# Comparative analysis of the metabolically active microbial communities in the rumen of dromedary camels under different feeding systems using total rRNA sequencing

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## ABSTRACT

Breakdown of plant biomass in rumen depends on interactions between bacteria, archaea, fungi, and protozoa; however, the majority of studies of the microbiome of ruminants, including the few studies of the rumen of camels, only studied one of these microbial groups. In this study, we applied total rRNA sequencing to identify active microbial communities in 22 solid and liquid rumen samples from 11 camels. These camels were reared at three stations that use different feeding systems: clover, hay and wheat straw (G1), fresh clover (G2), and wheat straw (G3). Bacteria dominated the libraries of sequence reads generated from all rumen samples, followed by protozoa, archaea, and fungi respectively. Firmicutes, Thermoplasmatales, *Diplodinium*, and *Neocallimastix* dominated bacterial, archaeal, protozoal and fungal communities, respectively in all samples. Libraries generated from camels reared at facility G2, where they were fed fresh clover, showed the highest alpha diversity. Principal co-ordinate analysis and linear discriminate analysis showed clusters associated with facility/feed and the relative abundance of microbes varied between liquid and solid fractions. This provides preliminary evidence that bacteria dominate the microbial communities of the camel rumen and these communities differ significantly between populations of domesticated camels.

**Subjects** Agricultural Science, Microbiology, Veterinary Medicine
**Keywords** Arabian camel, Rumen, Bacteria, Archaea, Fungi, Protozoa, Diversity, rRNA sequencing, Metatranscriptomics, Feeding regime

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## INTRODUCTION

Camels (*Camelus dromedaries*) can produce milk and meat in hot, arid and semi-arid regions and provide food security as the climate warms (*Samsudin et al., 2011*; *Faye, 2013*). Camels also provide textiles (fiber and hair) and are commonly used for transportation,

agriculture, tourism, racing (*Rabee et al., 2019*). The unique feeding behavior and the functional structure of digestive tract of these pseudo-ruminants is well adapted to deserts (*Kay & Maloiy, 1989*). The retention time of feed particles in the camel forestomach is longer than the retention time for true ruminants, which improves the efficiency of digestion (*Lechner-Doll & Engelhardt, 1989*). The feed ranchers provide camels, which ranges from forage in traditional pastures to concentrated supplements in intensive feedlots, influences the structure of the camel microbiome (*Faye, 2013*; *Henderson et al., 2015*).

The chemical composition of diet shapes fermentation in rumen. For instance, cellulolytic and hemicellulytic diets favor the fibrolytic microorganisms; while, starch and sugars favor the amylolytic (*Carberry et al., 2012*). Also, the microbial composition and diversity varies between liquid and solid rumen fractions, which might indicate different roles in rumen fermentation; for instance, plant-adherent microbiota might have a major role in fiber degradation (*Ren et al., 2020*).

Digestion in the camels depends on microbial fermentation in rumen (*Samsudin et al., 2011*) and the efficiency of this microbial fermentations is based on the interactions between a wide variety of microbial groups, including bacteria, archaea, fungi and protozoa (*Yanagita et al., 2000*; *Kamra, 2005*). Analysis of these microbial communities could lead to increases in animal productivity and reduction of greenhouse gas emissions (*Henderson et al., 2015*). Unlike other ruminants, camels can utilize thorny and low quality plants like shrubs with high lignocelulolytic content (*Samsudin et al., 2011*). Consequently, camel rumen microbes must have the capacity to degrade such poor-quality feeds (*Gharechahi et al., 2015*). However, the microbial community in the rumen of dromedary camel received less attention than other domesticated ruminants.

Recent development of next generation sequencing technologies provide a rapid method of microbial identification in rumen and overcome the intrinsic constraints of traditional culture-based methods (*Samsudin et al., 2011*; *Ishaq & Wright, 2014*). Most of assessments of microbial groups in the rumen have relied on amplicon sequencing, which target a specific variable region on 16S rRNA gene (*Li et al., 2016*). This approach needs a wide range of primers to study different microbial communities (*Kittelmann et al., 2013*). Therefore, primer selection and amplification conditions could bias the output (*Guo et al., 2015*; *Li et al., 2016*; *Elekwachi et al., 2017*).

Total RNA sequencing (RNA-Seq) offers the advantage of specifically targeting active microbes and avoids biases associated with primer selection and chimera generation in PCR (*Gaidos, Rusch & Ilardo, 2011*; *Guo et al., 2015*; *Li et al., 2016*). In addition, RNA-Seq approach is capable of identifying novel microbes as it is not reliant on primers for known microbes (*Li et al., 2016*). High-throughput metatranscriptomic sequencing provides a comprehensive understanding of biological systems by characterization of different groups of organisms in the same environment based on the sequencing of coding and noncoding RNA (*Elekwachi et al., 2017*). Total RNA-Seq was applied to investigate microbial communities in many different systems including, for example, human gut (*Qin et al., 2012*), and cow rumen (*Li et al., 2016*; *Elekwachi et al., 2017*).

Previous microbiome studies on camel rumen have characterized one or two microbial groups using classical or molecular approaches. For example, the protozoal community in camel rumen was studied heavily by conventional microscopic methods (*Ghali, Scott & Jassim, 2005*; *Baraka, 2012*). Regarding the anaerobic fungi, a new fungal genus, *Oontomyces* was isolated from the rumen of Indian camel (*Dagar et al., 2015*), and only one study investigated whole fungal community in the gut of camel (*Rabee et al., 2019*). Only three molecular-based studies are available on the bacterial community (*Samsudin et al., 2011*; *Bhatt et al., 2013*; *Gharechahi et al., 2015*). Furthermore, only one study classified rumen archaea (*Gharechahi et al., 2015*).

In the present study, total rRNA sequencing was applied to (1) get insight into the composition of active microbiota in the rumen of camels; (2) describe the distribution of microbial groups among solid and liquid rumen fractions; (3) assessing the heterogeneity of these microbial populations within different populations of domestic camels.

## MATERIALS AND METHODS

### Rumen samples

Rumen samples were collected from 11 adult dromedary camels reared at three stations that use different feeding systems. Camels in group G1 ($n$ = 3) were housed in the Maryout Research Station, Alexendria, Egypt and were fed on Egyptian clover hay (*Trifolium alexandrinum*), wheat straw and concentrates feed mixture. Camels in group G2 ($n$ = 6) were housed at the commercial farm in the Kom Hammada and fed on fresh Egyptian clover (100% high-quality forage diet) then slaughtered in the Kom Hammada slaughterhouse, Elbehera, Egypt. Camels of group G3 ($n$ = 2) were housed at the commercial farm in Cairo area and fed on wheat straw (100% low-quality forage diet) then were slaughtered in Pasateen slaughterhouse, Cairo, Egypt. Animals were kept on these diets for at least 1 month before the sampling time. The proximate analysis of feeds is illustrated in Table S1. Details regarding camel rumen samples in this study presented in Table S2. Rumen contents were strained immediately by two layers cheesecloth to separate the liquid and solid to form 22 samples, frozen using liquid nitrogen and stored at −80 °C before further processing (*Elekwachi et al., 2017*).
The project was approved and all samples were collected according to the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Sadat City, Egypt (Approval number: VUSC00003).

### RNA isolation, quality and quantity estimation and sequencing

The frozen rumen samples were ground using liquid nitrogen. About 0.5 g of frozen fine powder was used for total RNA isolation using Trizol-Reagent protocol (Invitrogen, Carlsbad, CA, USA), followed by RNA clean up using MEGA clear Kit (Invitrogen, Carlsbad, CA, USA). Total RNA quality and quantity were estimated using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and RNA 6000 Nano kit (Agilent Technologiess, Santa Clara, CA, USA, USA). One hundred nanogram of total RNA was reverse-transcribed into first strand cDNA and sequenced using Illumina rRNA MiSeq preparation kit (Illumina, San Diego, CA, USA) by Illumina MiSeq platform.

### Bioinformatic data analysis

The generated RNA sequence reads were analyzed using pipeline developed by *Elekwachi et al. (2017)*. Briefly, the sequence quality was checked using the FastQC program v. 0.11.4 (*Andrews, 2010*), then Trimmomatic program v. 0.35 (*Bolger, Lohse & Usadel, 2014*) was used to trim adaptors, barcodes, ambiguous and low quality reads. PEAR program v. 0.9.6 (*Zhang et al., 2014*) was used to merge read 1 and read 2 using default options. Then after, the hidden Markov models rRNA-HMM tool of the rapid analysis of multiple metagenomes with a clustering and annotation pipeline (RAMMCAP) (*Li, 2009*) was used to sort the reads into archaea and bacteria (16S, 23S), and eukaryote (18S, 23S) rRNA sequences. Merged sequence files were then sub-sampled as needed using MEME program v. 4.10.2 (*Bailey et al., 2009*). For each sample, 70,000 reads were run through the pipeline. For subsequent analysis steps, 20,000, 10,000, and 2,000 sequences were used for bacteria, eukaryote and archaea, respectively. Taxonomy binning for eukaryote and archaeal SSU rRNA sequences was performed using BLASTN. The sub-sampled query sequences were searched against the SILVA SSURef-111 database using an *e*-value of $1e^{-5}$. Bacterial SSU sequences were binned into operational taxonomic units (OTUs) using the "classify. seqs" command of Mothur v. 1.33.1 program (*Schloss et al., 2009*). The SSURef-108 gene and the SSURef-108b taxonomy databases were used. Principal co-ordinate analysis (PCoA) using Bray Curtis dissimilarity and alpha diversity indices (Chao1, Shannon and Inverse Simpson) were evaluated by Mothur (*Schloss et al., 2009*) based on sub-sampling of 70,000 reads per sample according the protocol "Community Structure Analysis Based on OTU Clustering" outlined in *Elekwachi et al. (2017)*.

### Statistical analyses

Data of relative abundance of bacterial phyla, protozoal genera, fungal genera and archaea genera and order Thermoplasmatales were tested for normality and homogeneity using Shapiro–Wilk test and variables that were deemed non-normal were then arcsine transformed. Linear Discriminate Analysis (LDA) and Bray Curtis Permutational Multivariate Analysis of Variance (PERMANOVA) tests depended on the relative abundance of bacterial phyla. All the protozoal, fungal and archaeal genera and the order Thermoplasmatales were used to show the differences in community structure and to compare the clustering of samples. Pearson correlation analysis was used to identify correlation within and between microbial communities and the correlation scores were visualized as a heatmap. The statistical analyses were performed using the SPSS v. 20.0 software package (*SPSS, 1999*) and PAST (*Hammer, Harper & Ryan, 2001*). Sequences were deposited to the sequence read archive (SRA) under the accession number: SRP107370.

