RESEARCH ARTICLE

# The giraffe (*Giraffa camelopardalis*) rumen microbiome

Michael Roggenbuck[1], Cathrine Sauer[2,3], Morten Poulsen[2], Mads F. Bertelsen[3] & Søren J. Sørensen[1]

[1]Department of Biology, Microbiology, University of Copenhagen, Copenhagen O, Denmark; [2]Department of Animal Science, Aarhus University, Tjele, Denmark; and [3]Copenhagen Zoo, Centre for Zoo and Wild Animal Health, Frederiksberg, Denmark

**Correspondence:** Søren J. Sørensen, Department of Biology, Microbiology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen O, Denmark. Tel.: +45 5182 7007; fax:+4535322040; e-mail: SJS@bio.ku.dk

Received 11 March 2014; revised 12 July 2014; accepted 14 July 2014. Final version published online 01 September 2014.

DOI: 10.1111/1574-6941.12402

Editor: Julian Marchesi

**Keywords**
rumen; microbiome; giraffe; wild life; diet.

## Abstract

Recent studies have shown that wild ruminants are sources of previously undescribed microorganisms, knowledge of which can improve our understanding of the complex microbial interactions in the foregut. Here, we investigated the microbial community of seven wild-caught giraffes (*Giraffa camelopardalis)*, three of which were fed natural browse and four were fed Boskos pellets, leafy alfalfa hay, and cut savanna browse, by characterizing the 16S rRNA gene diversity using 454 FLX high-throughput sequencing. The microbial community composition varied according to diet, but differed little between the ruminal fluid and solid fraction. The giraffe rumen contained large levels of the phyla of *Firmicutes* and *Bacteroidetes* independent of diet, while *Prevotella*, *Succinclasticium*, and *Methanobrevibacter* accounted for the largest abundant taxonomic assigned genera. However, up to 21% of the generated sequences could not been assigned to any known bacterial phyla, and *c.* 70% not to genus, revealing that the giraffe rumen hosts a variety of previously undescribed bacteria.

## Introduction

It has been estimated that there are 75.3 million wild and 3.57 billion domestic ruminants on earth belonging to six ruminant families of *Antilocapridae*, *Cervidae*, *Bovidae*, *Giraffidae*, *Moschidae*, and *Tragulidae* (Hackmann & Spain, 2010). The majority of research on the microbial community of ruminants has focused on livestock members of the *Bovidae*, while little is known about the microbiome of wild ruminants.

With a height of 5–6 m and a body weight of up to 1400 kg, the *Giraffa camelopardalis* (family *Giraffidae*) is one of the largest existing ruminants (Hall-Martin, 1977). The daily dry matter intake The word "circa" doesn't fit here. It needs to be as in the original submitted text "approximates" 1.6% and 2.1% of body weight, for males and females, respectively (Pellew, 1984).

In the wild, giraffes are browsers – ruminants that feed on leaves, shoots, fruits, flowers, and even twigs of many different species of trees and shrubs (Leuthold & Leuthold, 1972). Browse generally has higher crude protein and lignin contents than grasses and may also contain other secondary plant metabolites such as tannins (Shipley *et al.*, 1998). In contrast, grasses are usually richer in fibers.

Zoo diets are often designed to mimic the natural diet of an animal. As the wild diet items vary greatly in availability, palatability, and nutrient content throughout the year, it is, however, very difficult to replicate the natural diet in captivity. In addition, the nutrient requirements of most captive wildlife species are unknown, making it even more difficult to design an appropriate diet, and diet related problems have been reported for captive giraffes (Potter & Clauss, 2005; Clauss *et al.*, 2007). The impact of neither natural nor captive diets on the giraffe rumen microbiota has not been investigated.

Like other herbivores, the giraffe depend on symbiosis with microorganisms in the digestive system to utilize cellulose and hemicellulose. In the rumen, the central organ of foregut fermentation of large herbivores, microorganisms ferment fibers and produce along with several other metabolites volatile fatty acids – the major energy source of the host (Saengkerdsub & Ricke, 2013). This has driven coevolution between the animal host and the microorganisms, where microorganisms have specialized in utilizing specific organic compounds reflecting differences in composition of the diet ingested by the animal (Ley *et al.*, 2008a, b).



© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

FEMS MICROBIOLOGY ECOLOGY

Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

In this pilot study, we investigated the ruminal microbiota of seven giraffes fed with Boskos pellets, leafy alfalfa hay, and cut savanna browse.

However, due to the logistics of the project, only four animals (group I) were fed continuously as described above. The other three giraffes (group II) were first kept under the same conditions as group I, but 4–6 days prior to sampling fed natural browse from surrounding enclosure.

It has been demonstrated that diet composition alter the rumen microbiota of calves (Pitta *et al.*, 2010) and as the giraffes had eaten different diets for a longer period than the mean particle retention time of 40 h reported for giraffes (Clauss *et al.*, 1998), we hypothesized that the rumen microbiota would be distinct between feeding group I and II.

To investigate this, we characterized simultaneously the bacterial and archaeal communities of the solid and fluid fraction of rumen content of the giraffe via a culture independent approach using the 16S rRNA gene phylogenetic marker and 454 FLX Titanium high-throughput sequencing amplifying the hypervariable region of V3–V4. Our study gives first insights into the giraffe rumen microbiome.

## Material and methods

### Sample collection

Samples of solid and fluid reticulo-ruminal content were collected from seven juvenile giraffes (*G. camelopardalis*), six males and one female, weighing 491 ± 92 kg.

The giraffes originated from private game parks in the Republic of South Africa and were brought to a central facility as part of a physiological research project. Here, the animals received a diet of Boskos pellets [WES Enterprises (Pty) Ltd, South Africa], leafy alfalfa hay, and various species of fresh cut savannah browse for 1–2 months. Four animals (A, B, C, D) continued the diet described above (diet group I), whereas three giraffes (E, F, G) were fed on natural browse only in a large natural enclosure for 4–6 days before sampling (diet group II).

