

# Effect of Antibiotic Treatment on the Gastrointestinal Microbiome of Free-Ranging Western Lowland Gorillas (*Gorilla g. gorilla*)

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**Abstract** The mammalian gastrointestinal (GI) microbiome, which plays indispensable roles in host nutrition and health, is affected by numerous intrinsic and extrinsic factors. Among them, antibiotic (ATB) treatment is reported to have a significant effect on GI microbiome composition in humans and

other animals. However, the impact of ATBs on the GI microbiome of free-ranging or even captive great apes remains poorly characterized. Here, we investigated the effect of cephalosporin treatment (delivered by intramuscular dart injection during a serious respiratory outbreak) on the GI

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microbiome of a wild habituated group of western lowland gorillas (*Gorilla gorilla gorilla*) in the Dzanga Sangha Protected Areas, Central African Republic. We examined 36 fecal samples from eight individuals, including samples before and after ATB treatment, and characterized the GI microbiome composition using Illumina-MiSeq sequencing of the bacterial 16S rRNA gene. The GI microbial profiles of samples from the same individuals before and after ATB administration indicate that the ATB treatment impacts GI microbiome stability and the relative abundance of particular bacterial taxa within the colonic ecosystem of wild gorillas. We observed a statistically significant increase in Firmicutes and a decrease in Bacteroidetes levels after ATB treatment. We found disruption of the fibrolytic community linked with a decrease of *Ruminococcus* levels as a result of ATB treatment. Nevertheless, the nature of the changes observed after ATB treatment differs among gorillas and thus is dependent on the individual host. This study has important implications for ecology, management, and conservation of wild primates.

**Keywords** Gorilla · Antibiotics · Medical treatment · Gastrointestinal microbiome · Illumina MiSeq · Bacteria

## Introduction

The mammalian gastrointestinal (GI) microbiome is a complex system, with vital physiological and immunological roles. Production of vitamins and fermentation of non-digestible dietary components underscore the contribution of the GI microbiome to host metabolism and nutrition [1]. Similarly, the GI microbiome is an integral part of the host's immune system, protecting the host from infection by pathogenic microorganisms and constituting a mucosal defense barrier [1, 2].

It is widely known that administration of antimicrobial agents has significant effects on the composition and activity

of GI microbiome in humans and laboratory animals [3–8]. Antibiotic (ATB) treatment is used to suppress bacterial infections in humans and animals; however, it usually results in a decrease in the diversity of the gut microbiome, changes in microbial composition, and dysregulation of the host's immune homeostasis, causing higher susceptibility to other diseases [4, 9], as well as emergence of ATB-resistant strains [10, 11]. While the various ATBs target specific bacterial groups, non-target microbes may also be differentially impacted by ATB treatment [12] due to their exchanges of secondary metabolites or cross-feed products with the targeted species [9]. Even short-term administration of ATBs can result in long-term changes in the climax structure of the fecal microbiome of humans, altering the composition and relative abundance of specific taxa [4, 12].

Several studies have examined the impact of ATBs on the GI microbiome of various hosts, e.g., humans [4, 6–8], swine [13], and mice [14]. However, the impact of ATBs on the GI microbiome of non-human great apes remains unknown, despite the fact that various ATBs are often used in captive and sometimes even free-ranging great apes to treat various infections. For instance, wild mountain gorillas (*Gorilla beringei beringei*) from Volcanoes National Park in Rwanda have been treated with ATBs including ceftriaxone for respiratory diseases [15, 16]. Cefovecin has been used for treating respiratory outbreaks in captive chimpanzees (*Pan troglodytes*), western lowland gorillas (*Gorilla gorilla gorilla*), and Bornean orangutans (*Pongo pygmaeus*) [17].

Here, we opportunistically studied the effect of ATB treatment on a fully habituated group of wild western lowland gorillas in Dzanga Sangha Protected Areas (DSPA), Central African Republic, during a serious respiratory disease outbreak that occurred on February to April 2012. EXCEDE<sup>®</sup> (ceftiofur crystalline-free acid) Sterile Suspension and Ceftriaxone MYLAN<sup>®</sup> were the ATBs used to treat the gorillas in this study. Both are third-generation cephalosporin formulations with a broad spectrum activity against gram-positive and gram-negative bacteria, including  $\beta$ -lactamase-producing strains (Zoetis Inc., Madison, WI, USA; MYLAN<sup>®</sup>, Belgium). Like other cephalosporins, ceftiofur is bactericidal, via disruption of cell wall synthesis, which occurs in the peptidoglycan layer of the bacterial cell wall that maintains cell rigidity.  $\beta$ -lactam agents bind to penicillin-binding proteins (essential for synthesis of the bacterial wall), inhibiting cell wall synthesis and ultimately causing cell lysis [18]. EXCEDE is used for intramuscular administration in horses and other domestic species [19, 20]. Nevertheless, due to its prolonged duration of action, it is also increasingly used in wildlife species requiring less frequent injections to minimize handling and stress [21, 22]. The ATB treatment was administrated because of the highly productive (i.e., audible expectoration) nature of the coughs, which differed markedly from four previously observed respiratory outbreaks

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in DSPA-habituated gorilla groups, the prolonged history of symptoms in some individuals, and the observed spread of symptoms within the group. Moreover, there was a risk of a bacterial infection, possibly initiated by a viral infection, which, based on experience in mountain gorillas, may result in the sudden death of asymptomatic juveniles and infants [16]. The fully habituated status of the lowland gorillas allowed monitoring and collection of fecal samples from individuals before and after ATB administration, as well as subsequent assessment of the overall impact of ATB on the composition of GI microbiome.

