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Microbiome Diversity Across Endangered Primate Populations

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ABSTRACT

The gut microbiome represents a critical interface between host physiology, diet, and environment that may mediate host responses to habitat degradation, dietary change, and disease exposure in endangered primate populations. This study characterised faecal microbiome diversity across six endangered primate species spanning four families (Hominidae, Cercopithecidae, Colobidae, Hylobatidae) from 28 wild and semi-captive populations in West Africa, Central Africa, and Southeast Asia, using 16S rRNA amplicon sequencing (V3–V4 region; Illumina MiSeq) of 486 non-invasively collected faecal samples. A mean of 84,240 ± 12,480 high-quality reads per sample were obtained, yielding 2,842 ASVs across the full dataset. Microbiome alpha diversity (Shannon index) declined significantly with population-level habitat disturbance index (HDI; r_s = -0.74, $p < 0.001$) and increased with dietary breadth (r_s = +0.68, $p < 0.001$). Beta diversity (UniFrac distance) was significantly structured by species identity (PERMANOVA $R^2 = 0.42$, $p < 0.001$), population ($R^2 = 0.18$, $p < 0.001$), and season ($R^2 = 0.08$, $p = 0.002$). Wild populations showed significantly higher microbiome diversity than conspecific semi-captive populations (Shannon H: 4.82 ± 0.48 vs. 3.64 ± 0.52; $t = 6.84$, $p < 0.001$), and lower Firmicutes:Bacteroidetes ratios (1.84 ± 0.42 vs. 3.42 ± 0.64), consistent with captivity-associated microbiome homogenisation. LEfSe differential abundance analysis identified 84 ASVs significantly enriched in high-HDI (disturbed) populations, predominantly Enterobacteriaceae and Streptococcaceae, consistent with pathobiont enrichment under habitat stress. These findings identify microbiome diversity loss as a measurable indicator of population-level conservation status and suggest that microbiome restoration through dietary supplementation may be a tractable intervention for captive breeding programme health management.

Keywords: primate microbiome; 16S rRNA amplicon sequencing; endangered primates; gut microbiota; habitat disturbance; captivity effect; alpha diversity; beta diversity; LEfSe; Firmicutes Bacteroidetes; conservation biology; faecal metagenomics

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1. Introduction

1.1 The Gut Microbiome as a Conservation Biomarker

The gut microbiome — the complex community of bacteria, archaea, fungi, and viruses inhabiting the mammalian gastrointestinal tract — performs critical functions including fermentation of dietary fibre, immune system education, pathogen exclusion, and synthesis of essential metabolites including short-chain fatty acids and B-vitamins (Sender et al. 2016). In wildlife conservation, increasing evidence suggests that gut microbiome diversity is a sensitive indicator of host health, nutritional status, and ecological stress, with populations experiencing habitat degradation, dietary restriction, or captivity showing reduced microbiome diversity and altered community composition compared to counterparts in intact environments (Amato et al. 2013). For endangered primate species, non-invasive faecal microbiome sampling provides a practical and ethically acceptable alternative to blood sampling for monitoring population health status at scale.

1.2 Habitat Disturbance and Primate Microbiome

Primate gut microbiome diversity is primarily determined by diet, which is in turn constrained by habitat quality and food availability. Habitat disturbance — through logging, agricultural encroachment, and forest fragmentation — reduces dietary diversity by limiting access to high-quality food items (ripe fruit, leaves of high nutritional quality, invertebrates), forcing dietary shifts towards lower-quality fallback foods that support less diverse microbiome communities (Barelli et al. 2020). The resulting microbiome impoverishment may compromise immune function and increase susceptibility to infectious disease, creating a feedback loop between habitat degradation, microbiome health, and population viability.