Following anesthesia for physiological research, animals were euthanized and immediately underwent a thorough postmortem examination. Contents of the reticulo-rumen were sampled by collecting a handful of material from six different locations (see Fig. 1). Samples from all locations were mixed in a filter bag with 0.5-mm pore size (Grade Blender Bags, VWR, Denmark) to separate the fluid and the solid fractions. Immediately after sampling, the filter bag was stored at *c.* 5 °C for *c.* 2 h until the fluid and the solid part were separated. Thirty milliliter of each fraction was sampled and kept at −18 °C for up to 15 days until shipment on dry ice. After shipment, samples were stored at −80 °C until analysis.

### DNA preparation, sequencing and data treatment

Subsamples of 0.5 g of each sample were transferred to a tube together with 1 mL extraction buffer (50 mM Tris-HCl, 5 mM EDTA, 3% SDS) and sterile glass beads. Cells were mechanically disrupted by FastPrep (MP Biomedicals) 5.5 m $s^{-1}$ for 30 s. Cell debris was removed by centrifugation, and the supernatant was incubated in 465 μL ammonium acetate (5 M) for 5 min at 4 °C. The reaction was stopped by adding 2x volume of reaction mix of Guanidine–HCl (7 M) to the tube. The genomic DNA was purified using the Genomic Mini AX SOIL Spin Kit (A&A Biotechnology).

The hypervariable region V3–V4 of bacterial and archaeal 16S rRNA genes was simultaneously amplified using the primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) and the AccuPrime™ Pfx DNA Polymerase (Neefs *et al.*, 1991; Yu *et al.*, 2005). The PCR mix contained 2.5 μL 10x AccuPrime™ Buffer (dNTPs included), 1.25 μL of each primer (10 μM), 1 μL template and 0.15 μL AccuPrime™ Pfx DNA Polymerase plus sterile water with a final reaction volume of 25 μL. The reaction started with an initialization at 94 °C for 2 min, following 30 cycles of denaturation at 94 °C for 20 s, annealing at 56 °C for 30 s, and elongation at 68 °C for 40 s. The constructions of the amplicon libraries were divided into two steps to avoid biased amplification due to unspecific adaptor binding to the template (Berry *et al.*, 2011). The size of the PCR product was evaluated (fragment length; 466 bp) using gel electrophoresis. The fragment was then excised and purified using the Montage Gel Extraction Kit (Merck Millipore). In a second PCR of 15 cycles, adaptors were added to the amplicons using the same reaction conditions as mentioned above. After additional fragment size (526 bp) evaluation and excision, the amplicon concentration was measured using Qubit dsDNA HS Assay Kit (Invitrogen). The concentration was adjusted to $1.0\times E+08$ copies $\mu L^{-1}$ and sent for sequencing. The sequences were generated by the 454 FLX – Titanium at the 'National Danish High-Throughput DNA Sequencing Center' in Copenhagen, Denmark. The reads were trimmed for low quality (minimal quality score = 25) and denoised using the QIIME PIPELINE version 1.5.0 (Caporaso *et al.*, 2010; Quince *et al.*, 2011). Only sequences with a minimal length of 200 bp were considered. Chimeras were removed using the UCHIME algorithm (Edgar *et al.*, 2011). Operational taxonomic units (OTUs) were picked *de novo* from quality checked reads and clustered at 97% sequence similarity

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved



Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

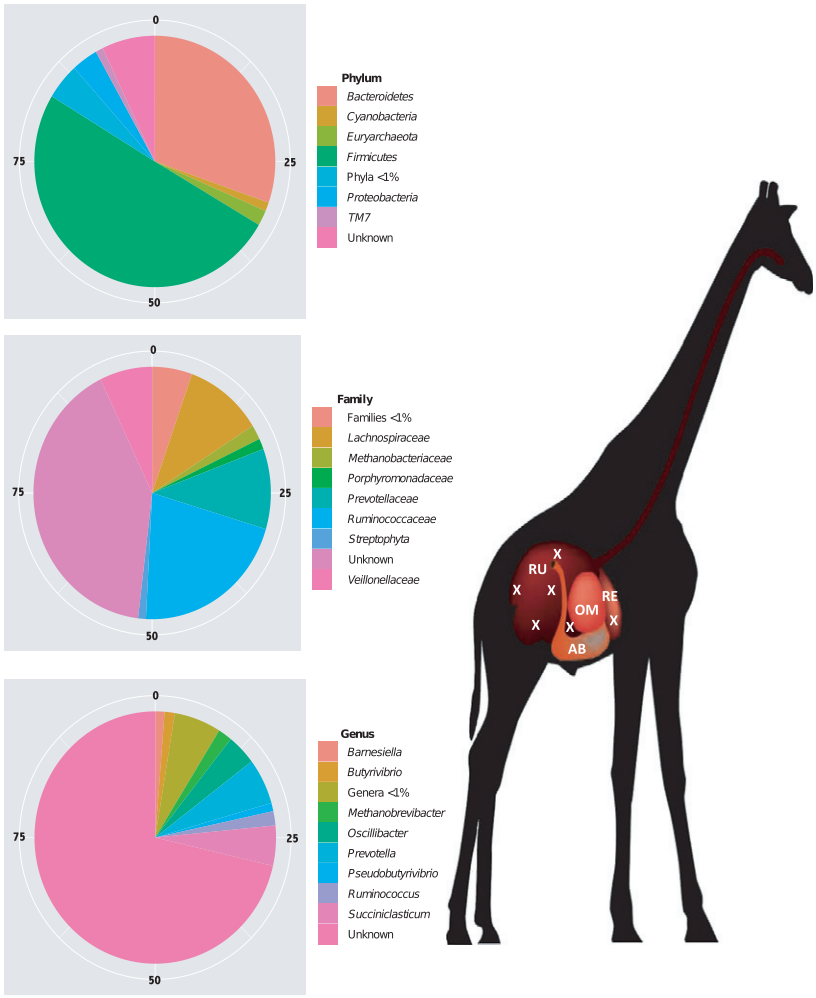


**Fig. 1.** Sampling points of reticulo-ruminal content of the giraffe and mean rumen microbial composition on phyla, family, and genus level. RU = rumen, RE = reticulum, OM = omasum, AB = abomasum, and X = locations of sampling. Both fluid and solid material was collected from each sampling point.

using UCLUST. Taxonomy was assigned using the RDP classifier (version 2.2) method with a bootstrap cutoff value of 0.8 and GREENGENES as reference database (Liu *et al.*, 2008). Variations between samples (diet group I vs. II and fluid vs. solid sample) were checked for normal distribution using the Shapiro–Wilk test (significance value $P < 0.05$). When normally distributed, we evaluated the variables with the Welch's *t*-test, if nonparametric, the Wilcoxon rank-sum test with the significance value of $P < 0.05$ was preferred. The microbial community composition was evaluated with the Bray–Curtis (BC) and the Euclidean matrix. The clustering was confirmed by the *R* value (0 = highly similar; 1 = highly dissimilar) of the analysis of similarity Insert here in brackets (Anosim) and significance permutation $P < 0.05$.