## Material and Methods

### Study Site and Sample Collection

The study was conducted in the Dzanga sector of the Dzanga-Ndoki National Park (part of the DSPA Complex), Central African Republic (Fig. S1). Fecal samples from eight individual gorillas from the fully habituated group Makumba (Table S1) were collected in the surroundings of the Bai Hokou research camp (2° 50' N, 16° 28' E) from February to April 2012. The time of fecal sample collection and date of ATB application in each individual are listed in Table 1. Thirty-two fecal samples composed of 13 samples collected before ATB treatment and 19 samples after ATB treatment were obtained. Four samples from two gorillas that were not subjects of ATB treatment were also collected. ATB treatment was given to the gorillas between March 10 and 13, 2012. Ceftriaxone (1 g, IM; MYLAN<sup>®</sup>, Belgium) was used for the first treatment dose of a gorilla named Mossoko, and subsequent treatments were with EXCEDE<sup>®</sup> (~1.4 mg CE/kg, IM, ceftiofur crystalline-free acid; Zoetis Inc., Madison, USA) for all other treated gorillas including Mossoko (for the second dose). Fecal samples were obtained non-invasively, immediately after defecation during gorilla follows. Samples (approximately 0.2 g of feces) were mixed with 1.5 ml of RNAlater (QIAGEN, CA, USA) and kept at room temperature for a maximum of 1 month before transport to the Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, where they were stored at -80 °C. Samples were shipped to the Institute for Genomic Biology, University of Illinois at Urbana-Champaign, USA, for DNA sequencing analyses in November 2012. The non-invasive sample collection was conducted according to the Convention on Human Rights and Biomedicine of the Council of Europe and Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes and meet the International Guiding Principles for Biomedical Research involving Animals, as issued by the Council for International Organizations of Medical Sciences (C.I.O.M.S., c/o WHO,

CH 1211 Geneva 27, Switzerland). The research adhered to the legal requirements of the Central African Republic. Importation of the samples to the EU was approved by the State Veterinary Authority of the Czech Republic. The sequencing was conducted under University of Illinois IACUC protocol 11046.

### Sample Processing and Sequencing Analyses

DNA from fecal samples was extracted using the Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's protocol. DNA was purified using QIAquick Gel Extraction Kit (QIAGEN, Germany). The V3 and V5 regions of 16S ribosomal ribonucleic acid (rRNA) were amplified by polymerase chain reaction (25 cycles of 98 °C (15 s), 65 °C (30 s), and 72 °C (30 s)) using the primer set 357f (5'-CCTACGGGAGGCAGCAG-3') and 926r (5'-CCGTCAATTCMTTTRAGT-3') tagged with identifying bar codes (Table S2). Because some of the obtained PCR amplicons were of low DNA concentration, we performed a second round of amplification on these amplicons, using the same primer set and PCR conditions. To determine the effect of the second round of amplification, we performed the same procedure in all amplicons, including those with strong DNA concentration. Amplicon purification was done using the QIAquick PCR Purification Kit (QIAGEN, Germany). Amplicon sequencing was performed using the Illumina MiSeq Platform at the Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign (Urbana, IL). Samples with <4000 reads after Illumina sequencing were removed from all subsequent analyses to avoid biases in diversity estimations. The reads were clustered into operational taxonomic units (OTUs) using the online tool IM-TORNADO as described in Jeraldo et al. [23]. Briefly, reads were filtered for quality, and non-overlapping pair-end reads were joined, concatenated, and sorted by cluster to find de novo OTUs using USEARCH and its UPARSE algorithm, which also removed chimeric sequences. Taxonomy assignments were carried out using Ribosomal Database Project's naive Bayesian classifier against the Greengenes 13\_5 database. Fully unclassified reads were then eliminated. After taxonomy assignment, reads were aligned and mapped against their corresponding set of unaligned. OTU representatives were defined using USEARCH at 97 % sequence identity to generate an OTU table. OTUs detected less than three times in a sample and/or occurring less than five reads in the entire data set were discarded to avoid including probable sequence artifacts in the analyses.

### Statistical Analyses

Relative abundance of each OTU and Bray-Curtis dissimilarity matrices were used for principal coordinate (PCO)

**Table 1** Timetable of samples of individual gorillas during respiratory outbreak

Individual/	February												March												April															
Date	17	22	23	24	25	26	27	28	29	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	24	25	2	3	7	14	15	16	19	22	24					
Infection day	1	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26																		
Makumba				x	x							x		x		x					x			x																
Malui									x											x				x																
Mopambi																				x				x																
Mossoko																				x		x	x																	
Tembo					x																																			
Mobangui					x				x																															
Sopo																																								
Kunga																																								

Dark grey day of ATB treatment, pink days after ATB treatment, x collection of sample. Detailed information about samples are provided in Table S1

analyses and analysis of similarities (ANOSIM) in PRIMER6 for Windows v.6.1.10 [24]. The stacked bar plots of microbial profiles were generated with QIIME v.1.7.0 [25]. Taxa characterizing samples from before and after ATB treatment were identified based on the frequency and relative abundance of taxa using indicator species analysis [26] in the labdsv R package [27, 28]. These analyses were performed at the microbial genus, family, and phylum levels for all samples collected before and after ATB treatment and also individually for the one individual with more than four samples collected before and after ATB treatment (the silverback male, Makumba). The vegan package of R [29] was used for the analysis of multivariate homogeneity of group dispersions, rarefied richness analysis, calculation of Shannon diversity index, and permutational analysis of variance (PERMANOVA). Boxplots, *t* tests, Wilcoxon’s rank sum tests, and one-way ANOVAs were calculated using stats package in R [28].