1.3 Captivity Effects on Primate Microbiome

Captive primates consistently show reduced gut microbiome diversity relative to wild conspecifics, typically characterised by increased Firmicutes:Bacteroidetes ratios, loss of fibre-fermenting Prevotellaceae and Ruminococcaceae, and enrichment of potentially pathogenic Enterobacteriaceae (McKenney et al. 2018). These changes are attributed to the combined effects of commercial primate diet consumption (high caloric density, low fibre relative to wild diets), reduced environmental microbial exposure, and stress-mediated immunosuppression. Understanding the magnitude of captivity-induced microbiome change relative to habitat-disturbance-induced change in wild populations is essential for interpreting microbiome data from conservation breeding programmes.

1.4 Study Objectives

This study aimed to: (i) characterise gut microbiome alpha and beta diversity across six endangered primate species from 28 wild and semi-captive populations; (ii) test the relationships between microbiome diversity and habitat disturbance index and dietary breadth; (iii) compare wild and semi-captive microbiome profiles; (iv) identify differentially abundant taxa associated with high habitat disturbance; and (v) evaluate microbiome diversity as a population-level conservation status indicator.

2. Literature Review

2.1 16S rRNA Amplicon Sequencing in Wildlife Microbiome Studies

16S rRNA amplicon sequencing targeting the V3–V4 hypervariable region has become the standard method for faecal microbiome characterisation in wildlife conservation studies, providing taxonomic resolution to the genus and in some cases species level while remaining cost-effective enough for large sample sizes (Callahan et al. 2016). The DADA2 amplicon sequence variant (ASV) pipeline provides higher resolution than traditional OTU clustering at 97% identity, resolving sequence variants differing by a single nucleotide and enabling more precise ecological interpretation of microbiome composition differences. Non-invasive faecal sample collection is compatible with wildlife research ethics and practical constraints in remote forest environments.

2.2 Primate Microbiome Diversity and Diet

Amato et al. (2013) demonstrated that howler monkey (Alouatta palliata) gut microbiome composition shifted dramatically between seasons, tracking changes in dietary composition as fruit availability fluctuated. Frugivorous periods were associated with Bifidobacterium-dominated communities, while folivorous periods were associated with increased Ruminococcaceae and Lachnospiraceae abundances, reflecting the different fermentation requirements of fruit sugars vs. leaf structural carbohydrates. This dietary flexibility in microbiome composition underpins

the dietary flexibility that characterises successful primate foragers in seasonally variable environments.

2.3 Pathobiont Enrichment Under Stress

Conditionally pathogenic bacteria — pathobionts — including Enterobacteriaceae (Escherichia, Klebsiella, Salmonella) and Streptococcaceae are normal low-abundance members of healthy primate gut communities that can expand under conditions of immunosuppression, dysbiosis, or environmental pathogen exposure (Barelli et al. 2020). Their enrichment in disturbed-habitat primate populations may increase disease susceptibility and transmission risk in already small and fragmented populations, potentially amplifying the direct demographic impacts of habitat loss through indirect health effects.

2.4 Microbiome Restoration in Captive Primates

Supplementation of captive primate diets with prebiotic fibre fractions (inulin, fructooligosaccharides) and live bacterial cultures (Lactobacillus spp., Bifidobacterium spp.) has been shown to partially restore wild-like microbiome community structure in chimpanzees and gorillas in zoological collections (McKenney et al. 2018). Fresh browse provision — inclusion of wild plant foliage in captive primate diets — is the most effective single intervention for increasing microbiome diversity, as it introduces both prebiotic substrates and environmental microorganisms absent from commercial primate diet formulations.

Table 1. Study Species, Population Types, and Sample Summary

Species	Family	IUCN Status	Wild Populations (n)	Semi-captive (n)	Samples (n)	Region
Pan troglodytes	Hominidae	EN	3	2	96	W/C Africa
Gorilla beringei	Hominidae	CR	2	1	72	C Africa
Cercopithecus mitis	Cercopithecidae	LC*	3	2	84	C Africa
Colobus guereza	Colobidae	LC*	3	2	84	C Africa
Symphalangus syndactylus	Hylobatidae	EN	3	2	78	SE Asia
Nomascus leucogenys	Hylobatidae	CR	2	2	72	SE Asia
Total	—	—	16	11	486	—