## Results

After quality check (material and methods), we received 30 400 sequences with an average length of 376 bp (min 204, max 400, Fig. 2 – length distribution). The additional denoising and chimera check removed 6077 sequences as primer bias indicated sequences, leaving 24 323 sequences for downstream analysis. The number of sequences of each animal varied from 448 to 4339.

We used rarefaction to randomly subsample the sequences and evaluate diversity, as rarefaction (Gotelli & Colwell, 2001) is a method widely used for characterizing microbial diversity (Koenig *et al.*, 2011; Koren *et al.*, 2013; Charlop-Powers *et al.*, 2014; Davenport *et al.*, 2014; Ding & Schloss, 2014).

We characterized the richness estimator (Chao1) and the diversity (Shannon) index to evaluate the depth of the generated sequences (Fig. 3) (Colwell & Coddington, 1994; Hill *et al.*, 2003). The richness and diversity curve did not reach the plateau of saturation for all samples at 500 sequences per sample. Thus, the most abundant taxa will be described but more rare species are potentially underrepresented. To remove bias by sequencing effort, we used a subset of 548 reads per sample. The


© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

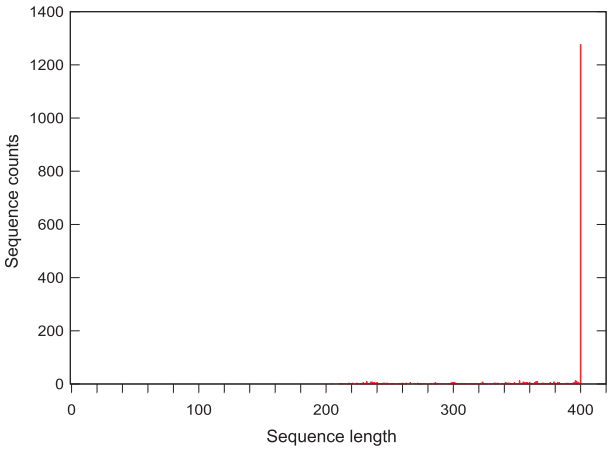


**Fig. 2.** Length distribution of representative sequences. Evaluated are the representative sequences (most abundant sequences of a given OTU) of the UCLUST picked OTUs used for taxonomy assignment.

subsampled OTU table contained *c.* 22% of the total generated sequences but more than 50% of the total OTUs. Therefore, two samples (Table 1) were disregarded as the sequences generated accounted for < 10 sequences. These samples were not part of the downstream analysis.

We observed in total 1692 OTUs spread throughout all samples. After subsampling, the OTUs table contained 878 OTUs. Of the subset OTUs, 45.9 % were uniquely associated to diet group I, 22.7% where shared between both diets and 31.7% were only observed in the samples of group II. Rumen solid and fluid shared 27.7% of all OTUs identified, whereas 29.5% were unique to solid and 42.8% unique to fluid. Additionally, every animal carried on average 28% (min 11%, max 54%) of unique OTUs (Table 1) not shared with other giraffes at 97% sequence identity and without dissimilarity between fluid or solid rumen content (Welch's *t*-test, $P = 0.905$) or the diet groups (Welch's *t*-test, $P = 0.688$).

## Diet shapes microbial communities

To investigate variations in microbial distribution between diet groups and ruminal fractions of the giraffe rumen, the diversity between the sampling sites was compared. No significant difference in richness (Chao1) or diversity (Shannon) indexes was found between neither feeding types nor ruminal fractions (Table 2).

Additionally, we observed if the samples were more similar in microbial composition within group I and II as well as in fluid or solid fraction, by generating an OTU relative abundance-based BC dissimilarity matrix. The principal coordinate analysis (PCoA; Fig. 4) revealed no distinct differences in the microbial distribution of solid or fluid fraction. Only the samples from animals A and B clustered according to sample fraction. However, giraffe E, F, and G, fed only on browse, grouped together in distance of animal A, B, C, and D, which had received Boskos pellets, alfalfa hay, and cut savanna browse.

## *Firmicutes* and *Bacteroidetes* most prevalent phyla

A total of 21 phyla were observed for all samples combined (Fig. 5). The majority of the relative sequence counts were assigned to *Firmicutes* (50%) and *Bacteroidetes* (30%). Furthermore, both ruminal fractions were composed of Proteobacteria (4%), *Cyanobacteria* (1%), *Actinobacteria* (1%), *Euryarchaeota* (2%), and the candidate division TM7 (1%) without significant variation

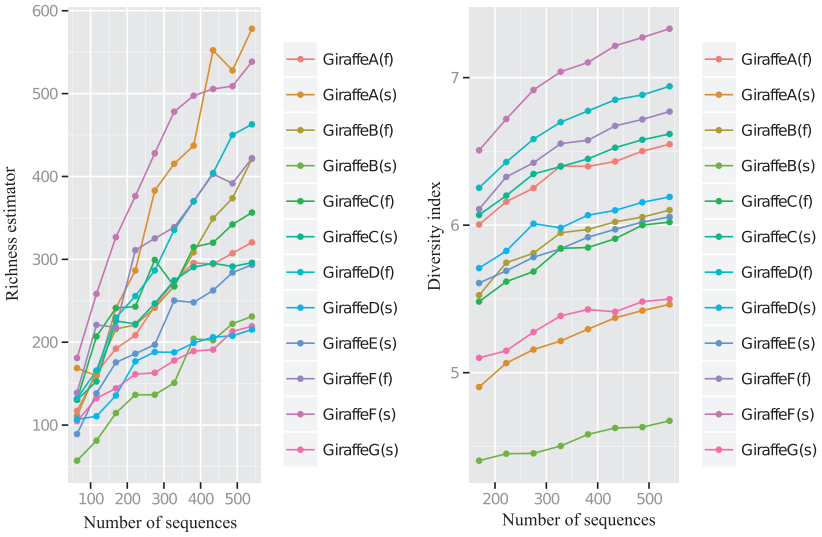


**Fig. 3.** Rarefaction curves of Chao1 richness and Shannon diversity index generated at even sequencing depth. (f) = fluid fraction and (s) = solid fraction. Fluid samples from giraffe A and C were not included in the analysis.