## RESULTS

### The composition and diversity of active microbial community

Total rRNA sequencing in 22 solid and liquid rumen samples from 11 camels resulted in a total of 3,958,591 reads with average of 359,872 ± 85,366 (mean ± standard error (SE)) reads per animal in the solid fraction (SF) and 3,386,392 reads with an average of

**Table 1 The relative abundance (%) of bacteria, archaea, protozoa and fungi and diversity indices.** The relative abundance (%) of bacteria, archaea, protozoa and fungi and OTU numbers and values of Shannon, Chao1 and Inverse Simpson indices in the ruminal solid (SF) and liquid (LF) fractions of dromedary camels fed a mixed ration (G1), high-quality forage (G2) and low-quality-forage (G3) (Mean ± Standard error (SE)).

| Item | G1 | G2 | G3 | Overall mean |
|---|---|---|---|---|
| Bacteria SF | 92 ± 1 | 89 ± 2 | 89 ± 2 | 90 ± 1 |
| Bacteria LF | 85 ± 4 | 91 ± 2 | 87 ± 8 | 88 ± 2 |
| Archaea SF | 2.3 ± 0.2 | 3.4 ± 0.4 | 2.2 ± 1.0 | 3.0 ± 0.3 |
| Archaea LF | 2.2 ± 0.2 | 2.8 ± 0.4 | 1.8 ± 0.2 | 2 ± 0.3 |
| Protozoa SF | 5 ± 1 | 7 ± 2 | 6 ± 2 | 6 ± 1 |
| Protozoa LF | 12 ± 4 | 6 ± 1.6 | 8 ± 5 | 8 ± 1.6 |
| Fungi SF | 0.15 ± 0.05 | 1 ± 0.3 | 3 ± 1 | 1 ± 0.4 |
| Fungi LF | 0.35 ± 0.1 | 0.5 ± 0.1 | 3 ± 3 | 1 ± 0.5 |
| OTUs SF | 1,012 ± 43 | 1,201 ± 38 | 1,135 ± 148 | 1,137 ± 39 |
| OTUs LF | 1,076 ± 26 | 1,229 ± 38 | 1,147 ± 53 | 1,172 ± 30 |
| Shannon SF | 6 ± 0.1 | 7 ± 0.10 | 7 ± 0.3 | 7 ± 0.1 |
| Shannon LF | 6.5 ± 0.06 | 7 ± 0.1 | 7 ± 0.1 | 7 ± 0.1 |
| Chao1 SF | 6,644 ± 650 | 9,329 ± 714 | 9,028 ± 1,985 | 8,542 ± 608 |
| Chao1 LF | 7,280 ± 521 | 10,839 ± 724 | 7,688 ± 625 | 9,295 ± 672 |
| Invsimpsone SF | 117 ± 14 | 863 ± 306 | 644 ± 398 | 620 ± 196 |
| Invsimpsone LF | 13 5± 21 | 983 ± 492 | 612 ± 142 | 684 ± 282 |

307,854 ± 60,989 reads per animal in the liquid fraction (LF). The sequence reads of bacteria dominated the active microbial community, followed by protozoa, archaea and fungi (Table 1). Relative abundance of protozoa was higher in liquid fraction of G1 (LF-G1), while relative abundance of bacteria was higher in solid fraction of G1 (SF-G1). The highest population of archaea was observed in G2 camels. Additionally, G3 camels showed the highest relative abundance of fungi (Table 1; Fig. S1). Number of OTUs and Alpha-diversity indices, Chao1, Shannon and Inverse Simpson, were higher in the rumen of LF-G2 samples (Table 1).

## Bacterial community

The composition of bacterial community varied little between groups and consisted of 12 phyla. The five most predominant phyla were Firmicutes, Bacteroidetes, Proteobacteria, Spirochaetes and Fibrobacteres, respectively (Table 2). Firmicutes dominated the bacterial community in all groups and was higher in G2 followed by G1 and G3 camels, respectively, and was also higher in SF compared to LF (Table 2). At the family level, Lachnospiraceae and Ruminococcuceae dominated the Firmicutes. In addition, six genera dominated this phylum, including *Butyrivibrio*, RFN8-YE57, *Ruminococcus*, vadinHA42, *Acetitomaculum* and *Blautia* (Fig. 1A; Table S3). The second largest phylum, Bacteroidetes, showed the highest relative abundance in G3 followed by G1 and G2 camels and was higher in LF than SF (Fig. 1A; Table S3). At the family level, Prevotellaceae,

**Table 2 Relative abundance (%) of bacterial phyla.** Relative abundance (%) of bacterial phyla in the ruminal solid (SF) and liquid (LF) fractions of camels fed a mixed ration (G1), high-quality forage (G2) and low-quality forage (G3) (Mean ± Standard Error (SE)).

| Bacterial Phylum | G1 | G2 | G3 | Overall mean |
|---|---|---|---|---|
| Firmicutes SF | 63 ± 2 | 65 ± 0.1 | 48 ± 10 | 60 ± 3 |
| Firmicutes LF | 46 ± 3 | 56 ± 2 | 45 ± 13 | 50 ± 3 |
| Bacteroidetes SF | 20 ± 1 | 15 ± 1 | 27 ± 8 | 19 ± 2 |
| Bacteroidetes LF | 31 ± 0.5 | 21 ± 1.5 | 31 ± 12 | 26 ± 3 |
| Proteobacteria SF | 5 ± 1 | 3.5 ± 0.3 | 3 ± 0.5 | 4 ± 0.3 |
| Proteobacteria LF | 6.5 ± 1 | 6 ± 2 | 3 ± 0.1 | 5.5 ± 1 |
| Spirochaetes SF | 3 ± 0.6 | 5 ± 1 | 6 ± 1.5 | 4.5 ± 0.6 |
| Spirochaetes LF | 3.7 ± 1 | 2.6 ± 0.5 | 5.6 ± 1 | 3.5 ± 0.5 |
| Fibrobacteres SF | 2.5 ± 0.6 | 4 ± 0.7 | 9 ± 1 | 4.5 ± 1 |
| Fibrobacteres LF | 1.6 ± 0.5 | 2.5 ± 1 | 7 ± 3 | 3 ± 1 |
| Actinobacteria SF | 2 ± 0.2 | 4.5 ± 0.3 | 1.5 ± 0.3 | 3 ± 0.5 |
| Actinobacteria LF | 1.5 ± 0.14 | 5.5 ± 1 | 1 ± 0.1 | 3.6 ± 10 |
| Lentisphaerae SF | 0.7 ± 0.03 | 0.7 ± 0.1 | 1.5 ± 0.2 | 1 ± 0.1 |
| Lentisphaerae LF | 3.2 ± 0.3 | 2 ± 0.5 | 3.2 ± 2 | 2.6 ± 0.4 |
| Tenericutes SF | 2 ± 0.4 | 1 ± 0.1 | 0.6 ± 0.3 | 1 ± 0.2 |
| Tenericutes LF | 3.7 ± 0.6 | 1.5 ± 0.3 | 0.4 ± 0.1 | 1.8 ± 0.4 |
| Verrucomicrobia SF | 0.3 ± 0.1 | 0.20 ± 0.1 | 0.6 ± 0.4 | 0.30 ± 0.1 |
| Verrucomicrobia LF | 2.2 ± 0.4 | 1 ± 0.3 | 1.3 ± 0.3 | 1.3 ± 0.3 |
| Chloroflexi SF | 0.4 ± 0.03 | 0.5 ± 0.06 | 0.24[a] | 0.4 ± 0.04 |
| Chloroflexi LF | 0.3 ± 0.03 | 0.3 ± 0.05 | 0.24[a] | 0.3 ± 0.02 |
| Cyanobacteria SF | 0.3 ± 0.04 | 0.3 ± 0.05 | 0.5[a] | 0.35 ± 0.04 |
| Cyanobacteria LF | 0.3 ± 0.05 | 0.3 ± 0.05 | 0.25[a] | 0.3 ± 0.03 |
| Elusimicrobia SF | 0.2 ± 0.05 | 0.15 | 0.3 ± 0.14 | 0.2 ± 0.04 |
| Elusimicrobia LF | 0.3 ± 0.07 | 0.2 ± 0.04 | 0.8 ± 0.4 | 0.4 ± 0.1 |

**Note:**
[a] The value was calculated from one animal.

BS11_ gut_ group, and Rikenellaceae dominated the Bacteroidetes; and at the genus level, Prevotella, RC9_gut_group dominated the Bacteroidetes. Proteobacteria, phylum showed a higher relative abundance in LF-G1 samples and was dominated by Succinivibrionaceae family and *Desulfovibrio* genus (Table 2; Fig. 1A; Table S3). The Spirochaetes phylum was higher in the SF-G3 and it was classified into two families including Spirochaetaceae and PL-11B10 and was dominated by *Treponema* genus. Fibrobacteres phylum was higher in SF-G3 (Table 2; Fig. 1A; Table S3). Actinobacteria were higher in SF-G2 samples, Tenricutes phylum was higher in LF-G1 samples and Lentisphaerae phylum, was about 3-fold higher in LF as relative to SF and accounted for a large population in the camels of G3 (Table 2). Additionally, several minor bacterial phyla were also observed in the rumen of camels such as Verrucomicrobia, Elusimicrobia, Cyanobacteria and Chloroflexi (Table 2).