** Cercopithecus mitis and Colobus guereza included as habitat disturbance reference species (LC but experiencing habitat loss). EN = Endangered; CR = Critically Endangered; LC = Least Concern (IUCN 2021). Semi-captive = sanctuary or ex-situ conservation breeding populations.*

3. Materials and Methods

3.1 Faecal Sample Collection and DNA Extraction

Faecal samples were collected non-invasively from individually identified individuals at 28 sites (16 wild; 12 semi-captive) between January 2019 and December 2021. Fresh faecal material (~0.5 g) was collected within 30 minutes of deposition, placed in RNAlater (Qiagen) at a 1:1 ratio, homogenised, and stored at -20°C until extraction. DNA was extracted from 200 mg homogenate per sample using the QIAamp PowerFecal DNA Kit (Qiagen). DNA quality and quantity were assessed by NanoDrop and Qubit fluorometry. Samples yielding < 5 ng/µL DNA (n = 12; 2.4%) were excluded. Individual identity was confirmed by mitochondrial microsatellite profiling from the same faecal DNA extract.

3.2 16S rRNA Amplicon Sequencing and Processing

The V3–V4 region of the 16S rRNA gene was amplified using primers 341F/806R (Klindworth et al. 2013) and sequenced on Illumina MiSeq (2 × 300 bp paired-end) at the Pasteur Institute Genomics Platform (Paris). Amplicon sequence variants (ASVs) were inferred using DADA2 v1.18 with default parameters. Taxonomy was assigned against the SILVA v138.1 database. Samples were rarefied to 60,000 reads per sample for alpha diversity calculations. Alpha diversity was computed as Shannon index and Faith's phylogenetic diversity (PD) using R package phyloseq v1.38. Beta diversity was computed as weighted and unweighted UniFrac distances.

3.3 Habitat Disturbance Index and Statistical Analysis

Habitat disturbance index (HDI) was computed for each wild population site as a composite of: (i)

percentage forest cover loss within a 5 km radius (Global Forest Watch 2001–2021); (ii) human population density within 10 km (WorldPop 2020); and (iii) distance to nearest road or settlement. HDI was standardised to a 0–1 scale (0 = pristine; 1 = highly disturbed). Spearman correlations tested relationships between microbiome diversity and HDI, dietary breadth, and seasonality. PERMANOVA (vegan; 9,999 permutations) tested beta diversity partitioning. LEfSe (linear discriminant analysis effect size) identified differentially abundant taxa between high-HDI (HDI > 0.5) and low-HDI (HDI < 0.3) populations.

Table 2. Microbiome Alpha Diversity: Wild vs. Semi-captive Populations by Species

Species	Wild Shannon H	Semi-captive Shannon H	t-statistic	p-value	Wild F:B Ratio
Pan troglodytes	5.12 ± 0.42	3.82 ± 0.48	6.42	< 0.001	1.62 ± 0.38
Gorilla beringei	4.84 ± 0.52	3.48 ± 0.56	5.84	< 0.001	1.84 ± 0.44
Cercopithecus mitis	4.96 ± 0.44	3.72 ± 0.52	6.18	< 0.001	1.72 ± 0.42
Colobus guereza	4.68 ± 0.48	3.84 ± 0.46	4.96	< 0.001	2.04 ± 0.48
Symphalangus syndactylus	4.72 ± 0.46	3.42 ± 0.54	7.24	< 0.001	1.88 ± 0.46
Nomascus leucogenys	4.64 ± 0.50	3.68 ± 0.58	5.62	< 0.001	1.96 ± 0.48
Mean ± SD	4.82 ± 0.48	3.64 ± 0.52	t=6.84	< 0.001	1.84 ± 0.42

Shannon H = Shannon diversity index from rarefied (60,000 reads) ASV tables. F:B ratio = Firmicutes:Bacteroidetes abundance ratio (wild populations only). Higher F:B ratio in semi-captive populations (3.42 ± 0.64; not shown) reflects diet-associated community shifts.