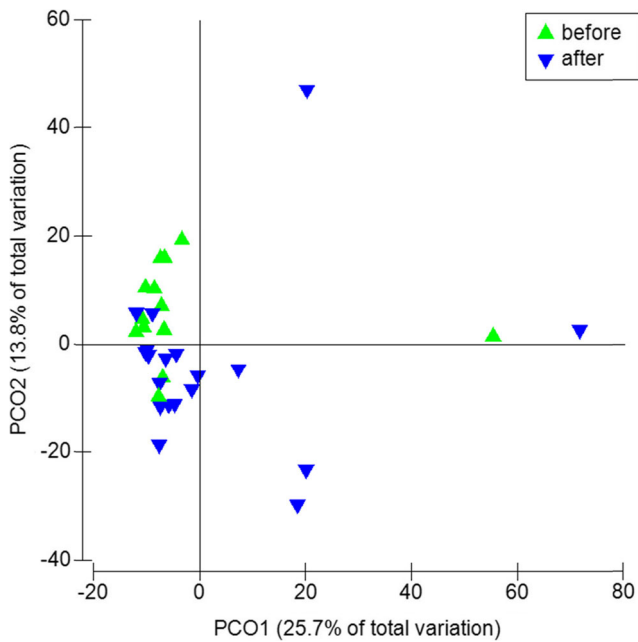
**Results**

We validated the two-round PCR approach with analyses of a subset of samples amplified by both a one-round and two-round PCR approach. We observed no statistically significant differences between the GI microbial profiles from 16S rRNA amplicons obtained using either one-round PCR or two-round PCR (Fig. S2) according to ANOSIM ( $R=0.005, p=0.378$ ) and PERMANOVA (pseudo  $F=1.501, p=0.105$ ). We did not detect statistically significant differences in GI bacterial diversity between samples processed by one-round PCR and two-round PCR according to the Shannon index ( $t$  test  $t=0.881, p=0.386$ ; Fig. S3) and rarefied richness analysis ( $t$  test  $t=0.657, p=0.516$ ). Thus, the set of samples processed by two-round PCR was used for all further analyses. In total, we obtained 16,165,656 high-quality 16S rRNA reads with a median sampling depth of 128,645 reads per sample (minimum 34,927 and maximum 1,158,244) after sequence quality control. Non-overlapping pair-end reads yielded a median sequence length of 450 nt (250 nt with R1 and 200 nt with R2).

PERMANOVA revealed small but significant differences before and after ATB treatment (pseudo  $F=1.968, p=0.021$ ; Fig. 1). The relative abundance of specific taxa changed after ATB treatment (Fig. S4a), with Firmicutes increasing and Bacteroidetes decreasing in most individuals (Fig. S4a and Table 2). Unclassified members of the Desulfovibrionales and Fibrobacteres increased after ATB treatment. This was also the case for *Coriobacterium*, while the opposite pattern was seen with *Ruminococcus* (decreased after ATB treatment; Table 2). We also observed changes in the relative abundance of minor bacterial phyla after ATB treatment; however, these were not statistically significant (Fig. S4b).

There were no significant differences in GI bacterial diversity in samples before and after ATB treatment from all gorillas analyzed collectively according to the Shannon index (ANOVA  $F=0.106, p=0.747$ ) and rarefied richness analysis (ANOVA  $F=1.093, p=0.306$ ). However, the microbiome of gorillas after ATB treatment was significantly more dispersed and less homogeneous (ANOVA  $F=7.86, p=0.009$ ; Fig. 2). Despite not detecting overall differences in alpha diversity, we found that the impact of ATB treatment on GI microbiome was largely dependent on the individual host. That is, when taking the host into account in the statistic models, individual gorilla was a significant factor explaining Shannon diversity (ANOVA  $F=2.282, p=0.077$ ; Table S3), rarefied richness (ANOVA  $F=2.791, p=0.0389$ ; Table S4), microbial homogeneity (ANOVA  $F=4.293, p=0.005$ ; Table S5), and overall community composition (PERMANOVA pseudo  $F=1.782, p=0.006$ ; Table S6) after ATB treatment.

