181 2017. A harp trap was placed at sunset immediately outside the cave opening and monitored
182 continually until the maximum number of bats that could be processed in a single night was
183 captured. The harp trap was used at Culebrones because the cave has a single, small opening,
184 that funnels hundreds of thousands of bats through a small space as bats emerge during and after
185 sunset.

Larva is a cold cave that is much smaller, cooler, and less structurally complex than Culebrones. Only a small number of bats (30-200) representing two species (*A. jamaicensis* and *E. fuscus*) roost in the cave. Bats were sampled from Larva on seven occasions from June to August of 2017, using two different techniques. After sunset, mist nets were placed along a trail outside of the cave entrance and were checked at least every ten minutes. Because few individuals were captured with mist nets, hand nets were used to capture bats inside the cave to increase sample sizes.

Río Encantado is home to Ramon Cave (18° 21.41' N, 66° 32.36' W), a large, cool cave known to support a single bat species, *A. jamaicensis* [33]. The cave is 10 km southeast of Mata de Plátano and is associated with an extensive underground river system. The underground river has many openings throughout its range, but only a single opening exists at this location. Habitats surrounding Ramon Cave are owned and protected by a non-profit organization (Para la Naturaleza). Bats were sampled at Río Encantado on six nights during July of 2017. A harp trap was placed near the cave entrance and mist nets were placed along the trail leading to the cave. Harp traps were monitored continually, and mist nets were checked at least every 10 minutes. Bats were captured from sunset until the maximum number of bats that could be processed in a single night was collected.

Aguas Buenas is a cool cave that is located 70 km southeast of Mata de Plátano (18° 14.01' N, 66° 6.30' W). *Artibeus jamaicensis*, *B. cavernarum*, *M. redmani*, *P. quadridens*, *E. fuscus*, and *L. borealis* have been recorded roosting in or flying near the cave [33]. Bats were captured at Aguas Buenas on four nights in July and August of 2017. The entrance to the cave is not easily accessible, as it is elevated above ground-level and blocked by a river. Consequently, bats were captured using mist nets at each of the two major flyways from the cave: along the trail

209 to the cave and across the river outside of the cave. Nets were opened at sunset and monitored at
210 least every 10 minutes until approximately 01:00 or until the maximum number of bats that
211 could be processed in a single night was collected.
212 Species identity, sex, reproductive status and mass were determined for each captured
213 individual prior to placement in a cotton holding bag. Separate, clean cotton-tipped swabs were
214 used to collect saliva from the mouth or feces from the rectum and anal region of each bat.
215 Swabs were placed in individual cryovials and sent to the University of Connecticut at -80 °C in
216 a dry ice shipper. All methods were approved by the University of Connecticut Institutional
217 Animal Care and Use Committee (IACUC, protocol A15-032).
218
219 **Microbiome Analysis**
220 DNA was extracted using DNeasy PowerSoil kit (Qiagen). Swabs were shaved off to maximize
221 DNA output using sterile surgical blades, carbon steel, Size 15 (Bard-Parker). The DNeasy
222 PowerSoil protocol was followed, but instead of vortexing the bead tubes a PowerLyzer 24 was
223 used (45 seconds at 2,000 RPM for 1 cycle) (Qiagen). The DNeasy PowerSoil extraction was
224 performed using a QIAcube (Qiagen).
225 The hypervariable V4 region of the 16S rRNA gene was amplified to characterize the
226 microbiome [40]. The universal 16S primers 515F/806R were used to PCR amplify the V4
227 region [41]. PCR was performed in triplicate, each reaction with a total volume of 25 µL. Each
228 reaction contained 12.5 µL Phusion High-Fidelity PCR Master Mix with HF Buffer 2X
229 concentration and 1 µL bovine serum albumin 20 mg/ml (New England BioLabs), 0.75 µL
230 forward primer 10 µM, 0.75 µL reverse primer 10 µM, and 10 µL of DNA/molecular grade
231 water. A total of 10 ng DNA was added per reaction. Thermocycler parameters were: denaturing

232 step at 95 °C for 3 min, followed by 30 cycles of 95 °C for 45 s, 50 °C for 60 s, 72 °C for 90 s,
233 and an extension step of 72 °C for 10 min. Subsequently, QIAxcel capillary electrophoresis
234 (Qiagen) was utilized to assess presence of PCR product and determine the V4 band
235 concentration for library pooling. PCR samples with similar concentrations (< 5 ng/µL, 5-10
236 ng/µL, > 10 ng/µL) were pooled together. Libraries clean-up was performed using GeneRead
237 Size Selection kit (Qiagen). Libraries were sequenced on an Illumina MiSeq at the UConn
238 Microbial Analysis, Resources, and Services facility. The reads were demultiplexed using the
239 Illumina BaseSpace sequence hub and FASTQ files were downloaded for further data analysis.
240 Data was analyzed in R [42] using the dada2 package [43] to process data and generate
241 Amplified Sequence Variants (ASV) and taxonomy tables. The forward and reverse reads were
242 trimmed to 240 and 200 bp, respectively, and truncated using Q=11 and no Ns were allowed.
243 The taxonomy was assigned to each ASV using silva_nr_v128. The phangorn package [44] was
244 used to generate phylogenetic trees from ASV tables. Further analyses and sample filtering were
245 performed in phyloseq [44]. Using the rarefy_even_depth function in phyloseq, microbiome
246 count data were rarefied to sequencing depths of 1,000, 5,000, and 10,000 reads. Data were
247 rarefied to these three levels to optimize microbiome sampling completeness, while trying to
248 maximize sample sizes for analyses of effects related to host sex, host species, host guild, and
249 geographical location. A sequencing depth of 1,000 reads was selected as minimum depth to
250 retain the greatest number of samples for analyses, but this level discards a large amount of data
251 from many samples and may include samples that are relatively poorly characterized. Increasing
252 sequencing depth reduces the number of samples that meet the minimum requirements, resulting
253 in reduced statistical power, but increases the relative completeness and number of rare ASVs

254 included in samples. This represents a trade-off of statistical power for confidence in the
255 characterization of the microbiome samples.
256
257 **Quantitative Analysis**
258 Separately for oral and rectal samples from each host individual, we quantified microbiome
259 biodiversity using four metrics based on ASVs: richness, Shannon diversity [46], Camargo's
260 evenness [47], and Berger-Parker dominance [48]. Hereafter, we refer to these metrics simply as
261 "richness", "diversity", "evenness", and "dominance", and use "biodiversity" to refer to the
262 general concept that comprises all 4 metrics. Each metric was expressed as Hill numbers, which
263 are transformations based on relative abundances [48, 49]. Within the context of ASVs, Hill
264 numbers are based on the relative number of reads that represent each ASV. Importantly, Hill
265 numbers for all metrics are on the same scale (i.e. from 1 to richness) and in the same units
266 (effective number of ASVs), which is defined as the number of equally abundant ASVs required
267 to achieve the empirical value of a metric. Greater values for any Hill number represent greater
268 biodiversity, including for dominance (i.e. larger values for Hill-transformed dominance indicate
269 low dominance and greater biodiversity).
270 We used a 2-way analysis of variance (ANOVA) with type II sums of squares to evaluate
271 effects of site (i.e. host population) and host sex for each host species that was represented by
272 more than 1 population. Site and host sex were model I (fixed) treatment factors. *Artibeus*
273 *jamaicensis* was captured at all three caves; *B*. *cavernarum*, *E*. *sezekorni* and *P*. *quadridens* were
274 captured at Mata de Plátano and Río Encantado; and *M*. *redmani* was captured at Mata de
275 Plátano and Aguas Buenas. For each host species without sufficient samples from multiple
276 caves, but with samples for each sex, we used a general linear model (GLMM) to evaluate

differences in microbiome biodiversity between males and females with host sex as a fixed effect and site as a random factor (i.e. model II treatment factor). Use of site as a random factor controlled for geographic variation to more powerfully evaluate differences between sexes in microbiome biodiversity

We used GLMMs to evaluate differences in microbiome biodiversity among host species for each guild (i.e. only among carnivorous species and only among herbivorous species) and between host guilds. Host species or host guild was a fixed effect and site was modeled as a random factor. Use of site as a random factor controlled for geographic variation to more powerfully evaluate species- or guild-level differences in microbiome biodiversity. For each GLMM that identified a significant difference in microbiome biodiversity between host species with a guild, we conducted a posteriori tests (Tukey's test with a Holm-Šidák adjustment) to identify consistent differences between all possible pairs of host species. Because such a posteriori tests are less powerful than their associated GLMM and are protected in the sense that a posteriori tests were only executed when GLMMs were significant ($\alpha \leq 0.05$), we considered $P \leq 0.10$ as evidence for significant pairwise differences.

