

# Gastrointestinal symbiont diversity in wild gorilla: A comparison of bacterial and strongylid communities across multiple localities

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## Abstract

Western lowland gorillas (*Gorilla gorilla gorilla*) are Critically Endangered and show continued population decline. Consequently, pressure is mounting to better understand their conservation threats and ecology. Gastrointestinal symbionts, such as bacterial and eukaryotic communities, are believed to play vital roles in the physiological landscape of the host. Gorillas host a broad spectrum of eukaryotes, so called parasites, with strongylid nematodes being particularly prevalent. While these communities are partially consistent, they are also shaped by various ecological factors, such as diet or habitat type. To investigate gastrointestinal symbionts of wild western lowland gorillas, we analysed 215 faecal samples from individuals in five distinct localities across the Congo Basin, using high-throughput sequencing techniques. We describe the gut bacterial microbiome and genetic diversity of strongylid communities, including strain-level identification of amplicon sequence variants (ASVs). We identified strongylid ASVs from eight genera and bacterial ASVs from 20 phyla. We compared

these communities across localities, with reference to varying environmental factors among populations, finding differences in alpha diversity and community compositions of both gastrointestinal components. Moreover, we also investigated covariation between strongylid nematodes and the bacterial microbiome, finding correlations between strongylid taxa and *Prevotellaceae* and *Rikenellaceae* ASVs that were consistent across multiple localities. Our research highlights the complexity of the bacterial microbiome and strongylid communities in several gorilla populations and emphasizes potential interactions between these two symbiont communities. This study provides a framework for ongoing research into strongylid nematode diversity, and their interactions with the bacterial microbiome, among great apes.

**KEY WORDS**

bacterial microbiome, gorilla, helminth, non-human primates, Strongylida

## 1 | INTRODUCTION

The Critically Endangered western lowland gorilla (*Gorilla gorilla gorilla*) shows continued population decline (Plumptre et al., 2008), primarily threatened by habitat destruction, human disturbance and hunting (Breuer et al., 2018; Plumptre et al., 2008; Strindberg et al., 2018). As large terrestrial herbivores they are keystone species for their ecosystem, particularly for seed dispersal in the tropical forests they inhabit (Petre et al., 2013; Petre et al., 2015). Additionally, western lowland gorillas are deemed important for their phylogenetically close relationship to humans (*Homo sapiens*), which increases the potential for pathogen transmission between these two species. This has been previously evidenced for both gastrointestinal bacteria (Rwego et al., 2008) and strongylid nematodes (Pafčo, Kreisinger, et al., 2019), one of the most common eukaryotic groups referred to as parasites among nonhuman primates (NHPs) (Pafčo et al., 2018).

The bacterial part of the gut microbiome (herein referred to as the bacterial microbiome) is important to host physiological functioning, playing critical roles in immune function, nutrition and development of the nervous system (Clayton, Gomez, et al., 2018; Kau et al., 2011; West et al., 2019). As a result, differences in gut bacterial microbiome communities, such as those resulting from individual differences (Pafčo, Sharma, et al., 2019), diet (Knight, 2015), stress (Vlčková et al., 2018) or antibiotics (Vlčková et al., 2016), may drastically impact host health (Kinross et al., 2011; Shreiner et al., 2015).

Similarly, strongylid infections are often asymptomatic in great apes but infections have been linked to clinical manifestation of disease (Cantacessi et al., 2012; Krief et al., 2008; Masi et al., 2012; Pit et al., 2001). Climatic changes, host density and host traits, such as sociality or body mass, are suspected to alter infections (Friant et al., 2016; Huffman et al., 1997; Petrželková et al., 2021). Consequently, ongoing habitat encroachment may increase the likelihood of clinical manifestations of parasite infections within gorillas, while gorillas also have potential to serve as zoonotic pathogen reservoirs for humans (Bittar et al., 2014; Raoult, 2012; Wolfe et al., 1998).

The gut bacterial microbiome of *Gorilla* spp. has been well explored in recent years, with a high diversity of consistent bacterial families commonly reported (Bittar et al., 2014; Campbell et al., 2020; Nishida & Ochman, 2019; Pafčo, Sharma, et al., 2019). The consistency of the gut microbiome within a host species, particularly with regard to alpha diversity, is shaped by evolutionary traits of ancestral host-microbial systems, whereas the beta diversity of the gut bacterial microbiome is more variable among hosts (Björk et al., 2019; Clayton, Gomez, et al., 2018). Compositional shifts of the gut bacterial microbiome occur in response to various environmental and host factors including diet, individual identity, sociality and habitat fragmentation (Amato et al., 2014; Gogarten et al., 2018; Moeller et al., 2016; Nagpal et al., 2018; Rudolph et al., 2022; Tung et al., 2015). The diversity of the gut bacterial microbiome composition is less known across gorilla distributions, with research on wild populations largely restricted to single locality studies. Recent evidence has suggested that geographical range is a significant discriminator of differences in the bacterial microbiome among wild gorillas (Gomez et al., 2015), suggesting that our knowledge of gorilla bacterial microbiomes may not be generalizable across wild populations.

Little is currently known about strongylid infections in gorillas. However, it is thought that strongylids occur in complex communities, including multiple species with differing rarity levels, as is typical within large terrestrial herbivores (Pafčo et al., 2018). Characteristically, epidemiological research of strongylids has focused on human and livestock infections (Cantacessi et al., 2012; Newton et al., 1998; Zajac, 2006); thus, our present understanding of strongylid communities in wildlife hosts is poor (Mclean et al., 2012; Walker & Morgan, 2014), particularly among NHPs (Krief et al., 2008; Pafčo, Kreisinger, et al., 2019). Although traditional coproscopic methods have confirmed a high prevalence of strongylid nematodes in gorillas (Pafčo et al., 2017; Sleeman et al., 2000), these methods are generally insufficient for species/genus-level identification. Previously implemented molecular methods, based on Sanger sequencing, have confirmed the occurrence of multiple strongylid species (namely of the genera *Necator* and *Oesophagostomum*) in

gorillas, including some shared with humans (Ghai et al., 2014; Hasegawa et al., 2014, 2017; Makouloutou et al., 2014). Yet, these methods typically rely on single larval specimens or species-specific primers (Pafčo et al., 2018), both of which are impractical for studying mixed DNA of entire communities.

Recent developments in high-throughput sequencing (HTS) have allowed complex communities of multiple species to be sequenced simultaneously, quickly and cost effectively, including detection of rare taxa (von Bubnoff, 2008). While both the bacterial microbiome and strongylid communities of lowland gorilla have been analysed through HTS methods (Hicks et al., 2018; Pafčo et al., 2018; Pafčo, Kreisinger, et al., 2019; Vlčková et al., 2016), their application is not widespread. Previously, the bacterial microbiome of lowland gorillas has been compared across populations and localities (Gomez et al., 2015; Moeller et al., 2013), yet no such comparison of their strongylid communities currently exists.

Furthermore, research into covariation and potential interactions between these two gastrointestinal components is only recently emerging (Kreisinger et al., 2015). The diversity of the bacterial microbiome has been shown to be both reduced (Holm et al., 2015; Houlden et al., 2015) and restored (Broadhurst et al., 2012) in the presence of helminth infections. While the exact mechanisms of how helminth infections may alter bacterial microbiome diversity are for the most part unknown (Filyk & Osborne, 2016), various theories have been suggested (Kreisinger et al., 2015). Helminths may compete for food resources, altering nutrient access of the bacterial microbiome, or secrete molecules that disrupt bacterial microbiome growth, such as mucus or antimicrobial peptides (Filyk & Osborne, 2016; Kreisinger et al., 2015). The resultant disruption to the bacterial microbiome can consequently influence host physiology. Existing evidence of bacterial microbiome–helminth interactions has primarily been founded on single-species, laboratory-based infections (Peachey et al., 2017), meaning these covariations are poorly characterized within wild populations (Kreisinger et al., 2015) where mixed infections of multiple helminth species occur (Pafčo et al., 2018). Helminth infection has partially explained bacterial microbiome variation in wild lemurs, though findings were not consistent when the studied populations were analysed separately (de Winter et al., 2020). In wild black howler monkeys (*Alouatta pigra*), differences in the community composition of gut bacteria were weakly associated with pinworm (*Trypanoxyuris*) infection, a gastrointestinal helminth, though a better understanding of the parasite-microbiome interplay was called for (Martínez-Mota et al., 2021).