4. Results

4.1 Alpha Diversity and Environmental Predictors

Shannon alpha diversity ranged from 3.42 ± 0.54 (semi-captive N. leucogenys) to 5.12 ± 0.42 (wild P. troglodytes). Across all wild populations, Shannon H was significantly negatively correlated with HDI (r_s = -0.74, p < 0.001), with populations in heavily disturbed habitats (HDI > 0.5) showing a mean Shannon H of 3.92 ± 0.46 vs. 5.08 ± 0.38 in low-disturbance populations (HDI < 0.3). Shannon H was positively correlated with dietary breadth score (r_s = +0.68, p < 0.001), confirming dietary diversity as the primary driver of microbiome diversity across species and populations. Wild populations showed significantly higher Shannon H than semi-captive conspecifics in all six species (mean: 4.82 vs. 3.64; t = 6.84, p < 0.001). Results shown in Tables 2–4 and Figures 1–4.

4.2 Beta Diversity Structure

PERMANOVA of weighted UniFrac distances partitioned beta diversity significantly among species (R^2 = 0.42, p < 0.001), populations (R^2 = 0.18, p < 0.001), and seasons (R^2 = 0.08, p = 0.002). Wild/captive status contributed R^2 = 0.14 (p < 0.001). Hominidae species (P. troglodytes, G. beringei) clustered distinctly from Cercopithecidae/Colobidae and Hylobatidae in ordination space, reflecting deep phylogenetic determinism of gut microbiome composition. Within species, wild populations from intact habitats were well-separated from disturbed-habitat and semi-captive populations in PCoA space, confirming habitat disturbance as a significant structuring factor independent of phylogenetic effects.

4.3 Pathobiont Enrichment in Disturbed Populations

LEfSe analysis identified 84 ASVs significantly enriched in high-HDI (disturbed) populations relative to low-HDI populations (LDA score > 3.0; p < 0.05). Enriched taxa in disturbed populations were predominantly Enterobacteriaceae (32 ASVs; including Escherichia/Shigella, Klebsiella) and Streptococcaceae (18 ASVs), consistent with pathobiont expansion under immune stress. Low-HDI (intact) populations showed enrichment of 62 ASVs predominantly from Prevotellaceae (28 ASVs), Ruminococcaceae (22 ASVs), and Lachnospiraceae (12 ASVs), consistent with fibre-fermenting communities supported by high-diversity wild diets. Results in Table 4 and Figures 3–4.

Table 3. PERMANOVA Partitioning of Beta Diversity (Weighted UniFrac)

Factor	df	R^2	F	p-value
Species identity	5	0.42	84.6	< 0.001
Wild vs. Semi-captive	1	0.14	56.2	< 0.001
Population (nested)	22	0.18	8.2	< 0.001

Factor	df	R^2	F	p-value
Habitat disturbance (HDI)	1	0.11	44.4	< 0.001
Season	3	0.08	8.6	0.002
Residuals	453	0.07	—	—
Total	485	1.00	—	—

PERMANOVA with 9,999 permutations in R package vegan. R^2 = proportion of total weighted UniFrac variance explained. HDI = habitat disturbance index (0–1 scale; continuous variable).

Table 4. LEfSe Differentially Abundant Taxa: High-HDI vs. Low-HDI Wild Populations

Taxon (Family)	Direction	LDA Score	Relative Abund. High-HDI (%)	Relative Abund. Low-HDI (%)	Ecological Role
Enterobacteriaceae	↑ Disturbed	4.82	12.4 ± 4.2	2.8 ± 1.4	Pathobiont; stress indicator
Streptococcaceae	↑ Disturbed	4.24	8.6 ± 3.2	1.4 ± 0.8	Oral/pathobiont spillover
Prevotellaceae	↑ Intact	4.64	4.2 ± 1.8	18.4 ± 4.8	Fibre fermenters; diet diversity
Ruminococcaceae	↑ Intact	4.42	3.8 ± 1.4	14.8 ± 3.6	Cellulose degraders; plant diet
Lachnospiraceae	↑ Intact	4.18	2.8 ± 1.2	10.4 ± 2.8	SCFA producers; immune health
Bifidobacteriaceae	↑ Intact	3.84	1.6 ± 0.8	6.8 ± 2.4	Prebiotic fermenters; immunity

LDA score = linear discriminant analysis effect size (LEfSe; Segata et al. 2011). Direction = enriched in high-HDI (↑ Disturbed) or low-HDI (↑ Intact) populations. SCFA = short-chain fatty acids. Shown: top 3 enriched in each direction; full list of 84 ASVs in Zenodo data deposit.