For all analytical approaches, oral and rectal microbiomes were evaluated separately for each sequencing depth (i.e. 1,000, 5,000, and 10,000 reads) and analyses were conducted separately for each metric of biodiversity. For analyses based on host foraging guild, all host species were included to best represent variation associated with all carnivorous or herbivorous hosts. Because sample sizes decreased with increasing sequencing depth, the number of host species sometimes declined with greater sequencing depth.

Accession Number

Sequencing data of V4 region of the 16S rRNA gene has been deposited in the NCBI Short Read Archive database under BioProject PRJNA602518 and accession numbers SRX7587313-7587772.

RESULTS

Oral and rectal samples were collected from 331 individual bats, representing 10 species: 3 insectivorous mormoopids (*M. blainvillii*, *P. quadridens*, *P. parnellii*), 1 insectivorous vespertilionid (*E. fuscus*), 1 piscivorous noctilionid (*N. leporinus*), 2 frugivorous phyllostomids (*A. jamaicensis*, *S. rufum*), 1 nectarivorous phyllostomid (*M. redmani*), and 2 generalist herbivore phyllostomid (*B. cavernarum*, *E. sezekorni*). Samples were obtained from 10 bat species at Mata de Plátano (155 individuals), 9 species (all but *S. rufum*) at Río Encantado (101 individuals), and 6 species (75 individuals) at Aguas Buenas (*P. parnellii*, *N. leporinus*, *A. jamaicensis*, *M. redmani*, *B. cavernarum*, and *E. sezekorni*). As the bats were released after sampling, we used swabs to sample microbiomes, especially for the smaller species whose size made it challenging to extract sufficient amounts of microbial DNA for analysis. We obtained reasonable representation of the microbiomes (i.e. sequence depths > 1,000 reads) from less than half of those samples. Specifically, 136, 111, and 94 oral samples yielded sequencing depths of at least 1,000, 5,000, and 10,000 reads, respectively; and 157, 122, and 106 rectal samples yielded sequencing depths of at least 1,000, 5,000, and 10,000 reads, respectively.

Oral microbiomes comprised 2,114, 2,282, and 1,973 ASVs in samples with sequencing depths of 1,000, 5,000, and 10,000 reads, respectively. Rectal microbiomes comprised 2,986, 4,035, and 4,026 ASVs in samples with sequencing depths of 1,000, 5,000, and 10,000 reads,

322 respectively. The reduction in number of ASVs between sequencing depths of 5,000 and 10,000
323 is due to the smaller number of samples available for analysis.

324 Bacteria represented over 98.8% of the ASVs in oral and rectal microbiomes from each
325 host species. Archaea comprised the remainder of the microbiomes, occurring in the oral
326 microbiomes of 8 of 10 host species (all but *N. leporinus* and *S. rufum*) and in the rectal
327 microbiomes of 9 of 10 host species (all but *S. rufum*).

328 In aggregate, 37 and 36 phyla were identified from oral and rectal microbiomes,
329 respectively; however, most of these taxa were represented by few ASVs and few reads of those
330 ASVs. Only 16 and 14 phyla were represented by at least 5 ASVs from oral and rectal samples,
331 respectively. Oral microbiomes were dominated by Actinobacteria (30.6% of all reads),
332 Bacteroidetes (15.4%), and Firmicutes (29.2%). Actinobacteria was the most abundant phylum
333 in oral microbiomes of 5 host species, including all 3 mormoopids, and 2 phyllostomids (a
334 nectarivore and frugivore), whereas Firmicutes was the most abundant phylum in oral
335 microbiomes of the remaining 5 host species, including the noctilionid, vespertilionid, and 3
336 phyllostomids (a frugivore and 2 generalist herbivores).

337 Rectal microbiomes were dominated by Actinobacteria (15.9% of all reads),
338 Bacteroidetes (9.8%), Firmicutes (19.2%), and Proteobacteria (43.3%). The dominant phylum in
339 rectal microbiomes (Proteobacteria) represented only 0.4% of oral microbiomes, but was the
340 most abundant phylum in the rectal microbiomes of 9 host species, except for *E. fuscus*, for
341 which Actinobacteria was the most abundant taxon.

342 Biodiversity was highly variable among individuals within each host species regardless
343 of sequencing depth. Using sequencing depth of 1,000 as an example, maximum richness from
344 an individual host for oral microbiomes was 3 to 38 (mean of 11) times greater than the

345 minimum richness within host species. Similarly, maximum richness of rectal microbiomes from
346 an individual host was 3 to 33 (mean of 9) times greater than the minimum within host species.
347 Similar variation was observed within each host species for oral diversity (maximum 3 to 56
348 times that of the minimum, with a mean of 17) rectal diversity (maximum 3 to 57 times that of
349 the minimum, with a mean of 20), oral evenness (maximum 2 to 55 times that of the minimum,
350 with a mean of 17), rectal evenness (maximum 3 to 58 times that of the minimum, with a mean
351 of 21), oral dominance (maximum 2 to 18 times that of the minimum, with a mean of 7), and
352 rectal dominance (maximum 2 to 11 times that of the minimum, with a mean of 6).

353 The oral microbiome exhibited greater biodiversity than did the rectal microbiome in four
354 host species, including 2 insectivorous mormoopids (*M. blainvillii* and *P. parnellii*) that harbor
355 high microbiome biodiversity and 2 frugivorous phyllostomids (*A. jamaicensis* and E. *sezekorni*)
356 that harbor low microbiome biodiversity (Table 1). In general, biodiversity of the more
357 biodiverse microbiome (oral or rectal) was less than twice as great as its companion microbiome;
358 however, *E. fuscus* (an insectivore) harbored rectal microbiomes that were more than 4 times as
359 biodiverse as its oral microbiomes.

360 As expected, microbiome biodiversity increased as sequencing depth increased (Table 1).
361 Insectivores had both the least (*E. fuscus*) and greatest (*Pteronotus* spp.) oral microbiome
362 biodiversity. In contrast, frugivores (*A. jamaicensis*, *E. sezekorni*) had the least rectal
363 microbiome biodiversity, and nectarivores (*M. redmani*) and insectivores (*Pteronotus* spp.) had
364 the greatest rectal microbiome biodiversity (Table 1).

365 Host sex did not exhibit effects on oral or rectal microbiome richness (Table 2); however,
366 at least one effect of sex on oral microbiome diversity, evenness, or dominance was found in *B.*
367 *cavernarum* and on rectal microbiome diversity, evenness, or dominance in *M. blainvillii*, *P.*

368 *quadridens*, and *A. jamaicensis* (Table 2). Consistent effects of site on oral microbiomes only
369 manifested for *A. jamaicensis*, which had the greatest number of samples. At least one metric of
370 rectal microbiome biodiversity responded to site for *A. jamaicensis* (richness), *E. sezekorni*
371 (richness, evenness, and dominance), and *P. quadridens* (richness, diversity, evenness, and
372 dominance) (Table 2). Oral and rectal microbiome biodiversity was greater from *A. jamaicensis*
373 at Río Encantado than from *A. jamaicensis* at Mata de Plátano or Aguas Buenas (Fig. 3). For host
374 species (*B. cavernarum*, *E. sezekorni* and *P. quadridens*) with sufficient sample sizes only at Río
375 Encantado and Mata de Plátano, differences in oral microbiome biodiversity did not manifest
376 between sites; however, rectal microbiome biodiversity was typically greater at Río Encantado
377 than at Mata de Plátano (Fig. 4). Microbiome biodiversity from *M. redmani* did not differ
378 between sites.

379 Within each host guild, host species differed in oral microbiome biodiversity at each
380 sequence depth; however, interspecific host differences in rectal microbiome biodiversity
381 decreased with increasing sequence depth. We have stronger evidence for consistent species-
382 specific differences in oral microbiome biodiversity within each guild than for species-specific
383 difference in rectal microbiome biodiversity within each guild (Table S1). In contrast, no
384 evidence suggests that guild-specific differences in oral microbiome biodiversity exist, whereas
385 rectal microbiome biodiversity differed significantly between guilds (Table 3). Rectal
386 microbiome biodiversity in carnivores was about twice that found in herbivores (Table 1; Fig. 5).
387

388 **DISCUSSION**

389 Considerable intraspecific variation characterized microbiome biodiversity, even after
390 controlling for geography or sex of the host individual. These results mirror those for fecal and

391 gastrointestinal microbiomes from vespertilionid bats of Slovenia [51] and from emballonurid,
392 molossids, mormoopid, phyllostomid, and vespertilionid bats from Costa Rica [14], for which
393 variation among conspecific hosts was high. This suggests that studies relying on a few samples
394 per host species [9, 13] do not accurately capture variation in microbiome biodiversity or
395 composition that naturally occurs within populations. Consequently, ecological conclusions
396 based on such small samples may not be reliable, as estimates of biodiversity may not be
397 accurate (especially richness) and statistical power to detect differences in any metric would be
398 quite low.