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved



Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

**Table 1.** Sequence and OTU distribution per individual sample

| Sample ID | Raw sequences | Sequences after denoising and chimera check | Total OTUs (shared and unique) | Unique OTUs per animal in % |
|---|---|---|---|---|
| GiraffeA(f) | 633 | 561 | 172 | 30 |
| GiraffeA(s) | 5521 | 4339 | 643 | 52 |
| GiraffeB(f) | 1365 | 1189 | 269 | 33 |
| GiraffeB(s) | 4634 | 3750 | 279 | 21 |
| GiraffeC(f) | 4601 | 3818 | 449 | 26 |
| GiraffeC(s) | 898 | 746 | 227 | 20 |
| GiraffeD(f) | 4979 | 4011 | 660 | 32 |
| GiraffeD(s) | 596 | 448 | 144 | 11 |
| GiraffeE(f) | 2 | 2 | 2 | – |
| GiraffeE(s) | 984 | 796 | 190 | 18 |
| GiraffeF(f) | 954 | 759 | 257 | 23 |
| GiraffeF(s) | 809 | 638 | 304 | 44 |
| GiraffeG(f) | 2 | 2 | 2 | – |
| GiraffeG(s) | 4422 | 3339 | 319 | 30 |

The table shows impact of quality trimming and chimera check on the sequences generated and the OTU survey. Distinguished are the seven giraffes (Sample ID) A–G together with the corresponding ruminal fluid (f) and solid fraction (s). Giraffe A–D is diet group I, whereas giraffe E–G belong to diet group II. Total OTUs are all observations found in the individual animal. Unique OTUs in % is the fraction of bacteria or archaea found in the specific giraffe and not shared with other individuals.

**Table 2.** Diversity comparison of ruminal fluid vs. solid fraction and diet group I vs. II

| Diversity index | Fluid mean [min; max] | Solid mean [min; max] | Ruminal fraction comparison *W*; *P*-value | Diet group I mean [min; max] | Diet group II mean [min; max] | Diet comparison *W*; *P*-value |
|---|---|---|---|---|---|---|
| Chao1 richness estimator | 396; [320; 462] | 338; [215; 578] | 25; 0.2677 | 360 [215; 578] | 368 [219; 538] | 20; 0.570 |
| Shannon diversity estimator | 6.5; [6.0; 6.9] | 6.0; [4.7; 7.3] | 24; 0.3434 | 6.1 [4.7; 6.9] | 6.4 [5.5; 7.3] | 16; 1 |

Listed are the nonparametric Chao1 richness (compares OTUs only once observed against OTUs found exactly twice) and the Shannon diversity index (relates OTU counts and their respective relative occurrences in the complex community). Differences were investigated with Wilcoxon rank-sum test (*W*) $P < 0.05$.

(Wilcoxon, $P > 0.05$; Fig. 1). However, despite the difference in clustering between the diet groups, there were no significant variations on phyla level (Wilcoxon, $P > 0.05$). The phyla of *Acidobacteria*, *Chloroflexi*, *Crenarchaeota*, *Fibrobacteres*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospira*, *Planctomycetes*, *Spirochetes*, *Synergistetes*, *Tenericutes*, *Verrucomicrobia*, candidate phylum WS3 and candidate division SR1 were detected to each constitute below 1% relative abundance. *c.* 7% of the relative sequences were not assigned to any known bacterial phyla. There was no variation in read length of the unassigned representative sequences compared with the ones identified (Wilcoxon, $W = 82\,069$, $P = 0.101$). This indicates that the length of the reads is sufficient to receive at least phylum taxonomic information. Furthermore, there was no difference in sequence length between the diet groups or the ruminal fractions (Wilcoxon, $P > 0.05$).

## Majority of the sequences were not assigned to genus

On the phylogenetic family level only 53% of the sequences were assigned to known taxa of *Ruminococcaceae* (21%), *Lachnospiraceae* (11%), *Prevotellaceae* (10%), *Veillonellaceae* (7%), *Methanobacteriaceae* (2%), *Porphyromonadaceae* (1%), and *Streptophyta* (1%). Furthermore, only 28% of all sequences were assigned to genus level. Of 78 genera, 24 were observed only in the solid and 23 only in the fluid content, whereas 31 genera were found in both fractions. The most abundant genera (identified), independent of ruminal fraction, were *Prevotella* (6%), *Succiniclasticum* (5%), *Oscillibacter* (4%), *Methanobrevibacter* (2%), *Ruminococcus* (1%), *Barnesiella* (1%), and *Pseudobutyrivibrio* (1%). Besides the absence of *Coprococcus* and *Ruminobacter* in feeding group II, there was no further diet-dependent


© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

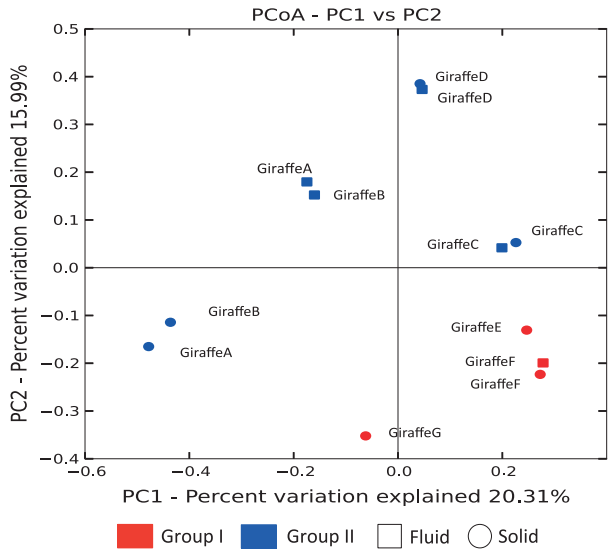


**Fig. 4.** Principal coordinate analysis of the giraffe's rumen. Compared are fluid and solid rumen samples based on BC relative dissimilarity matrix. Principal coordinate 1 (PC1) plotted against Principal coordinate 2 (PC2) explained the largest variance between the samples. Animals E and G were missing the fluid phase for comparison. The cluster analysis revealed that the samples grouped by diet (ANOSIM, $R = 0.317$, $P = 0.05$) but not by ruminal fraction ($R = 0.028$, $P = 0.562$).

variation on genus level between the feeding groups. Furthermore, a comparison of the ruminal fractions revealed higher levels of *Oscillibacter* in the fluid phase.