Here, we use an HTS approach previously developed and optimized by our team (Pafčo et al., 2018) to analyse two components of gastrointestinal symbionts of wild western lowland gorillas. We relied on ITS-2 metabarcoding for strain-level profiling of strongylid communities, alongside 16S rRNA gene metabarcoding for generating gut bacterial profiles. We replicate and apply this technique to five geographically distinct gorilla populations (Loango National Park, Campo Ma'an National Park, Dja Faunal Reserve, Odzala-Kokoua National Park and Dzanga-Sangha Protected Areas), describing and comparing the diversity of the gastrointestinal bacterial

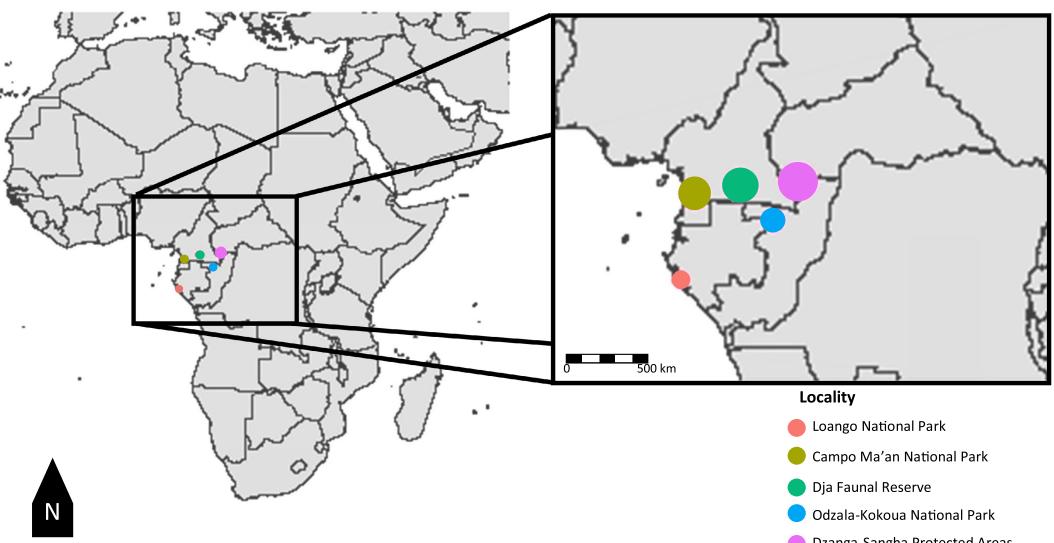
microbiome and strongylid communities across populations. We use this whole community approach to investigate cooccurrence between these two gastrointestinal components, examining species-specific covariation of strongylids and bacteria. We hypothesize that both the communities would show similarity across populations, with alpha diversity conserved through host-symbiont co-evolution. However, we predict compositional differences in the gut bacterial microbiome, more so than in strongylid communities, in response to environmental variation across localities. This leads us to a second hypothesis assuming that the localities with higher similarity in habitat type and thus environmental factors, such as Dja and Dzanga-Sangha, will show more similar gastrointestinal symbiont communities. Accordingly, we expect a more unique community composition of symbionts at Loango, the locality with the most distinct habitat type. Last, we hypothesize that geographically distant populations will show greater divergence of symbiont communities.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

A total of 215 individual faecal samples were collected from western lowland gorillas in five localities across four countries within the Congo Basin (Figure 1): Loango National Park, Gabon (N = 8); Campo Ma'an National Park, Cameroon (N = 46); periphery of Dja Faunal Reserve, Cameroon (N = 61); Odzala-Kokoua National Park, Republic of Congo (N = 20); and Dzanga-Sangha Protected Areas, Central African Republic, (N = 80). Sample collection occurred within protected areas at all localities, except in the case of Dja, where sampling occurred on the periphery of the national park and hence anthropogenic disturbance was more apparent. Localities show some habitat differences, with Loango a coastal lowland forest with savannah patches and extensive swamps (Head et al., 2012) and Campo Ma'an an evergreen forest (Matthews & Matthews, 2004). Dja and Dzanga-Sangha are both evergreen swamp forest (Dupain et al., 2004; Sak et al., 2013), while Odzala-Kokoua is characterized by evergreen forest with areas of forest-savannah mosaic (Molina-Vacas et al., 2020). Some environmental factors are consistent across localities, such as temperature, with averages ranging from 22 to 29°C (Dupain et al., 2004; Head et al., 2012; Mbenoun Masse et al., 2018; Medjibe et al., 2011; Molina-Vacas et al., 2020). All sites experience two wet seasons yearly, but average annual rainfall varies across localities as follows: Loango 2215 mm (Head et al., 2012), Campo Ma'an 2800 mm (Mbenoun Masse et al., 2018), Dja 1600 mm (Dupain et al., 2004), Odzala-Kokoua 1200 mm (Molina-Vacas et al., 2020) and Dzanga-Sangha 1400 mm (Medjibe et al., 2011). The described differences in habitat and climate among localities probably cause variation in other environmental factors, such as diet diversity and food availability, among surveyed gorilla populations, though empirical and comparable data for all are lacking.

Except for habituated gorilla groups in Dzanga-Sangha, all faecal samples were collected from gorilla night nests early in the morning



**FIGURE 1** Geographical locations of sampled western lowland gorillas, whereby marker size is scaled to the number of samples collected from that locality [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

(within 3 h from the time we suspected the gorillas had left the nests). One sample was collected per nest to ensure every sample corresponded to a unique but unidentified individual; if faeces of two distinct sizes were found at one nest it was assumed to be a mother with infant and two samples were taken. Faecal samples of habituated gorillas were collected after defecation when the gorillas relocated. The samples were immediately fixed in 96% ethanol and stored at environmental temperature before being shipped to the Department of Pathology and Parasitology at the University of Veterinary Sciences Brno, Czech Republic, where the samples were stored at  $-20^{\circ}\text{C}$  prior to DNA isolation.

## 2.2 | DNA isolation and sequencing

We extracted total genomic DNA from faecal samples using the PowerSoil DNA isolation kit (MO BIO Laboratories, Qiagen) under strict conditions to minimize contamination. For strongylid communities, we used the forward primer Strongyl\_ITS-2\_F (ACGTCTGGTTCAAGGTTG) and the reverse Strongyl\_ITS-2\_R (ATGCTTAAGTTCAGCGGGTA) for PCR (polymerase chain reaction) amplification of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA. We have previously shown low PCR bias and consistent community profiling of strongylid nematodes with these primers (Pafčo et al., 2018). For bacterial communities, we used the primer pair S-D-Bact-0341-b-S-17 (CCTACGGGNNGGCWGCA) and S-D-Bact-0785-a-A-21 (GACTACHVGGGTATCTAATCC) targeting the hypervariable V3 and V4 regions of 16S rDNA (Klindworth et al., 2013).

We used a two-step-PCR approach to generate HTS sequencing libraries, employing Fluidigm Access Array primer design (Pafčo

et al., 2018). We duplicated samples with different tag primer barcodes, providing two technical replicates for all analyses. We used nuclease-free water and DNA extracted from strongylid-negative human faeces as negative controls and synthetic DNA templates as positive controls (Pafčo et al., 2018). For bacteria we used nuclease-free water as a negative control. We sequenced the final library using the Illumina MiSeq platform, utilizing Miseq Reagent Kit version 2 ( $2 \times 250$ -bp pair-end reads, 500 cycles).

## 2.3 | Bioinformatics and data assembly

We trimmed gene-specific primers using SKEWER (Jiang et al., 2014) and assembled paired-end reads using PEAR version 0.9.6 (Zhang et al., 2014). We then eliminated low-quality (expected error rate  $>1$ ) reads and denoised the data set using DADA2 (Callahan et al., 2016) to identify strongylid and bacterial amplicon sequence variants (ASVs). To avoid inflation of the diversity by PCR/sequencing artefacts that were not corrected by DADA2, we considered only those ASVs that were consistently present in both technical duplicates for a given sample (Pafčo et al., 2018). We implemented the naive Bayesian RDP classifier (Wang et al., 2007) in the DADA2 pipeline to taxonomically assign ASVs. For strongylid ASVs, we constructed the reference sequence training data set based on strongylid ITS-2 sequences downloaded (February 10, 2020) from the NCBI nr/nt database (200 top BLASTN hits for each of our ASVs, environmental samples were excluded). For bacterial ASVs, our reference training data set was a 16S rRNA reference database downloaded from SILVA version 138 and formatted for DADA2 (McLaren & Callahan, 2021).