399 Greater microbiome biodiversity in a host species could arise in two ways: 1) an increase
400 in the number of Phyla or Classes of microbes found in the microbiome, or 2) an increase in the
401 number of ASVs that belong to the same Phyla or Classes of microbes (i.e. not an increase in
402 higher level taxonomic biodiversity). For both oral and rectal microbiomes, the latter scenario
403 occurred. Host species with greater microbiome biodiversity (e.g. *P. parnellii*, *P. quadridens*, *M.*
404 *redmani*) typically harbored more ASVs belonging to the same Phyla as those present in hosts
405 with low microbiomes biodiversity. Archaea richness and Bacteria richness at the host species
406 level (i.e. data combined for all hosts belonging to the same species) were highly correlated (oral,
407 $R = 0.928$, $P < 0.001$; rectal, $R = 0.690$; $P = 0.027$). Similarly, pairwise correlations between
408 richness values of different Phyla at the host species level indicate positive associations
409 predominate (i.e. an increase in microbiome richness is associated with an increase in richness
410 for most of the Phyla present). In oral microbiomes, 70% of pairwise correlations of Phylum
411 relative abundances at the host species level were strongly positive ($R > 0.50$), and in rectal
412 microbiomes 56% of pairwise correlations of Phylum abundances at the host species level were

413 strongly positive (R > 0.50). Such correlations also characterize vespertilionid, rhinolophid, and
414 miniopterid bats from Slovenia [51].
415
416 **Effects of host sex**
417 Host sex could affect microbiome biodiversity of bat hosts due to differences in social
418 organization or diet. Harems, comprising several adult females with 1 adult male, are common
419 social structures for noctilionid [52] and phyllostomid bats [53], whereas maternity colonies,
420 comprising adult females and their offspring, are common in mormoopid [54] and vespertilionid
421 [55] bats. In contrast, most adult males are solitary in both of these social systems. In addition,
422 the diets of male and female bats differ during some seasons, especially during periods of
423 pregnancy and lactation when females target food sources that are higher in energy and protein
424 [56,57]. Despite sampling during the reproductive season, when these sex-based ecological
425 differences manifest most strongly, we found little evidence of differences between sexes based
426 on oral or rectal microbiome biodiversity (Table 2). When evidence of differences in microbiome
427 biodiversity did manifest (i.e. in oral microbiomes of *B. cavernarum* and rectal microbiomes of
428 *A. jamaicensis*), those differences were in the relative abundances of the ASVs (diversity,
429 evenness, or dominance) in the microbiomes and not in the number of ASVs (richness). Fecal
430 microbiomes from 12 species of vespertilionid bat from Slovenia failed to reveal differences
431 between the sexes [51].
432
433 **Effects of geographical location**
434 Despite the potential for environmental factors (e.g. roost environment, abundance and diversity
435 of hosts in the roost) to affect oral microbiome biodiversity, only *A. jamaicensis* exhibited site-

436 specific differences in oral microbiome biodiversity (Table 2; Figs 2 & 3). These differences
437 may be related to population size or to host species diversity in associated roosts. Oral
438 microbiomes from *A. jamaicensis* in Río Encantado had the greatest biodiversity, whereas those
439 from Mata de Plátano (Larva Cave) had the lowest biodiversity. The population of *A.*
440 *jamaicensis* at Río Encantado was greater than at other locations, and especially compared to
441 Mata de Plátano. Moreover, the number of bats and bat species was much greater at other caves
442 than at Larva, where *A. jamaicensis* roosts at Mata de Plátano. Of course, populations sizes
443 differed among sites for other host species without significant differences in oral microbiome
444 biodiversity. This suggests that host abundance may not be the major factor determining oral
445 microbiome biodiversity. In general, intraspecific variation in oral microbiome composition and
446 biodiversity is high and may rival interspecific variation.
447 Rectal microbiomes of each host species exhibited site-specific variation in biodiversity
448 (Table 2; Figs 2 & 3). In *A. jamaicensis*, rectal microbiomes exhibited patterns similar to those
449 observed for oral microbiomes, with greater biodiversity associated with larger populations from
450 roosts with greater bat species richness. In contrast, rectal microbiomes from *E. sezekorni* and *P.*
451 *quadridens* exhibited greater biodiversity from Río Encantado than from Mata de Plátano, with
452 the former harboring fewer individuals than the latter. Host abundance or biodiversity may not
453 have direct effects on microbiome biodiversity, but may serve as proxies for important ecological
454 factors. For example, bat abundance or diversity may be related to the diversity or abundance of
455 dietary items or habitat types used by resident bats, and the diversity of diet or habitat may
456 influence spatial patterns of microbiome biodiversity. Alternatively, microbiome biodiversity
457 within sites may represent legacies or factors such as the effects of hurricane-induced
458 disturbances on bat populations and communities [39]. Although confident identification of

459 causal mechanisms that drive spatial variation in microbiome biodiversity is challenging and
460 beyond the scope of this study, our results strongly suggest that spatial variation must be
461 considered when evaluating aspects of microbiome biodiversity, especially for rectal
462 microbiomes.
463
464 **Effects of host species or guild on biodiversity of oral microbiomes**
465 Within each host guild, species-specific differences characterized biodiversity of oral
466 microbiomes. In contrast, guild-specific differences did not characterize oral microbiomes (Table
467 3). This combination of results indicates that oral microbiome biodiversity is unrelated to host
468 diet for Puerto Rican bats. For carnivores, nearly all pairwise comparisons of oral microbiome
469 biodiversity between host species were significant (Table S1), suggesting distinct oral
470 microbiome biodiversity for each carnivorous species. In contrast, pairwise differences in oral
471 microbiome biodiversity among herbivorous bat species were primarily driven by differences
472 between *M. redmani* (most diverse oral microbiome) and other herbivores.
473 Patterns of oral microbiome biodiversity may be structured by processes similar to those
474 of microbiomes from other mucosal surfaces (e.g. nose, mouth, vagina, lungs, gastrointestinal
475 tract). The microbiome of the mucosal lining of the intestines directly interacts with the host
476 immune system through receptors in the intestinal epithelia [58]. The direct sampling of the
477 intestinal mucosa showed a strong relationship between intestinal microbiome composition and
478 host phylogeny in Belizean bats [14]. The species-specific biodiversity observed for oral
479 microbiomes within each guild of bats in Puerto Rico likely represents a similar co-evolutionary
480 association between hosts and their microbiomes. The carnivores represent 3 families of bats
481 (Mormoopidae, Vespertilionidae, and Noctilionidae), which likely contribute to the

482 preponderance of significant pairwise differences in the biodiversity of oral microbiomes. In
483 contrast, the lower frequency of pairwise differences in oral microbiome biodiversity among
484 herbivorous species likely arises because they represent a single family (Phyllostomidae) of bats.
485
486 **Effects of host species or guild on biodiversity of rectal microbiomes**
487 Species-specific differences with host guilds exhibited 2 patterns: (1) species-specific differences
488 were more consistent at lower sequencing depths than at greater sequencing depths and (2)
489 species-specific differences were observed more consistently between species of herbivore than
490 between species of carnivore (Table 3). In contrast, consistent differences in biodiversity
491 occurred between the rectal microbiomes of carnivorous and herbivorous foraging guilds (Table
492 3). In concert, these results suggest that the biodiversity of rectal microbiomes is related to host
493 diet. Regardless of metric, the biodiversity of rectal microbiomes of carnivorous bats (mostly
494 insectivores) were nearly twice as great as those from herbivorous (mostly frugivores) bats
495 (Table 1; Fig. 5). Importantly, the lack of species-specific differences in biodiversity within host
496 foraging guilds in some cases does not suggest that the composition of rectal microbiomes does
497 not differ among species within a guild. Indeed, microbe composition may differ among host
498 species within a guild, with different microbe taxa performing the same function in different host
499 species. However, the number of microbial taxa that a host supports may be contingent on the
500 general diet of the host species (i.e. the number and kinds of functions a host requires of its
501 microbiome). This is consistent with findings from a soil and plant microbiome assembly
502 experiment in which metacommunities contained fixed fractions of coexisting families that were
503 determined by the available carbon source [59]. Despite consistent higher level (Familial)