All archaeal sequences (1060 sequences, constituting *c.* 2% of all sequences in the study) belonged to the family *Methanobacteriaceae*, specifically to the genera *Methanobrevibacter* (98% of the sequences) and *Methanosphera*. Almost all methanogenic sequences (97%) were observed in the ruminal solid phase of two giraffes (A and B) of diet group I. However, there was no overall difference of *Methanobrevibacter* relative abundance between the diet groups or the ruminal fractions (Table 2). This observation is independent of sequencing depth due to the fact that the two giraffes had higher levels of *Methanobrevibacter* compared with other giraffes (for example the solid phase of animal C, or the fluid phase of animal D) with similar or larger read counts per sample (Table 1).

To determine which OTUs caused the similarity and dissimilarity among the animals on the two diets, we compared the individual community profiles by generating two-sided dendrograms (Fig. 6. The giraffes did not cluster according to fluid and solid fraction as expected from the PCoA plot (Fig. 4). We observed three groups with similarities in microbial composition (Fig. 6). In cluster 1, giraffe B (only fluid fraction), C, and D (fluid and solid) grouped together by sharing *c.* 50% of the displayed OTUs. In cluster 2, the solid content of giraffe E, F, and both fraction sites of giraffe G shared only *c.* 26% of OTUs. Cluster 3 was composed of giraffe A (fluid and solid) and B (solid) appeared to be most different from the other giraffes. Both animals carried large amounts of *Methanobrevibacter* (12%), and *Succiniclasticum* (30%), in relative abundance. Most importantly, the giraffes of cluster 1 and 3 received Boskos pellets, cut browse, and alfalfa hay (diet group I), whereas cluster 2 represents the giraffes browsing naturally in the period of 4–6 days before sampling (diet group II). We observed several OTUs with significantly different levels between both feeding groups explaining the diet based clustering in Fig. 4, however, the majority of these OTUs were assigned to different taxonomic levels hindering taxonomic level based comparison between the diets. There were no significant variations between the ruminal fractions of the major OTUs shown in Fig. 6 (Wilcoxon, $P > 0.05$).

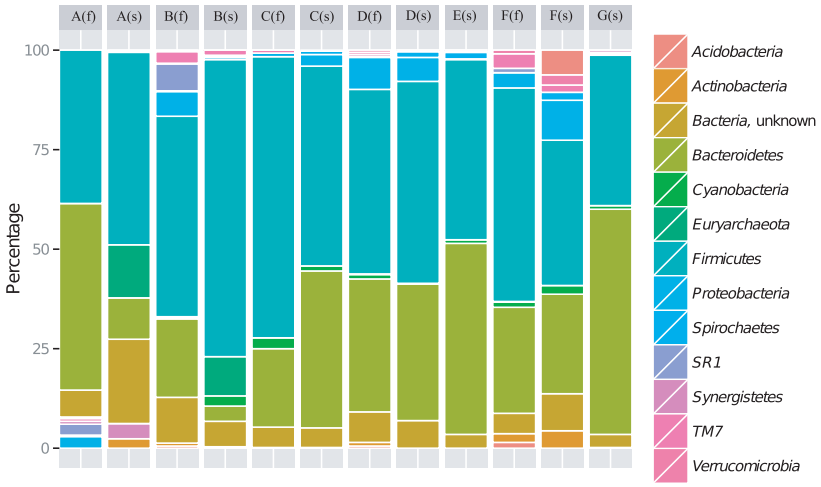


**Fig. 5.** Phyla distribution. Observations are displayed as stacked bar charts for individual giraffes (*x*-axis) against the taxa abundance (*y*-axis). Phyla below 1% relative abundance are not shown.

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved



Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

## Discussion

Herbivores have adapted to live in symbiosis with microorganisms with the ability to metabolize plant material, thereby being able to retrieve energy from otherwise indigestible organic material. One of the most developed symbiotic systems is the reticulo-rumen of ruminants (Hume & Warner, 1980; Hackmann & Spain, 2010). This organ is a fermentation vat where the host provides buffered anaerobic conditions necessary for the microorganisms to utilize cellulose-rich plant material. The rumen content can be divided into two phases of rumen fluid (mix of host fluids, microorganisms, and metabolites) and solid material (feed particles and fiber-adhered microorganisms) (Chen *et al.*, 2008). It has previously been reported that members of the complex microbial community have different substrate specificities creating differences between the two ruminal phases in the community composition in cattle (Cho *et al.*, 2006).

In this study, no significant variation in the microbial distribution was found between the fluid and solid fractions (Fig. 4, Table 2). Instead, variation in microbial composition was higher among animals than between the solid and fluid fraction of the rumen content of each individual animal (Figs 4 and 6).