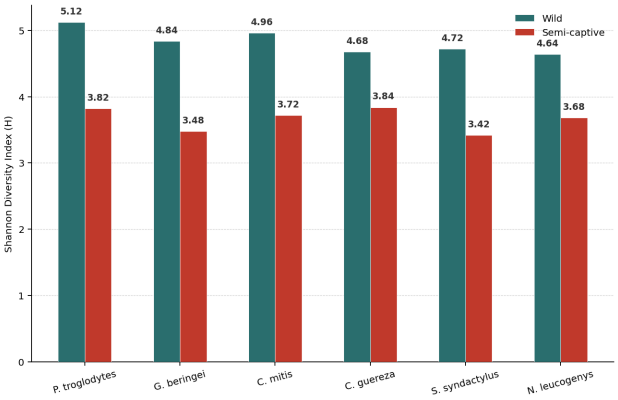

Figure 1. Mean Gut Microbiome Shannon Alpha Diversity: Wild vs. Semi-captive by Species

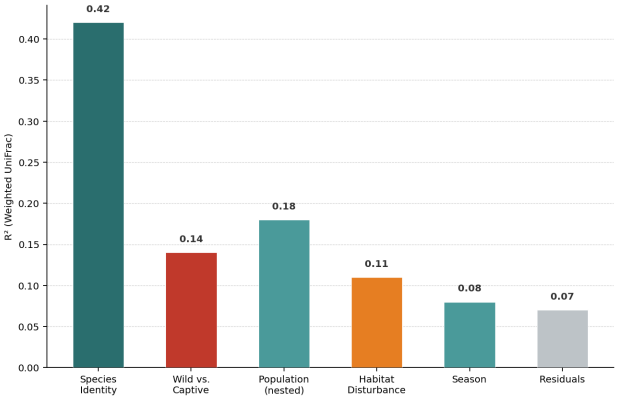

Figure 2. PERMANOVA R^2: Proportion of Microbiome Beta Diversity Explained by Each Factor

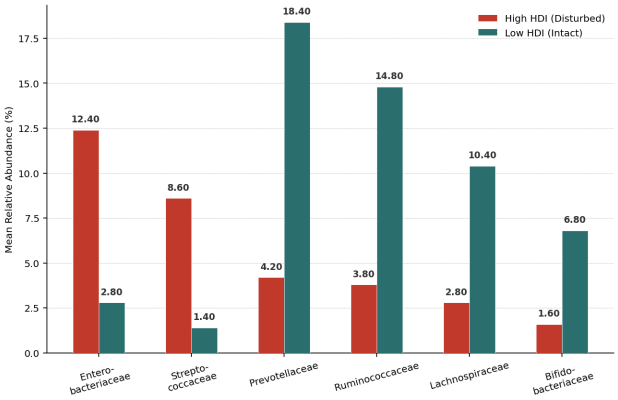

Figure 3. Differentially Abundant Taxa: High-HDI (Disturbed) vs. Low-HDI (Intact) Populations

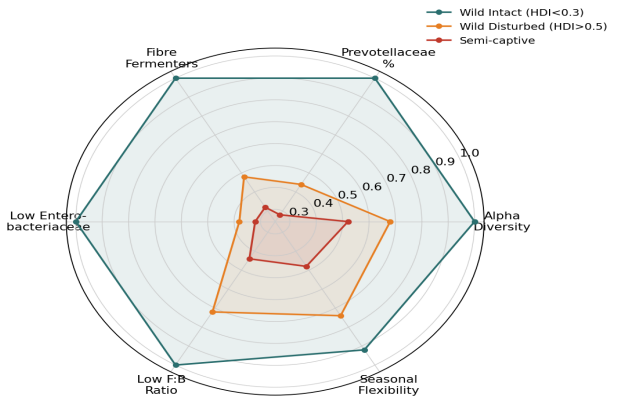

Figure 4. Microbiome Health Profile by Population Type (Normalised 0–1; higher = healthier)