The analysis of samples from the silverback male (Makumba), the individual from which we obtained the highest number of samples before and after ATB treatment (six samples before and six samples after), indicated that bacterial community composition changed significantly after ATB administration (PERMANOVA pseudo  $F=1.679, p=0.043$ ). The silverback’s gut microbiome was significantly more variable (Wilcoxon’s rank sum test  $W=3, p=0.015$ ; Fig. 3a) and more dispersed (Fig. 3b) in the samples collected after ATB treatment reflecting the instability of microbiome. We also observed a possible recovery of the silverback’s GI



**Fig. 1** PCO plot of bacterial community in samples collected before and after ATB treatment

microbiome (Fig. 3a). Microbial profiles in Makumba's samples collected before ATB treatment were very similar to those in samples collected shortly after ATB treatment (from 2 to 12 days after treatment; three samples). However, microbial profiles in Makumba's sample collected 25 days after treatment differed from the others. Microbial profiles in two of his

samples collected even later (33 and 37 days after treatment) were again similar to those in samples collected before ATB treatment (Fig. 3a). Differences in GI bacterial alpha diversity according to the Shannon index (Wilcoxon's rank sum test  $W=32$ ,  $p=0.026$ ) and rarefied richness analysis (Wilcoxon's rank sum test  $W=0$ ,  $p=0.002$ ) were also significant, indicating that the GI microbiome of the silverback after ATB treatment was less diverse. Analysis of changes in the relative abundance of specific taxa showed that Bacteroidetes decreased significantly in Makumba after ATB treatment (Fig. 4 and Table 3). At the family level, Bacteroidaceae and Spirochaetaceae also decreased in abundance, whereas Anaerolinaceae increased after ATB treatment. At the genus level, *Ruminococcus*, *Lachnobacterium*, *Sphaerochaeta*, and *Mycoplasma* significantly decreased after ATB administration (Table 3). Similar trends were observed in samples from the remaining gorillas of the group. However, the impact of ATB treatment on the relative abundances of individual taxa varied in each host (Fig. 5).

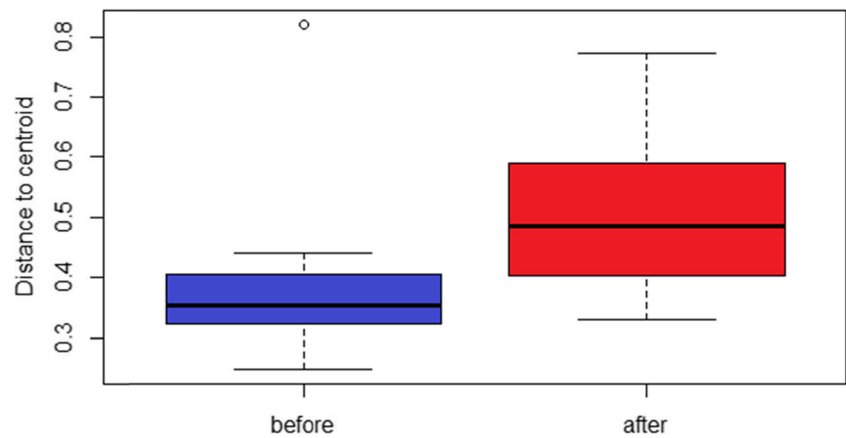
Microbiome compositions in two individuals that did not receive ATB treatment (the blackback male, Kunga, and the infant, Sopo) were also assessed (Fig. S5). We detected approximately similar levels of all indicator taxa in samples collected during the respiratory outbreak before the ATB treatment of the silverback Makumba and in blackback Kunga. However, differences in levels of indicator taxa were observed specifically in case of *Ruminococcus* in the period after ATB treatment (Fig. S6).

**Table 2** Indicator taxa with mean relative abundances in samples before and after ATB treatment

Taxonomic rank	Indicator taxa before ATB	Indicator value	Probability	Mean relative abundance % (SD)	
				Before ATB	After ATB
Phyla	Bacteroidetes	0.61	0.005	39.39*** (14.03)	24.69 (11.20)
Family	Unclassified (Mollicutes)	0.86	0.002	0.15 (0.46)	0.01 (0.02)
Family	Unclassified (Deltaproteobacteria)	0.47	0.027	0.02 (0.05)	0.01 (0.03)
Genus	Unclassified (Mollicutes)	0.86	0.001	0.15 (0.46)	0.01 (0.02)
Genus	<i>Ruminococcus</i>	0.66	<0.05	0.43** (0.34)	0.22 (0.22)
Genus	Unclassified (Deltaproteobacteria)	0.47	0.01	0.02 (0.05)	0.01 (0.03)
Taxonomic rank	Indicator taxa after ATB	Indicator value	Probability	Mean relative abundance % (SD)	
				Before ATB	After ATB
Phyla	Firmicutes	0.6	0.003	27.24 (4.8)	40.55*** (14.82)
Family	Coriobacteriaceae	0.73	0.05	2.41 (1.81)	8.01 (13.01)
Family	Unclassified (Desulfovibrionales)	0.57	0.045	0.01 (0.02)	0.06* (0.11)
Family	Unclassified (Fibrobacteres)	0.40	0.049	0	0.01* (0.01)
Genus	<i>Coriobacterium</i>	0.64	<0.05	0.17 (0.07)	0.34** (0.27)
Genus	Unclassified (Desulfovibrionales)	0.57	<0.05	0.01 (0.02)	0.06* (0.11)
Genus	Unclassified (Fibrobacteres)	0.40	<0.05	0	0.01* (0.01)