During filtering processes, we removed all unclassified ASVs, ITS-2 ASVs not assigned to Strongylida and 16S rRNA gene ASVs

not assigned at the phylum level. We then removed samples with no ASVs, excluding seven samples from the ITS-2 data set and three from the 16S rRNA gene data set.

## 2.4 | Statistical analysis

We first investigated variation of alpha diversity, independently for strongylid nematodes and bacterial microbiomes, through the number of different ASVs per sample. We then tested the effect of locality on ASV diversity using generalized linear models (GLMs) with quasipoisson error distribution (based on residual deviation) (stats R package; R Core Team, 2013). To account for potential nondetection of rare variants (due to low sequencing depth), we included a log-scaled number of sequences per sample as a covariate. We used Tukey post hoc comparisons to test the effect of locality factorially.

We investigated differences in beta diversity across localities through ANOSIM-based community compositional dissimilarities (vegan R package; Oksanen et al., 2019), defined as prevalence and relative representation of ASVs, again separately for strongylid nematodes and gut bacterial microbiomes. We visualized clustering with principal coordinate analysis (PCoA) for Bray–Curtis dissimilarities (McMurdie & Holmes, 2014), accounting for ASV relative abundances, as well as a binary version of Jaccard dissimilarities, considering only ASV presence/absence (phyloseq R package; McMurdie & Holmes, 2013). We also implemented permdisp2 tests (betadisper function—vegan R package) of multivariate homogeneity to test if interindividual variation in community composition differs between localities (Anderson, 2006). Significance was tested using permutation tests with the R function permute. For strongylid communities, we used the mvabund R package (Wang et al., 2012) to test the significance of community-wide differences through multivariate negative binomial GLMs, using transformed ASV counts to identify individual strongylid ASVs that showed a significant response to the effect of locality. For gut bacteria, we applied the same methods to the entire 16S rRNA gene data set after grouping ASVs at the phylum level as well as to the 20 most abundant genera.

To assess covariation between strongylid communities and bacterial microbiomes we explored co-occurrence networks. Due to differences in bacterial microbiomes across localities, we tested each locality independently, also excluding Loango and Odzala-Kokoua due to insufficient data set sizes. Therefore, to assess the covariation between strongylids and bacterial microbiomes in gorillas we only tested samples from Dzanga-Sangha Protected Areas, Campo Ma'an National Park and Dja Faunal Reserve. We used Mantel tests to assess the overall correlation between bacterial microbiomes and strongylid community matrices (vegan R package; Oksanen et al., 2019). We then calculated similarity measures (Spearman's correlation for compositional data) through bootstrap and permutation matrices (CCREPE R package; Bielski & Weingart, 2021). Network visualization was completed in CYTOSCAPE, an opensource software (Shannon et al., 2003), with edge colour depicting the nature of the interaction.

## 2.5 | Ethics statement

The research complied with the legal requirements of the Central African Republic, Republic of Congo, Gabon and Cameroon. The samples were collected noninvasively, adhering to research protocols and regulations at all research sites.

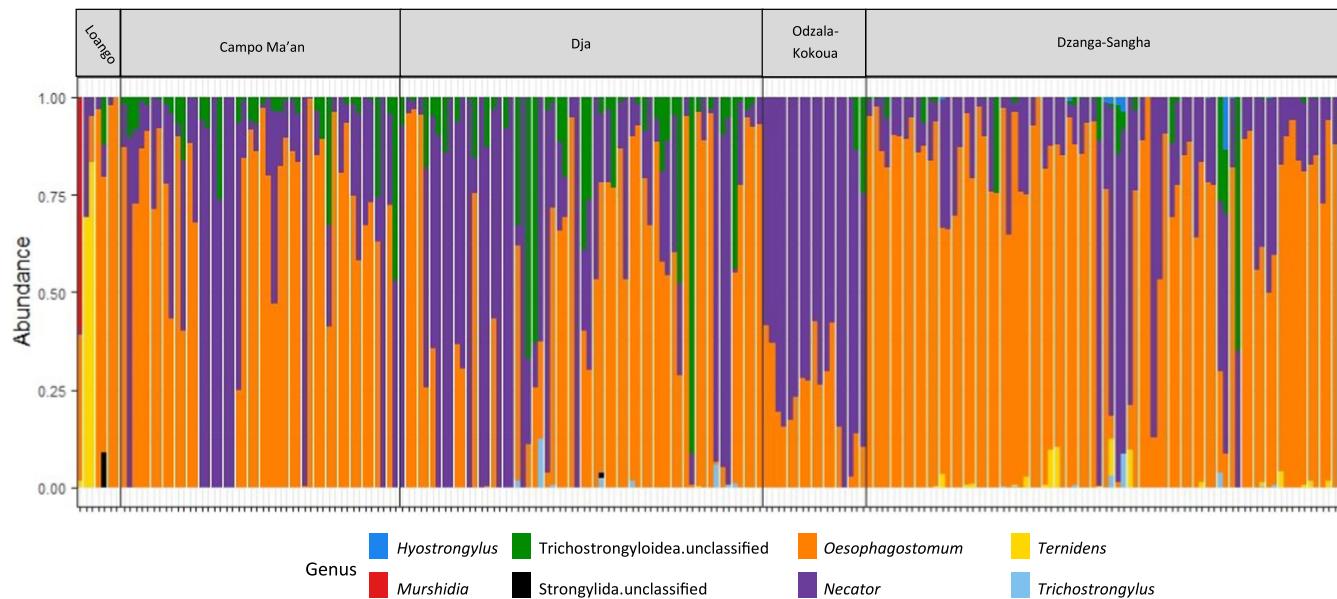
## 3 | RESULTS

Our sequencing data comprised 3,614,791 ITS-2 (averaging  $17,379 \pm 1223$  SEM per sample) and 3,178,311 16S rRNA gene (averaging  $14,992 \pm 607$  SEM per sample) high-quality reads. In total, strongylid nematode ASVs (GenBank accession nos. ON178442–ON178657) from eight genera and gut bacterial ASVs from 20 phyla were identified. Within individual hosts, strongylid nematodes were found in complex communities consisting of multiple species, with dominant (*Oesophagostomum* and *Necator*) and rarer genera identifiable (Figure 2); not all species were identified at all localities (Table 1). All but three strongylid ASVs, from two families, were identified to the genus level. The bacterial microbiome was dominated by Firmicutes, with high abundances of Actinobacteria and Bacteroidetes (Figure 3a). Selection of the 20 most abundant genera corresponded to 22.7% of 16S rRNA gene ASVs and revealed *Prevotella* (7.2% of the total 16S rRNA gene ASVs), *Rikenellaceae* RC9 gut group (4.8%) and *Senegaliimassilia* (4.7%) as the dominant genera (Figure 3b). The 20 most abundant genera were completely absent in five samples, all from Dja.

The number of strongylid ASVs per sample (alpha diversity) differed significantly only at Dja compared to other localities ( $p = .002$ ). Tukey post hoc tests revealed Dja exhibited fewer strongylid ASVs per sample compared to Campo Ma'an ( $p = .011$ ), but showed no significant differences with other localities ( $p > .3$  for all pairwise comparisons, Figure 4). The number of bacterial ASVs per sample varied across all localities, with Tukey post hoc tests showing significant differences (Figure 4). Dja had the fewest bacterial ASVs per sample compared to all other localities ( $p < .001$  for all pairwise comparisons, Figure 4).