Of the 74 genera observed, only seven were observed exclusively in libraries generated from a specific facility, including uncultured *Marinilabiaceae* (Bacteroidetes), *Quinella*

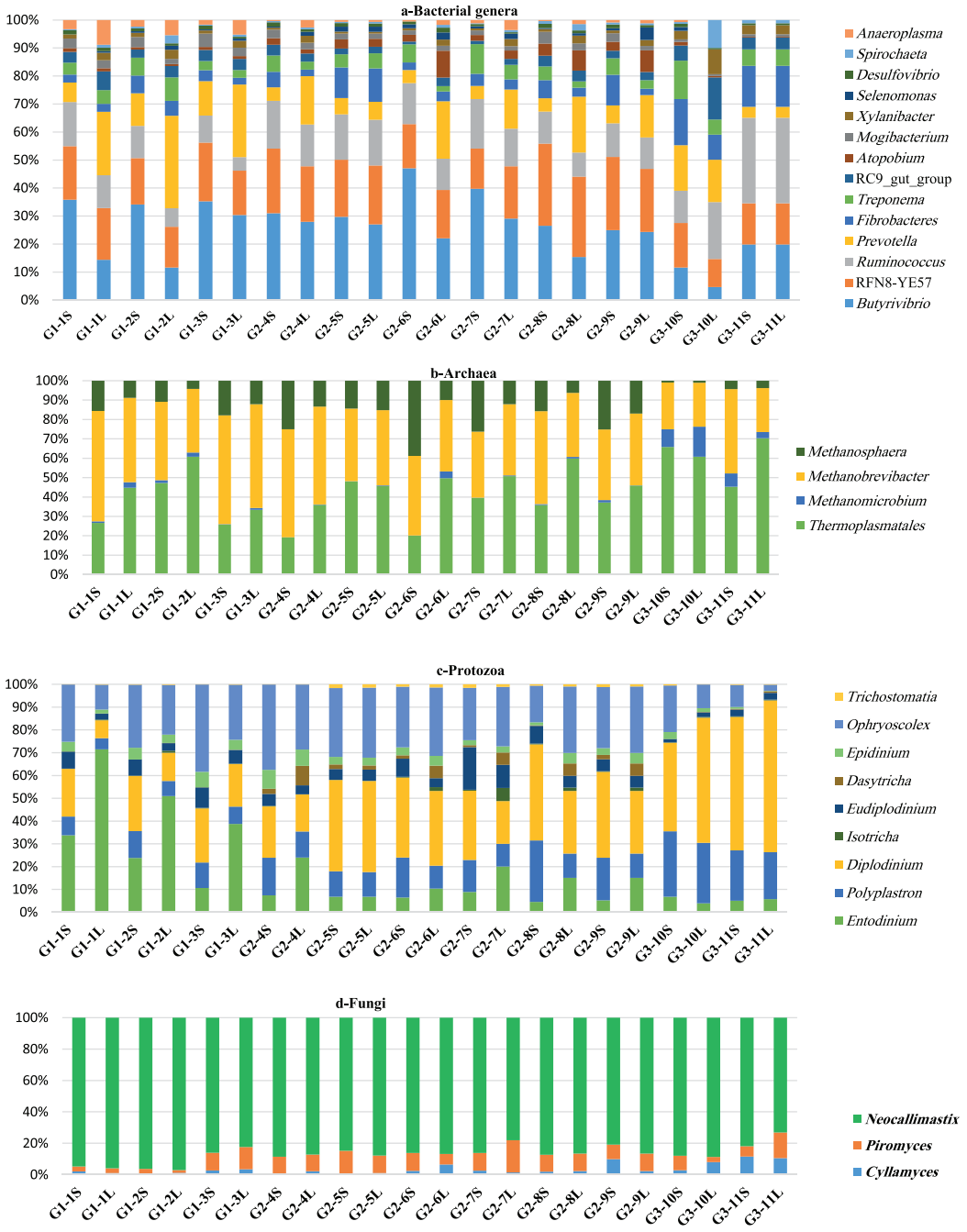


**Figure 1 The relative abundance of microbial groups.** Comparison of relative abundance of genera of the microbiota in dromedary camel. bacterial (A), archaeal (B), protozoal (C) and fungi (D) in ruminal solid (SF) and liquid (LF) fractions of camels under different feeding systems.

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(Firmicutes) and *Streptococcus* (Firmicutes) that were observed only in G2 and G3 camels. *Ruminobacter* (Proteobacteria) was observed only in G1 and G2 camels. On the other hand, *Arcobacter* and *Succinivibrio* within phylum Proteobacteria were observed only in G1 camels and *Betaproteobacteria* (Proteobacteria) was observed only in G3 camels.

**Table 3 Relative abundance (%) of archaeal orders and genera.** Relative abundance (%) of archaeal orders and genera observed in the ruminal solid (SF), and liquid (LF) fractions of camels under different feeding systems. Animals in G1 fed a mixed ration, animal in G2 fed high-quality forage and animal in G3 fed low quality-forage (Mean ± Standard Error (SE)).

| Archaea | G1 | G2 | G3 | Overall mean |
|---|---|---|---|---|
| Thermoplasmatales SF | 33 ± 7 | 33 ± 4 | 55 ± 10 | 37 ± 4 |
| Thermoplasmatales LF | 46 ± 8 | 48 ± 3 | 67 ± 5 | 51 ± 3 |
| *Methanomicrobium* SF | 1 ± 0.3 | 0.3 ± 0.2 | 8 ± 1 | 2 ± 0.9 |
| *Methanomicrobium* LF | 2 ± 0.5 | 1 ± 0.5 | 9 ± 6 | 3 ± 1 |
| *Methanobrevibacter* SF | 51 ± 5 | 42 ± 3 | 34 ± 9 | 43 ± 3 |
| *Methanobrevibacter* LF | 43 ± 5 | 39 ± 2.4 | 23 ± 0.01 | 37 ± 2 |
| *Methanosphaera* SF | 15 ± 2 | 24 ± 3 | 3 ± 1 | 18 ± 3 |
| *Methanosphaera* LF | 8 ± 2 | 12 ± 1.5 | 2.5 ± 1 | 9.5 ± 1.5 |
| *Methanobacterium* SF | 0.05 | 0.06 | 0 | ND |
| *Methanobacterium* LF | 0.2 ± 0.02 | 0.1 ± 0.02 | 0 | ND |

**Note:**
ND: Non Determined.

Moreover, many unclassified bacteria were observed across samples and accounted for 39% of total bacterial reads. Most of these unclassified bacterial reads were observed in phylum Firmicutes and Bacteroidetes.

## Archaeal community

Reads that classified as archaea were further classified to three orders within the phylum Euryacheota: Thermoplasmatales, Methanobacteriale and Methanomicrobial. Thermoplasmatales dominated the archaeal community and showed the highest population in LF-G3 samples, this order was not classified out of order level (Table 3; Fig. 1B). Reads that classified in the Methanobacteriale were further classified to family Methanobacteriacea that includes three genera: *Methanobrevibacter*, *Methanophera* and *Methanobacterium. Methanobrevibacter* is the second largest contributor in archaeal population and was higher in SF-G1 samples. *Methanosphaera* exhibited higher relative abundance in SF-G2 samples. *Methanobacterium* was absent in G3 camels; however, a small proportion of this genus was found in the camels of G1 and G2. *Methanomicrobium* genus, which belongs to order Methanomicrobiales and family Methanomicrobiaceae was the least contributor in archaeal population and was more prevalent in LF-G3 samples (Table 3; Fig. 1B).

## Protozoal community

Reads that classified as protozoa were further classified to two families: Ophryoscolecidae and Isotrichidae (Table 4). Reads that classified in the Ophryoscolecidae were further classified to seven genera, *Diplodinium, Ophryoscolex, Entodinium, Polyplastron, Eudiplodinium, Epidinium* and *Trichostomatia*. Reads that classified in the Isotrichidae were further classified to two genera, *Dasytricha* and *Isotricha*. The variation among the camels in protozoal population was clearly observed and seemed to be higher than other microbial communities; however, the protozoal community composition was similar

**Table 4 Relative abundance (%) of protozoal genera.** Relative abundance (%) of protozoal genera in the ruminal solid (SF) and liquid fraction (LF) of camels under different feeding systems. Animals in G1 fed a mixed ration, animals in G2 fed high-quality forage and animals in G3 fed low-quality forage (Mean ± SE).

| Protozoa | G1 | G2 | G3 | Overall mean |
|---|---|---|---|---|
| *Entodinium* SF | 23 ± 6 | 6.5 ± 0.6 | 6 ± 1 | 11 ± 3 |
| *Entodinium* LF | 54 ± 10 | 15 ± 2.5 | 5 ± 1 | 24 ± 6 |
| *Polyplastron* S F | 10 ± 1 | 17.5 ± 2 | 25 ± 3 | 17 ± 2 |
| *Polyplastron* LF | 6 ± 1 | 11 ± 0.2 | 24 ± 3 | 12 ± 2 |
| *Diplodinium* S F | 23 ± 1 | 35 ± 3 | 49 ± 10 | 34 ± 3 |
| *Diplodinium* LF | 13 ± 3 | 27 ± 3 | 61 ± 6 | 29 ± 5 |
| *Eudiplodinium* SF | 8 ± 0.6 | 8 ± 2 | 2 ± 0.7 | 7 ± 1 |
| *Eudiplodinium* LF | 4 ± 1 | 5.5 ± 1 | 2.5 ± 0.5 | 4.5 ± 0.6 |
| *Epidinium* SF | 5 ± 0.8 | 4 ± 1 | 2 ± 1 | 4 ± 0.1 |
| *Epidinium* LF | 3 ± 0.8 | 4.5 ± 0.6 | 1 ± 0.7 | 3.5 ± 0.5 |
| *Ophryoscolex* SF | 30 ± 4 | 27 ± 3 | 15 ± 5 | 26 ± 2.5 |
| *Ophryoscolex* LF | 19 ± 4 | 29 ± 0.6 | 6.5 ± 4 | 22 ± 3 |
| *Trichostomatia* SF | 0.1 ± 0.02 | 1 ± 0.25 | 0.3 ± 0.15 | 1 ± 0.2 |
| *Trichostomatia* LF | 0.2 ± 0.04 | 1 ± 0.2 | 1 ± 0.1 | 1 ± 0.2 |
| *Isotricha* SF | 0.2 ± 0.04 | 0.3 ± 0.05 | 0.3 ± 0.004 | 0.3 ± 0.03 |
| *Isotricha* LF | 0.5 ± 0.2 | 2 ± 0.9 | 0.3 ± 0.01 | 1 ± 0.5 |
| *Dasytricha* SF | 0.04 ± 0.01 | 1.5 ± 0.3 | 0.2 ± 0.15 | 1 ± 0.3 |
| *Dasytricha* LF | 0.1 ± 0.002 | 5.5 ± 0.8 | 0.5 ± 0.3 | 3 ± 1 |

among the camels (Table 4; Fig. 1C). The most dominant protozoal genera were *Diplodinium, Ophryoscolex* and *Entodinium*. Camels in G1 had the highest population of *Entodinium* and *Epidinium*. Camels in G2 had the greatest population of *Eudiplodinium, Ophryoscolex, Isotricha* and *Dasytricha* and camels in G3 had the greatest population of *Diplodinium, Polyplastron* and *Trichostomatia*. On the sample fraction level, solid fraction had a higher representation of *Ophryoscolex, Polyplastron, Eudiplodinium, Epidinium* and *Diplodinium*, while liquid fraction had a higher representation of *Entodinium, Isotricha* and *Dasytricha* (Table 4; Fig. 1C).