Significant differences based on ANOVA, \*\*\*0.01, \*\*0.05, and \*0.1

**Fig. 2** GI microbiome dispersion of all gorillas before and after ATB treatment ( $p = 0.009$ , ANOVA)

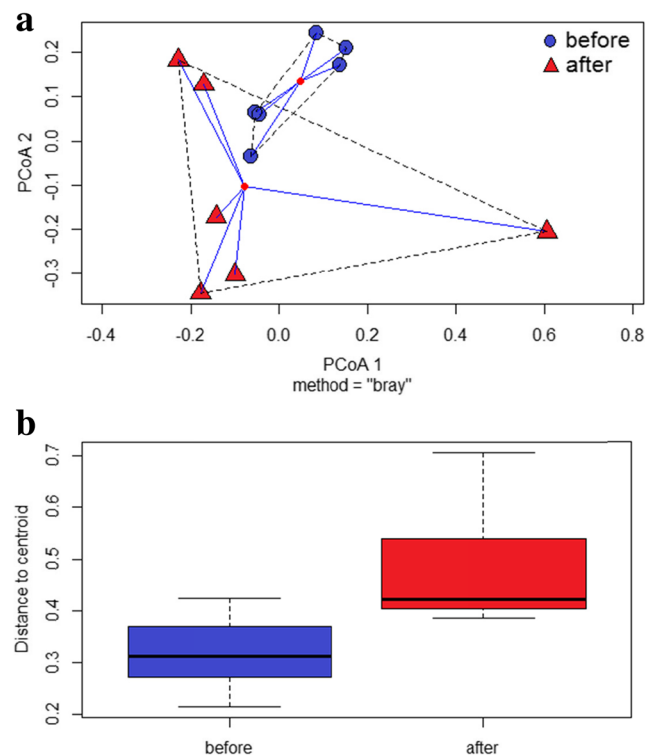


## Discussion

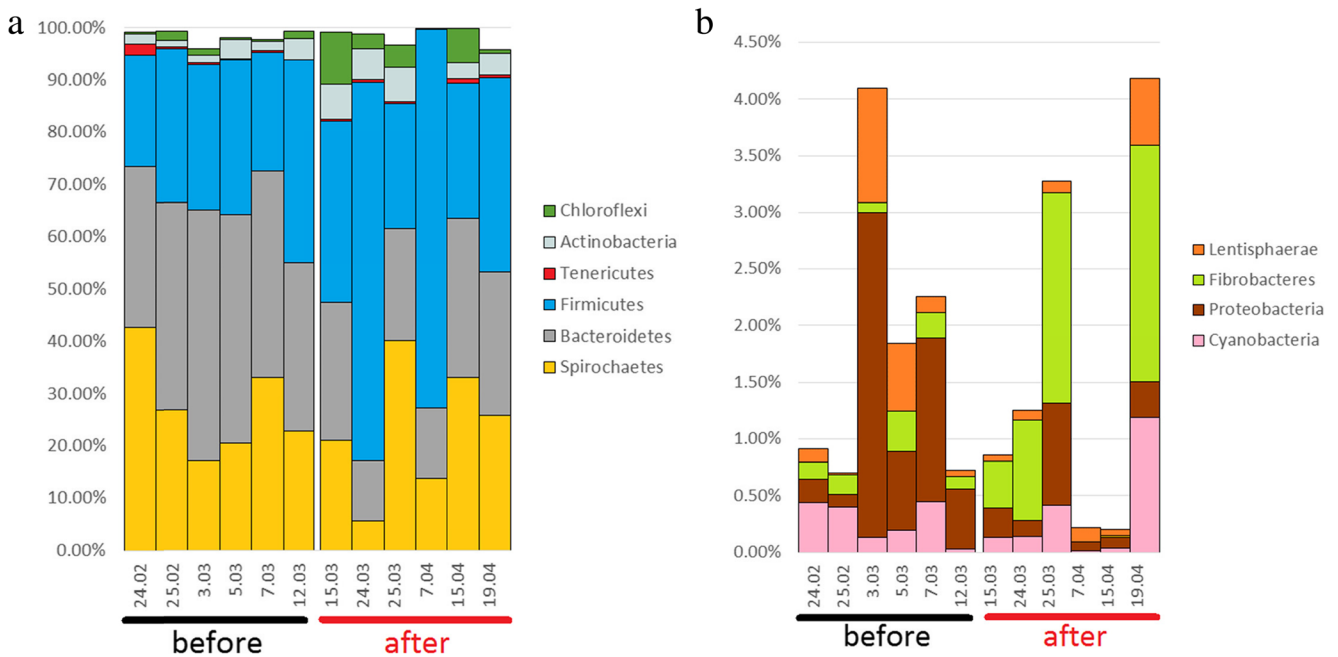
We showed that ATB treatment with ceftiofur has a detectable impact on the GI microbiome of wild western lowland gorillas, mainly affecting the stability of the microbial community and the relative abundance of particular taxa. We also showed that the effect of ATB administration on diverse GI bacteria differed among individual hosts. This may be a reflection of the significant inter-individual variability observed in the GI microbiome of humans and non-human primates [30–33]. This baseline variation appears to be affected by continual intrinsic and extrinsic selective factors that vary among individuals (age, gender, and host genotype) or specific mutualistic interactions that stabilize community composition or diet [31, 34–36]. Moreover, based on our experience, ATB dosing is challenging in free-ranging animals and this variation may also be a factor. The dosage of ATBs is determined by an estimate of individual mass, which could result in a different dosage per kilogram in each individual. In addition, although it is always visually confirmed that administration of ATB injection was successful, there is a possibility that an individual did not receive the full dose of treatment, such as when the injection is more subcutaneous than intramuscular or if some of the ATBs are not fully injected and might spray out the dart when it comes out of the gorilla. We acknowledge that the impact of ATB on GI microbiome of gorillas may also have been affected, to some extent, by sampling limitations and variations in sampling points. This may be the case of Mossoko, Mopambi, and Tembo. However, the fact that we observed steady, host-specific microbiome patterns in Makumba, Malui, and Mobangui (Fig. 5) implies that microbiome responses to ATB treatment in these individuals are still host dependent.