Although we observed 24 genera only found in the fluid fractions and 23, including the often fiber-associated genus *Fibrobacter*, only in the solid phase, these genera

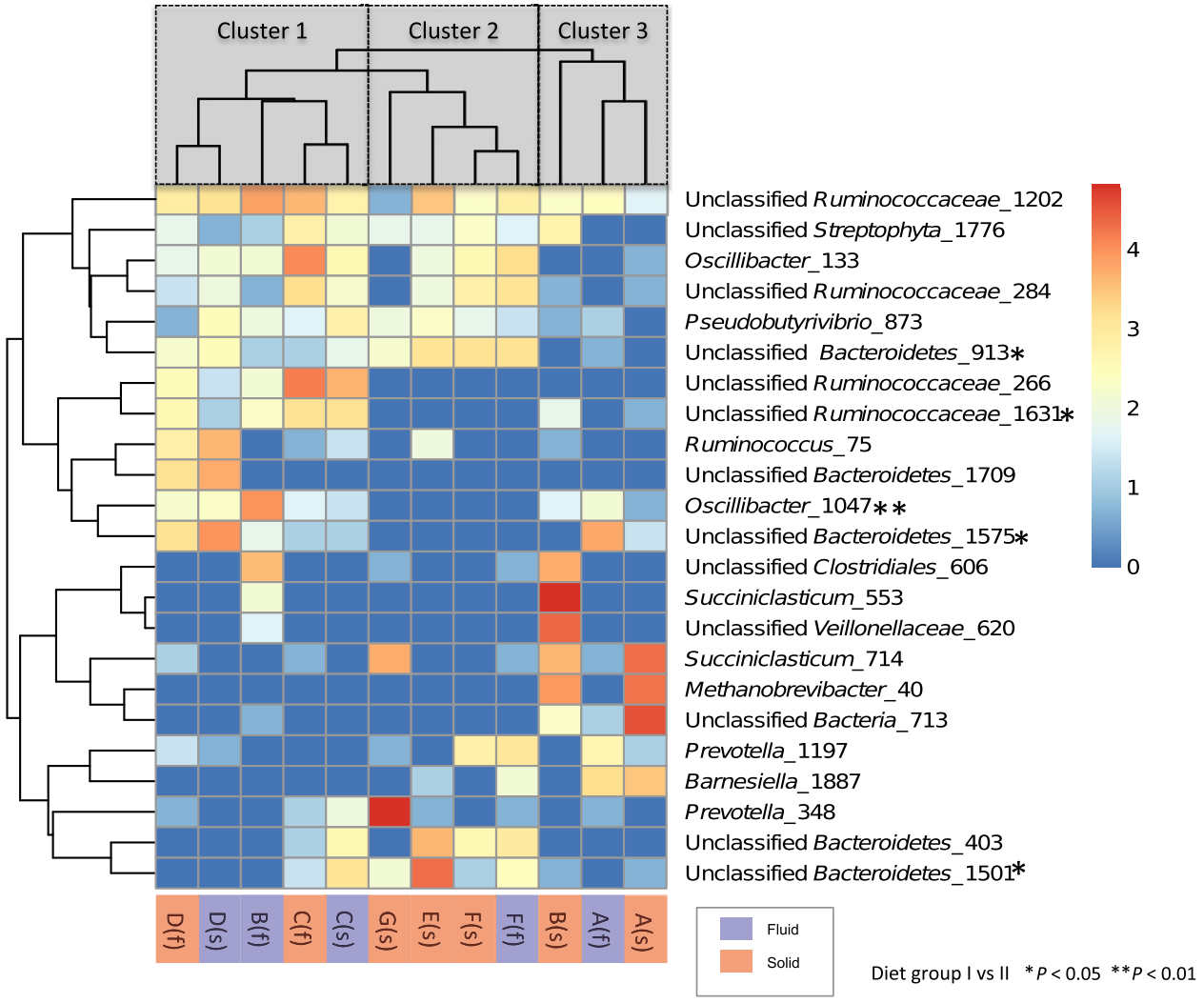


**Fig. 6.** Individual microbial community profiles. The profiles are based on a two-sided Euclidean distance dendrogram of the most abundant OTUs (>50 sequence counts throughout all samples) at even sequencing depth and log-transformed. Shown are animal (*x*-axis) vs. OTUs (*y*-axis). There were no significant differences in relative OTU abundances between the ruminal fractions (Wilcoxon, $P > 0.05$). (f) = fluid fraction and (s) = solid fraction.



© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

**Table 3.** Genus observations in rumen fluid and solid fraction as well as in diet group I and II (shown are only genera which accounted for a minimum of 1% of the received sequences in at least one animal)

| Genus | Fluid mean abundance [min; max] in % | Solid mean abundance [min; max] in % | Variation by ruminal fraction *W*; *P*-value | Diet group I [min; max] in % | Diet group II [min; max] in % | Variation by diet *W*; *P*-value |
|---|---|---|---|---|---|---|
| *Barnesiella* | 1.5 [0; 4.9] | 1.1 [0; 5.7] | 16; 0.868 | 1.5 [0, 5.7] | 0.6 [0; 1.82] | 17; 0.931 |
| *Butyrivibrio* | 0.8 [0.2; 1.3] | 1.8 [0; 4.6] | 25.5; 0.221 | 0.9 [0; 2.0] | 2.3 [1.1; 4.6] | 5.5; 0.088 |
| *Coprococcus* | 0.8 [0; 1.8] | 0.5 [0; 1.6] | 11.5; 0.362 | 0.9 [0.18; 1.8] | 0 [0; 0] | 32; 0.007 |
| *Methanobrevibacter* | 0.1 [0; 0.2] | 3.1 [0; 12.4] | 15; 0.715 | 2.8 [0; 12.4] | 0 [0; 0.0] | 21; 0.340 |
| *Moryella* | 0.5 [0; 2.4] | 0.2 [0; 0.7] | 20; 0.728 | 0.1 [0; 0.4] | 0.7 [0; 2.4] | 6; 0.084 |
| *Oscillibacter* | 6.8 [1.8; 11.7] | 1.9 [0; 4] | 4; 0.034 | 4.76 [0.4; 11.7] | 2.3 [0; 4.9] | 21; 0.441 |
| *Paraprevotella* | 1.1 [0; 2.7] | 0.5 [0; 2.2] | 12.5; 0.458 | 0.9 [0; 2.7] | 0.4 [0; 0.9] | 17; 0.931 |
| *Prevotella* | 5.8 [2; 13] | 5.9 [0.5; 26.8] | 10.5; 0.289 | 3.8 [0.5; 12.9] | 10.3 [2.4; 26.8] | 7; 0.147 |
| *Pseudobutyrivibrio* | 0.7 [0.2; 1.1] | 1.4 [0; 2.7] | 26; 0.192 | 0.9 [0; 2.7] | 1.3 [0.9; 1.6] | 9.5; 0.306 |
| *Pyramidobacter* | 0.2 [0; 0.7] | 0.3 [0; 1.1] | 14; 0.602 | 0.2 [0; 1.1] | 0.2 [0; 0.54] | 16; 1 |
| *Ruminobacter* | 0.7 [0; 2.2] | 0.5 [0; 2.7] | 12; 0.396 | 0.8 [0; 2.7] | 0 [0; 0] | 30; 0.017 |
| *Ruminococcus* | 1.8 [0.4; 4.4] | 1.8 [0; 6.9] | 15; 0.743 | 2.5 [0.9; 6.9] | 0 [0.5; 1.3] | 7; 0.147 |