## Anaerobic rumen fungal community

Reads that classified as rumen fungi were further classified to two phyla: Neocallimastigomycota and Chytridiomycota. Reads that classified in the Neocallimastigomycota were further classified to family Neocallimasticeceae that includes three genera, *Neocallimastix, Piromyces* and *Cyllamyces*. *Neocallimastix* dominated the fungal community, followed by *Piromyces* and *Cyllamyces* (Table 5; Fig. 1D). These anaerobic fungal genera represented >99.5% of the fungal population. In addition, reads that classified in the Chytridiomycota were further classified to family Spizellomycetaceae that includes genus *Spizellomyces*, which was noted in a very small proportion (<0.5%) (Table 5). *Neocallimastix* was more abundant in the SF-G1 samples

**Table 5 Relative abundance (%) of fungal genera.** Relative abundance (%) of fungal genera in the ruminal solid (SF) and liquid fraction (LF) of camels under different feeding systems. Camels in G1 fed a mixed ration, animals in G2 fed high-quality forage, and animals in G3 fed low-quality forage (Mean ± SE).

| Fungi | G1 | G2 | G3 | Overall mean |
|---|---|---|---|---|
| *Spizellomyces* SF | 0 | 0.1 | 0.02 | ND |
| *Spizellomyces* LF | 0.3 ± 0.1 | 0.3 ± 0.1 | 0 | ND |
| *Cyllamyces* SF | 2 ± 0.6 | 3 ± 1.5 | 7 ± 4 | 3.5 ± 1 |
| *Cyllamyces* LF | 2 ± 0.8 | 3 ± 0.8 | 10 ± 1 | 4 ± 1 |
| *Piromyces* SF | 6 ± 3 | 12 ± 0.7 | 8 ± 1 | 9 ± 1 |
| *Piromyces* LF | 6 ± 4 | 12 ± 2 | 10 ± 6 | 10 ± 2 |
| *Neocallimastix* SF | 92 ± 3 | 85 ± 1 | 85 ± 3 | 87 ± 1 |
| *Neocallimastix* LF | 92 ± 4 | 85 ± 1.5 | 81 ± 7 | 86 ± 2 |

**Note:**
ND: Non Determined.

while *Piromyces* and *Cyllamyces* were more abundant in LF-G2 and SF-G3 respectively (Table 5; Fig. 1D).

## Effect of feeding system and facility on the composition of microbial communities

Multivariate analysis separated libraries by feeding system and housing facility distinctly (Figs. 2 and 3). Also, bacteria, dominated by Firmicutes, drove differences between animals (Fig. 3). Furthermore, *Entodinium*, Thermoplasmatales, *Neocallimastix* drove differences in protozoal, archaeal and fungal communities, respectively. PERMANOVA analysis revealed that the difference between camel groups was significant ($P < 0.01$) in all microbial groups (Table S4). Pairwise comparison between camel groups based on Bonferroni-corrected *P*-value demonstrated that the difference was significant ($P < 0.05$) between camels of G2 and G3 in bacterial and archaeal communities (Table S4). Moreover, the difference was significant between the three groups in the protozoal community ($P < 0.05$), whereas, in the fungal community, the difference was significant only between camels in group G1 and G2 (Table S4).

## Pearson correlation between microbes in the rumen of dromedary camel

Pearson correlation analysis (Figs. 4A and 4B), revealed many significant positive and negative correlations ($P < 0.05$). For example, in active bacteria, Bacteroidetes correlated positively with *Cyllamyces* and negatively with *Butyrivibrio*, *Methanosphaera* and *Trichostomatia*. Prevotellaceae correlated positively with *Neocallimastix* and *Entodinium* and negatively with Ruminococcaceae, *Methanosphaera* and *Diplodinium*. *Fibrobacteres* correlated positively with *Cyllamyces, Methanomicrobium*, Thermoplasmatales and *Diplodinium* and negatively with *Methanosphaera, Epidinium*, Ruminococcaceae and *Butyrivibrio*. Firmicutes correlated positively with *Methanosphaera* and negatively with *Piromyces*, Thermoplasmatales and *Methanomicrobium*.

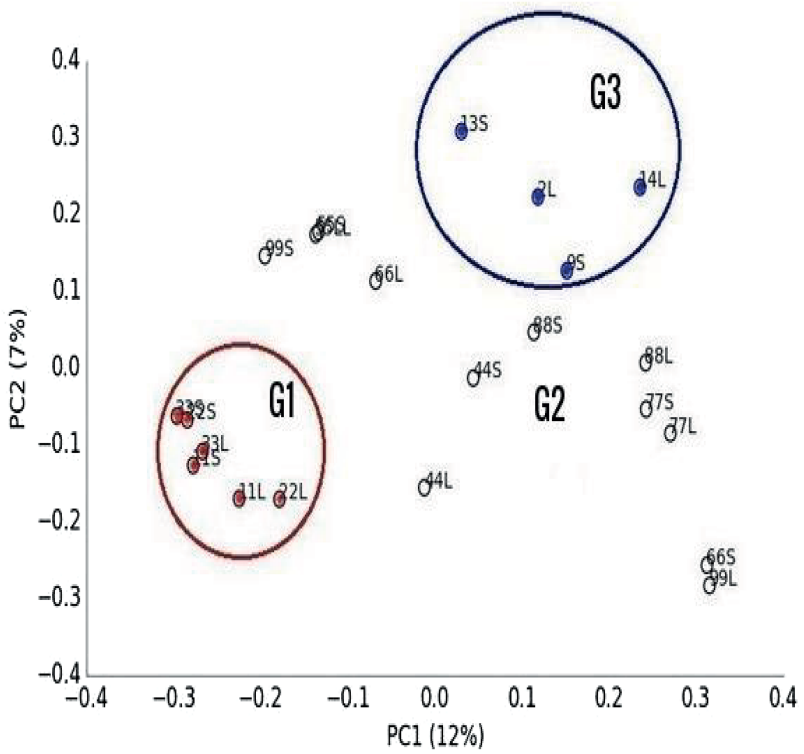


**Figure 2 Principal Co-ordinated analysis.** Principal Co-ordinated analysis derived from OTUs from 22 ruminal liquid (LF) and solid (SF) samples distributed on three camel groups. G1 camels (red circles), G2 (white circle) and G3 (blue circles). Full-size DOI: 10.7717/peerj.10184/fig-2

In active archaea, Thermoplasmatales correlated positively with *Diplodinium* and negatively with *Methanobrevibacter* and *Methanosphaera*. In active protozoa, there was a negative correlation between *Polyplastron*, *Entodinium*, *Ophryoscolex* and *Epidinium*. In active fungi, a negative correlation was observed between *Cyllamyces*, *Neocallimastix* and *Piromyces* and between *Piromyces* and *Entodinium*.

## DISCUSSION

Rumen microbes can ferment a wide variety of feed components, including cellulose, xylan, amylose and protein and produce volatile fatty acids that provide the animal with approximately 70% of daily energy requirements (*Bergman, 1990*; *Henderson et al., 2015*). Fermentation by rumen microbes also generates methane, which contributes to global warming and represents 2–12% loss of feed energy for the animal (*Johnson & Ward, 1996*; *Carberry et al., 2012*; *Jami, White & Mizrahi, 2014*). Investigation of these microbial communities could improve our understanding of their function in fiber digestion and lead to practices that maximize the efficiency of ruminal fermentation and minimize greenhouse gas release (*Lee et al., 2012*).

In this study, camel groups were fed different diets and reared in different locations. The diversity and relative abundance of microbial communities varied between camel groups, which was supported by the results of PCoA, LDA and PERMANOVA analyses. This result agrees with the results of studies of other ruminants (*Henderson et al., 2015*).

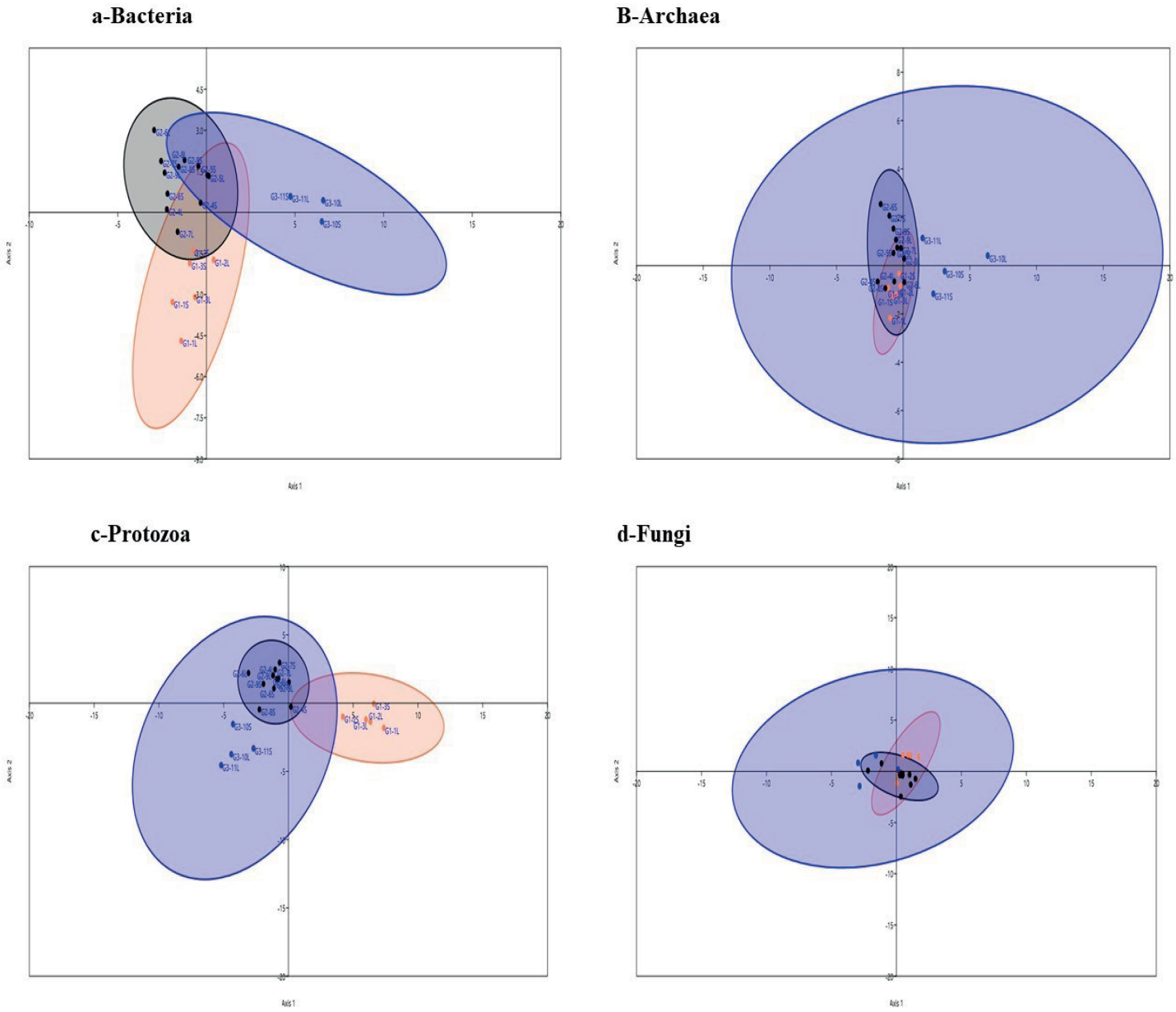


**Figure 3 Linear Discriminant analysis.** Linear Discriminant analysis of microbial communities in the samples based on the relative abundance of genera of active bacteria (A), archaea (B), protozoa (C) and fungi (D) in ruminal solid (SF), and liquid (LF) fractions of camels under three feeding systems, G1 (black dots), G2 (blue squares) and G3 (coral triangles). Full-size DOI: 10.7717/peerj.10184/fig-3