Gut microbiome diversity in samples of the silverback significantly decreased after ATB treatment. The same patterns were observed in other gorillas of different ages; however, we could not detect statistically significant differences, perhaps due to the smaller sample size. Therefore, we highlight both

the challenges encountered when sampling wild apes and the difficulty of obtaining these kinds of samples during challenging situations such as a disease outbreak and administering of ATBs. When following groups of wild habituated lowland gorillas, the easiest individual to follow and to obtain distinguishable samples from is always the silverback male (due to the size and social role within the group), which explains why we had the highest number of samples from Makumba. Females and infants are much more difficult to sample, even under normal conditions. Thus, the results from the samples of these animals should be interpreted with caution. Nonetheless, the reduction of GI microbial diversity observed is concordant



**Fig. 3** GI microbiome dispersion of Makumba before and after ATB treatment (a) in multivariate space and (b) in boxplots ( $p = 0.002$ , Wilcoxon's rank sum test)



**Fig. 4** Taxonomic profiles of major (a) and minor (b) phyla in samples before and after ATB treatment for the silverback Makumba

with other studies in humans and mice regardless of ATB type [6–8, 37]. Decrease in microbial diversity may suggest that displacement of microorganisms sensitive to ATBs provides space for resistant strains to overgrow and dominate the colonic niche [7]. A collateral effect of this bacterial community turnover might have also been reflected by the less

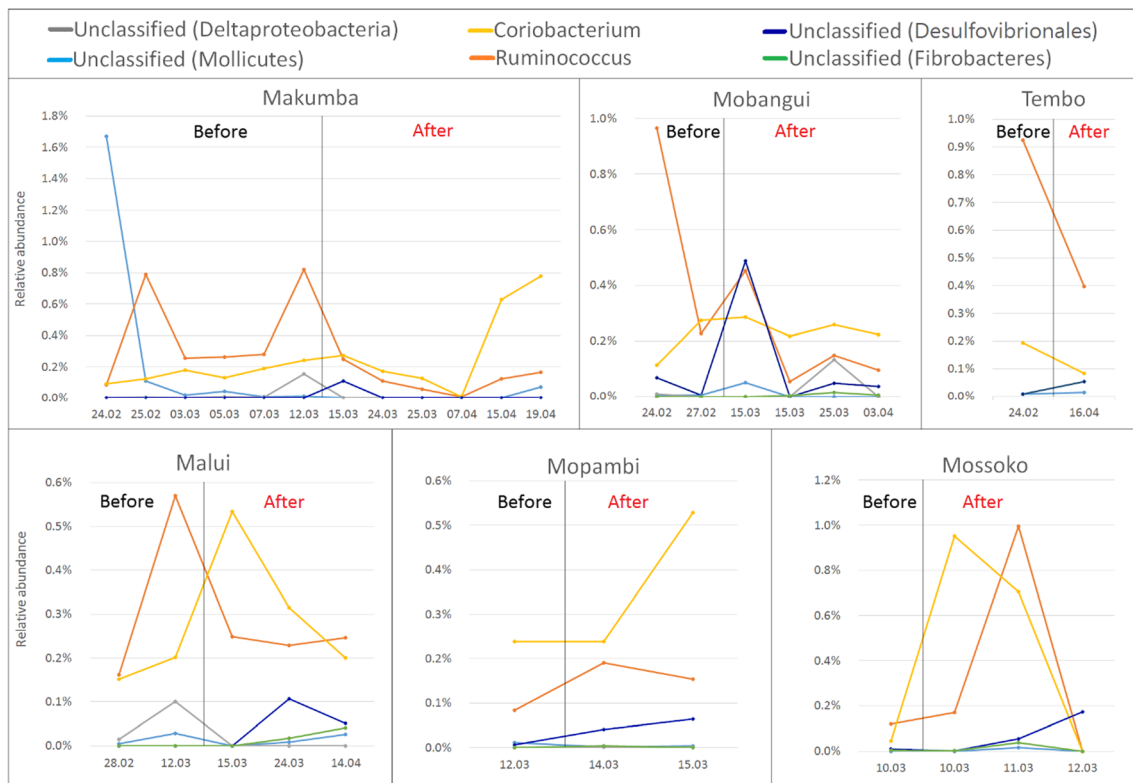
homogeneous and more dispersed microbiome observed in samples from all gorillas collected after ATB treatment.