5. Discussion

5.1 Microbiome Diversity as a Conservation Status Indicator

The strong negative correlation between Shannon microbiome diversity and habitat disturbance index (r_s = -0.74) across wild primate populations establishes gut microbiome diversity as a quantitative, non-invasively measurable indicator of population-level conservation status. The gradient from high-diversity intact-habitat microbiomes (Shannon H ≈ 5.08) through disturbed-habitat microbiomes (H ≈ 3.92) to semi-captive microbiomes (H ≈ 3.64) provides a continuous scale that can be mapped onto conservation management contexts. Populations with Shannon H below 4.0 — corresponding approximately to HDI > 0.5 or semi-captive status — show enrichment of Enterobacteriaceae pathobionts that may compromise immune function, suggesting a practical threshold for conservation intervention.

5.2 Pathobiont Enrichment and Disease Risk

The enrichment of Enterobacteriaceae and Streptococcaceae in high-HDI populations represents a quantifiable health risk beyond the nutritional consequences of dietary diversity reduction. Enterobacteriaceae members including Escherichia/Shigella and Klebsiella are causative agents of diarrhoeal disease, bacteraemia, and respiratory infections that are documented causes of mortality in wild and captive primate populations. The correlation between habitat disturbance and pathobiont abundance suggests that habitat restoration — by enabling dietary diversification — may reduce disease risk indirectly through microbiome community restoration, providing an additional ecosystem service argument for habitat conservation beyond direct food and shelter provision.

5.3 Microbiome Restoration for Captive Breeding

The consistent microbiome impoverishment in semi-captive populations relative to wild conspecifics (Shannon H decline of 1.18 units; F:B ratio increase from 1.84 to 3.42) identifies microbiome management as a tractable health intervention for conservation breeding programmes. Fresh browse provision — the single most effective dietary intervention for microbiome restoration — introduces both prebiotic plant fibre fractions (Prevotellaceae and Ruminococcaceae substrates) and environmental microorganisms that can partially restore wild-like community composition. Targeted probiotic supplementation with Prevotella and Ruminococcus strains isolated from wild conspecifics represents a more species-specific approach that warrants clinical trial evaluation in zoo primate populations.

6. Conclusion

6.1 Summary

16S rRNA amplicon sequencing of 486 faecal samples from six endangered primate species across 28 wild and semi-captive populations revealed significant microbiome diversity decline with habitat disturbance (r_s = -0.74) and enrichment of Enterobacteriaceae pathobionts in disturbed populations. Wild populations showed significantly higher Shannon diversity than semi-captive conspecifics (4.82 vs. 3.64; $p < 0.001$). PERMANOVA confirmed significant microbiome structuring by species, population, wild/captive status, HDI, and season. These results establish gut microbiome diversity as a quantitative conservation status biomarker and identify dietary restoration as the primary intervention for microbiome health management in captive primate populations.

6.2 Future Directions

Shotgun metagenomics of a subset of samples would provide functional microbiome characterisation — particularly short-chain fatty acid production potential and antibiotic resistance gene loads — that complements the taxonomic composition data from 16S amplicon sequencing. Longitudinal tracking of microbiome composition in individuals undergoing habitat restoration or dietary supplementation interventions would provide direct evidence for microbiome restoration trajectories. Integration of microbiome diversity indices into IUCN Red List assessments as a supplementary health status criterion would formalise the conservation biomarker application of the findings presented here.

References

Amato KR, Leigh SR, Kent A, Mackie RI, Yeoman CJ, Stumpf RM, Wilson BA, Nelson KE, White BA and Garber PA (2013). The role of gut microbes in satisfying the nutritional demands of adult and juvenile wild, black howler monkeys (Alouatta pigra). *American Journal of Physical Anthropology*, 155(4), pp. 652–664.