504 structure, these assembled microbiomes exhibited great variation in taxonomic composition with
505 the same functions performed in each microbiome but done so by different confamilial taxa.
506 Microbiomes associated with the digestive system from insectivorous bats are more
507 biodiverse than their herbivorous counterparts in Guatemala [9], Mexico [13], and Puerto Rico
508 (Tables 1 and 3). Greater microbiome biodiversity in carnivorous bats contrasts with theory
509 based on the study of a wide array of mammals (e.g. ruminants, primates, carnivores). Three
510 general predictions have been postulated [60]: (1) herbivorous hosts should have the most
511 complex gut morphologies and most diverse microbiomes; (2) carnivorous hosts should have the
512 most simple gut morphologies and the least biodiverse microbiomes; and (3) omnivorous hosts
513 should have intermediate levels of gut complexity and microbiome biodiversity. Regardless of
514 diet, all bats have shorter intestines and shorter food-retention times compared to similarly sized
515 non-volant mammals as an adaptation for flight [32, 61]. Nonetheless, herbivorous bats still have
516 slightly larger intestines than do carnivorous counterparts of similar size [62]. In contrast to non-
517 volant herbivorous mammals that feed primarily on leaves or grass, herbivorous bats generally
518 consume nectar and fruits that are poor sources of energy and nutrients, and that primarily
519 contain simple sugars and carbohydrates, resulting in brief retention times (i.e. < 60 minutes)
520 [63, 64]. Moreover, herbivorous bats rely on paracellular absorption for > 70% of their glucose
521 absorption, which may explain why these bats have relatively depauperate rectal microbiomes
522 [32, 65]. In contrast, the high protein, lipid, and nutrient content of insectivorous diets may result
523 in high microbiome biodiversity due to the variety of carbon and energy sources available [13].
524

525 **Conclusions**

526 High variation in microbiome diversity among individuals of the same species suggests that
527 individual-level host traits may affect the associated microbiome. Although initial, descriptive
528 studies may provide new insights from few samples per host species, research designed to
529 explore the ecological dynamics of microbiomes should account for such variation by increasing
530 the number of samples collected from host populations. Despite effects of host ecology and
531 evolutionary history on microbiome biodiversity, microbiome composition and biodiversity are
532 also affected by spatial phenomena, primarily via host-environment interactions. Future work
533 should investigate the roles of environmental factors that mediate microbiome biodiversity to
534 decouple these effects from those associated with host ecology and evolution.
535
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543
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707

Figure Legends

Fig. 1 Hierarchical design of statistical analyses. Yellow shapes indicate analyses: circle, General Linear Mixed-Effects Model (GLMM); square, Analysis of Variance (ANOVA). Numbers indicate particular statistical analyses that compare groups: 1, guilds; 2 herbivorous species; 3, carnivorous species; and 4-11, combinations of sex and cave or only sex for each species. Numbers in parentheses equal the number of treatment levels in a factor. Abbreviations are: Arja, *Artibeus jamaicensis*; Brca, *Brachyphylla cavernarum*; Erses, *Erophylla sezekorni*; More, *Monophyllus redmani*; Stru, *Stenoderma rufum*; Mobl, *Mormoops blainvillii*; Ptpa, *Pteronotus parnellii*; Ptqu, *Pteronotus quadridens*; Nole, *Noctilio leporinus*; and Epfu, *Eptesicus fuscus*. Only 1 sample was collected from *S.rufum*; therefore, this species was omitted from the interspecific comparison within the herbivore guild.

Fig. 2 Map of the Caribbean showing the location of Puerto Rico within the Antilles as well as the location of the three collection localities in Puerto Rico.

Fig. 3 Aspects of biodiversity (richness, black bars; Shannon diversity, dark gray bars; Camargo's evenness, light gray bars; Berger-Parker dominance, white bars) expressed as Hill numbers based separately on oral and rectal microbiomes for *Artibeus jamaicensis* at each of three sites (i.e. Aguas Buenas, Mata de Plátano, and Río Encantado) at sequencing depths of 1,000, 5,000, or 10,000 reads. In general, metrics of biodiversity for oral microbiomes were least at Mata de Plátano compared to other sites. For rectal microbiomes, only richness differed among sites, with Río Encantado exhibiting the greatest biodiversity. See Table 1 for details.

Fig. 4 Aspects of biodiversity (richness, black bars; Shannon diversity, dark gray bars; Camargo's evenness, light gray bars; Berger-Parker dominance, white bars) expressed as Hill numbers based separately on oral and rectal microbiomes for *Brachyphylla cavernarum*,

731 *Erophylla sezekorni*, and *Pteronotus quadridens* from each of two sites (i.e. Mata de Plátano and
732 Río Encantado) at sequencing depths of 1,000, 5,000, or 10,000 reads. No significant differences
733 between sites characterized the aspects of biodiversity of the oral microbiome, whereas aspects
734 of rectal microbiome biodiversity were generally greater at Río Encantado than at Mata de
735 Plátano. See Table 1 for details.

736 **Fig. 5** Aspects of biodiversity (richness, black bars; Shannon diversity, dark gray bars;
737 Camargo's evenness, light gray bars; Berger-Parker dominance, white bars) expressed as Hill
738 numbers based separately on oral and rectal microbiomes for carnivorous and herbivorous bats at
739 sequencing depths of 1,000, 5,000, or 10,000 reads. In general, metrics of biodiversity did not
740 differ between foraging guilds for the oral microbiome, whereas metrics of biodiversity were
741 significantly greater in carnivores than in herbivores for the rectal microbiome. See Table 1 for
742 details.

743

Table 1 Mean biodiversity of oral and rectal microbiomes for each of 10 bat species in Puerto Rico as well as for all bats in each of two foraging guilds (carnivores and herbivores) regardless of species. Biodiversity was quantified using each of four metrics based on Amplified Sequence Variants (richness, Shannon diversity, Camargo evennes, Berger-Parker dominance) and expressed as Hill numbers. Guild-level values are bold

Foraging guild Family	Richness		Shanon diversity		Carmargo evenness		B-P dominance	
Species (oral, rectal sample sizes)	Oral	Rectal	Oral	Rectal	Oral	Rectal	Oral	Rectal
	1,000 reads							
Carnivores	**47.80**	**66.70**	**14.18**	**21.95**	**13.66**	**20.19**	**3.28**	**4.65**
Mormoopidae								
Mormoops blainvillii (2, 6)	60.00	35.17	6.55	11.14	10.34	9.53	1.57	3.46
Pteronotus parnellii (7, 11)	106.29	91.36	31.00	24.66	31.55	24.80	5.43	4.40
Pteronotus quadridens (8, 8)	78.63	92.63	26.91	44.65	26.67	37.73	5.13	8.76
Noctilionidae								
Noctilio leporinus (12, 11)	24.67	51.64	7.00	15.92	5.55	13.87	2.74	4.49
Vespertilionidae								
Eptesicus fuscus (11, 10)	11.18	54.30	2.75	13.94	2.26	14.43	1.48	2.54
Herbivores	**32.93**	**31.17**	**10.57**	**7.01**	**8.94**	**6.83**	**3.17**	**2.20**
Phyllostomidae								
Artibeus jamaicensis (51, 61)	24.90	22.25	7.81	4.36	6.29	4.12	2.92	1.80
Brachyphylla cavernarum (20, 19)	29.35	41.47	8.38	8.62	6.72	8.15	3.04	2.63
Erophylla sezekorni (10, 26)	52.20	32.65	18.09	7.98	15.59	8.17	4.31	2.23
Monophyllus redmani (14, 4)	54.57	88.50	18.86	29.94	17.38	28.84	3.54	5.61
Stenoderma rufum (1, 1)	18.00	112.00	4.52	20.93	3.51	24.89	1.96	3.76