The shifts in the levels of Firmicutes and Bacteroidetes, the two main phyla in the mammalian GI microbiome [31], are consistent with results of Welling et al. [3] and Pérez-Cobas et al. [6], who studied the impact of broad spectrum ATB in

**Table 3** Indicator taxa with mean relative abundances in samples of Makumba before and after ATB treatment

Taxonomic rank	Indicator taxa before ATB	Indicator value	Probability	Mean relative abundance % (SD)	
				Before ATB	After ATB
Phyla	Bacteroidetes	0.64	0.004	38.96*** (6.61)	21.75 (7.86)
Family	Unclassified (Mollicutes)	0.96	0.016	0.31*** (0.67)	0.01 (0.03)
	Unclassified (Deltaproteobacteria)	0.83	0.017	0.03*** (0.06)	0
	Bacteroidaceae	0.83	0.024	0.78** (0.80)	0.16 (0.11)
	Spirochaetaceae	0.71	0.028	3.09*** (1.54)	1.26 (0.70)
	Genus	Unclassified (Mollicutes)	0.96	0.01	0.31*** (0.67)
Genus	Bacteroides	0.83	0.036	0.73** (0.81)	0.15 (0.10)
	Unclassified (Deltaproteobacteria)	0.83	0.015	0.03*** (0.06)	0
	<i>Ruminococcus</i>	0.78	0.035	0.42*** (0.31)	0.12 (0.08)
	<i>Mycoplasma</i>	0.74	0.039	0.09** (0.11)	0.01 (0.03)
	<i>Lachnobacterium</i>	0.74	0.025	0.37** (0.20)	0.13 (0.12)
	<i>Sphaerochaeta</i>	0.70	0.03	3.09*** (1.54)	1.26 (0.70)
Taxonomic rank	Indicator taxa after ATB	Indicator value	Probability	Mean relative abundance % (SD)	
				Before ATB	After ATB
Family	Anaerolinaceae	0.82	0.048	0.88 (0.64)	4.02* (3.73)

Significant differences based on Wilcoxon’s rank sum test, \*\*\*0.01, \*\*0.05, and \*0.1



**Fig. 5** Changes in relative abundances of six indicator taxa at the genus level during the sampling period in five studied gorillas. Plots are divided into two parts (*black line*), denoting samples collected before and after ATB treatment

humans. On the contrary, Panda et al. [7] detected a decrease of Firmicutes and an increase of Bacteroidetes after treatment with fluoroquinolones and  $\beta$ -lactams. Shifts in the ratios of these phyla have been associated with many physiological parameters, including obesity and an increased capacity to harvest energy from dietary sugars [38, 39]. It is unclear whether the increased Firmicutes:Bacteroidetes ratios in gorillas after ATB treatment affect their energetic landscape. However, these shifts, as well as the emergence and decrease of other taxa, may reflect succession of particular bacterial community members and/or their metabolic functions after microbial taxa were affected by treatment.

Along with decreases in Bacteroidetes after ATB treatment, we observed an increase in relative abundance of Coriobacteriaceae, Chloroflexi (Anaerolinaceae), and Fibrobacteres. The Coriobacteriaceae family, also previously reported in chimpanzees [40] and humans [34], has been associated with energy metabolism, particularly with lipid turnover [36]. Chloroflexi has been detected in western lowland gorillas, eastern lowland gorillas, chimpanzees, bonobos [32], humans [41], and even ruminants [42]. This taxon is reported to contribute significantly to fermentative metabolism and increased organic matter conversion in other natural environments [43]. Fibrobacteres are important members of fibrolytic bacterial communities in mammalian guts, especially in cattle and pigs [44, 45], and are well known for their ability to

degrade refractory plant structural polysaccharides [46]. Desulfovibrionales, which also increased after ATB treatment, has been reported in GI microbiome of ruminants, humans, and howler monkeys [42, 47, 48]. It has been demonstrated that members of Desulfovibrionales (e.g., *Desulfovibrio*, also detected in this study) have an important role in short-chain fatty acid (SCFA) turnover in the colon [49]. They may promote a shift in the metabolism of sugars, altering the synthesis by saccharolytic microorganisms of hydrolytic enzymes involved in carbohydrate breakdown [50].

A significant decrease in the relative abundance of *Ruminococcus* after ATB treatment was observed in all gorillas, with the exception of gorillas Mopambi and Mossoko, who were only sampled within a few days after treatment. Moreover, Mossoko was the only one receiving a second dose of ATB; however, we did not have available samples collected after the second treatment with EXCEDE<sup>®</sup>. Nevertheless, because of the similar characters of both ATBs (both are third-generation cephalosporins), we expected similar effect on the GI microbiome. Contrary to our results, Looft et al. [13] reported that treatment with an antibiotic feed additive containing chlortetracycline, sulfamethazine, and penicillin caused increases in the relative abundance of *Ruminococcus* in the GI tract of swine. *Ruminococcus* spp. may be vital in cellulose breakdown in the gut ecosystem of hosts such as chimpanzees [40], mountain gorillas [51], humans [52], equines [53], and



pigs [44]. Fibrolytic bacteria comprise part of a complex network in which different species play particular roles in fiber degradation. For example, both *Ruminococcus* and *Fibrobacter* are primary degraders of insoluble plant fiber, but degradation is performed differently, i.e., using different carbohydrate-processing modules by members of each genus [46].