Differences were investigated with Wilcoxon rank-sum test (*W*) $P < 0.05$.

accounted for < 1% and were not found in all fluid or solid fractions (Kobayashi *et al.*, 2008). The minor variation in the abundance of *Oscillibacter* between the ruminal fractions can either be the result of a small sample size and/or be the result of differences in diet composition between giraffes.

Diet was previously demonstrated to affect the composition of the rumen microbiome in cattle (Fernando *et al.*, 2010) with impact on the microbial phyla distribution.

The phyla of *Bacteroidetes* (together with *Firmicutes*) contain some of the primary fiber-degrading bacteria in the rumen, and the abundance of these taxa therefore have a great effect on the ability of the host animal to utilize fiber-rich diet items (Brulc *et al.*, 2009), thus it is not surprising that the diet composition impacts on the microbial community.

In giraffes, a ratio of *Firmicutes* to *Bacteroidetes* of 50 : 30 was observed across ruminal fractions and diets. This is similar to the ratios reported for wild goats (fluid 56 : 38; solid 40 : 39) (Cunha *et al.*, 2011), ruminants of intermediate feeding type (i.e. eating both browse and grass) and forage-mix fed cattle (43 : 33, whole rumen content) (Petri *et al.*, 2013). Henderson *et al.* (2013), however, found that the occurrence of *Firmicutes* and *Bacteroidetes* in cattle and sheep are dependent of the DNA extraction method. The abundance of *Firmicutes*, for example, was favored by bead beating in contrast to a phenol–chloroform DNA extraction without bead beating. Thus, direct comparison between rumen studies needs to be taken with caution. Interestingly, the same study revealed larger levels of *Bacteroidetes* compared with *Firmicutes* in sheep (independent of DNA extraction method) concluding that *Firmicutes* is not the dominant phyla in all ruminants.

Furthermore, no difference between the two diet groups on phylum level, diversity and richness was found, although a diet-dependent clustering in microbial composition was observed. Giraffes E, F, and G (diet group II), grouped closer together than giraffes A, B, C, D (diet group I), fed pellets with alfalfa hay and browse. Giraffes E, F, and G initially received the same diet as A, B, C, and D, but were given the opportunity to browse naturally for 4–6 days before sampling.

Recent investigations show that the human gut microbiota significantly diverges within 1 day of change of diet (David *et al.*, 2013), however, we can only speculate how fast the microbial community of the reticulo-rumen alternates after a shift in diet composition. Considering that the mean retention time of particles in giraffes has been reported to be *c.* 40 h (Clauss *et al.*, 1998), it appears likely that the variation of cluster 2 vs. 1 and 3 resulted from giraffes E, F, and G feeding only on browse.

An average 28% of the OTUs were unique to the individual animal but the majority of the OTUs observed were shared between the giraffes despite the large difference in abundance. It has previously been shown that the microbial gut communities in vertebrates cluster highly according to the preferred diet (Ley *et al.*, 2008a, b). Thus, it seems likely that the variations in microbial composition between giraffes were feed driven and that the biomes of cluster 2 most closely reflect those of free ranging giraffes.

Approximately 70% of all generated sequences from the giraffe rumen microbiome could not be assigned to genus level, and as much as 21% of all sequences could not be assigned even at phylum level. Although *Coprococcus* and *Ruminobacter* levels varied significantly between the feeding groups (Table 3), the major differ-

Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

ences were observed for OTUs assigned to different taxonomic levels (Fig. 6). As metabolic specialization of microorganisms may be very diverse even at the taxonomic level of order, family or genus, our data reveal only little functional information about the microbial communities in the giraffe. Hence, studying the microbial communities of wild ruminants at a deeper taxonomic level is crucial to compare microbial functionality of wild and domestic ruminants. Hopefully, this will be possible as more data from these environments become available from future studies.

## Conclusion

This is to the best of our knowledge, the first study of the rumen microbiome of the giraffe. Our results indicate that diet of the giraffe might be a key driving force in shaping the microbial diversity of the rumen. Future studies are needed to investigate differences in composition of microbial communities of browsers and grazers by examining animals receiving carefully controlled diets. Furthermore, our samples contained large amounts of novel bacteria. We believe that the giraffe is a reservoir for never described microbial communities deserving further characterization. Additionally, fungi and protozoa of the giraffe reticulo-rumen were not investigated here and need future attention.

## Acknowledgements

The authors would like to thank the Danish Cardiovascular Giraffe Research project (DaGIR) for the opportunity to collect the samples and Michael Petersen from Copenhagen Zoo for designing the giraffe graphic of Fig. 1. The study was funded by Det Strategiske Forskningsråd (DSF) from the Novenia project. The research of M.P. was granted by the Danish Agency for Science, Technology and Innovation through an individual Postdoc stipend and C.S. by a PhD scholarship from Aarhus University. The authors declare that they have no competing interests.

## Ethical approval

Permission to euthanize the animals after experimentation was granted by the Gauteng Province of South Africa.

## Authors' contribution

M.R., C.S., M.F.B., and S.J.S. designed the study. C.S. and M.P planned the sampling. C.S. carried out the animal sampling. M.R. extracted DNA from samples and generated 16S RNA gene data, and initial data analysis. All authors participated in data interpretation and drafting the manuscript.

## References

Berry D, Mahfoudh KB, Wagner M & Loy A (2011) Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Appl Environ Microbiol* **77**: 7846–7849.