744

745

Table 1 Continued

Foraging guild / Family / Species (oral, rectal sample sizes)	Richness		Shanon diversity		Carmargo evenness		B-P dominance	
	Oral	Rectal	Oral	Rectal	Oral	Rectal	Oral	Rectal
	5,000 reads							
Carnivores	**61.62**	**122.93**	**11.39**	**24.98**	**12.25**	**25.63**	**2.67**	**4.60**
Mormoopidae								
Mormoops blainvillii (0, 2)	--	22.50	--	1.94	--	1.90	--	1.16
Pteronotus parnellii (5, 9)	200.40	170.00	40.05	29.45	44.64	32.72	4.60	4.72
Pteronotus quadridens (2, 1)	180.93	329.00	34.64	149.16	40.40	123.14	5.08	25.51
Noctilionidae								
Noctilio leporinus (11, 8)	33.00	96.25	5.91	18.96	4.79	18.24	2.42	4.83
Vespertilionidae								
Eptesicus fuscus (11, 9)	16.09	99.00	2.77	17.19	2.31	19.55	1.48	2.70
Herbivores	**43.59**	**49.03**	**9.68**	**7.03**	**8.44**	**7.62**	**2.94**	**2.11**
Phyllostomidae								
Artibeus jamaicensis (46, 53)	32.61	38.98	8.24	6.13	6.69	7.13	2.98	1.62
Brachyphylla cavernarum (18, 14)	46.17	75.29	8.63	9.08	7.07	10.10	3.10	2.52
Erophylla sezekorni (8, 24)	57.75	48.71	5.28	9.38	6.16	10.53	1.81	2.28
Monophyllus redmani (9, 1)	82.89	86.00	23.67	24.37	22.69	23.84	3.46	4.66
Stenoderma rufum (1, 1)	35.00	185.00	4.63	24.41	3.69	30.04	2.94	4.01

Table 1 Continued

Foraging guild / Family / Species (oral, rectal sample sizes)	Richness		Shanon diversity		Carmargo evenness		B-P dominance	
	Oral	Rectal	Oral	Rectal	Oral	Rectal	Oral	Rectal
	10,000 reads							
Carnivores	**66.62**	**153.24**	**11.04**	**22.02**	**12.20**	**25.12**	**2.64**	**3.70**
Mormoopidae								
Mormoops blainvillii (0, 1)	--	28.00	--	2.59	--	2.34	--	1.28
Pteronotus parnellii (1, 7)	231.75	225.43	45.76	30.80	50.68	36.56	6.42	4.47
Pteronotus quadridens (4, 0)	208.00	--	10.79	--	38.08	--	1.56	--
Noctilionidae								
Noctilio leporinus (11, 6)	36.27	113.17	5.86	15.45	4.76	16.18	2.39	4.09
Vespertilionidae								
Eptesicus fuscus (10, 7)	19.80	133.29	2.88	21.63	2.41	24.60	1.51	2.95
Herbivores	**42.29**	**57.35**	**7.43**	**7.14**	**6.35**	**7.89**	**2.71**	**2.11**
Phyllostomidae								
Artibeus jamaicensis (40, 49)	33.48	46.92	7.74	4.95	6.33	5.18	2.85	1.86
Brachyphylla cavernarum (17, 12)	52.76	95.58	8.73	10.08	7.17	11.38	3.05	2.70
Erophylla sezekorni (7, 23)	66.86	53.43	5.24	9.56	6.60	10.89	1.75	2.25
Monophyllus redmani (3, 0)	45.33	--	2.00	--	2.25	--	1.43	--
Stenoderma rufum (1)	36.00	200.00	4.62	23.55	3.66	29.52	2.01	4.07

Table 2 Results (*P* -values) of 1-way generalized linear mixed-effects models (for analyses of host sex only with site as a model II treatment factor) or 2-way analyses of variance with type II sums of squares (for analyses of site and host sex) evaluating the effects of site or host sex on microbiome biodiversity. Analyses were conducted separately for each combination biodiversity metric, sample type (oral or rectal), and sequencing depth. Significant results ($P \leq 0.05$) are bold

Host species	Oral microbiomes			Rectal microbiomes		
Sequence depth / Biodiversity index	Site	Sex	Site x sex	Site	Sex	Site x sex
Mormoops blainvillii						
1,000						
Richness	--	--	--	--	**0.001**	--
Shannon diversity	--	--	--	--	0.053	--
Camargo evenness	--	--	--	--	**0.020**	--
B-P dominance	--	--	--	--	0.281	--
Pteronotus parnellii						
1,000						
Richness	--	0.368	--	--	0.682	--
Shannon diversity	--	0.359	--	--	0.816	--
Camargo evenness	--	0.397	--	--	0.939	--
B-P dominance	--	0.443	--	--	0.648	--
5,000						
Richness	--	0.561	--	--	0.852	--
Shannon diversity	--	0.714	--	--	0.815	--
Camargo evenness	--	0.709	--	--	0.668	--
B-P dominance	--	0.586	--	--	0.953	--
10,000						
Richness	--	--	--	--	0.599	--
Shannon diversity	--	--	--	--	0.897	--
Camargo evenness	--	--	--	--	0.873	--
B-P dominance	--	--	--	--	0.867	--
Pteronotus quadridens						
1,000						
Richness	--	0.097	--	**0.002**	0.418	0.059
Shannon diversity	--	0.723	--	**0.001**	**0.041**	0.135
Camargo evenness	--	0.492	--	**0.001**	0.056	0.070
B-P dominance	--	0.574	--	**0.013**	0.099	0.443

Table 2 Continued

Host species	Oral microbiomes			Rectal microbiomes		
Sequence depth / Biodiversity index	Site	Sex	Site x sex	Site	Sex	Site x sex
Noctilio leporinus						
1,000						
Richness	--	0.395	--	--	0.264	--
Shannon diversity	--	0.538	--	--	0.980	--
Camargo evenness	--	0.461	--	--	0.734	--
B-P dominance	--	0.911	--	--	0.876	--
5,000						
Richness	--	0.709	--	--	0.519	--
Shannon diversity	--	0.713	--	--	0.945	--
Camargo evenness	--	0.800	--	--	0.945	--
B-P dominance	--	0.487	--	--	0.902	--
10,000						
Richness	--	0.726	--	--	--	--
Shannon diversity	--	0.746	--	--	--	--
Camargo evenness	--	0.926	--	--	--	--
B-P dominance	--	0.316	--	--	--	--
Artibeus jamacensis						
1,000						
Richness	**0.049**	0.697	0.161	**0.045**	0.293	0.926
Shannon diversity	**0.001**	0.947	0.146	0.476	**0.030**	0.704
Camargo evenness	**0.004**	0.996	0.109	0.246	0.103	0.926
B-P dominance	**0.006**	0.714	0.982	0.966	**0.012**	0.246
5,000						
Richness	0.169	0.406	0.113	**0.009**	0.788	0.999
Shannon diversity	**0.007**	0.965	0.150	0.319	**0.036**	0.817
Camargo evenness	**0.022**	0.903	0.118	0.141	0.140	0.712
B-P dominance	**0.012**	0.724	0.970	0.845	**0.007**	0.624
10,000						
Richness	0.364	0.211	0.205	**0.003**	0.741	0.951
Shannon diversity	**0.016**	0.525	0.254	0.230	**0.034**	0.735
Camargo evenness	0.065	0.454	0.214	0.098	0.139	0.695
B-P dominance	**0.016**	0.922	0.911	0.725	**0.007**	0.458

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Table 2 Continued

Host species	Oral microbiomes			Rectal microbiomes		
Sequence depth						
Biodiversity index	Site	Sex	Site x sex	Site	Sex	Site x sex
Brachyphylla cavernarum						
1,000						
Richness	0.386	0.512	0.913	--	0.576	--
Shannon diversity	0.436	**0.027**	0.582	--	0.881	--
Camargo evenness	0.239	**0.041**	0.777	--	0.583	--
B-P dominance	0.694	**0.028**	0.536	--	0.259	--
5,000						
Richness	0.824	0.858	0.781	--	0.579	--
Shannon diversity	0.409	**0.014**	0.755	--	0.794	--
Camargo evenness	0.213	**0.028**	0.990	--	0.561	--
B-P dominance	0.770	**0.031**	0.616	--	0.440	--
10,000						
Richness	0.432	0.395	0.519	--	0.453	--
Shannon diversity	0.352	**0.020**	0.759	--	0.653	--
Camargo evenness	0.182	**0.041**	0.939	--	0.476	--
B-P dominance	0.651	**0.018**	0.827	--	0.388	--
Erophylla sezekorni						
1,000						
Richness	0.167	0.674	0.744	0.074	0.248	0.098
Shannon diversity	0.073	0.890	0.754	0.099	0.270	0.227
Camargo evenness	0.072	0.970	0.812	0.066	0.251	0.151
B-P dominance	0.130	0.598	0.429	**0.045**	0.107	0.263
5,000						
Richness	--	0.392	--	0.078	0.194	0.093
Shannon diversity	--	0.555	--	0.103	0.273	0.207
Camargo evenness	--	0.345	--	0.057	0.225	0.124
B-P dominance	--	0.821	--	0.071	0.130	0.286
10,000						
Richness	--	0.338	--	**0.030**	0.102	**0.025**
Shannon diversity	--	0.696	--	0.065	0.211	0.120
Camargo evenness	--	0.325	--	**0.028**	0.152	0.055
B-P dominance	--	0.495	--	0.061	0.122	0.222

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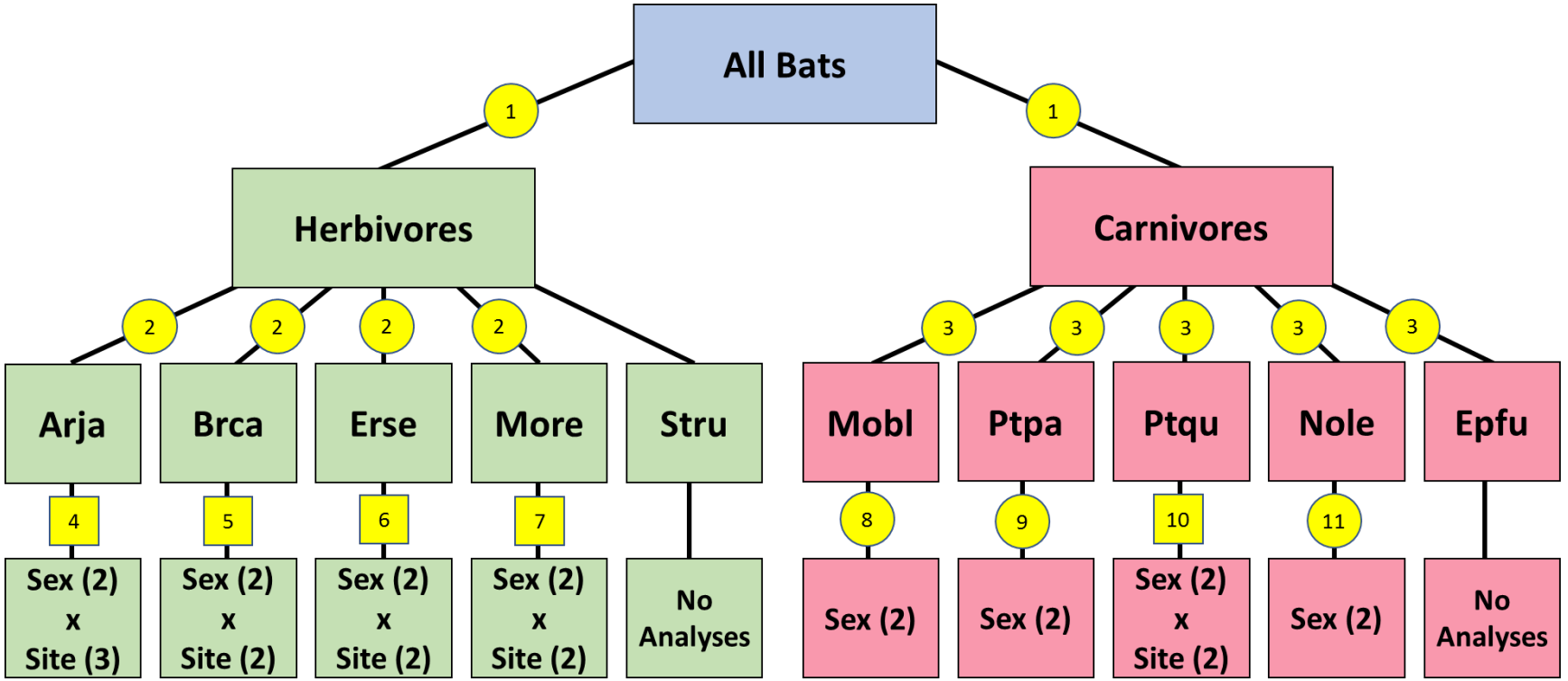
Table 2 Continued

Host species	Oral microbiomes			Rectal microbiomes		
Sequence depth / Biodiversity index	Site	Sex	Site x sex	Site	Sex	Site x sex
Monophyllus redmani						
1,000						
Richness	0.059	0.782	0.700	--	--	--
Shannon diversity	0.088	0.448	0.467	--	--	--
Camargo evenness	0.085	0.540	0.555	--	--	--
B-P dominance	2.957	0.546	0.564	--	--	--
5,000						
Richness	0.113	0.969	0.841	--	--	--
Shannon diversity	0.164	0.707	0.735	--	--	--
Camargo evenness	0.160	0.824	0.829	--	--	--
B-P dominance	0.193	0.570	0.689	--	--	--
10,000						
Richness	--	--	--	--	0.504	--
Shannon diversity	--	--	--	--	0.493	--
Camargo evenness	--	--	--	--	0.107	--
B-P dominance	--	--	--	--	0.681	--

Table 3 Results (*P*-values) of general linear mixed-effects models evaluating the effect of host species or host guild on microbiome biodiversity. Effect of host species was evaluated separately for each guild. Species and guild were model 1 treatment factors (i.e. fixed effects) and cave was a model II treatment factor (i.e. random effects). Analyses were conducted separately for each combination of biodiversity metric, sample type (oral or rectal), and sequencing depth. Significant results ($P \leq 0.05$) are bold

Sequencing depth	Comparison of species within guilds				Comparison between guilds	
	Oral microbiome		Rectal microbiome			
Biodiversity index	Carnivores	Herbivores	Carnivores	Herbivores	Oral microbiome	Rectal microbiome
1,000						
Species richness	**< 0.001**	**0.005**	0.116	**< 0.001**	0.061	**< 0.001**
Shannon diversity	**< 0.001**	**0.048**	**0.009**	**< 0.001**	0.296	**< 0.001**
Camargo evenness	**< 0.001**	**0.016**	**0.029**	**< 0.001**	0.120	**< 0.001**
B-P dominance	**0.002**	0.567	**0.006**	**< 0.001**	0.832	**< 0.001**
5,000						
Species richness	**< 0.001**	**0.008**	0.143	**0.011**	0.188	**< 0.001**
Shannon diversity	**< 0.001**	**0.005**	**< 0.001**	0.064	0.691	**< 0.001**
Camargo evenness	**< 0.001**	**0.003**	**0.009**	0.073	0.343	**< 0.001**
B-P dominance	**< 0.001**	0.122	**< 0.001**	**0.024**	0.367	**< 0.001**
10,000						
Species richness	**< 0.001**	**0.034**	0.363	**0.005**	0.094	**< 0.001**
Shannon diversity	**< 0.001**	**0.015**	0.589	0.095	0.829	**< 0.001**
Camargo evenness	**< 0.001**	0.205	0.534	0.075	0.266	**< 0.001**
B-P dominance	**< 0.001**	**0.025**	0.575	**0.049**	0.093	**< 0.001**

Figure 1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Carnivores
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3
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Arja
Brca
Erse
More
Stru
Mobl
Ptpa
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Sex (2) x Site (3)
Sex (2) x Site (2)
Sex (2) x Site (2)
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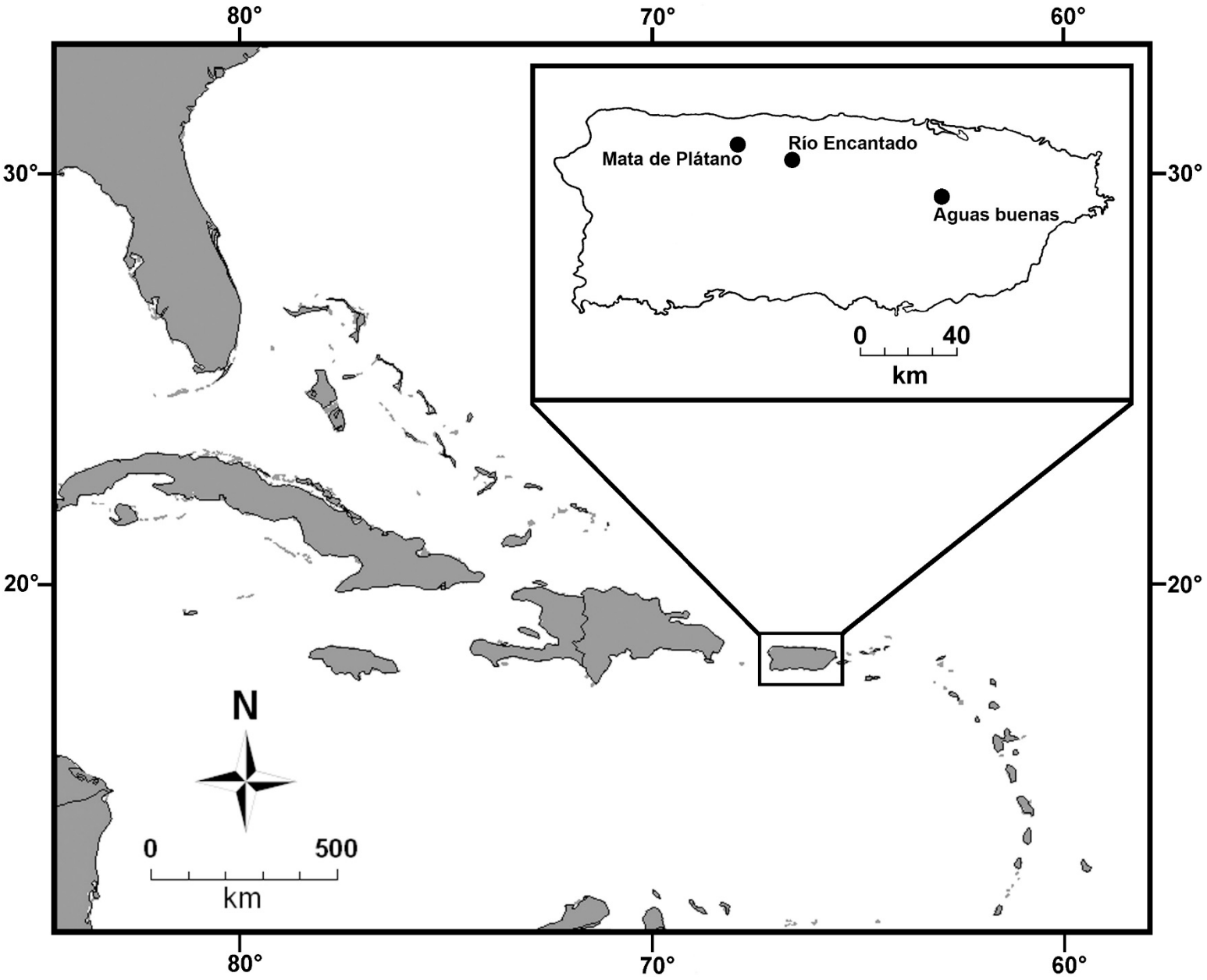
758

Figure 2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70°
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Mata de Plátano
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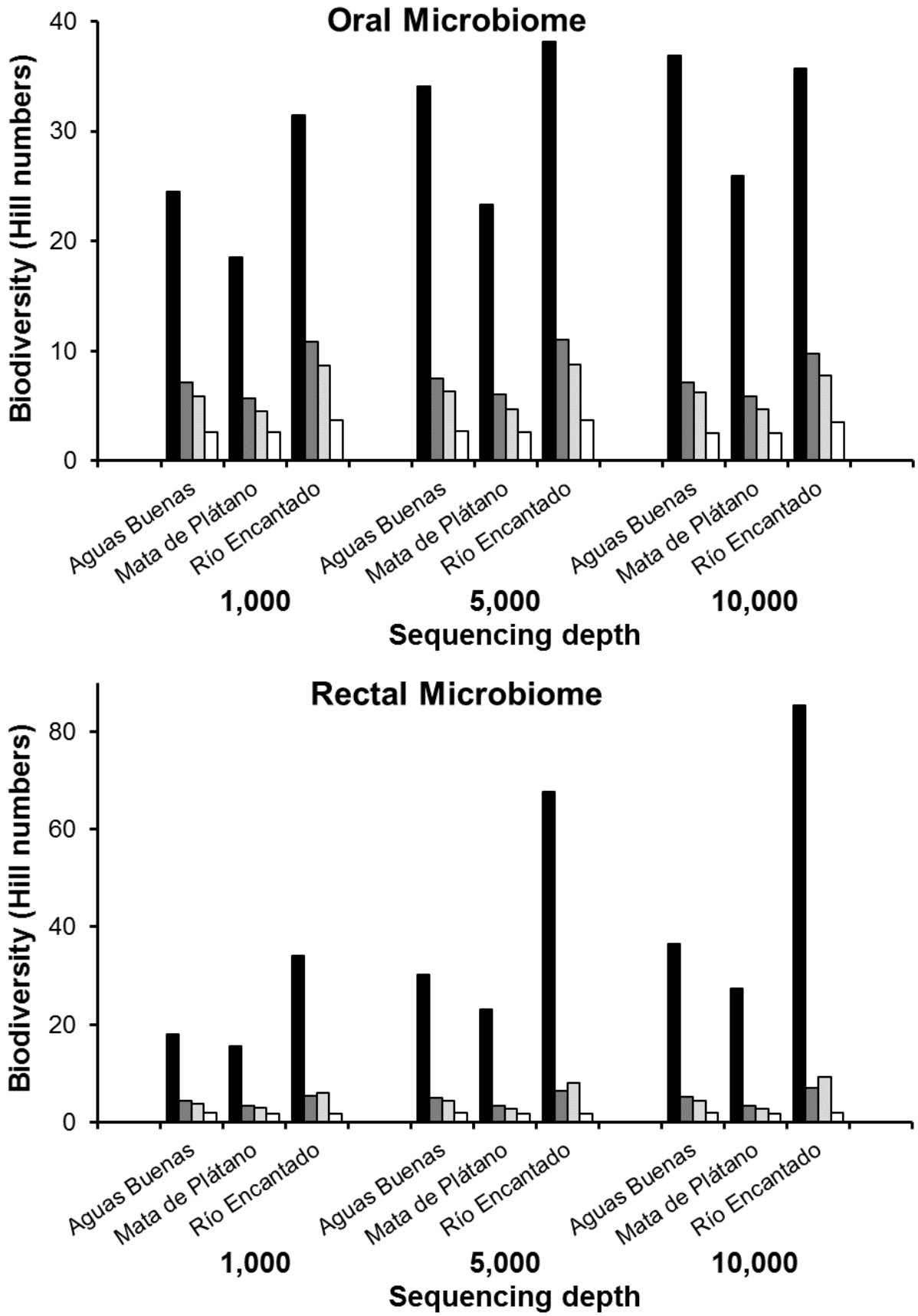
Figure 3



760

Figure 4

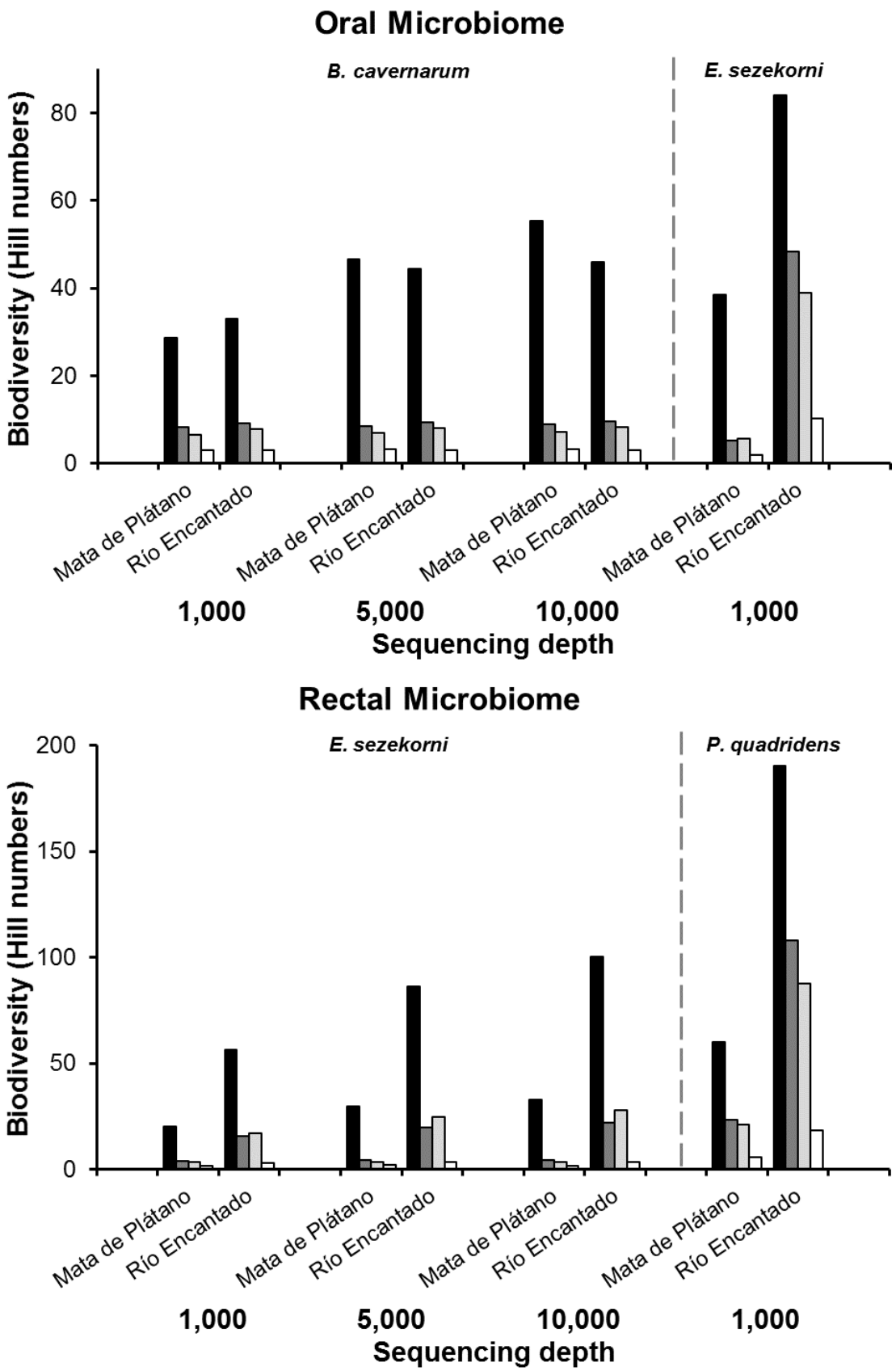


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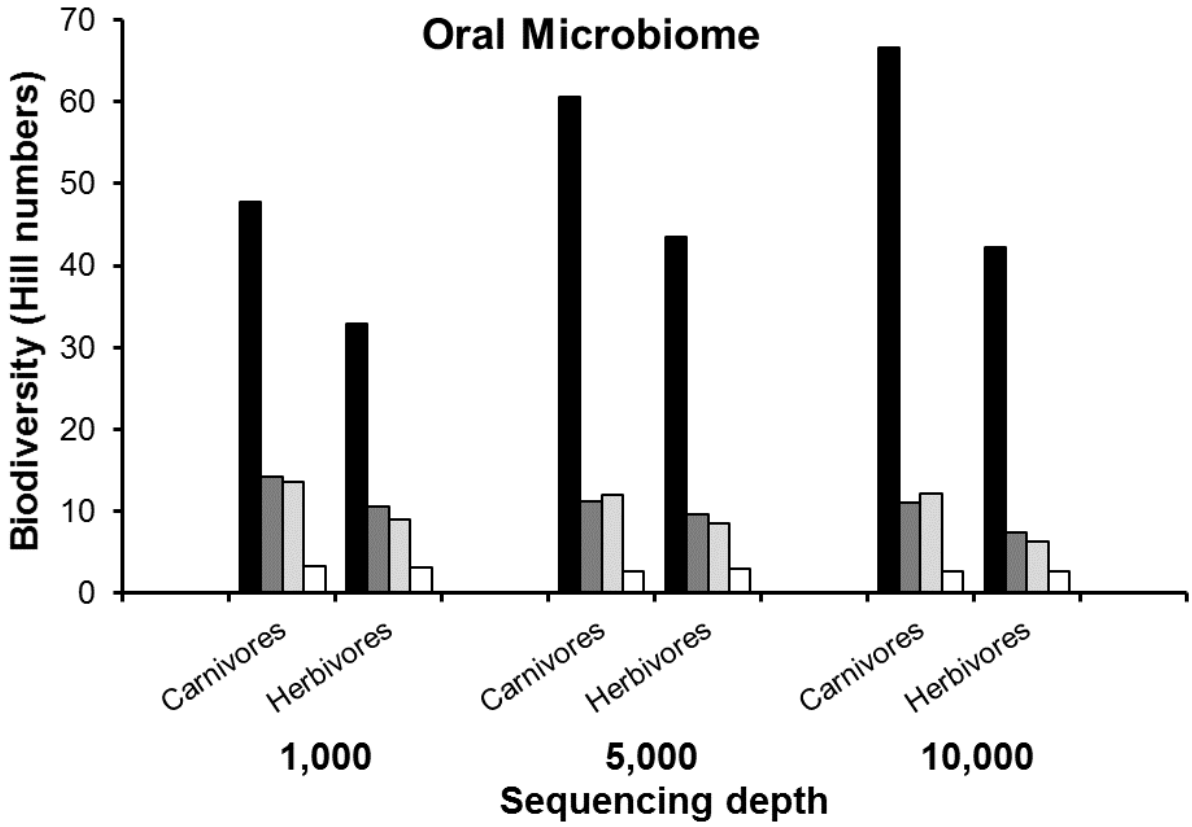
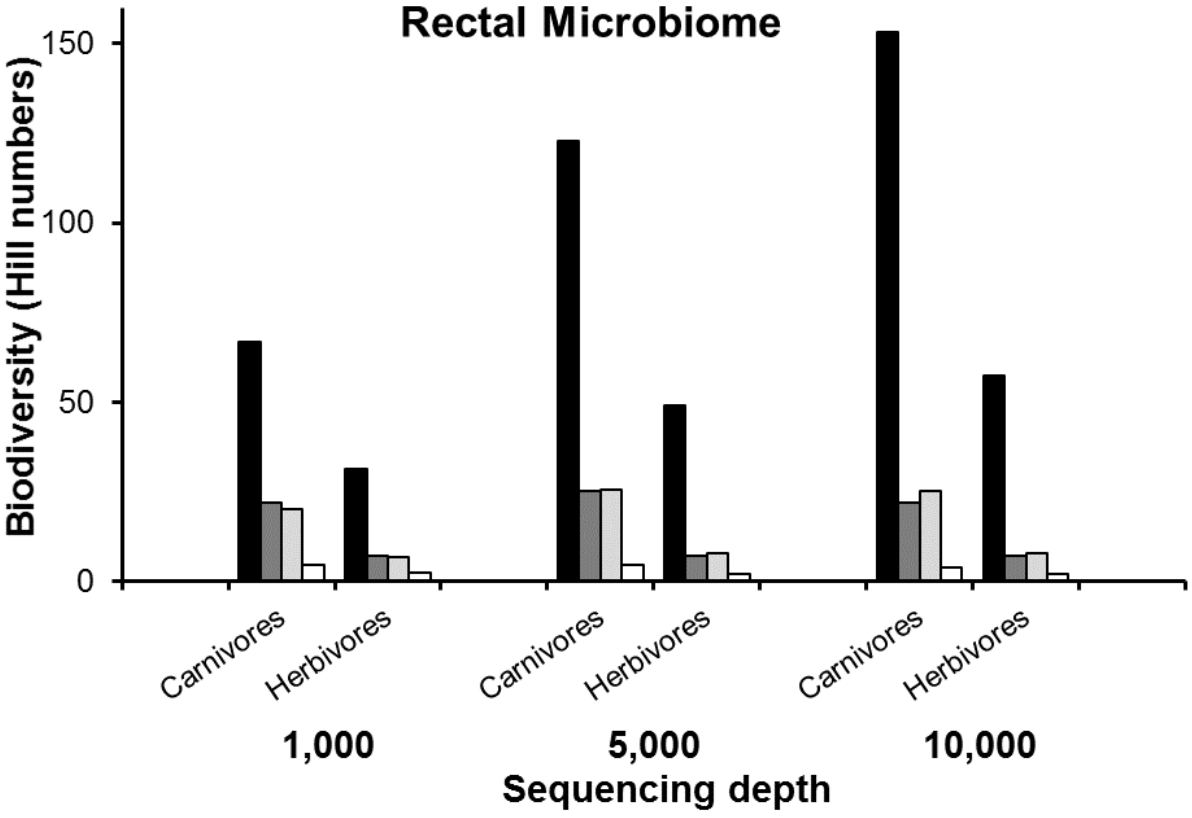
Figure 5





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