Camels in the present study were fed on different forages; Egyptian clover and wheat straw (Table S1). Egyptian clover is the most balanced and nutritious fodder widely used for feeding camels (*Carberry et al., 2012*; *Bakheit, 2013*; *Shrivastava et al., 2014*), which might supported the high microbial diversity in G2 camels compared to other groups (Table 1). This was consistent with previous studies on cows (*Pitta et al., 2010*; *Shanks et al., 2011*; *Kumar et al., 2015*). Highly degradable carbohydrates support bacterial and protozoal growth (*Dijkstra & Tamminga, 1995*; *Kumar et al., 2015*), which could demonstrate their higher population in G1 camels. Additionally, higher bacterial

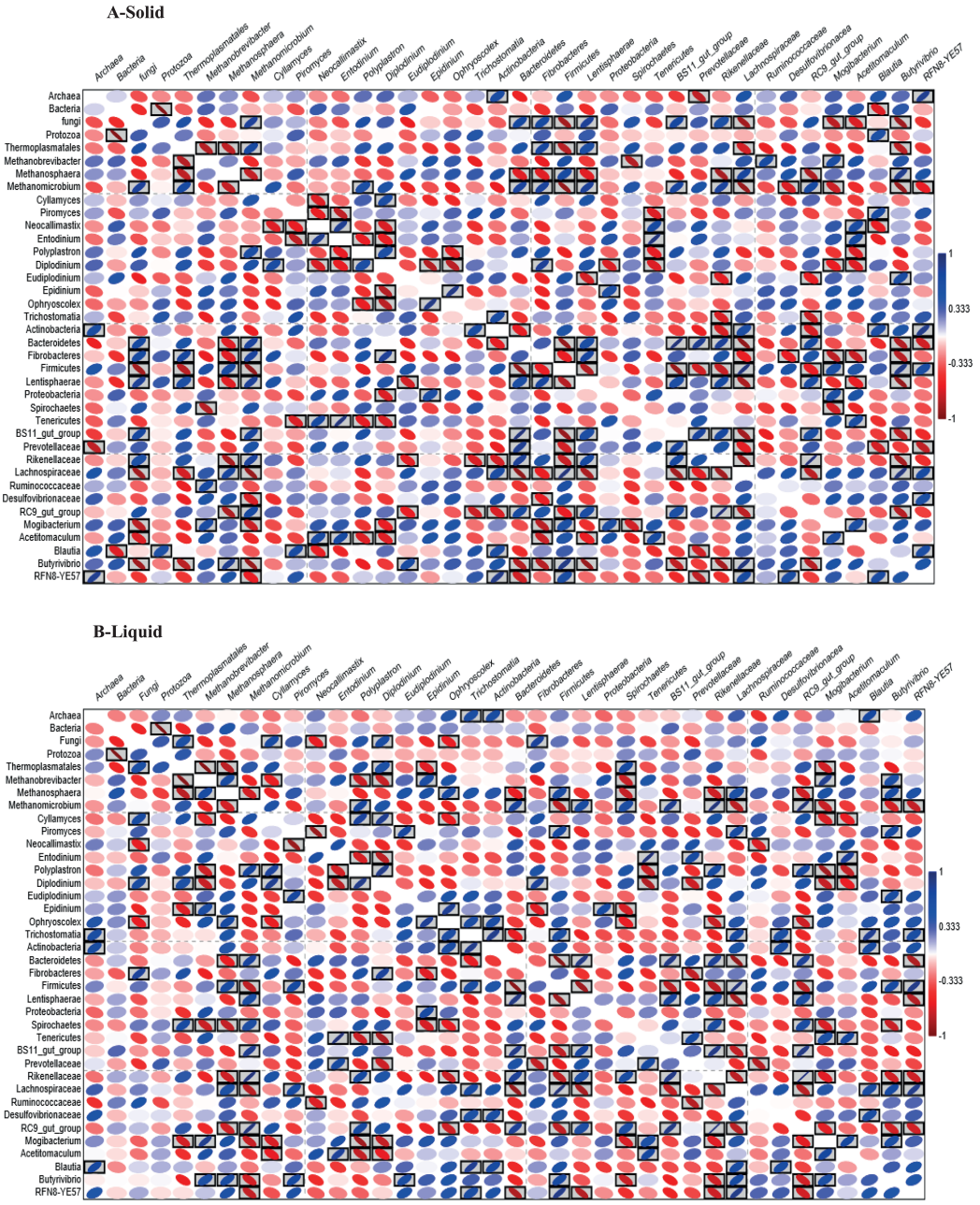


**Figure 4 Heatmap based on Pearson correlation.** Heatmap based on Pearson correlation coefficients between and within the relative abundance of bacteria, archaea, protozoa and fungi in solid (A) and liquid (B) rumen fractions of dromedary camel. The black boxed ellipses refer to the significant correlations at $P < 0.05$. Full-size DOI: 10.7717/peerj.10184/fig-4

population slows the fungi growth (*Stewart, Duncan & Richardson, 1992*; *Orpin & Joblin, 1997*), which was illustrated by low fungal population in G1 camels.

## Bacterial community

Firmicutes phylum was more abundant than Bacteroidetes and both phyla comprised >75% of all bacterial reads (Table 2), which agrees with studies of camels

(*Samsudin et al., 2011*), Surti Buffalo (*Pandya et al., 2010*) and muskoxen (*Salgado-Flores et al., 2016*). The majority of Firmicutes' members have a potential role in fiber digestion, which might illustrate their higher population in G2 camels that were fed on high-quality forage and also in solid fraction. The high proportion of Ruminococcaceae and Lachnospiraceae supports this speculation (*Pitta et al., 2014a*; *Nathani et al., 2015*). *Blautia* and *Acetitomaculum* genera have a key role as reductive acetogens (*Le Van et al., 1998*; *Yang et al., 2016*) and varied among the camel groups in this study. This supports the observation that manipulation of diet can enhance reductive acetogenesis in rumen and minimize methanogenesis (*Le Van et al., 1998*).

Bacteroidetes were higher in samples collected from animals reared in the station that used low-quality feed (G3), which was similar to results on cattle (*Pitta et al., 2014b*). The phylum was dominated by family Prevotellaceae, which confirms *Gharechahi et al. (2015)*. Members of Bacteroidetes possess diverse enzymes that can target cellulose, pectin and soluble polysaccharides released in the liquid phase (*Mackenzie et al., 2015*). Additionally, *Prevotella* genus produces propionate that is used for energy by the host (*Nathani et al., 2015*). We speculate that Bacteroidetes species contribute to the adaptation of camels to arid conditions.

The RC9_gut_group found in this study belongs to uncultured genera and was found also in the Rhinoceros hindgut (*Bian et al., 2013*). Unclassified Bacteroidetes specialize in lignocellulose degradation (*Mackenzie et al., 2015*), which could support their high proportion in G3 camels. Fibrobacteres was higher (3.1%) in this study compared to the other findings on camels (*Gharechahi et al., 2015*); this phylum is the principal cellulolytic bacteria in the rumen (*Ransom-Jones et al., 2012*; *Nathani et al., 2015*), which might illustrate its higher relative abundance in solid fraction and in the rumen of G3 camels that fed on wheat straw (Table 2). The members of Proteobacteria were lower in G2 and G3 camels that were fed on diet rich in fiber contents. These findings highlighted this phylum's function as a protein-degrading bacteria (*Liu et al., 2017*). The abundance of *Treponema* was higher in the solid fraction and in G3 camels (Fig. 1A). *Treponema* is the dominant genus in Spirochaetes phylum and it is fiber-associated bacteria, which could indicate to its cellulytic and xylanolytic activities (*Ishaq & Wright, 2012*).

The dominant bacterial genera in this study were *Butyriovibrio*, RFN8-YE57, *Ruminococcus*, *Prevotella*, *Fibrobacter*, *Treponema* and VadinHA. These genera were higher in the SF except RFN8-YE57 compared to the LF; this finding was consistent with a study on camels (*Gharechahi et al., 2015*), and confirms that solid-attached microbes could play a major role in ruminal fiber digestion (*Jewell et al., 2015*; *Noel et al., 2017*).

### *Most of Elusimicrobia in this study*

Most of Elusimicrobia observed in this study have yet to be cultured; some members of this phylum were isolated from the termite's gut that degrades cellulose (*Herlemann et al., 2009*). Therefore, we speculate that this phylum has a role in fiber digestion and that might illustrate their high proportion in G3 camels. Actinobacteria observed also in the rumen of moose and some members of this phylum have acetogenic activities

(*Ishaq et al., 2015*). Some members of *Victivallis* within Lentisphaerae phylum were involved in cellobiose degradation (*Zoetendal et al., 2003*).

Unclassified bacteria in our study (39% of total bacterial reads) were less than the percentage found in a study of muskoxen (54%) (*Salgado-Flores et al., 2016*). The presence of unclassified bacteria in the gut was commonly observed (*Gruninger, McAllister & Forster, 2016*) and could be a result of the presence of new bacteria that ferment plant biomass (*Salgado-Flores et al., 2016*) or related to short reads were generated from RNA-Seq (*Li et al., 2016*).

## Archaeal community

Since some archaea produce $CH_4$ from $H_2$ and $CO_2$, this phyla may control methane emission from ruminants (*Hook, Wright & McBride, 2010*). Additionally, acetate produced in fiber breakdown provides a methyl group for methanogenesis; therefore, alteration of diet shifts the structure of methanogen populations (*Hook, Wright & McBride, 2010*; *Tapio et al., 2017*), which could demonstrate the variation in the relative abundance of archaea between camel groups. Camels of the second group (G2) that were fed fresh clover, showed the highest archaeal population (Table 2) and archaeal community was dominated by Thermoplasmatales, a methylotrophic methanogens order (Table 3), which was consistent with the results on cattle (*Carberry et al., 2014*) and camels (*Gharechahi et al., 2015*). Thermoplasmatales produce methane from methyl amine and supplementing of animal's diet with rapeseed oil decreases the abundance of this order, making it a high potential target in future strategies to mitigate methane emissions (*Poulsen et al., 2013*). The *Methanobrevibacter, Methanosphaera*, *Methanomicrobium* and *Methanobacterium* (Table 4) dominated the reads classified as archaea in this study, which agrees with trends reported for beef cattle (*Carberry et al., 2014*). *Methanobrevibacter* dominated the methanogens in other ruminant (*Henderson et al., 2015*) and was associated with high methane emissions (*Tapio et al., 2017*). Moreover, *Methanomicrobium* was higher in the camels of G3 that were fed on poor quality forage, which was similar to results of buffalo (*Franzolin & Wright, 2016*), and in vitro (*Wang et al., 2018*). In rumen, *Methanomicrobium* converts $H_2$ and/or formate into $CH_4$ (*Leahy et al., 2013*). The abundance of Thermoplasmatales was also negatively correlated with *Methanobrevibacter*, which is consistent with previous results (*Danielsson et al., 2017*; *McGovern et al., 2017*).