### 3.1 | Variation in strongylid community composition

The composition of strongylid communities showed significant interspecific variation between localities (ANOSIM—Jaccard Index:  $R = 0.274$ ,  $p = .001$ ; Bray–Curtis dissimilarities:  $R = 0.255$ ,  $p = .001$ ). These communities also showed variation in dispersion among localities (ANOVA—Jaccard Index:  $F = 3.915$ ,  $p = .006$ ; Bray–Curtis dissimilarities:  $F = 14.241$ ,  $p = 0.001$ ). For Jaccard's Index, this variation in dispersion was attributed to differences between Loango and two other sites: Dzanga-Sangha ( $p = .011$ ) and Odzala-Kokoua ( $p = .015$ ), as determined through Tukey's post hoc tests ( $p > .06$  for all other pairwise comparisons). For Bray–Curtis dissimilarities,



**FIGURE 2** Community composition of strongylid nematodes of western lowland gorillas in five localities, with each column representing the relative abundances of ITS-2 amplicon sequence variants per sample [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 1** Strongylid nematode species identified in western lowland gorillas in five localities

	Loango	Campo Ma'an	Dja	Odzala-Kokoua	Dzanga-Sangha
Hyostrongylus sp. <sup>a</sup>	—	—	—	5.9	15.4
Murshidia sp. <sup>b</sup>	14.3	—	—	—	—
Necator americanus	—	32.6	33.3	76.5	44.9
Necator gorillae	71.4	97.8	96.7	100.0	96.2
Necator sp. Type III	42.9	37.0	13.3	11.8	6.4
Oesophagostomum bifurcum	14.3	—	—	—	—
Oesophagostomum stephanostomum	71.4	80.4	81.7	94.1	93.6
Oesophagostomum sp. MC-2015	—	—	1.7	—	—
Ternidens deminutus	42.9	—	1.7	—	23.1
Trichostrongyloidea sp. MM-2016b	14.3	80.4	76.7	17.6	41.0

Note: Values represent percentage prevalence.

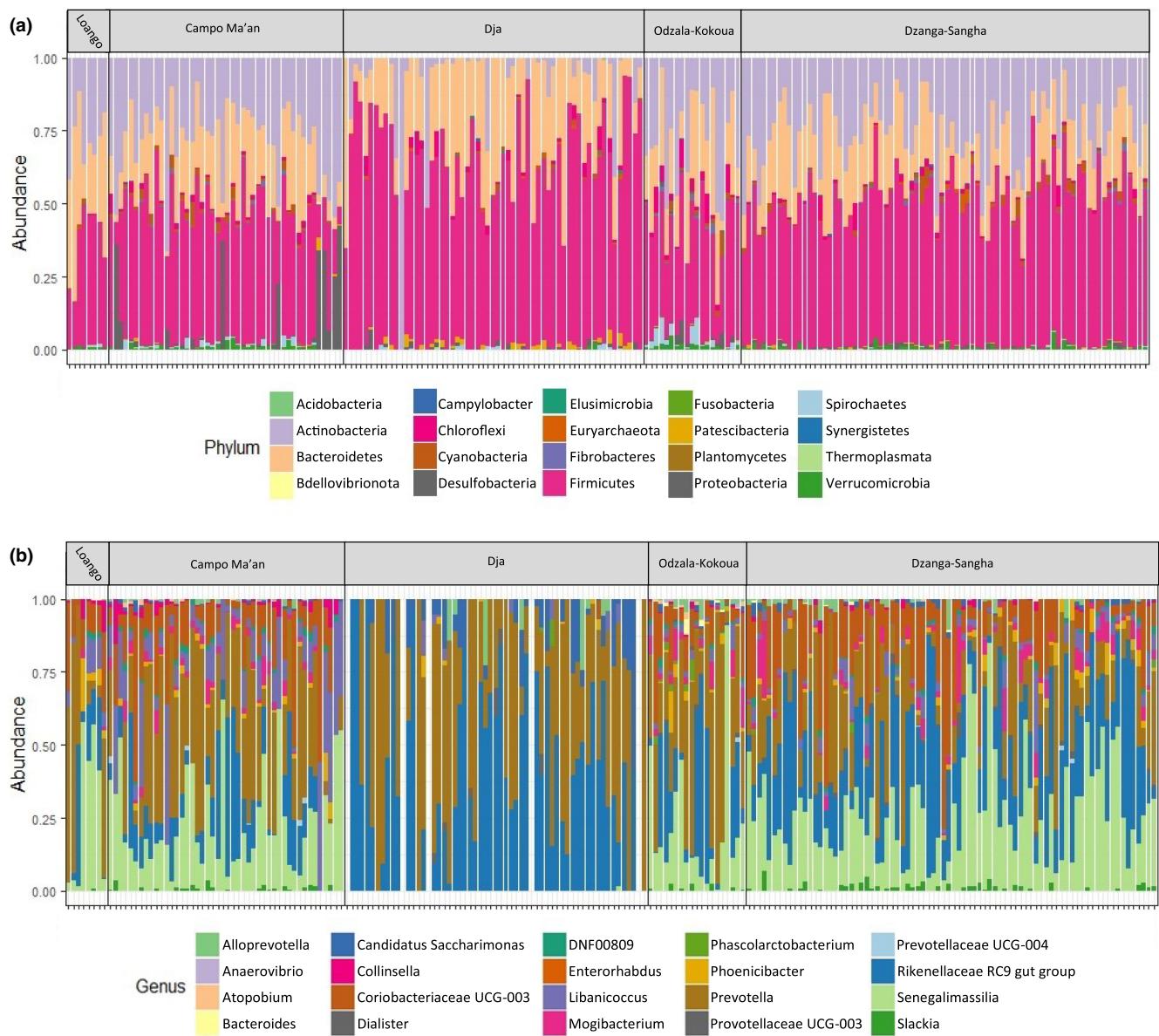
<sup>a</sup>Closest sequence *H. rubidius* (~93%–100% identity), ASVs may represent *H. kigeziensis*.

<sup>b</sup>Closest sequence *M. africana* (~96%–97% identity), ASVs may represent different species.

Tukey's post hoc tests revealed numerous localities varying in dispersion (Table 2).

Visual inspection of both Jaccard and Bray–Curtis PCoA ordination plots (Figure 5) indicated Dzanga-Sangha and Campo Ma'an overlapped more closely in ordination space, though Campo Ma'an showed two clusters with Bray–Curtis. Samples from Dja were spread more generally and thus overlapped somewhat with samples from Odzala-Kokoua and Loango. Mvabund testing identified 21 ITS-2 ASVs as the main drivers of these differences in strongylid community composition between localities (Figure S1). These included only three common ASVs, those which were identified at all localities (Trichostrongyloidea sp. MM-2016b and two Necator gorillae). Eight ASVs were exclusive to a given locality and Loango was the most unique locality with 15 of these 21 ASVs absent from all samples.

Of the three ASVs identified as unclassified Strongylida, unable to be assigned at the genus level, one showed a 100% identity match with a *Libyostrongylus* environmental sample (GenBank JX159807), and the other two a 99.63% match with the same sequence, each differing by one nucleotide base pair. However, these ASVs more probably represent *Paralibyostrongylus*, as *Libyostrongylus* is believed to be a trichostrongylid nematode of ostriches (McKenna, 2011) while *Paralibyostrongylus* was previously described in mountain gorillas (Durette-Desset et al., 1992). Two of these ASVs, assigned as unclassified Strongylida, also showed a 98% identity match with *Paralibyostrongylus* (GenBank LC512866.1). However, the remainder, assigned to unclassified Trichostrongyloidea, showed lower sequence similarity with *Paralibyostrongylus*, and instead showed a 99% identity match with Trichostrongyloidea sp. (GenBank



**FIGURE 3** Community composition of the gut bacterial microbiome of western lowland gorillas in five localities, expressed at: (a) the phylum level and (b) the genus level (of the 20 most abundant genera). Each column represents the relative abundances of 16S rRNA gene amplicon sequence variants per sample [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

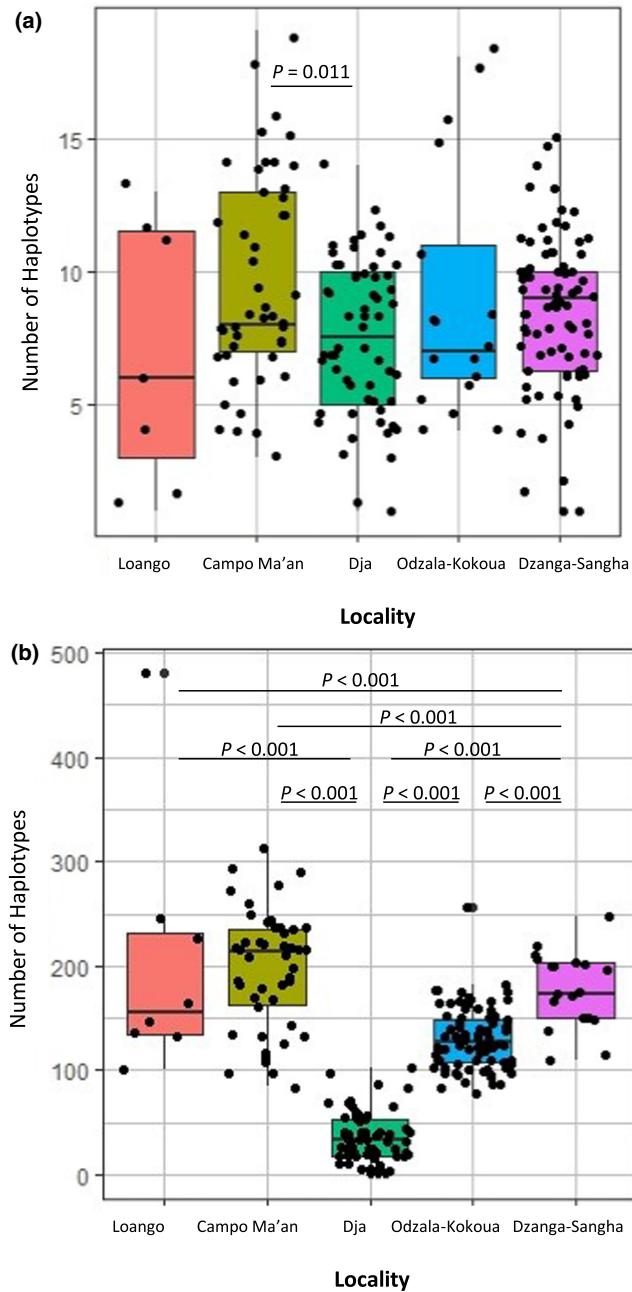
LC185220), a Trichostrongyloidea culture from chimpanzees, which aligned most closely with the genus *Libyostrongylus*. However, morphological study of Trichostrongyloidea adult worms from chimpanzees was called for to identify the true genus (McLennan et al., 2017).

### 3.2 | Variation in bacterial microbiome composition

Bacterial communities showed significant variation between localities (ANOSIM–Jaccard Index:  $R = 0.699$ ,  $p = .001$ ; Bray–Curtis dissimilarities:  $R = 0.605$ ,  $p = .001$ ) as well as variation in dispersion among localities (ANOVA–Jaccard Index:  $F = 5.122$ ,  $p = .003$ ;

Bray–Curtis dissimilarities:  $F = 7.998$ ,  $p = .001$ ). For Jaccard's Index, this variation in dispersion was attributed to differences between only Dja and Dzanga-Sangha ( $p < .001$ ;  $p > .1$  for all other pairwise comparisons), determined through Tukey's post hoc tests. With Bray–Curtis dissimilarities, variation in dispersion was attributed to differences between Dja and two other localities: Campo Ma'an ( $p = .023$ ) and again Dzanga-Sangha ( $p < .001$ ) ( $p > .06$  for all other pairwise comparisons).

Visual inspection of PCoA ordination plots (Figure 5) generally showed that each locality occupies separate ordination spaces, with the exception of Loango and Campo Ma'an, which overlapped in ordination space. Some patterns differed between the two ordination methods, with Odzala-Kokoua and Campo Ma'an showing similarities



**FIGURE 4** Amplicon sequence variant diversity of gastrointestinal symbionts detected in faecal samples of western lowland gorillas from five localities, represented by (a) number of strongylid nematodes and (b) gut bacteria [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

in the case of Bray–Curtis but not Jaccard. Analysis of differentially abundant ASVs identified 13 phyla as significant drivers of interspecific differences between localities (Figure S2). Only eight of these phyla were present at all localities, with one phylum, Planctomycetes, present only at Odzala-Kokoua. Odzala-Kokoua appeared to show a high proportion of reads for Desulfobacteria and Spirochaetes, relative to other localities, while the proportion of reads for Patescibacteria was high at Dja relative to other localities, compensating for absence of other phyla. At the genus level, 15 of the 20 most abundant genera were identified as significant drivers of diversity (Figure S3), many of which were absent or in low abundance at Dja.

### 3.3 | Strongylid–bacterial microbiome covariation

All three localities where covariation of strongylid and bacterial communities was tested indicated weak but significant covariation of community matrices (Table 3). All showed covariation of various strongylid taxa and ASVs within the Prevotellaceae and Rikenellaceae bacterial families, with some consistency across localities. At both Campo Ma'an and Dzanga-Sangha, there were numerous bidirectional covariations of Prevotellaceae ASVs with *Oesophagostomum* and *N. gorillae* ASVs (Figure S4). Interestingly, at Dzanga-Sangha the majority of Prevotellaceae and *N. gorillae* covariations were negative, differing from the more bidirectional pattern seen at Campo Ma'an. Both localities also showed covariation between Prevotellaceae and Trichostrongyloidea and between Rikenellaceae and *Oesophagostomum*. Additionally, covariations were observed at Dzanga-Sangha between Rikenellaceae and *Ternidens* and between Prevotellaceae and *Ternidens*, *Hyostrongylus* and *Necator americanus*. At Dja, covariations were primarily observed between Rikenellaceae with *Oesophagostomum* and *N. gorillae*, with Trichostrongyloidea also showing covariation with Rikenellaceae.

## 4 | DISCUSSION

We examined faecal samples from wild western lowland gorillas in five localities to assess the diversity of their gastrointestinal symbionts and assess possible covariation between selected prokaryote and eukaryote communities. We apply a community profiling method based on HTS amplicon sequencing. Note that taxonomic resolution may be limited due to the short length of the resulting sequences and that the observed diversity may be inflated and biased due to sequencing errors, PCR artefacts and PCR biases (Ambardar et al., 2016; Kircher & Kelso, 2010). However, as we have shown in our previous study (Pafčo et al., 2018), these stochastic effects have negligible impact on the community profiles presented in our current study thanks to the use of technical duplicates for each sample. Moreover, the taxonomic marker we used does not appear to introduce significant PCR biases, at least within the order Strongylida (Pafčo et al., 2018), while providing finer taxonomic resolution than more commonly used markers for ribosomal genes. Focusing on the samples from multiple populations, spread throughout the Congo Basin, we investigated these symbiont communities within individuals from habitats differing in ecological settings, including various vegetation types, sympatric species and climates. Our reported similarities and differences highlight the general consistency in the gastrointestinal symbionts of western lowland gorillas across localities.