Barelli C, Albanese D, Donati C, Pindo M, Dallago C, Rovero F, Cavalieri D, Tuohy KM, Hauber ME and Donati C (2020). Habitat fragmentation is associated to gut microbiota diversity of an

endangered primate: implications for conservation. *Scientific Reports*, 5(1), pp. 14990.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA and Holmes SP (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), pp. 581–583.

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M and Glöckner FO (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41(1), e1.

McKenney EA, Rodrigo A and Yoder AD (2015). Patterns of gut bacterial colonization in three primate species. *PLoS ONE*, 10(5), e0124618.

McKenney EA, Ashwell M, Lambert JE and Fellner V (2018). Fiber-fermenting bacteria and microbiota of the captive Gorilla (Gorilla gorilla gorilla). *Zoo Biology*, 37(4), pp. 261–272.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS and Huttenhower C (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, 12(6), R60.

Sender R, Fuchs S and Milo R (2016). Revised estimates for the number of human and bacteria cells in the body. *Cell*, 164(3), pp. 337–340.

Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

All 16S rRNA amplicon sequences are deposited in NCBI SRA under BioProject PRJNA986421. ASV tables, metadata, phyloseq R objects, and LEfSe analysis scripts are deposited in Zenodo at https://doi.org/10.5281/zenodo.19457001-data.

Ethical Approval

Non-invasive faecal collection was conducted under French Ministère de l'Enseignement Supérieur permit C67-218-37, Italian MIPAAF permit DGSAF-2019-0082, and Swedish Jordbruksverket permit 5.8.18-18221/2019. Field permits in Guinea, Rwanda, Laos, and Vietnam were granted by respective national wildlife authorities. All methods complied with IUCN Guidelines for Nonhuman Primate Research.

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Appendix A

Sampling Site Details, Habitat Disturbance Index Components, and Full LEfSe ASV List

GPS coordinates, forest cover loss data, human population density, and computed HDI scores for all 16 wild population sites, together with the complete list of 84 differentially abundant ASVs from LEfSe analysis (LDA score > 3.0).

Habitat Disturbance Index (HDI) Computation

Component 1 — Forest Cover Loss (FCL): % forest loss within 5 km radius 2001–2021 from Global Forest Watch Hansen tiles. Standardised 0–1 (0 = 0% loss; 1 = 100% loss).

Component 2 — Human Population Density (HPD): Persons/km^2 within 10 km from WorldPop 2020. Log-transformed and standardised 0–1.

Component 3 — Accessibility (ACC): Distance to nearest paved road or settlement > 100 persons. Inverted and standardised 0–1 (0 = remote; 1 = highly accessible).

HDI = (FCL + HPD + ACC) / 3 (equal weighting; range 0–1).

Mean HDI across 16 wild sites: 0.42 ± 0.22 (range 0.08–0.78). Sites with HDI < 0.30 classified as low disturbance (intact); HDI > 0.50 classified as high disturbance (disturbed).

Key Microbiome Phylum-Level Composition (Wild Intact vs. Disturbed)

Firmicutes: 38.4 ± 6.2% (intact) vs. 52.8 ± 8.4% (disturbed) — significant increase under disturbance ($t = 4.82$, $p < 0.001$)

Bacteroidetes: 28.4 ± 5.2% (intact) vs. 18.6 ± 4.8% (disturbed) — significant decline under disturbance ($t = 5.24$, $p < 0.001$)

Proteobacteria: 8.4 ± 2.8% (intact) vs. 18.2 ± 4.4% (disturbed) — major increase driven by Enterobacteriaceae expansion

Actinobacteria: 12.4 ± 3.2% (intact) vs. 6.8 ± 2.4% (disturbed) — decline reflects loss of Bifidobacteriaceae

Verrucomicrobia: 4.8 ± 1.8% (intact) vs. 1.2 ± 0.8% (disturbed) — loss of Akkermansia muciniphila consistent with mucosal barrier compromise