## Protozoal community

The majority of protozoal reads were classified as *Diplodinium*, *Ophryoscolex*, *Entodinium*, *Polyplastron*, *Eudiplodinium* and *Epidinium* (Table 4). Similar findings were observed on different ruminants (*Baraka, 2012*). Feed appeared to influence the relative abundance of protozoa, as reported previously for cattle (*Hristov et al., 2001*; *Weimer, 2015*); however, we cannot differentiate the effects of feed from facility. *Diplodinium* dominated protozoal community and was prevalent in G3 camels, which highlighted the cellulolytic activity of this genus (*Coleman et al., 1976*). Some species of genus *Diplodinium*, such as *Diplodinium cameli*, were discovered in, and are unique to, the rumen of Egyptian

camel (*Kubesy & Dehority, 2002*). In addition, *Entodinium* was higher in G1 camels that were fed on concentrates feed mixture that increase the protozoa. Also, this genus predominates rumen of camels (*Selim et al., 1999*; *Ghali, Scott & Jassim, 2005*) and cattle (*Carberry et al., 2012*). Moreover, *Kittelmann & Janssen (2011)* showed that the *Polyplastron* was the dominant genus in cattle. On the function level, all the genus *Eudiplidinum*, *Epidinum* and *Diplodinum* have cellulolytic activity (*Coleman et al., 1976*), whereas, *Polyplastrone* and *Epidinium* have a xylanolytic activity (*Devillard et al., 1999*; *Béra-Maillet et al., 2005*).

## Anaerobic rumen fungal community

The highest fungal population was observed in the solid fraction and rumen of G3 camels (Table 1). These findings were in agreement with the results of studies stated that the fiber-based diets stimulated the fungal growth (*Orpin, 1977*; *Roger et al., 1993*; *Kamra, 2005*; *Haitjema et al., 2014*). This could explain the low fungal population in G1 camels in our study. Moreover, the longer retention time and neutral pH in camel's forestomach (*Russell & Wilson, 1996*) make it more suitable for the survival of rumen fungi. *Neocallimastix* dominated the fungal community and was higher in the G1 camels, which was similar to other results on sheep and camels (*Kittelmann et al., 2013*, *Rabee et al., 2019*). This genus produces enzymes capable of hydrolyzing cellulose, xylan and starch (*Pearce & Bauchop, 1985*). *Cyllamyces* that was observed in small population, has the ability to degrade poor-quality feeds (*Sridhar, Kumar & Anadan, 2014*), which might explain its high population in solid fraction and G3 camels. *Piromyces* was the second dominant genus in the camel rumen of this study and produces cellulolytic and xylanolytic enzymes (*Teunissen et al., 1992*). Therefore, this genus was most abundant in rumen collected from the G2 group of camels. The genus *Spizellomyces* is closely related to Chytridiomctes (*Bowman et al., 1992*), and common in grassland and crop soil (*Lozupone & Klein, 2002*, *Kittelmann et al., 2012*). Thus, contamination of forages by soil could explain the presence of this fungus in camel rumen.

## Correlation between rumen microbes

Interactions between rumen microbes drive feed degradation and methane formation in the rumen, which influence the animal production and the environment (*Williams et al., 1994*; *Lee et al., 2012*; *Henderson et al., 2015*). Positive and negative correlations were observed within and between microbial communities in this study (Fig. 4). Methanogens colonize protozoa and this relationship enhances methane formation (*Newbold, Lassalas & Jouany, 1995*). Additionally, fibrolytic bacteria produce hydrogen and methyl groups that methanogens use for growth (*Johnson & Johnson, 1995*), which demonstrated positive correlations found between *Fibrobacteres* and some methanogens. Also, positive correlation between methylotrophic *Methanosphaera* and *Lachnospiraceae* that has been implicated in pectin degradation and provides methanol as a substrate for the methylotrophs (*Dehority, 1969*). On the other hand, *Prevotella* is a hydrogen utilizer and produces propionate that impact the methanogenesis in the rumen negatively

(*Pitta et al., 2014a*; *Liu et al., 2017*), which illustrates negative correlation between Prevotellaceae and archaea.

Since the rumen anaerobic fungi produce abundant $H_2$ through the fermentation of carbohydrate; they can interact positively with $H_2$ utilizers such as archaea, Prevotellaceae, *Blautia* and *Acetitomaculum* (*Orpin & Joblin, 1997*; *Le Van et al., 1998*; *Yang et al., 2016*; *Liu et al., 2017*). Additionally, anaerobic fungi penetrate plant tissue, providing an increased surface area for bacterial colonization (*Orpin & Joblin, 1997*), which could explain positive correlation between fungi and both *Butyrivibrio* and *Fibrobacteres*. However, some bacteria and protozoa prey on fungal zoospores (*Morgavi et al., 1994*), which demonstrated the negative correlation between both *Neocallimastix* and *Piromyces* with *Diplodinium* and *Entodinium*. Furthermore, *Ruminococcus* produces compounds that inhibit the growth of rumen fungi (*Stewart, Duncan & Richardson, 1992*), which supports the negative correlation between *Neocallimastix* and Ruminococcaceae. *Polyplastron* predates upon other protozoa like *Epidinium, Eudiplodinium, Diplodinium*, and *Ostracodinium* (*Eadie, 1967*).

## CONCLUSIONS

The microbial community in camel rumen was diverse and similar in composition between the groups of camels. The majority of camel rumen microbes (bacteria, fungi, and protozoa) were fibrolytic or have a possible role in fiber digestion, which might illustrate the ability of camel to live in desert harsh conditions under poor feeds. Moreover, the structure of microbial community in rumen of camels was similar to other ruminants.

## ADDITIONAL INFORMATION AND DECLARATIONS

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The authors received no funding for this work.

### Competing Interests

The authors declare that they have no competing interests.

### Author Contributions

- Alaa Emara Rabee conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Robert Forster conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Chijioke Elekwachi performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Ebrahim Sabra conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, sample collection, and approved the final draft.
- Mebarek Lamara analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

## Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Sadat City, Egypt approve the study (VUSC00003).

## Data Availability

The following information was supplied regarding data availability:

Data is available at the SRA database: SRP107370.

## Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.10184#supplemental-information.

# REFERENCES

**Andrews S. 2010.** FastQC a quality control tool for high throughput sequence data. *Available at https://www.bioinformatics.babraham.ac.uk/projects/fastqc/*.

**Bakheit BR. 2013.** Egyptian clover (*Trifolium alexandrinum*) breeding in Egypt: a review. *Asian Journal of Crop Science* **5(4)**:325–337 DOI 10.3923/ajcs.2013.325.337.

**Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. 2009.** MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Research* **37**:W202–W208 DOI 10.1093/nar/gkp335.

**Baraka TA. 2012.** Comparative studies of rumen pH, total protozoa count, generic and species composition of ciliates in camel, buffalo, cattle, sheep and goat in Egypt. *Journal of American Science* **8**:448–462.

**Bergman EN. 1990.** Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* **70(2)**:567–590 DOI 10.1152/physrev.1990.70.2.567.

**Béra-Maillet C, Devillard E, Cezette M, Jouany J, Forano E. 2005.** Xylanases and carboxymethylcellulases of the rumen protozoa *Polyplastron multivesiculatum*, *Eudiplodinium maggii* and *Entodinium sp. FEMS Microbiology Letters* **244(1)**:149–156 DOI 10.1016/j.femsle.2005.01.035.

**Bhatt VD, Dande SS, Patil NV, Joshi CG. 2013.** Molecular analysis of the bacterial microbiome in the forestomach fluid from the dromedary camel (*Camelus dromedarius*). *Molecular Biology Reports* **40(4)**:3363–3371 DOI 10.1007/s11033-012-2411-4.

**Bian G, Ma L, Su Y, Zhu W. 2013.** The microbial community in the feces of the white Rhinoceros (*Ceratotherium simum*) as determined by barcoded pyrosequencing analysis. *PLOS ONE* **8(7)**:e70103 DOI 10.1371/journal.pone.0070103.

**Bolger AM, Lohse M, Usadel B. 2014.** Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30(15)**:2114–2120 DOI 10.1093/bioinformatics/btu170.

**Bowman BH, Taylor JW, Brownlee AG, Lee J, Lu SD, White TJ. 1992.** Molecular evolution of the fungi: relationship of the Basidiomycetes, Ascomycetes, and Chytridiomycetes. *Molecular Biology and Evolution* **9**:285–296 DOI 10.1093/oxfordjournals.molbev.a040720.

**Carberry CA, Waters SM, Kenny DA, Creevey CJ. 2014.** Rumen methanogenic genotypes differ in abundance according to host residual feed intake phenotype and diet type. *Applied and Environmental Microbiology* **80(2)**:586–594 DOI 10.1128/AEM.03131-13.

**Carberry CA, Kenny DA, Han S, McCabe MS, Waters SM. 2012.** Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. *Applied and Environmental Microbiology* **78(14)**:4949–4958 DOI 10.1128/AEM.07759-11.

**Coleman GS, Laurie JI, Bailey JE, Holdgate SA. 1976.** The cultivation of cellulolytic protozoa isolated from the rumen. *Journal of General Microbiology* **95(1)**:144–150 DOI 10.1099/00221287-95-1-144.

**Dagar SS, Kumar S, Griffith GW, Edwards JE, Callaghan TM, Singh R, Nagpal AK, Puniya AK. 2015.** A new anaerobic fungus (*Oontomyces anksri gen. nov., sp. nov.*) from the digestive tract of the Indian camel (*Camelus dromedarius*). *Fungal Biology* **119(8)**:731–737 DOI 10.1016/j.funbio.2015.04.005.

**Danielsson R, Dicksved J, Sun L, Gonda H, Müller B, Schnürer A, Bertilsson J. 2017.** Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. *Frontiers in Microbiology* **8**:226 DOI 10.3389/fmicb.2017.00226.