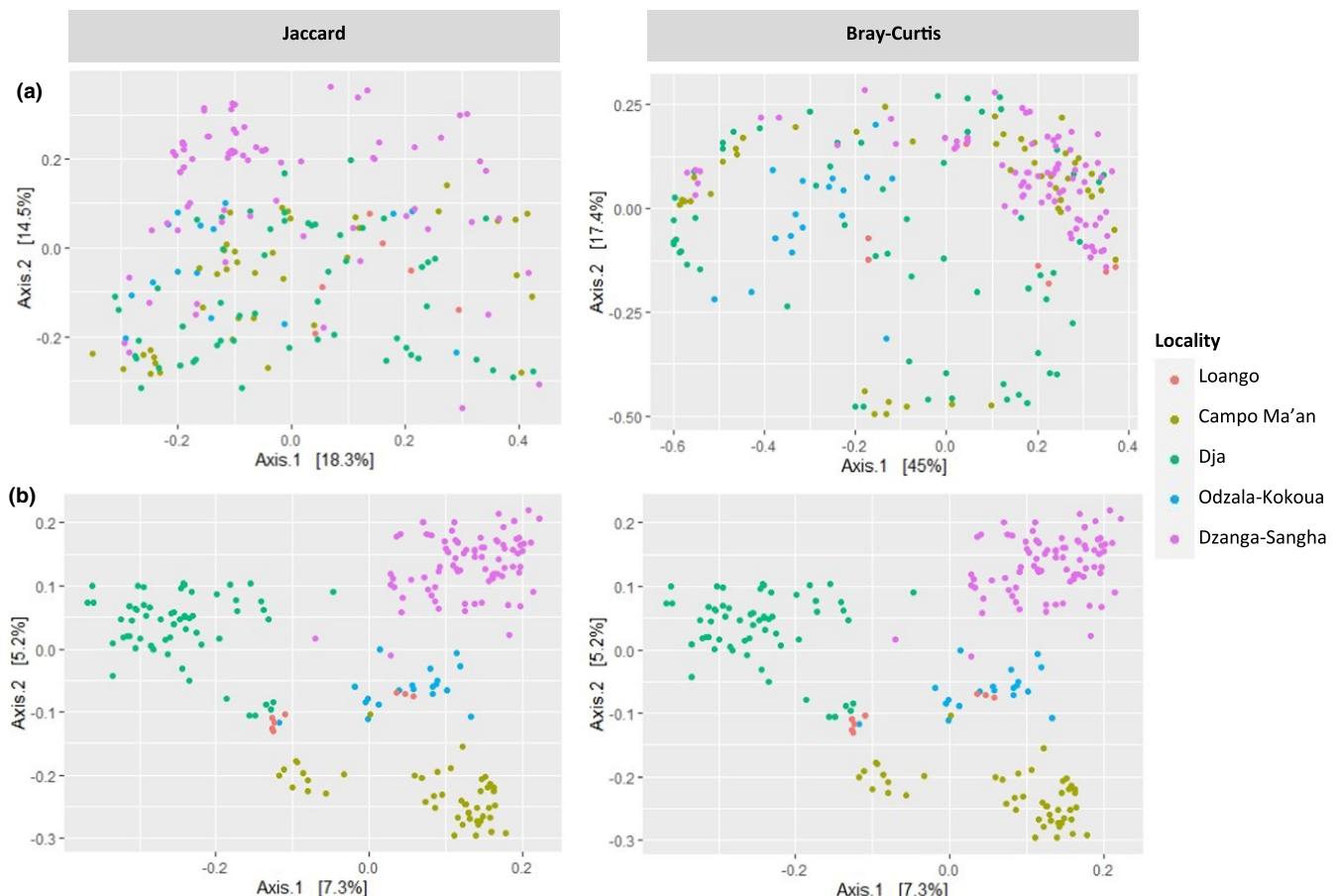
### 4.1 | Strongylid community consistency

The analysed strongylid communities were consistent across localities, as predicted, aligning with previous reports in western lowland gorillas (Pafčo et al., 2018; Pafčo, Kreisinger, et al., 2019). *Necator* and *Oesophagostomum* dominated complex communities consisting

**TABLE 2** Significance (*p*) values of dispersion variation in strongylid nematode communities of western lowland gorillas from five localities, based on Tukey's post hoc tests of bray–Curtis dissimilarities

	Campo Ma'an	Dja	Odzala-Kokoua	Dzanga-Sangha
Loango	0.829	0.998	0.023*	0.016*
Campo Ma'an		0.502	0.013*	<0.001*
Dja			<0.001*	<0.001*
Odzala-Kokoua				0.995

\*Significant result (*p* < .05).



**FIGURE 5** Principal coordinates analysis (PCoA) plots of beta diversity among (a) strongylid nematode communities and (b) gut bacterial microbiome of western lowland gorillas in five localities, assessed through Jaccard's index (amplicon sequence variant presence–absence) and bray–Curtis dissimilarities (amplicon sequence variants relative abundance). Convergent dots indicate similarities in community composition [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

of several genera. However, *Murshidia* has been previously identified only in other NHP hosts (Pafčo, Kreisinger, et al., 2019), including the closely related mountain gorilla (*Gorilla beringei beringei*) (Freeman et al., 2004). These observations are helpful to establish key baseline data on strongylid communities of western lowland gorillas, identifying core genera (*Necator* and *Oesophagostomum*) that are regionally common and locally abundant, with satellite species varying among populations (Hanski, 1982). Our research highlights that previous reports from a single locality (Pafčo et al., 2018; Pafčo, Kreisinger, et al., 2019) are analogous to diversity patterns observed at other localities, suggesting core genera are consistent within lowland gorillas.

The number of strongylid ASVs per sample showed little variation across localities, probably reflecting the similar (yet not identical) climates, habitat type and sympatric species at all localities (Dupain et al., 2004; Head et al., 2012; Mbenoun Masse et al., 2018; Molina-Vacas et al., 2020; Sak et al., 2013). Dja was the only locality to show a difference, with significantly fewer ASVs than Campo Ma'an, probably reflecting high sampling yield at these two sites, as opposed to higher effect size compared to other localities. It seems environmental factors have more impact on shaping NHP strongylid communities than geography alone, with Campo Ma'an and Dja the geographically nearest localities. As such, it is important to consider variation in environmental factors between these two

**TABLE 3** Mantel r values for community matrices of gut bacterial microbiome and strongylid nematodes of western lowland gorillas from three localities

Locality	r	p
Campo Ma'an	.141	.041
Dja	.1602	.001
Dzanga-Sangha	.332	.001

localities and how this variation shapes strongylid communities. These two localities have similar vegetation, both defined as lowland evergreen forests of the Congo Basin (Bekhuis et al., 2008; Dupain et al., 2004), as well as analogous soil and hydrological characteristics (Peh et al., 2011; Tchouto et al., 2006) suggesting that habitat type or diet are unlikely to be the cause of this difference. In both localities the lowland gorilla populations interact with analogous sympatric species, including other great ape species (chimpanzees—*Pan troglodytes*) and large terrestrial herbivores (African forest elephants—*Loxodonta cyclotis*) (Bekhuis et al., 2008; Dupain et al., 2004). The two localities do differ in rainfall, with mean annual rainfall lower in Dja, reported at around 1500 mm (Dupain et al., 2004) compared to over 2500 mm in Campo Ma'an (Mbenoun Masse et al., 2018). While reduced rainfall at Dja could mean absence of less environmentally resistant strongylid species, with strongylids typically requiring a moist external environment for development (Kalousová et al., 2014), this is unlikely to be the cause of ASV absence at Dja due to its complex hydrological network (Peh et al., 2011). We would also expect other localities to show reduced diversity to Campo Ma'an if rainfall was an environmental factor of major influence, but this was not observed.

Consequently, we suspected the reduced diversity at Dja is a reflection of higher anthropogenic disturbance, with unprotected areas previously linked with loss of gastrointestinal symbiont richness in wild NHPs (Barelli et al., 2020). Dja was the only locality at which sampling occurred outside of the national park, where anthropogenic disturbance is more common (Tagg et al., 2015). Anthropogenic disturbance, in the way of illegal hunting and wildlife-trade, are theorized as the main drivers of the drastic decline of gorilla populations in Dja, declining over 80% since 1995 (Bruce, Ndjassi, et al., 2018). Naturally, gorilla density has also fallen, estimated at 0.24 individuals per km<sup>2</sup>, lower than density estimates within other protected areas of this region (Bruce, Ndjassi, et al., 2018). Specifically, gorilla density is estimated at 1.25 individuals per km<sup>2</sup> in some regions of Campo Ma'an (Nzooh Dongmo et al., 2015). The reduced host density at Dja may lower transmission risk of strongylids at this locality (Morand & Poulin, 1998), explaining the reduced diversity observed in our analysis. However, it is important to note that gorilla density estimates were not specific to our sample areas.

Despite its low strongylid diversity, Dja showed the least clustering of strongylid communities during ordination, spreading much of the ordination space and partially overlapping with all other localities. Campo Ma'an and Dzanga-Sangha showed similar ordination patterns, suggesting similar strongylid nematode community

composition. Differences in community composition between localities, primarily driven by *Necator* and *Oesophagostomum* ASV variation, as expected for core genera, suggests an influence of environmental factors in shaping the beta diversity of gorilla strongylid communities. One such factor may be gorilla and also sympatric chimpanzee densities, as they commonly host similar strongylid communities (Pafčo, Kreisinger, et al., 2019), with density variations reported across localities (Bermejo, 1999; Blom et al., 2001; Furuichi et al., 1997; Matthews & Matthews, 2004). Another ASV recognized as a driver of differences showed closest identity match with *Hyostrongylus rubidus*, a parasite of domesticated pigs (Davidson et al., 1968; Kendall et al., 1969). While some identified *Hyostrongylus* ASVs were more distant from *H. rubidus*, others differed by only a few single nucleotide polymorphism sites or were even identical to the *H. rubidus* sequence (AJ251121), meaning transmission from Suidae (pigs, hogs, boars) cannot be excluded. *Hyostrongylus* ASVs were present at only two localities, Dzanga-Sangha and Odzala, suggesting sympatric Suidae may occur at higher densities within gorilla habitat at these localities and highlighting a potential environmental factor which shapes strongylid communities.

Loango appeared to show quite a distinctive strongylid community composition, as hypothesized, with many driver ASVs not present at this locality and untypically high relative abundance of *Ternidens deminutus*, a species described in primates including humans (Amberson & Schwarz, 1952; Schindler et al., 2005). Loango was also the only locality where *Murshidia* ASVs were identified. The unique habitat type (coastal forest-savannah) of Loango compared to other localities supports the role of environmental factors in shaping strongylid community compositional differences. *Murshidia* presence may highlight the role of sympatric host species in shaping strongylid communities, with all *Murshidia* ASVs showing closest identity match with *M. africana*, a parasite of African savannah elephants (*Loxodonta africana*) (Chel et al., 2020; Mclean et al., 2012). However, *M. africana* is yet to be documented in African forest elephants (*Loxodonta cyclotis*) (Kinsella et al., 2004), the elephant species documented at all localities (Bekhuis et al., 2008; Blom, 2000; Bruce, Amin, et al., 2018; Bruce, Ndjassi, et al., 2018; Head et al., 2012; Molina-Vacas et al., 2020). *Murshidia* ASVs identified at Loango are probably a closely related taxon to *M. africana*, potentially a subspecies, but limited availability of reference sequences prevents firmer conclusions.

## 4.2 | Gut bacterial microbiome variation

The bacterial microbiome of studied populations, dominated by Firmicutes, Actinobacteria and Bacteroidetes, aligned with previous reports on wild lowland gorilla bacterial microbiomes (Bittar et al., 2014; Gomez et al., 2015; Hicks et al., 2018; Nishida & Ochman, 2019), although some report Chloroflexi as a dominant phylum (Campbell et al., 2020; Gomez, Rothman, et al., 2016; Gomez, Petrzelkova, et al., 2016). Members of these phyla have important roles in digestion of dietary starches and fibre (Gomez, Rothman,

et al., 2016; Gomez, Petrzelkova, et al., 2016; Gomez et al., 2015; Magne et al., 2020; Martens et al., 2009; O Sheridan et al., 2016). While the dominant taxa appeared consistent, bacterial microbiome ASV richness varied across multiple localities, with Dja having significantly fewer ASVs per sample than all other localities, similar to the observed pattern among strongylid communities. This variation may result from various environmental factors which differ among localities, such as diet, a previously identified driver of gut bacterial microbiome composition in *Gorilla* (Gomez, Rothman, et al., 2016; Gomez, Petrzelkova, et al., 2016), with fibre content previously identified as influencing microbiome composition (Gomez, Rothman, et al., 2016; Gomez, Petrzelkova, et al., 2016; Nagpal et al., 2018). In localities with savanna patches (Loango and Odzala-Kokoua) we suspect that the gut bacterial microbiome is influenced by higher fibre intake resulting from reduced frugivory (increased folivore, leaf-eating, behaviours), with lower fruit availability in savannah habitats (Isbell & Young, 1996). Anthropogenic disturbance may be another environmental factor that shapes the observed gut bacterial microbiome. It would explain the low bacterial microbiome diversity at Dja, which is the only locality outside of a protected area (Ashley & Mbile, 2005; Le Gouar et al., 2009; Rabanal et al., 2010; Sak et al., 2013). Habitat degradation has been previously associated with less diverse gut microbiomes in black howler monkeys (*Alouatta pigra*) (Amato et al., 2013).

Interestingly, Loango and Odzala-Kokoua were the only two sites to show similarity in dispersion patterns, clustering together during ordination, perhaps reflecting shared presence of savannah patches (and consequently gorilla dietary shifts) within the habitat mosaics here. The differences observed among other localities highlight the potential of environmental factors in shaping beta diversity of the gut bacterial microbiome. These variables can include geography or diet, both previously linked to variation in the *Gorilla* gut bacterial microbiome (Gomez et al., 2015). Numerous individual factors may also shape composition of the gut bacterial microbiome in NHPs, such as age, sex, sociality and health status (Clayton, Gomez, et al., 2018; Gogarten et al., 2018; Moeller et al., 2016; Pafčo, Sharma, et al., 2019). Physiological stress, assessed through faecal glucocorticoid metabolites, has also shown to be associated with gut microbiome composition in lowland gorillas (Vlčková et al., 2018), highlighting the many ecological drivers of dynamism in NHP gut bacterial microbiomes (Clayton, Gomez, et al., 2018). While individual factors may shape differences among localities, they can also shape intersite variation of symbiont communities (Gomez et al., 2015).

Numerous drivers of bacterial microbiome composition differences were identified at both the genus and the phylum level, including both dominant (Firmicutes, Actinobacteria, Bacteroidetes) and rarer phyla. Many of these probably reflect dietary differences between localities, with numerous taxa identified for their roles in digestion of various dietary compounds, such as pectin, sugars and fats (Fujio-Vejar et al., 2017; Gharechahi et al., 2020; Gomez, Rothman, et al., 2016; Gomez, Petrzelkova, et al., 2016; Méndez-Salazar et al., 2018; Murphy et al., 2021; Ransom-Jones et al., 2012). As such, we speculate the community composition of the gut

bacterial microbiome is more sensitive to variation of environmental factors compared with strongylid communities. Due to the isolated nature of the studied populations, it should not be overlooked that host genetics may also shape these differences in the gut bacterial microbiome, with heritability of the gut bacterial microbiome previously suggested in humans and other NHPs (Goodrich et al., 2014; Grieneisen et al., 2021; Zhao et al., 2016).

The role of other identified taxa in the bacterial microbiome is less well understood, though some, such as Planctomycetes, Patescibacteria and Euryarchaeota, have been linked to pathogenic occurrences (Aghnatiros & Drancourt, 2016; Cayrou et al., 2013; Horz & Conrads, 2010; Shin et al., 2015). Bacteria of the genus *Treponema* are part of the normal gut microbiome of gorillas (Campbell et al., 2020; Levin et al., 2021) and humans (Schnorr et al., 2014). Generally, these members of *Treponema* are not pathogenic and probably support their host in digesting fibres. The higher relative abundance of *Treponema* reads in samples from Odzala-Kokoua, relative to other localities, can be explained by the presence of a yaws bacterium in the gorilla population at this location. Although *Treponema pallidum* subsp. *pertenue* is associated with skin lesions (Chuma et al., 2020), the bacterium causes systemic infection and is frequently detected in faecal samples (Chuma et al., 2019). Ingestion during grooming activities must be considered a factor contributing to the higher number of *Treponema* reads in gorilla groups infected with yaws.

### 4.3 | Strongylid and gut bacterial microbiome covariation

We identified numerous strongylid nematode and bacteria ASVs showing patterns of covariation which were consistent, in part, across localities, highlighting the overlap of niche occupation within the mammalian gut. The majority of strongylid nematodes showing covariation with the bacterial microbiome were from just two genera: *Necator* and *Oesophagostomum*. This probably reflects their dominant abundance within the strongylid communities, unanimously across all localities. Interactions between helminth occurrence and gut bacterial microbiomes have been previously reported for numerous taxa, with most research focused on humans and animal models, such as mice or rats (Cortés et al., 2019; Peachey et al., 2017; Reynolds et al., 2015). Limited research has previously been done on characterizing these interactions in NHPs (Broadhurst et al., 2012; de Winter et al., 2020; Loke & Lim, 2015).

We identified covariation between strongylids and ASVs from two bacterial families, Prevotellaceae and Rikenellaceae. Prevotellaceae has previously shown shifts in community composition in association with various helminth infections in mice, pigs and ungulates (Cortés et al., 2019; Leung et al., 2018; Peachey et al., 2017). Occurrence of this bacterial family is connected to carbohydrates, simple sugars and high-fibre diets (Lee et al., 2014), linked to fermentative breakdown of digestible plant matter (Gomez, Rothman, et al., 2016; Gomez, Petrzelkova, et al., 2016; Gomez et al., 2019). Consequently,

reduced carbohydrate metabolism due to helminth infection may explain associated reductions in carbohydrate-utilizing bacteria, such as Prevotellaceae (Leung et al., 2018), as seen at Campo Ma'an and Dzanga-Sangha. We suspect no covariation of strongylids and Prevotellaceae was observed at Dja due to the low diversity of the gut bacterial microbiome observed here, suggesting environmental factors can indirectly shape covariation patterns. This alludes to the lesser-known potential impacts of anthropogenic disturbance, with Dja sampling occurring on the periphery of the protected area where the mounting pressure of human activities is more apparent than at other localities.

Covariations at Dja were restricted to strongylids and Rikenellaceae, with limited covariations being observed between Rikenellaceae and strongylids at other localities. Nematode infection has been previously associated with shifts in Rikenellaceae in equids (Peachey et al., 2019) and experimentally in mice (Guiver et al., 2022; Holm et al., 2015), with helminth infection linked to dysbiosis, the disruption of microbiota homeostasis. We suspect some level of covariation is consistent with the gut bacterial microbiome and strongylid nematodes both existing as established and stable communities within the host gut. However, variation of environmental factors which shift parasite epidemiology, such as reduced diversity or increased parasitic burden, can drive bacterial microbiome deviation and result in dysbiosis (Guiver et al., 2022), hence shifting observed covariation and explaining differences seen across localities. Dysbiosis of the gut bacterial microbiome in response to nematode infection has been previously proven as reversible (Afrin et al., 2019), supporting the dynamic nature of strongylid and gut bacterial microbiome covariations. Moreover, environmental factors have also been shown to affect interactions between the gut bacteria and the gut mycobiome (the fungal communities) in primates (Sharma et al., 2022), further highlighting the dynamic nature of symbiont interactions within the primate gut.

Various mechanisms have been proposed for the interactions between gastrointestinal helminths and bacteria, including both direct and indirect. Directly, gastrointestinal bacteria and strongylid nematodes may compete for nutrition (Ling et al., 2020), though unlikely for our observed covariations of *Necator*, which feed directly on host blood (Ranjit et al., 2009). More probably, *Necator* influences the gut bacterial microbiome through epithelial barrier disruption (Reynolds et al., 2015), as these worms penetrate the mucosa to feed (Ranjit et al., 2009). *Oesophagostomum* can influence the gut bacterial microbiome through similar mechanisms due to nodule formation within the colonic wall during development (Gasser et al., 2005). Direct interaction may also occur through physical contact or chemical exposure to secretions (Ling et al., 2020). Indirectly, helminth infection may alter the bacterial microbiome through an influence on immune homeostasis of the host, whether by altering inflammatory modulation, mucus production or antimicrobial peptide production (Ling et al., 2020; Reynolds et al., 2015). Alternatively, bacterial microbiome composition can alter parasite colonization success, replication and virulence through variation in the physical gut landscape (Leung et al., 2018; Moyat et al., 2022),

suggesting that the establishment of strongylid communities may vary dependent on the bacterial microbiome. It is also possible, but unlikely, that observed covariations do not reflect interactions between strongylid nematodes and the bacterial microbiome but in fact echo specific bacterial and strongylid taxa showing harmonized responses to extraneous environmental variables (Reynolds et al., 2015).

Despite many proposed mechanisms for these interactions, clear understanding is still lacking due to their complex nature, highlighted by the variation in interactions we observed across localities. Research is also limited through dependence on faecal samples, whereby smaller-scale interactions on a local level probably go undetected (Reynolds et al., 2015). This is further convoluted by particular bacteria or strongylid taxa occurring only in specific regions of the gut. Faecal sampling cannot determine the localization of adult worms within the host gut, but spatial niche separation of nematodes within large herbivore guts has been previously demonstrated in equids (Stancampiano et al., 2010). In humans *Oesophagostomum* occupies niches within the caecum and colon, while *Necator* occupies the jejunum (part of the small intestine) and *Ternidens* the colon (Glendinning et al., 2014). Naturally, we may expect strongylids to interact only with bacteria inhabiting the same spatial niche of the host gut. Ongoing research in this area is important to help better understand the complex interactions among various members of the gut ecosystem, upon which good gut health and metabolism is reliant. A better understanding of the factors shaping the pathology of helminth infections is also necessary (Peachey et al., 2017).

## 5 | IMPLICATIONS AND CONCLUSIONS

The gastrointestinal symbiont communities of wild western lowland gorillas are comparable across localities in terms of dominant taxa, suggesting a certain extent of consistency in the host-symbiont system, as hypothesized. We also identified variation in both alpha and beta diversity. This highlights the complex nature of these communities, shaped by various environmental factors as hypothesized, including vegetation type, human disturbance and occurrence of sympatric species. The identification of strongylid nematodes in gorillas most closely identifying with those reported in pigs and elephants highlights the importance of sympatric species in understanding parasite epidemiology and calls for further research on the parasite communities of all sympatric wildlife hosts. Monitoring of these strongylid communities is also important due to the zoonotic potential of some taxa, with numerous strongylid ASVs previously identified in humans, including *Necator americanus*, *Oesophagostomum bifurcum* and *Ternidens deminutus* (Amberson & Schwarz, 1952; Pit et al., 2001; Wolfe et al., 1998).

The variation of gastrointestinal symbiont communities may reflect shifts beneficial to the host individuals, with different bacterial taxa believed to aid digestion of particular food types. Alternatively, this variation may be detrimental to host health or an

indication of host illness, with both strongylid infections and bacterial microbiome variation previously linked to poor host health in NHPs (Amato et al., 2013; Clayton, Al-Ghalith, et al., 2018; Krief et al., 2008; Petrželková et al., 2021; Terio et al., 2018). Applying the HTS methodologies presented in this research to strongylid communities of other NHPs, principally great apes, provides an exciting opportunity to better understand how ecological factors may erode primate-strongylid evolutionary relationships. The complex nature of bacterial and strongylid communities within the gastrointestinal system restricts our knowledge of potential consequences resulting from their changes. Future research combining collection of ecological data, such as diet, vegetation type and climatic conditions, with identification of gastrointestinal symbionts by HTS will allow a greater understanding of these complex communities and drivers of their variation.

## AUTHOR CONTRIBUTIONS

K.J.P., B.C., T.F., S.K., T.B., U.M., S.M., E.F., N.T., N.W., M.H.S. and B.P. facilitated data collection in the various field sites. B.M. designed the study, carried out laboratory work, conducted statistical analysis and drafted the manuscript. B.P. supervised the study and its design, co-ordinating both field collection and laboratory work. J.K. preformed the bioinformatics, advised on statistical approaches and, together with A.G., provided valuable guidance on the microbiome-related sections of this paper. K.J.P. significantly improved the manuscript and funded the study jointly with D.M. All authors revised and gave final approval for publication of the manuscript.

## CONFLICT OF INTEREST

All authors declare no conflict of interest.

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## OPEN RESEARCH BADGES



This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at under the accession number of the whole project PRJEB52974 (available at: <https://www.ebi.ac.uk/ena/browser/view/PRJEB52974>).

## DATA AVAILABILITY STATEMENT

Sequencing data, for both strongylids (ITS-2) and bacteria (16S rRNA), are archived in the European Nucleotide Archive under the accession number of the whole project PRJEB52974 (available at: <https://www.ebi.ac.uk/ena/browser/view/PRJEB52974>). Accession numbers for each sample are available alongside related metadata in Table S1, which includes geographical location and sampling period. Individual strongylid ITS-2 sequences have also been uploaded to GenBank under the accession numbers available in Table S2. Benefits Generated: This research involved collaboration of scientists working in multiple countries, with collaborators from all surveyed localities included as co-authors. The research addresses valuable questions, and the results will be shared with all research teams involved as well as the wider scientific community, as detailed above.

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