It is likely that the emergence of specific taxa with fiber degradation and fermentative roles, such as *Fibrobacter* at the expense of *Ruminococcus*, taxa related to the Bacteroidaceae, and Deltaproteobacteria, may contribute to the absence of important nutrient-processing roles after some taxa have been affected after ATB treatment. Moreover, relative abundances of *Lachnobacterium* from the Lachnospiraceae family and *Sphaerochaeta* from the Spirochaetaceae family, the common members of human and animal GI microbiome [54–56], significantly decreased in samples collected after ATB treatment.

If the taxa with potential carbohydrate-processing roles dissipate and re-emerge after ATB treatment, then the GI microbiome of gorillas may be relatively resilient to extrinsic disturbances. This is a key consideration since fiber and carbohydrate processing and fermentation are critical functions in the GI tract of wild gorillas, especially when they rely heavily on fiber for nutrient and energy harvesting during low-fruit seasons, as was the case for this gorilla group during the study period [54, 57]. Indeed, disruption of the complex fibrolytic bacterial community at the GI level may lead to an inability to process high-fiber diets, with subsequent impact on the welfare of the host [52]. For instance, in equines, altered patterns of fiber degradation can result in illness and even death, caused by factors such as excessive accumulation of gas or decreased motility leading to intestinal impaction [58, 59].

Interestingly, we also recorded a significant decrease in the relative abundance of *Mycoplasma* in the silverback Makumba's samples collected after ATB treatment. *Mycoplasma* spp. are responsible for a variety of diseases, including contagious pleuropneumonia, in humans, domestic animals, wildlife, and non-human primates [60, 61]. Detected decreases in *Mycoplasma* levels, however, cannot be the direct result of the antibiotic treatment because cephalosporins act by inhibiting cell wall synthesis and are therefore ineffective against bacteria lacking a cell wall such as *Mycoplasma*.

Contrary to reports in humans [62–64], we did not detect any opportunistic pathogenic bacteria such as *Clostridium*, *Klebsiella*, or *Staphylococcus*, commonly linked with deleterious effects from ATB treatment or subsequent ATB-associated diarrhea. Moreover, no evident side effects were observed in treated gorillas (only gorilla Mossoko showed a runny stool once on the day of the second ATB treatment). This may suggest that the GI microbiome of wild apes may be less susceptible to ATBs compared to those of humans. However, as there have been no previous ATB treatments in the studied gorilla group and as the frequency of ATB

administration is a key factor in boosting the emergence of potential pathogens and ATB-resistant bacteria, we suggest that additional studies should focus on apes more often treated with ATBs (e.g., mountain gorillas or captive great apes) [15, 16] or longer-term studies in lowland gorillas with more frequent sampling. However, this scenario is hardly attainable with wild lowland gorillas due to the rare administration of ATB treatment, dispersed groups, and challenging field/research conditions.

Although interpretations of results based on comparisons with untreated animals are limited due to the low sample size from untreated individuals, we were able to confirm the effect of ATB treatment on the microbial composition when comparing the silverback Makumba and blackback Kunga (Fig. S6). In this regard, the fact that trends in the levels of all indicator taxa were similar for both males in samples collected before ATB treatment, but not after, is not only support for the effect of ATB on the gut microbiome of wild apes but also support for successful administration of ATB treatment. This is also supported by the fact that all animals successfully recovered from the outbreak. Nonetheless, to further validate the influence of ATBs on the gut microbiome of great apes and a possible resilience or recovery, more controlled studies with increased sample sizes are needed.

## Conclusions

Despite the challenging sampling conditions and limitations covering the whole treatment period in all studied gorillas (including those not receiving treatment), our study is the first to describe how ATB treatment affects GI bacterial communities in wild apes. In summary, our results showed significant changes in the GI microbial composition caused by ATB treatment with differences dependent on individual host and potential resilience of the gorilla GI microbiome in terms of metabolic capacity. The consequences of the changes may not be immediately clear; however, as shown in the human GI microbiome, ATBs do appear to have long-term impacts [4]. Although no serious health problems were observed after ATB treatment in the studied gorillas and all individuals recovered fully from the outbreak, further research is warranted on the pervasive and persisting effect of ATBs in wild apes after ATB administration, including the presence and spread of ATB-resistant strains. Additionally, future studies should assess whether the effect of ATB treatment described in this study may be generalized to other ATB types. These data provide important conservation implications for the considerations of ATB treatment in wild animals. These results are suggestive and point to the need for more controlled sampling. Until then, we urge caution in wild ape ATB treatments and stress the need for proper risk assessment and further study.

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**Compliance with Ethical Standards** The non-invasive sample collection was conducted according to the Convention on Human Rights and Biomedicine of the Council of Europe and Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes and meet the International Guiding Principles for Biomedical Research Involving Animals, as issued by the Council for International Organizations of Medical Sciences (C.I.O.M.S., c/o WHO, CH 1211 Geneva 27, Switzerland). The research adhered to the legal requirements of the Central African Republic. Importation of the samples to the EU was approved by the State Veterinary Authority of the Czech Republic. The sequencing was conducted under University of Illinois IACUC protocol 11046.

**Conflict of Interest** The authors declare that they have no conflict of interests.

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