**Dehority BA. 1969.** Pectin-fermenting bacteria isolated from the bovine rumen. *Journal of Bacteriology* **99(1)**:189–196 DOI 10.1128/JB.99.1.189-196.1969.

**Devillard E, Newbold CJ, Scott KP, Forano E, Wallace RJ, Jouany JP, Flint HJ. 1999.** A xylanase produced by the rumen anaerobic protozoan *Polyplastron multivesiculatum* shows close sequence similarity to family 11 xylanases from Gram-positive bacteria. *FEMS Microbiology Letters* **181(1)**:145–152 DOI 10.1111/j.1574-6968.1999.tb08837.x.

**Dijkstra J, Tamminga S. 1995.** Simulation of the effects of diet on the contribution of rumen protozoa to degradation of fibre in the rumen. *British Journal of Nutrition* **74(5)**:617–634 DOI 10.1079/BJN19950166.

**Eadie JM. 1967.** Studies on the ecology of certain rumen ciliate protozoa. *Journal of General Microbiology* **49(2)**:175–194 DOI 10.1099/00221287-49-2-175.

**Elekwachi CO, Wang Z, Wu X, Rabee A, Forster RJ. 2017.** Total rRNA-Seq analysis gives insight into bacterial, fungal, protozoal and archaeal communities in the rumen using an optimized RNA isolation method. *Frontiers in Microbiology* **8**:1814 DOI 10.3389/fmicb.2017.01814.

**Faye B. 2013.** Camel farming sustainability: the challenges of the camel farming system in the XXIth century. *Journal of Sustainable Development* **6(12)**:74–82 DOI 10.5539/jsd.v6n12p74.

**Franzolin R, Wright AG. 2016.** Microorganisms in the rumen and reticulum of buffalo (*Bubalus bubalis*) fed two different feeding systems. *BMC Research Notes* **9(1)**:243 DOI 10.1186/s13104-016-2046-y.

**Gaidos E, Rusch A, Ilardo M. 2011.** Ribosomal tag pyrosequencing of DNA and RNA from benthic coral reef microbiota: community spatial structure, rare members and nitrogen-cycling guilds. *Environmental Microbiology* **13(5)**:1138–1152 DOI 10.1111/j.1462-2920.2010.02392.x.

**Ghali MB, Scott PT, Jassim RAM. 2005.** Effect of diet change on population of rumen protozoa in dromedary camel. *Recent Advances in Animal Nutrition in Australia* **15**:27A.

**Gharechahi J, Zahiri HS, Noghabi KA, Salekdeh GH. 2015.** In-depth diversity analysis of the bacterial community resident in the camel rumen. *Systematic and Applied Microbiology* **38(1)**:67–76 DOI 10.1016/j.syapm.2014.09.004.

**Gruninger RJ, McAllister TA, Forster RJ. 2016.** Bacterial and archaeal diversity in the gastrointestinal tract of the orth American Beaver (*Castor canadensis*). *PLOS ONE* **11(5)**:e0156457 DOI 10.1371/journal.pone.0156457.

**Guo J, Cole J, Zhang Q, Brown C, Tiedje J. 2015.** Microbial community analysis with ribosomal gene fragments from shotgun metagenomes. *Applied and Environmental Microbiology* **82(1)**:157–166 DOI 10.1128/AEM.02772-15.

**Haitjema CH, Solomon KV, Henske JK, Theodorou MK, O'Malley MA. 2014.** Anaerobic gut fungi: advances in isolation, culture, and cellulolytic enzyme discovery for biofuel production. *Biotechnology and Bioengineering* **111(8)**:1471–1482 DOI 10.1002/bit.25264.

**Hammer Ø, Harper DAT, Ryan PD. 2001.** PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**:9.

**Henderson G, Cox F, Ganesh S, Jonker A, Young W, Janssen PJ. 2015.** Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports* **5(1)**:14567 DOI 10.1038/srep14567.

**Herlemann DPR, Geissinger O, Ikeda-Ohtsubo W, Kunin V, Sun H, Lapidus A, Hugenholtz P, Brune A. 2009.** Genomic analysis of "*Elusimicrobium minutum*", the first cultivated representative of the phylum "*Elusimicrobia*" (formerly termite group 1). *Applied and Environmental Microbiology* **70(9)**:2841–2849 DOI 10.1128/AEM.02698-08.

**Hook SE, Wright ADG, McBride BW. 2010.** Methanogens: methane producers of the rumen and mitigation strategies. *Archaea* **2010(2)**:945785 DOI 10.1155/2010/945785.

**Hristov AN, Ivan M, Rode LM, McAllister TA. 2001.** Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. *Journal of Animal Science* **79(2)**:515–524 DOI 10.2527/2001.792515x.

**Ishaq SL, Wright AG. 2012.** Insight into the bacterial gut microbiome of the North American moose (*Alces alces*). *BMC Microbiology* **12(1)**:212 DOI 10.1186/1471-2180-12-212.

**Ishaq SL, Wright ADG. 2014.** High-throughput DNA sequencing of the ruminal bacteria from moose (*Alces alces*) in Vermont, Alaska, and Norway. *Microbial Ecology* **68(2)**:185–195 DOI 10.1007/s00248-014-0399-0.

**Ishaq S, Sundset M, Crouse J, Wright A. 2015.** High-throughput DNA sequencing of the moose rumen from different geographical locations reveals a core ruminal methanogenic archaeal diversity and a differential ciliate protozoal diversity. *Microbial Genomics* **1(4)**:e000034 DOI 10.1099/mgen.0.000034.

**Jami E, White BA, Mizrahi I. 2014.** Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. *PLOS ONE* **9(1)**:e85423 DOI 10.1371/journal.pone.0085423.

**Jewell KA, McComirck C, Odt CL, Weimer PJ, Suen G. 2015.** Ruminal bacterial community composition in dairy cows is dynamic over the course of two lactations and correlates with feed efficiency. *Applied and Environmental Microbiology* **18(14)**:4697–4710 DOI 10.1128/AEM.00720-15.

**Johnson KA, Johnson DE. 1995.** Methane emissions from cattle. *Journal of Animal Science* **73(8)**:2483–2492 DOI 10.2527/1995.7382483x.

**Johnson DE, Ward GM. 1996.** Estimates of animal methane emissions. *Environmental Monitoring and Assessment* **42(1–2)**:113–141 DOI 10.1007/BF00394046.

**Kamra DN. 2005.** Rumen microbial ecosystem. *Current Science* **89**:124–135.

**Kay RNB, Maloiy MO. 1989.** Digestive secretions in camels. *Options Méditerranéennes—Série Séminaires: n. 2* **1989**:83–87.

**Kittelmann S, Naylor GE, Koolaard JP, Janssen PH. 2012.** A proposed taxonomy of anaerobic fungi (Class Neocallimastigomycetes) suitable for large-scale sequence-based community structure analysis. *PLOS ONE* **7(5)**:e36866 DOI 10.1371/journal.pone.0036866.

**Kittelmann S, Seedorf H, Waters WA, Clemente JC, Knight R, Gordon J, Janssen PH. 2013.** Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. *PLOS ONE* **8(2)**:e47879 DOI 10.1371/journal.pone.0047879.

**Kittelmann S, Janssen PH. 2011.** Characterization of rumen ciliate community composition in domestic sheep, deer, and cattle, feeding on varying diets, by means of PCR-DGGE and clone libraries. *FEMS Microbiology Ecology* **75(3)**:468–481 DOI 10.1111/j.1574-6941.2010.01022.x.

**Kubesy AA, Dehority BA. 2002.** Forestomach ciliate Protozoa in Egyptian dromedary camels (*Camelus dromedarius*). *Zootaxa* **51(1)**:1–12 DOI 10.11646/zootaxa.51.1.1.

**Kumar S, Indugu N, Vecchiarelli B, Pitta DW. 2015.** Associative patterns among anaerobic fungi, methanogenic archaea, and bacterial communities in response to changes in diet and age in the rumen of dairy cows. *Frontiers in Microbiology* **6**:781 DOI 10.3389/fmicb.2015.00781.

**Le Van TD, Robinson JA, Ralph J, Greening RC, Smolenski WJ, Leedle JA, Schaefer DM. 1998.** Assessment of reductive acetogenesis with indigenous ruminal bacterium populations and Acetitomaculum ruminis. *Applied and Environmental Microbiology* **64(9)**:3429–3436 DOI 10.1128/AEM.64.9.3429-3436.1998.

**Leahy S, Kelly W, Ronimus R, Wedlock N, Altermann E, Attwood G. 2013.** Genome sequencing of rumen bacteria and archaea and its application to methane mitigation strategies. *Animal* **7(s2)**:235–243 DOI 10.1017/S1751731113000700.

**Lechner-Doll M, Engelhardt WV. 1989.** Particle size and passage from the forestomach in camels compared to cattle and sheep fed a similar diet. *Journal of Animal Physiology and Animal Nutrition* **61(1–5)**:120–128 DOI 10.1111/j.1439-0396.1989.tb00091.x.

**Lee HJ, Jung JY, Oh YK, Lee SS, Madsen EL, Jeon CO. 2012.** Comparative survey of rumen microbial communities and metabolites across one Caprine and three Bovine groups, using bar-coded pyrosequencing and $^{1}$H nuclear magnetic resonance spectroscopy. *Applied and Environmental Microbiology* **78(17)**:5983–5993 DOI 10.1128/AEM.00104-12.

**Li W. 2009.** Analysis and comparison of very large metagenomes with fast clustering and functional annotation. *BMC Bioinformatics* **10(1)**:359 DOI 10.1186/1471-2105-10-359.

**Li F, Henderson G, Sun X, Cox F, Janssen PH, Guan LL. 2016.** Taxonomic assessment of rumen microbiota using total RNA and targeted amplicon sequencing approaches. *Frontiers in Microbiology* **7**:987 DOI 10.3389/fmicb.2016.00987.

**Liu K, Xu Q, Wang L, Guo W, Zhou M. 2017.** The impact of diet on the composition and relative abundance of rumen microbes in goat. *Asian-Australasian Journal of Animal Sciences* **30(4)**:531–537 DOI 10.5713/ajas.16.0353.

**Lozupone CA, Klein DA. 2002.** Molecular and cultural assessment of chytrid and *Spizellomyces* populations in grassland soils. *Mycologia* **94(3)**:411–420 DOI 10.1080/15572536.2003.11833206.

**Mackenzie AK, Naas AE, Kracun SK, Schuckel J, Fangel JU, Agger JW, Willats WG, Eijsink VG, Pope PB. 2015.** A polysaccharide utilization locus from an uncultured Bacteroidetes phylotype suggests ecological adaptation and substrate versatility. *Applied and Environemental Microbiology* **81(1)**:187–195 DOI 10.1128/AEM.02858-14.

**McGovern E, McCabe MS, Cormican P, Popova M, Keogh K, Kelly AK, Kenny DA, Waters SM. 2017.** Plane of nutrition affects the phylogenetic diversity and relative abundance of transcriptionally active methanogens in the bovine rumen. *Scientific Reports* **7(1)**:13047 DOI 10.1038/s41598-017-13013-y.

**Morgavi DP, Sakurada M, Mizokami M, Tomita Y, Onodera R. 1994.** Effects of ruminal protozoa on cellulose degradation and the growth of an anaerobic ruminal fungus, *Piromyces sp* strain OTS1, in vitro. *Applied and Environmental Microbiology* **60(10)**:3718–3723 DOI 10.1128/AEM.60.10.3718-3723.1994.

**Nathani NM, Patel AK, Mootapally CS, Reddy B, Shah SV, Lunagaria PM, Kothari RK, Joshi CG. 2015.** Effect of roughage on rumen microbiota composition in the efficient feed

converter and sturdy Indian Jaffrabadi buffalo (*Bubalus bubalis*). *BMC Genomics* **16(1)**:1116 DOI 10.1186/s12864-015-2340-4.

**Newbold CJ, Lassalas B, Jouany JP. 1995.** The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. *Letters in Applied Microbiology* **21(4)**:230–234 DOI 10.1111/j.1472-765X.1995.tb01048.x.

**Noel SJ, Attwood GT, Rakonjac J, Moon CD, Waghorn GC, Janssen PH. 2017.** Seasonal changes in the digesta-adherent rumen bacterial communities of dairy cattle grazing pasture. *PLOS ONE* **12(3)**:e0173819 DOI 10.1371/journal.pone.0173819.

**Orpin CG. 1977.** The rumen flagellate *Piromonas communis*: its life cycle and invasion of plant material in the rumen. *Journal of General Microbiology* **99(1)**:107–117 DOI 10.1099/00221287-99-1-107.

**Orpin CG, Joblin KN. 1997.** The rumen anaerobic fungi. In: Hobson PN, Stewart CS, eds. *The Rumen Microbial Ecosystem*. London: Blackie Academic and Professional, 140–184.

**Pandya PR, Singh KM, Parnerkar S, Tripathi AK, Mehta HH, Rank DN, Kothari RK, Joshi CG. 2010.** Bacterial diversity in the rumen of Indian Surti buffalo (*Bubalus bubalis*), assessed by 16S rDNA analysis. *Journal of Applied Genetics* **51(3)**:395–402 DOI 10.1007/BF03208869.

**Pearce PD, Bauchop T. 1985.** Glycosidases of the rumen anaerobic fungus *Neocallimastix frontalis* grown on cellulosic substrates. *Applied and Environmental Microbiology* **49(5)**:1265–1269 DOI 10.1128/AEM.49.5.1265-1269.1985.

**Pitta DW, Kumar S, Veiccharelli B, Parmar N, Reddy B, Joshi CG. 2014a.** Bacterial diversity associated with feeding dry forage at different dietary concentrations in the rumen contents of Mehshana buffalo (*Bubalus bubalis*) using 16S pyrotags. *Anaerobe* **25**:31–41 DOI 10.1016/j.anaerobe.2013.11.008.

**Pitta DW, Parmar N, Patel AK, Indugu N, Kumar S, Prajapathi KB, Patel AB, Reddy B, Joshi C. 2014b.** Bacterial diversity dynamics associated with different diets and different primer pairs in the rumen of Kankrej cattle. *PLOS ONE* **9(11)**:e111710 DOI 10.1371/journal.pone.0111710.

**Pitta DW, Pinchak E, Dowd SE, Osterstock J, Gontcharova V. 2010.** Rumen bacterial diversity dynamics associated with changing from Bermuda grass hay to grazed winter wheat diets. *Microbial Ecology* **59(3)**:511–522 DOI 10.1007/s00248-009-9609-6.

**Poulsen M, Schwab C, Jensen B, Engberg R, Spang A, Canibe N, Højberg O, Milinovich G, Fragner L, Schleper C, Weckwerth W, Lund P, Schramm A, Urich T. 2013.** Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen. *Nature Communications* **4(1)**:1428 DOI 10.1038/ncomms2432.

**Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J. 2012.** A metagenome wide association study of gut microbiota in type 2 diabetes. *Nature* **490(7418)**:55–60 DOI 10.1038/nature11450.

**Rabee AE, Forster RJ, Elekwachi CO, Kewan KZ, Sabra EA, Shawket SM, Mahrous HA, Khamiss OA. 2019.** Community structure and fibrolytic activities of anaerobic rumen fungi in dromedary camels. *Journal of Basic Microbiology* **49(1)**:1–10 DOI 10.1002/jobm.201800323.

**Ransom-Jones E, Jones DL, McCarthy AJ, McDonald JE. 2012.** The fibrobacteres: an important phylum of cellulose-degrading bacteria. *Microbial Ecology* **63(2)**:267–281 DOI 10.1007/s00248-011-9998-1.

**Ren Q, Si H, Yan X, Liu C, Ding L, Long R, Li Z, Qiu Q. 2020.** Bacterial communities in the solid, liquid, dorsal, and ventral epithelium fractions of yak (*Bos grunniens*) rumen. *MicrobiologyOpen* **9(2)**:e963 DOI 10.1002/mbo3.963.

**Roger V, Bernalier A, Grenet E, Fonty G, Jamot J, Gouet P. 1993.** Degradation of wheat straw and maize stem by a monocentric and a polycentric rumen fungus, alone or in association with rumen cellulolytic bacteria. *Animal Feed Science and Technology* **42(1–2)**:69–82 DOI 10.1016/0377-8401(93)90024-E.

**Russell JB, Wilson DB. 1996.** Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *Journal of Dairy Science* **79(8)**:1503–1509 DOI 10.3168/jds.S0022-0302(96)76510-4.

**Salgado-Flores A, Bockwoldt M, Hagen L, Pope P, Sundset M. 2016.** First insight into the faecal microbiota of the high Arctic muskoxen (*Ovibos moschatus*). *Microbial Genomics* **2(7)**:e000066 DOI 10.1099/mgen.0.000066.

**Samsudin AA, Evans PN, Wright AD, Al Jassim R. 2011.** Molecular diversity of the foregut bacteria community in the dromedary camel (*Camelus dromedarius*). *Environmental Microbiology* **13(11)**:3024–3035 DOI 10.1111/j.1462-2920.2011.02579.x.

**Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009.** Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75(23)**:7537–7541 DOI 10.1128/AEM.01541-09.

**Selim HM, Imai S, ElSheik AK, Attia H, Okamot E. 1999.** Rumen ciliate protozoal fauna of native sheep, Friesian cattle and Dromedary camel in Libya. *Journal of Veterinary Medical Science* **61(3)**:303–305 DOI 10.1292/jvms.61.303.

**Shanks OC, Kelty CA, Archibeque S, Jenkins M, Newton RJ, McLellan SL, Huse SM, Sogin ML. 2011.** Community structures of fecal bacteria in cattle from different animal feeding operations. *Applied and Environmental Microbiology* **77(9)**:2992–3001 DOI 10.1128/AEM.02988-10.

**Shrivastava B, Jain KK, Kalra A, Kuhad RC. 2014.** Bioprocessing of wheat straw into nutritionally rich and digested cattle feed. *Scientific Reports* **4(1)**:6360 DOI 10.1038/srep06360.

**SPSS. 1999.** *Statistical package for social science "Release 15*. Chicago: SPSS INC.

**Sridhar M, Kumar D, Anadan S. 2014.** *Cyllamyces icaris sp. nov*., a new anaerobic gut fungus with nodular sporangiophores isolated from Indian water buffalo (*Bubalus bubalis*). *International Journal of Current Research and Academic Review* **2**:7–24.

**Stewart CS, Duncan SH, Richardson AJ. 1992.** The inhibition of fungal cellulolysis by cell-free preparations from Ruminococci. *FEMS Microbiology Letters* **97(1–2)**:83–87 DOI 10.1111/j.1574-6968.1992.tb05444.x.

**Tapio I, Snelling TJ, Strozzi F, Wallace RJ. 2017.** The ruminal microbiome associated with methane emissions from ruminant livestock. *Journal of Animal Science and Biotechnology* **8(1)**:7 DOI 10.1186/s40104-017-0141-0.

**Teunissen MJ, Dekort GM, Opdencamp HM, Huistveld HJ. 1992.** Production of cellulolytic and xylanolytic enzymes during growth of the anaerobic fungus *Piromyces* sp. on different substrates. *Journal of General Microbiology* **138(8)**:1657–1664 DOI 10.1099/00221287-138-8-1657.

**Wang K, Nan X, Chu K, Tong J, Yang L, Zheng S, Zhao G, Jiang L, Xiong B. 2018.** Shifts of hydrogen metabolism from methanogenesis to propionate production in response to replacement of forage fiber with non-forage fiber sources in diets *in vitro*. *Frontiers in Microbiology* **9**:2764 DOI 10.3389/fmicb.2018.02764.

**Weimer PJ. 2015.** Redundancy, resilience, and host specificity of the ruminal microbiota: implications for engineering improved ruminal fermentations. *Frontiers in Microbiology* **6**:296 DOI 10.3389/fmicb.2015.00296.

**Williams AG, Withers SE, Naylor GE, Joblin KN. 1994.** Effect of heterotrophic ruminal bacteria on xylan metabolism by the anaerobic fungus *Piromyces communis*. *Letters in Applied Microbiology* **19(2)**:105–109 DOI 10.1111/j.1472-765X.1994.tb00917.x.

**Yanagita K, Kamagata Y, Kawaharasaki M, Suzuki T, Nakamura Y, Minato H. 2000.** Phylogenetic analysis of methanogens in sheep rumen ecosystem and detection of *Methanomicrobium mobile* by fluorescence in situ hybridization. *Journal Bioscience, Biotechnology, and Biochemistry* **64(8)**:1737–1742 DOI 10.1271/bbb.64.1737.

**Yang CL, Mi L, Hu XL, Liu JX, Wang JK. 2016.** Investigation into host selection of the cecal acetogen population in rabbits after weaning. *PLOS ONE* **11(7)**:e0158768 DOI 10.1371/journal.pone.0158768.

**Zhang J, Kobert K, Flouri T, Stamatakis A. 2014.** PEAR: a fast and accurate Illumina paired-end read merger. *Bioinformatics* **30(5)**:614–620 DOI 10.1093/bioinformatics/btt593.

**Zoetendal E, Plugge CM, Akkermans ADL, Vos WM. 2003.** *Victivallisvadensis gen. nov*., sp. nov., a sugar-fermenting anaerobe from human faeces. *International Journal of Systematic and Evolutionary Microbiology* **53(1)**:211–215 DOI 10.1099/ijs.0.02362-0.