Brulc JM, Antonopoulos DA, Miller ME *et al.* (2009) Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. *P Natl Acad Sci USA* **106**: 1948–1953.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336.

Charlop-Powers Z, Owen JG, Reedy BVB, Ternei MA & Brady SF (2014) Chemical-biogeographic survey of secondary metabolism in soil. *P Natl Acad Sci USA* **111**: 3757–3762.

Chen X, Whang JK, Wu YM & Liu JX (2008) Effects of chemical treatments of rice straw on rumen fermentation characteristics, fibrolytic enzyme activities and populations of liquid-and solid-associated ruminal microbes *in vitro*. *Anim Feed Sci Technol* **141**: 1–14.

Cho SJ, Cho KM, Shin EC *et al.* (2006) 16S rDNA analysis of bacterial diversity in three fractions of cow rumen. *J Microbiol Biotechnol* **16**: 92–101.

Clauss M, Deutsch M, Lechner-Doll M, Flach EJ & Tack C (1998) Passage rate of fluid and particle phase in captive giraffe (*Giraffa camelopardalis*). *Adv Ethol* **33**: 98.

Clauss M, Franz-Odendaal TA, Brasch J, Castell JC & Kaiser T (2007) Tooth wear in captive giraffes (*Giraffa camelopardalis*): mesowear analysis classifies free-ranging specimens as browsers but captive ones as grazers. *J Zoo Wildl Med* **38**: 433–445.

Colwell RK & Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. *Philos Trans R Soc Lond B Biol Sci* **345**: 101–118.

Cunha IS, Barreto CC, Costa OY, Bomfim MA, Castro AP, Kruger RH & Qurino BF (2011) *Bacteria* and *Archaea* community structure in the rumen microbiome of goats *Capra hircus* from the semiarid region of Brazil. *Anaerobe* **17**: 118–124.

Davenport ER, Mizrahi-Man O, Michelini K, Barreiro LB, Ober C & Gilard Y (2014) Seasonal variation in human gut microbiome composition. *PLoS One* **9**: e90731.

David LA, Maurice CF, Carmody RN *et al.* (2013) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**: 559–563.

Ding T & Schloss PD (2014) Dynamics and associations of microbial community types across the human body. *Nature* **509**: 357–360.

Edgar RC, Haas BJ, Clemente JC, Quince C & Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Fernando SC, Purvis HT 2nd, Najar FZ, Sukharnikov LO, Krehbiel CR, Nagaraja TG, Roe BA & Desilva U (2010)

Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024



© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

Rumen microbial population dynamics during adaptation to a high-grain diet. *Appl Environ Microbiol* **76**: 7482–7490.

Gotelli NJ & Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* **4**: 379–391.

Hackmann T & Spain J (2010) Invited review – Ruminant ecology and evolution: perspectives useful to ruminant livestock research and production. *J Dairy Sci* **93**: 1320–1334.

Hall-Martin AJ (1977) Giraffe weight estimation using dissected leg weight and body measurements. *J Wildl Manag* **41**: 740–745.

Henderson G, Cox F, Kittelmann S, Miri VH, Zethof M, Noel SJ & Janssen PH (2013) Effect of DNA extraction methods and sampling techniques on the apparent structure of cow and sheep rumen microbial communities. *PLoS One* **8**: e74787.

Hill TC, Walsh KA, Harris JA & Moffett BF (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbiol Ecol* **43**: 1–11.

Hume I & Warner A (1980) Evolution of microbial digestion in mammals, in Digestive physiology and metabolism in ruminants. *Oecologia* **78**: 665–684.

Kobayashi Y, Shinkai T & Koike S (2008) Ecological and physiological characterization shows that *Fibrobacter succinogenes* is important in rumen fiber digestion—review. *Folia Microbiol* **53**: 195–200.

Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT & Ley RE (2011) Succession of microbial consortia in the developing infant gut microbiome. *P Natl Acad Sci USA* **108**: 4578–4585.

Koren O, Knights D, Gonzales A, Waldron L, Segata N, Knight R, Huttenhower C & Ley RE (2013) A guide to enterotypes across the human body: meta-analysis of microbial community structures in human microbiome datasets. *PLoS Comput Biol* **9**: e1002863.

Leuthold BM & Leuthold W (1972) Food habits of giraffe in Tsavo National Park, Kenya. *Afr J Ecol* **10**: 129–141.

Ley RE, Hamady M, Lozupone C *et al.* (2008a) Evolution of mammals and their gut microbes. *Science* **320**: 1647–1651.

Ley RE, Lozupone CA, Hamady M, Knight R & Gordon JI (2008b) Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat Rev Microbiol* **6**: 776–788.

Liu Z, DeSantis TZ, Andersen GL & Knight R (2008) Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Res* **36**: e120.

Neefs JM, Van De Peer Y, Hendriks L & Wachter RD (1991) Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Res* **19**: 1987–2015.

Pellew RA (1984) Food consumption and energy budgets of the giraffe. *J Appl Ecol* **21**: 141–159.

Petri RM, Schwaiger T, Penner GB, Beauchemin KA, Forster RJ, McKinnon JJ & McAllister TA (2013) Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well as during and after an acidotic challenge. *PLoS One* **8**: e83424.

Pitta DW, Pinchak WE, Dowd S *et al.* (2010) Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. *Microb Ecol* **59**: 511–522.

Potter JS & Clauss M (2005) Mortality of captive giraffe (*Giraffa camelopardalis*) associated with serous fat atrophy: a review of five cases at Auckland Zoo. *J Zoo Wildl Med* **36**: 301–307.

Quince C, Lanzen A, Davenport RJ & Turnbaugh PJ (2011) Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* **12**: 38.

Saengkerdsub S & Ricke SC (2013) Ecology and characteristics of methanogenic *Archaea* in animals and humans. *Crit Rev Microbiol* **40**: 97–116.

Shipley L, Blomquist S & Danell K (1998) Diet choices made by free-ranging moose in northern Sweden in relation to plant distribution, chemistry, and morphology. *Can J Zool* **76**: 1722–1733.

Yu Y, Lee C, Kim J & Hwang S (2005) Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol Bioeng* **89**: 670–679.

Downloaded from https://academic.oup.com/femsec/article/90/1/237/2680515 by guest on 20 March 2024

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved