



Bacteroidetes and Firmicutes Drive Differing Microbial Diversity and Community Composition Among Micro-Environments in the Bovine Rumen

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Ruminants are a critical human food source and have been implicated as a potentially important source of global methane emissions. Because of their unique digestive physiology, ruminants rely upon a symbiotic relationship with the complex and rich community of microorganism in the foregut to allow digestion of complex carbohydrates. This study used 16S rRNA gene sequencing to investigate the composition of microbial communities from three rumen micro-environments of cattle fed identical diets: (1) free fluid, (2) the fibrous pack, and (3) the mucosa. Community composition analysis revealed that while a phylogenetic core including the most abundant and most common ruminal taxa (members of Bacteroidetes and Firmicutes) existed across micro-environments, the abundances of these taxa differed significantly between fluid- and mucosa-associated communities, and specific lineages were discriminant of individual micro-environments. Members of Firmicutes, specifically Clostridiales, Lachnospiraceae, Mogibacteriaceae, Christenellaceae, and Erysipelotrichaceae were significantly more abundant in fluid communities, while members of Bacteroidetes, namely Muribaculaceae and Prevotellaceae were more abundant in mucosa-associated communities. Additionally, Methanobacteriaceae, a family of methanogenic Archaea, was more abundant in fluid-associated communities. A set of four more diverse lineages were discriminant of pack-associated communities that included Succinivibrionaceae, RFP12 (Verruco-5), Fibrobacteraceae, and Spirochaetaceae. Our findings indicate that different ecological niches within each micro-environment have resulted in significant differences in the diversity and community structure of microbial communities from rumen fluid, pack, and mucosa without the influence of diet that will help contextualize the influence of other environmental factors.

Keywords: 16S rRNA gene sequencing, cattle, rumen, microbiome, core microbial community

INTRODUCTION

Ruminant livestock are a critical source of food for humans worldwide, with the population of cattle alone estimated to be over 1.5 billion (1). Ruminants are also of environmental significance as they release considerable amounts of methane into the atmosphere (2). Characterized by their unique method of plant digestion, ruminants rely on a complex consortium of microorganisms to partially digest plant polysaccharides in their forestomach, or rumen before it enters the glandular “true” stomach. Thus, the microbial community within the rumen plays an essential role in the health of the animal by providing a major source of nutrients (3), and the microbiota of the rumen are critical to the productivity of this important food source and to potential significance of methane emissions.

The rumen is a rich and diverse microbial ecosystem largely composed of anaerobic bacteria, protozoa, anaerobic fungi, methanogenic archaea, and phages. Bacteria represent the most abundant and diverse taxonomic group, and it is the bacterial members of the rumen microbial community that primarily drive the degradation and fermentation of plant fibers and proteins into digestible compounds such as volatile fatty acids and microbial proteins (4, 5). Ruminal archaea are mostly limited to methanogenic members of the phylum Euryarchaeota, and more specifically the classes Methanobacteria, Methanomicrobia, and Thermoplasmata. Typically, ruminal microbial communities are predominately made up of starch and sugar degrading organisms (5), though it is well-established that diet exerts a strong influence on ruminal microbial community composition (6–8). Despite this, abundant ruminal taxa are remarkably consistent across individual animals regardless of their diet or location. In a meta-analysis characterizing ruminal microbial communities in 742 individual ruminants from across the world, the top 30 most abundant microbial genera were found in over 90% of samples (9).

The microbiota of the rumen as a whole has been well-characterized, but relatively few studies have investigated differences in community structure between ruminal components. While there are a large number of ecological niches available, broadly speaking rumen microorganisms can be free-living or particle-attached in ruminal fluid, attached to the fibrous pack, or attached to the ruminal mucosa (10). Differences in diversity and community structure have been identified between liquid and solid-associated ruminal communities (5, 11, 12), but comparisons to mucosa- and pack-associated microbial communities are limited (13, 14). Mucosa-associated microbial communities in the rumen have also been described temporally (15) and been compared to ruminal pack-associated communities (16), but there is little knowledge about which microbial taxa are discriminant of ruminal fluid, pack, and mucosa-associated communities.

This study utilized 16S rRNA gene sequencing to characterize the diversity and composition of microbial communities of different locations within the rumen: within the ruminal fluid, fibrous pack, and those found on the mucosa. It was designed to test if the previously described ruminal phylogenetic core is present across ruminal micro-environments, and to

discover which microbial taxa were discriminant of these micro-environments. We hypothesized that core rumen taxa would be present across all three micro-environments, but that the composition of fluid, pack, and mucosa-associated microbial communities would differ.

MATERIALS AND METHODS

Cattle Population

Twelve crossbred feedlot steers (450 kg; ~3.0 years of age) fitted with ruminal fistulas, were utilized in this study. All cattle in this study were adjusted to a high energy finishing diet consisting of ~90% concentrate and 10% roughage (1.43 NEg MCal/kg—**Supplementary Table S1**) for 4 weeks (28 d) before sample collection. Animals were managed and sampled in accordance with Colorado State University's (CSU) Institutional Animal Care and Use Committee (IACUC) approval (Protocol 16-6550A). Steers were housed at CSU's Agricultural Research, Development and Education Center.

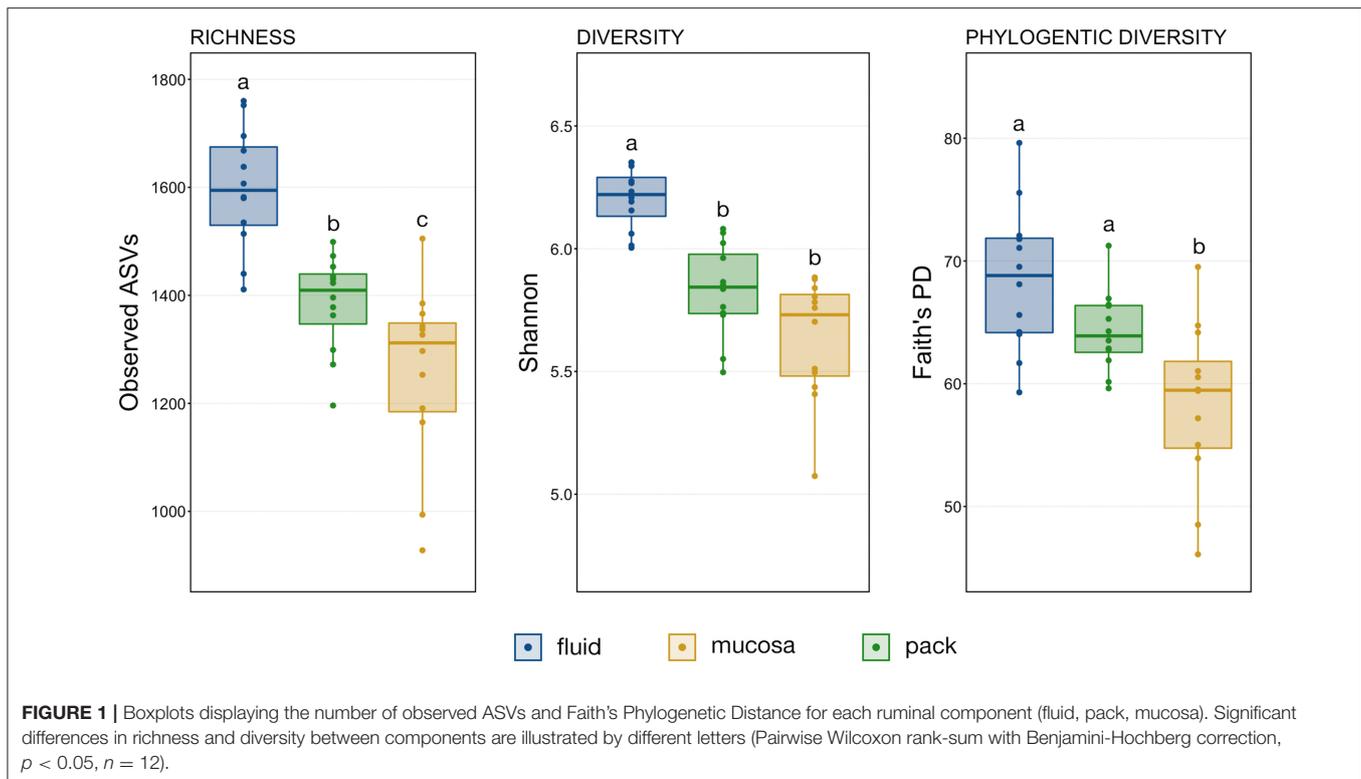
Rumen Content Collection

At the completion of the diet adjustment period, samples from three micro-environments within the rumen were collected *via* ruminal fistulas from each steer ~1 h after morning feeding: the aqueous portion of the rumen (fluid; $n = 12$), a bolus of the ruminal fibrous pack (pack; $n = 12$), and a swab of the ruminal mucosa (mucosa; $n = 12$). The bolus of fibrous material was sampled by collecting 85 g from the center of the solid portion of the rumen, and the ruminal mucosa was sampled by running a sterile swab across the ruminal wall. Fluid was collected by compressing the fibrous bolus and capturing the resulting liquid. After collection, samples were immediately placed on ice, transported to the laboratory and stored at -80°C at the laboratory until DNA isolation.

DNA Isolation, 16S rRNA Library Preparation and Sequencing

Genomic DNA was isolated from fluid and mucosal swab samples using a QIAamp PowerFecal DNA Kit (Qiagen, Hilden, Germany) from 0.16 to 0.18 g of material, while a DNeasy PowerMax Soil Kit was used to isolate DNA from 2 to 5 g fibrous pack samples. Following isolation, DNA was quantified ($\text{ng } \mu\text{L}^{-1}$) and assayed for quality (A_{260}/A_{280}) using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA).

Amplicon library preparation and sequencing was carried out by Novogene Corporation Inc (Chula Vista, CA, USA). The V4 region of the 16S rRNA gene was amplified with the 515f (5' – GTG CCA GCM GCC GCG GTA A – 3') and 806r (5' – GCA CTA CHV GGG TWT CTA AT – 3') primer pair and Novogene's proprietary amplification conditions, which include no template PCR negative controls. Following successful PCR, amplicon libraries were prepared and pooled using Novogene's proprietary process and sequenced on an Illumina HiSeq 2500 instrument (Illumina Inc., San Diego, CA, USA) using 2×250 bp paired-end chemistry. The number of sequenced reads ranged from 188,276 to 219,029, with an average of 209,279 reads per



sample. The average sequencing depth did not differ between the three micro-environments (Pairwise Wilcoxon rank-sum + Benjamini-Hochberg correction for multiple comparisons, $p > 0.05$, $n = 12$).

Bioinformatics

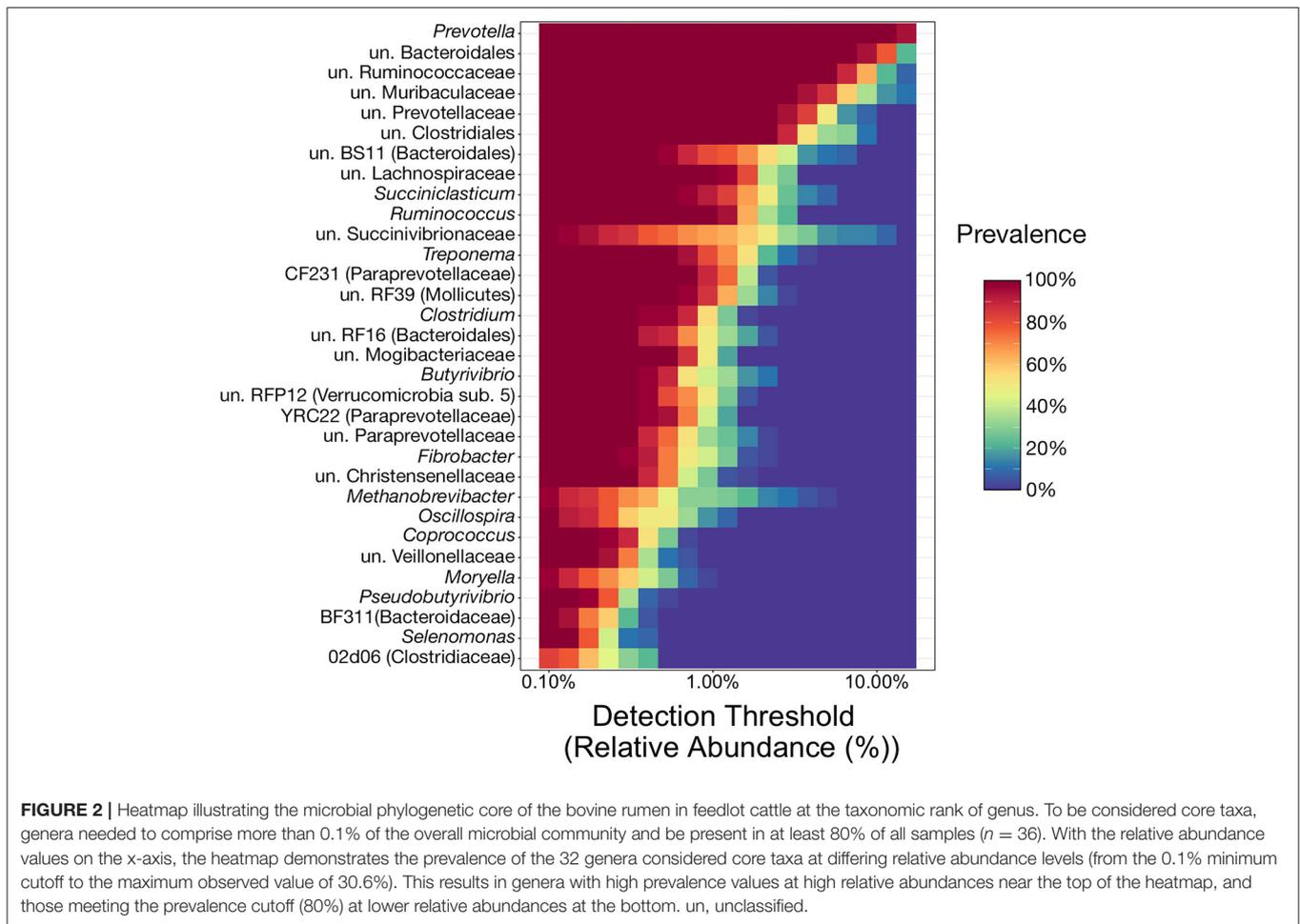
Demultiplexed paired-end reads were imported into QIIME2 version 2020.11 (16) and DADA2 was used to filter reads for quality, remove chimeric sequences, merge overlapping paired-end reads, and generate amplicon sequence variants (ASVs) (17). Forward reads were trimmed at 17 bp and truncated at 249 bp, while reverse reads were trimmed at 21 bp and truncated at 249 bp. Taxonomy was assigned using a Naïve Bayes classifier trained on the Greengenes version 13_8 99% OTUs database (18), where sequences had been trimmed to include only the base pairs from the V4 region bound by the 515f/806r primer pair. Reads that mapped to chloroplast and mitochondrial sequences were filtered from the representative sequences and ASV table using the “filter_taxa” function, and a midpoint-rooted phylogenetic tree was then generated using the “q2-phylogeny” pipeline with default settings, which was used to calculate phylogeny-based diversity metrics. Data and metadata were then imported into phyloseq (19) using the “import_biom” and “import_qiime_sample_data” functions and merged into a phyloseq object. Richness (Observed ASVs), Shannon's diversity, and Faith's Phylogenetic Diversity (FPD) were calculated for all samples with phyloseq and the “estimate_pd” function from the btools package. Samples were then proportionally transformed to the lowest total ASV count of 101,175 and

beta-diversity was analyzed using weighted, generalized, and unweighted UniFrac distances (20, 21). From these distances, non-metric multidimensional scaling (NMDS) was performed and plotted, and a permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences in community structure using the vegan (22) and pairwiseAdonis (23) packages. To ensure significant differences were not the result of unequal dispersion of variability between groups, permutational analyses of dispersion (PERMDISP) were conducted for all significant PERMANOVA outcomes using the vegan package. Hierarchical clustering was performed using Ward's agglomeration clustering method (24) on generalized UniFrac distances and the “hclust” function. Further, the relative abundances of ASVs within each sample were calculated and plotted using phyloseq. The proportion of reads mapping to each taxonomic rank are displayed in **Supplementary Table S1**.

Rumen Phylogenetic Core and Discriminant Lineages

The core microbiota of the rumen was identified at the taxonomic rank of genus. A detection limit (minimum allowed relative abundance at the level of genus) of 0.1% and a minimum prevalence (proportion of samples the genus was present in) of 80% were used as cutoffs, and the “core” function within the package microbiome was used to calculate and plot a heatmap of core community members.

To identify taxa discriminant of each micro-environment (i.e., fluid, pack, mucosa), linear discriminant analysis effect size (LEfSe) was performed using the LEfSe tool (25) with default



settings, except for using a more stringent alpha value (0.01) and a higher logarithmic LDA score threshold (4.0) due to small sample size. Because of the low proportion of reads classified at the rank of genus (40.4%; **Supplementary Table S1**) families with a mean relative abundance $>0.5\%$ across all samples were considered, with the factor “micro-environment” set as class, and an “one-against-all” strategy was applied. The relative abundances of the families considered discriminant of any of the three micro-environments were then individually visualized and compared.

Statistical Analyses

Unless specified otherwise, R version 3.6.3 (26) was used for statistical analysis of data. Pairwise Wilcoxon rank-sum tests were performed with a Benjamini-Hochberg correction (PW+BH) for multiple comparisons. Differences in beta diversity were tested using pairwise PERMANOVA with a Benjamini-Hochberg correction for multiple comparisons and 9,999 permutations. Additionally, pairwise PERMDISPs were carried out for all significant PERMANOVA outcomes using 9,999 permutations to test for differences in the variability of dispersions. Except for the LefSe analysis, an alpha of 0.05 was used as the cutoff to reject the null hypothesis and for differences to be considered statistically significant.

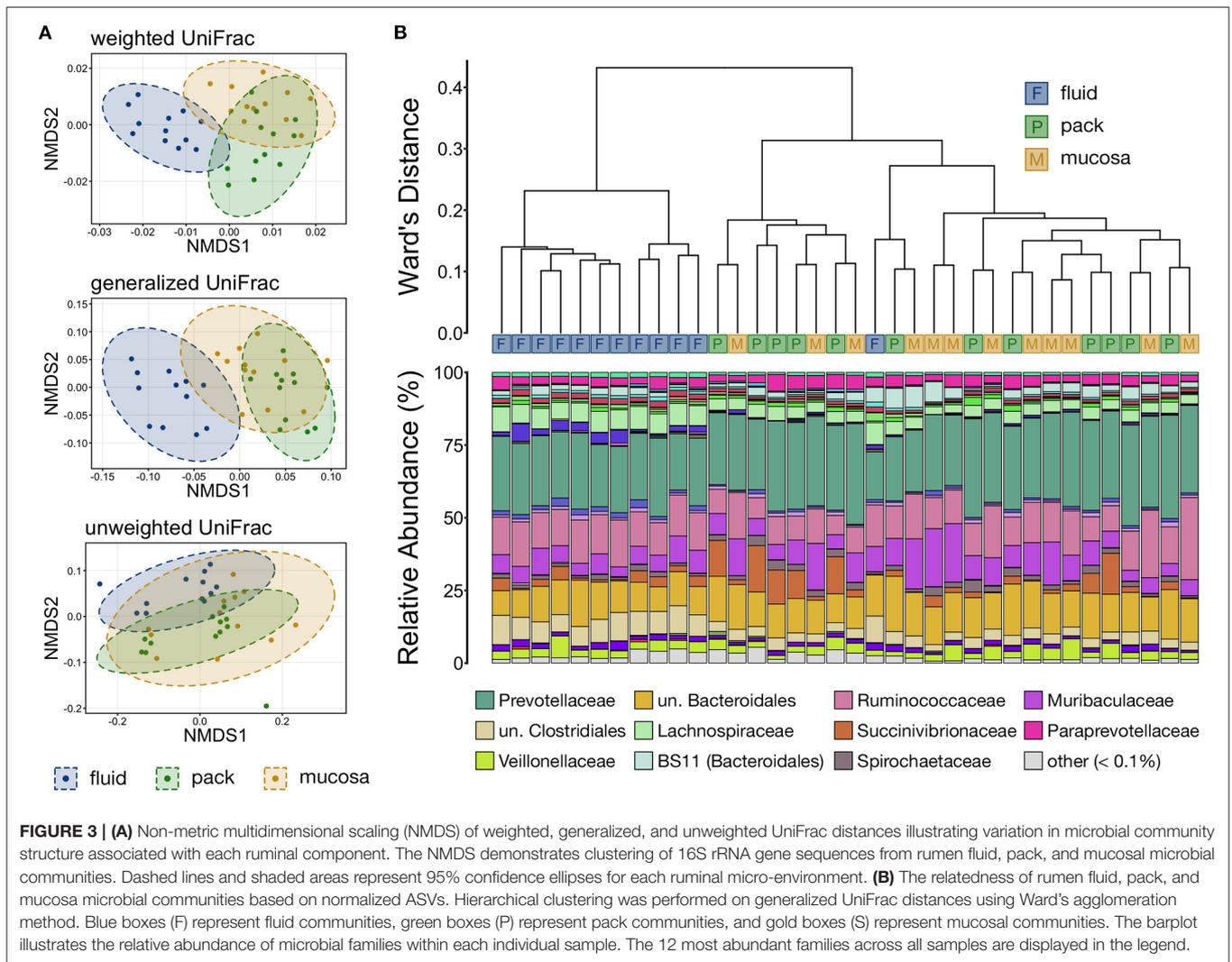
Data Availability

All sequence reads were made available through the BioProject PRJNA749669 at the National Center for Biotechnology Information’s Sequence Read Archive.

RESULTS

Microbial Richness and Diversity

A comparison of observed ASVs was used to test for differences in richness among the ruminal components, and comparisons of Shannon’s index and Faith’s Phylogenetic Distance (FPD) were used to test for differences in diversity. The trend was the same for richness, diversity, and phylogenetic diversity, with fluid being the richest and the most diverse followed by pack and then mucosa (**Figure 1**). However, statistical significance differed between metrics. Microbial communities within the rumen fluid were richer than ruminal pack and mucosa communities and pack communities were richer than mucosa (**Figure 1**; PW+BH, $n = 12$, $p < 0.05$). Fluid communities were also more diverse than pack and mucosa communities, which did not differ significantly from each other (**Figure 1**; PW+BH, $n = 12$, $p < 0.05$). However, the phylogenetic diversity of ruminal fluid and pack communities were not different, and both were more



phylogenetically diverse than mucosal communities (**Figure 1**; PW+BH, $n = 12$, $p < 0.05$).

Rumen Phylogenetic Core

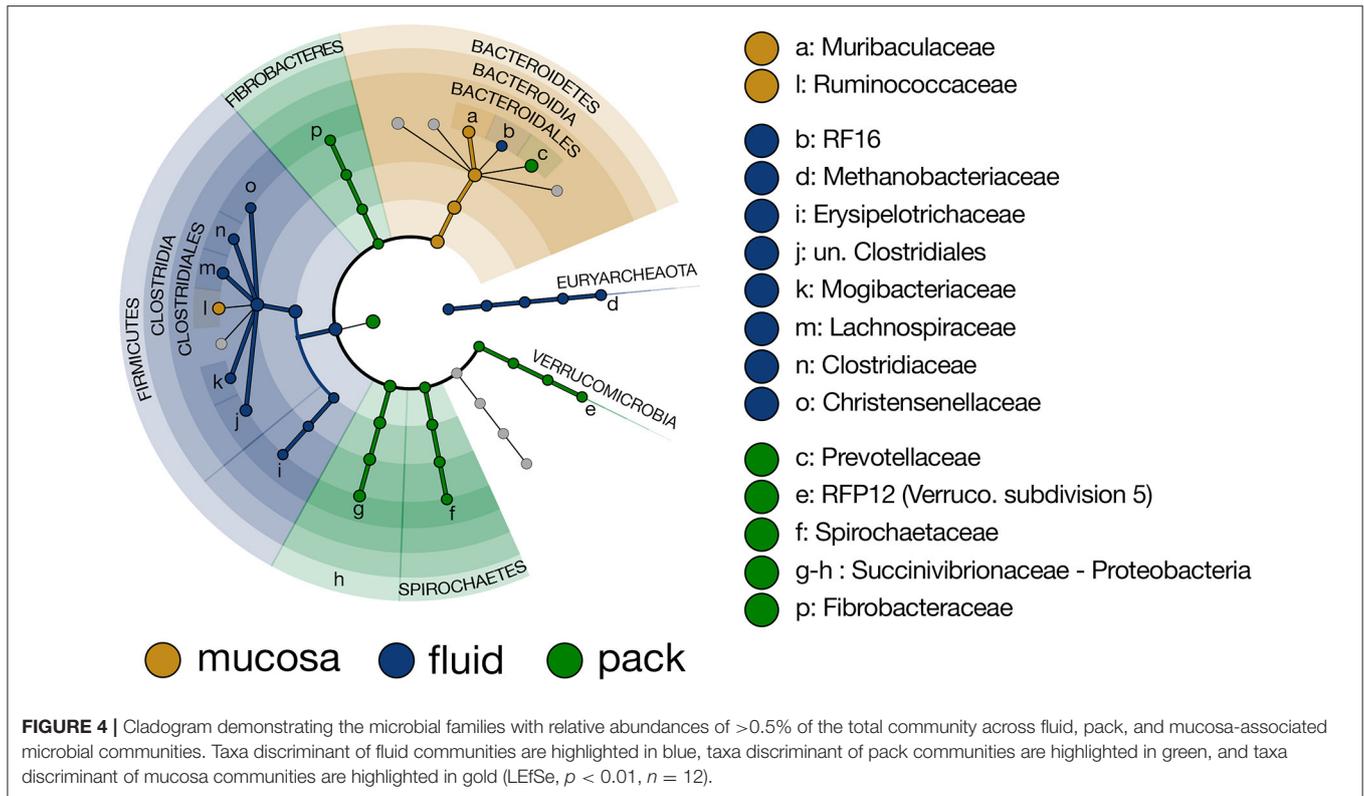
Of the 117 genera above the detection threshold of 0.1% relative abundance, 32 (27%) were present in at least 80% of samples from all three ruminal components across every individual animal, and thus were considered to represent the phylogenetic core (**Figure 2**). Of the genera within the phylogenetic core, 22 (69%) of them belong to the phyla Bacteroidetes or Firmicutes, and specifically the orders Bacteroidales or Clostridiales. Genera from these two orders were the most abundant members of the phylogenetic core, with 9 of the top 10 most abundant core genera belonging to these two orders (**Figure 2**). Interestingly, of the 32 core genera, all 32 were present in 100% of all samples.

Microbial Community Structure of Ruminal Components

Differences in overall microbial community structure among the three ruminal components was analyzed using NMDS,

hierarchical clustering, and PERMANOVA. The composition of ruminal fluid, pack, and mucosa communities were different from each other when compared using all three UniFrac distances (**Supplementary Table S1**; pairwise PERMANOVA, $n = 12$, $p < 0.05$). Non-significant PERMDISP tests confirmed that significant PERMANOVA results were the result of differences in community structure and not due to unequal dispersions of variance (**Supplementary Table S1**; pairwise PERMDISP, $p > 0.05$). Visualization with NMDS illustrated that microbial communities from each of the three ruminal components were distinct, and that communities became more similar when compared using less weight on abundant lineages (i.e., from weighted to unweighted UniFrac). Further, fluid and pack communities appear to be more tightly clustered and distinct from each other, while mucosa communities were slightly more varied (**Figure 3A**).

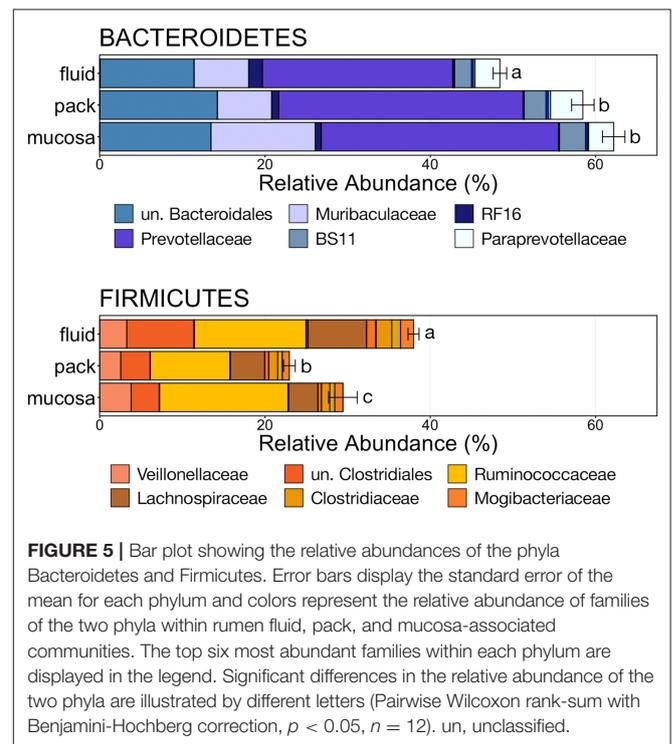
Hierarchical clustering based on generalized UniFrac values revealed that fluid communities were most similar to other fluid communities and formed a fluid-specific clade, with the exception of one fluid community that formed a clade



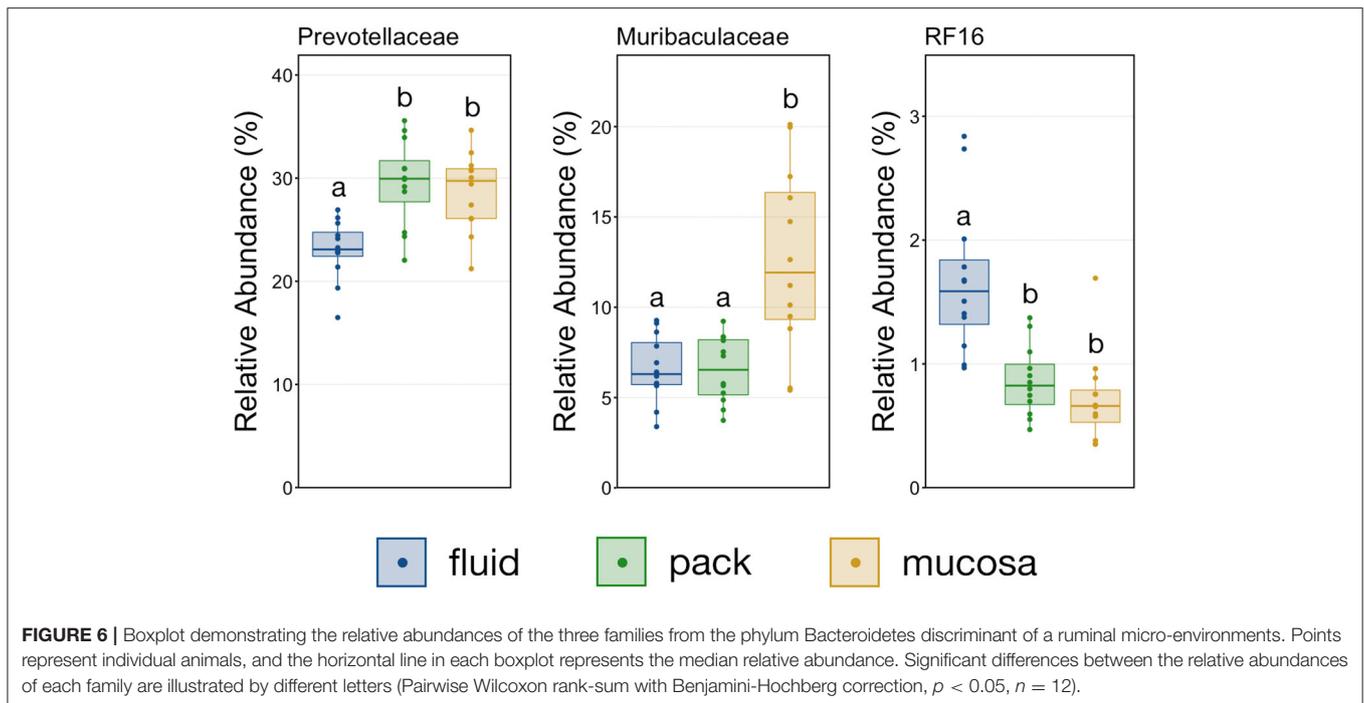
with pack and mucosa communities collected from the same individual animal (Figure 3B). Ruminal pack and mucosa communities were more intermixed, however both pack and mucosa communities were slightly more likely to cluster with other communities from the same ruminal component (i.e., pack or mucosa). The dichotomy between rumen fluid and pack or mucosa communities was primarily the result in differences in the abundances of members of Bacteroidales (higher in pack/mucosa-associated communities) and Clostridiales (higher in fluid-associated communities) (Figure 3B).

Ruminal Component-Specific Discriminant Lineages

To more closely investigate taxa that were responsible for the differing community structure among the three ruminal components, linear discriminant analysis effect size (LEfSe) was used to identify taxonomic lineages that were differentially abundant between rumen fluid, pack, and mucosa communities. Results show that six lineages of Firmicutes (unclassified Clostridiales, Lachnospiraceae, Clostridiaceae, Mogibacteriaceae, Christensenellaceae, and Erysipelotrichaceae) were discriminant of fluid communities. Five of the six lineages included members of Clostridiales. Further, Methanobacteriaceae (Euryarchaeota) and RF16 (Bacteroidales) were discriminant of fluid-associated communities (Figure 4). Muribaculaceae (Bacteroidales) and Ruminococcaceae (Clostridiales) were discriminant of mucosa-associated communities (Figure 4), though Ruminococcaceae being discriminant of mucosal communities is misleading. Ruminococcaceae was in similar abundance in fluid and



mucosa-associated communities (see Figure 8 below), and its designation as mucosa-discriminant was an artifact of the “one-against-all” comparison strategy coupled with a lower abundance in pack communities. A set of four more diverse



lineages including RFP12 (Verrucomicrobia), Spirochaetaceae (Spirochaetes), Succinivibrionaceae (Proteobacteria), and Fibrobacteraceae (Fibrobacteres) were discriminant of pack communities (**Figure 4**).

To further characterize the Bacteroidetes-Firmicutes dichotomy among fluid, pack, and mucosa communities, the relative abundances of each phylum and the families comprising them were directly compared. The abundance of members of Bacteroidetes was higher in mucosa- and pack-associated communities than fluid communities (**Figure 5**; PW+BH, $n = 12$, $p < 0.05$). Differences in Bacteroidetes were largely the result of differences within the families of Prevothellaceae and Muribaculaceae (**Figure 5**), which represented the two most abundant families of Bacteroidetes. Prevothellaceae was less abundant in fluid communities compared to both pack and mucosa, while Muribaculaceae was more abundant in mucosa-associated communities vs. both fluid and pack (**Figure 6**; PW+BH, $n = 12$, $p < 0.05$). RF16 (Bacteroidales) was more abundant in fluid communities (**Figure 6**; PW+BH, $n = 12$, $p < 0.05$), although its relative abundance was far lower than that of Prevothellaceae and Muribaculaceae in all three micro-environments.

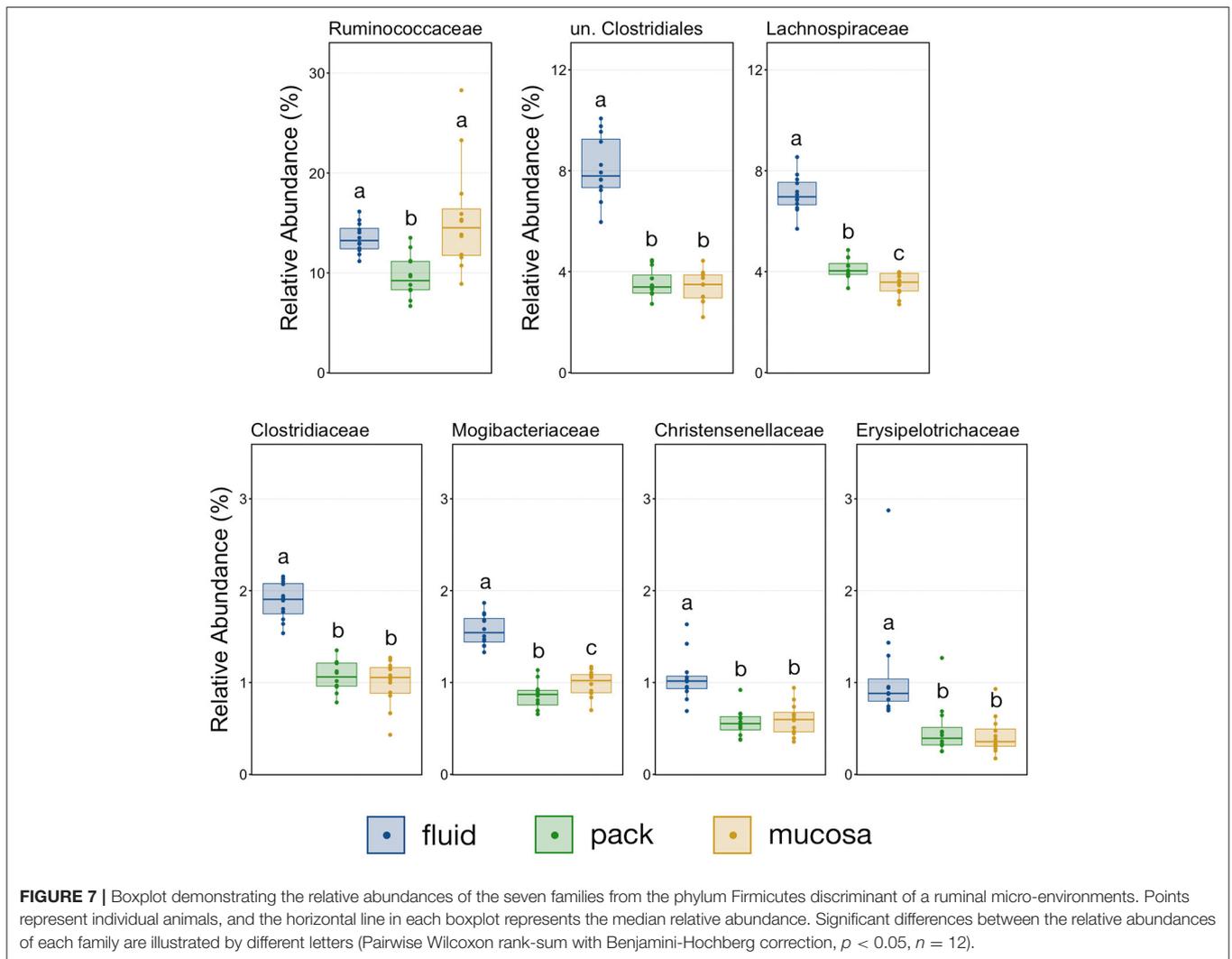
The relative abundance of Firmicutes was different among each of the three ruminal components, with the phylum being more abundant in fluid communities than both pack and mucosa-associated communities, and less abundant in mucosa-associated communities than pack (**Figure 5**; PW+BH, $n = 12$, $p < 0.05$). The differential abundance of Firmicutes between ruminal components was largely the result of differences in the relative abundance unclassified Clostridiales and Lachnospiraceae (**Figure 5**). A total of six families within Firmicutes (unclassified Clostridiales, Lachnospiraceae,

Clostridiaceae, Mogibacteriaceae, Christensenellaceae, Erysipelotrichaceae) were more abundant in fluid communities than both pack and mucosa-associated communities, while Ruminococcaceae was more abundant in fluid and mucosa-associated communities than in pack (**Figure 7**; PW+BH, $n = 12$, $p < 0.05$).

With the exception the Methanobacteriaceae, which was discriminant of fluid-associated communities (**Figure 4**), every discriminant lineage outside of Firmicutes and Bacteroidetes was from pack-associated communities. Succinivibrionaceae, RFP12, Spirochaetaceae, and Fibrobacteraceae were all discriminant of pack-associated communities and belong to the phyla Proteobacteria, Verrucomicrobia, Spirochaetes, and Fibrobacteres, respectively (**Figure 5**). Pack-associated communities had higher relative abundances of Succinivibrionaceae, Spirochaetaceae (virtually entirely made up of *Treponema*), Fibrobacteraceae, and RFP12 than fluid and mucosa-associated communities, which had similar abundances (**Figure 8**; PW+BH, $n = 12$, $p < 0.05$). The family Methanobacteriaceae was in higher abundance in fluid-associated communities than both pack and mucosa-associated communities (**Figure 8**; PW+BH, $n = 12$, $p < 0.05$).

DISCUSSION

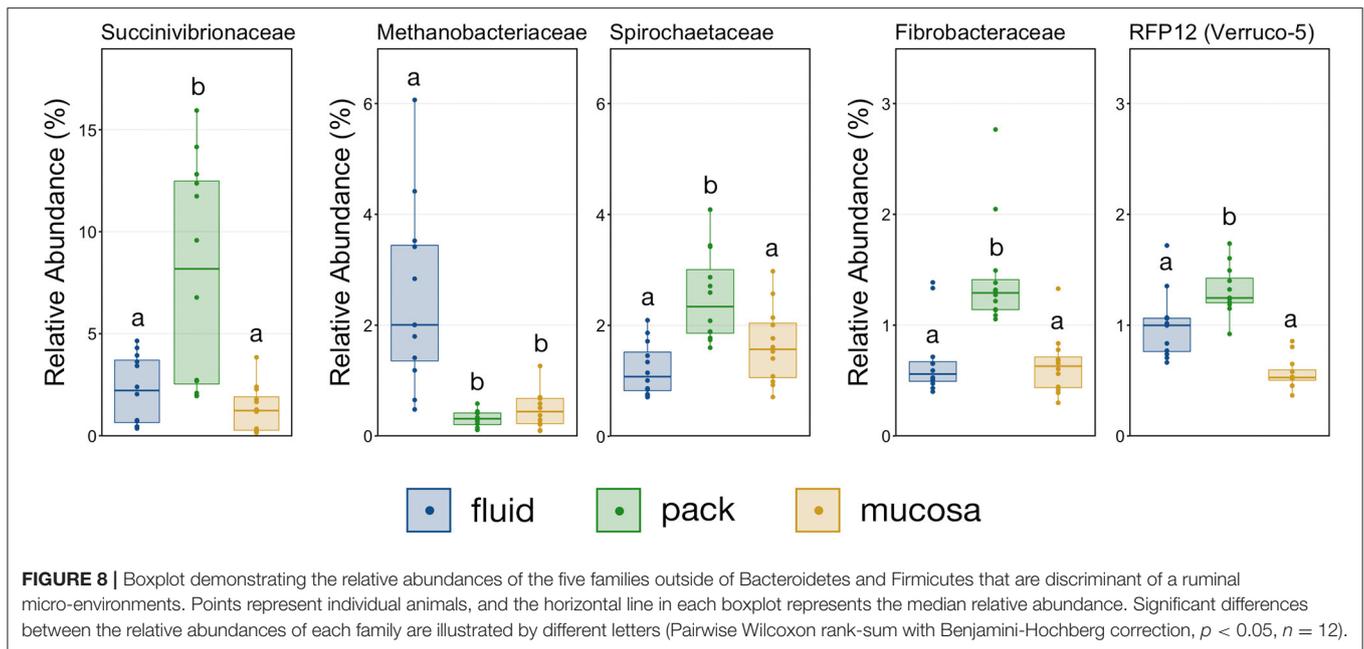
This study used 16S rRNA gene sequencing to investigate the localization of microbiota within the bovine rumen and to identify microbial taxa discriminant of its micro-environments. Results demonstrated that rumen fluid contained the richest and most diverse microbial communities, while mucosa-associated communities were the least diverse. Importantly, while a large proportion of microbial taxa were present in every ruminal fluid,



pack, and mucosa-associated community, the proportions of these taxa differed significantly among ruminal components. Members of the phylum Firmicutes were disproportionately predominant within fluid communities as compared to pack and mucosa-associated communities, while members of the phylum Bacteroidetes were disproportionately under-represented in fluid communities. Further, pack-associated communities contained discriminant lineages from the phyla Proteobacteria, Verrucomicrobia, Spirochaetes, and Fibrobacteres. Diet-induced shifts within rumen microbial communities are well-established (5–8) and due to the consistent diet throughout this study, these results provide a baseline for the diversity and community composition of microbial communities associated with specific ruminal micro-environments (i.e., fluid, pack, and mucosa) that can be used to contextualize the influence of environmental factors such as diet.

Rumen fluid functions as a transportation and growth medium for digestive microorganisms and as a result it could be expected that the majority of microbial taxa found within the rumen as a whole would be present in the fluid even if in

very low abundance. Indeed, fluid communities in this study were the richest, but diversity was similar in fluid and pack-associated communities. Higher richness, but similar diversity suggests that ruminal fluid contains more types of microorganisms, but the additional types are either rare or closely related. Interestingly, mucosa-associated communities in this study were both less rich and less diverse, which contradicts previous research that found mucosal communities to be most diverse (13). However, multiple differences in methodology (i.e., different primers, lower sequencing depth, rarefied data, OTU/ASV picking method) could explain the differences. For example, the previous study had considerably lower sequence depth [490,001 processed sequences across 89 samples (5,505 sequences per sample)] as compared to this study [4,571,185 processed sequences across 36 samples (126,977 sequences per sample)]. Further, the previous study performed OTU clustering with an unusually low similarity cutoff (95%), while this study denoised sequences to produce ASVs without the need to cluster, which provides a higher resolution examination of the community structure (17). Differences in community coverage (i.e., sequencing depth) and



sequence processing could explain the contradictory relationship between micro-environments.

A large number of studies have used amplicon sequencing to characterize the rumen's phylogenetic core and illustrated the overwhelming dominance of the phyla Bacteroidetes and Firmicutes, and more specifically members of orders Bacteroidales and Clostridiales (9, 27–29). Here, we demonstrated that these two orders remain very abundant and were found in 100% of samples collected from ruminal fluid, pack, and mucosa-associated communities. Our finding that 27% of taxa were included in the phylogenetic core across the three micro-environments falls within the range of previous research investigating the ruminal phylogenetic core in whole-rumen samples (29–31). The shared core between each micro-environment is in itself largely uninteresting, as it only illustrates the presence of microbial taxa and not abundance.

Despite the most abundant taxa being dominant in all three micro-environments, fluid, pack, and mucosa-associated microbial communities were different when compared with and without weight being placed on abundance, suggesting that both abundant and rare taxa contribute to the differences. Variations in community composition in communities associated with liquid- and solid-fractions of different ruminal micro-environments have been well-described (5, 12, 13) and this study confirms this. Further, our results illustrate that the difference between liquid- and solid-associated fractions is much larger than that between solid- and mucosa-associated communities. The dichotomy between free-living and attached microbial communities is a long-established fact in environmental microbial ecology (32–34) and here we demonstrate this likely holds true in the rumen as well.

The primary drivers of the liquid-solid dichotomy in the rumen were members of the phyla Bacteroidetes and

Firmicutes. In particular, members of Muribaculaceae (formerly S24-7) and Prevotellaceae (both families of Bacteroidetes) were more abundant in solid attached communities, with Muribaculaceae abundance being the highest in mucosal communities. A study of the Muribaculaceae family showed that its members have high functional diversity with regards to complex carbohydrate degradation, and contain protective mechanisms against benzoate and macrolide antibiotics (35). However, the family is relatively poorly characterized and why it is over-represented in the mucosa specifically should be the focus of future investigation. Contrastingly, a set of six families within Firmicutes were significantly more abundant in fluid-associated communities. The most abundant of these belonged to the Clostridiales (unclassified Clostridiales, Lachnospiraceae, and Clostridiaceae). Lachnospiraceae is a well-characterized butyrate producer (36), which is an important energy source for rumen epithelial cells and plays a role in regulating rumen barrier function (37). Lachnospiraceae produce butyrate through the degradation of plant fibers (38), and would benefit from access to the pack. Additionally, ruminal fluid itself contains small fiber particles and would provide an environment where Lachnospiraceae and other butyrate producers would be in contact with both a fiber-source and the epithelium where the butyrate would be used as an energy source. Comparisons of the abundance of Bacteroidetes and Firmicutes between liquid and solid rumen communities have produced mixed results; either reporting very small differences between fluid and solids (5), or more drastic differences similar to ours (13).

It should also be noted that the samples were collected from cattle that had permanent fistulas placed through the skin into the rumen. Accessing the rumen *via* these fistulas meant that mucosal samples were taken from the surface by swab, and it was not possible to determine whether the mucosa was bathed with

ruminal fluid during collection, which could represent a source of cross-contamination between micro-environments. However, it is unlikely that these factors would have been fully responsible for creating systematic differences among the microbiota found in the different ruminal fractions that were studied. Another consideration is that fluid samples here were collected from fluid extracted from the pack, which could potentially yield different communities than free fluid found in the ventral rumen. One additional potentially confounding factor in this study was the use of different extraction methods for fluid and solid communities (pack and mucosa-associated), which has been demonstrated to influence 16S rRNA amplicon sequencing and subsequent community composition results in ruminal communities (39, 40). While two extraction kits were used, they both used mechanical shearing (bead-beating) without phenol and both kits utilize the same chemistries, which eliminates the largest source of extraction bias (39). Despite this, community structure (i.e., relative abundance of different taxa) may have been influenced by extraction method and resulted in differences in relative abundance values. However, we believe any influence was likely minimal and would not change the conclusions drawn from the results of this study.

Multiple lineages outside Bacteroidetes and Firmicutes were also discriminant of individual micro-environments. With the exception of Methanobacteriaceae, a methanogenic Euryarchaeota, lineages outside the two major phyla were discriminant of pack-associated communities. Members of Spirochaetes are known to be involved with the degradation of soluble fibers (41), and members of Fibrobacteraceae are widely described ruminal flora capable of degrading plant-based cellulose (42, 43). It follows that members of Spirochaetaceae and Fibrobacteraceae would be over-represented in communities attached to the fibrous pack. Members of the family Succinivibrionaceae ferment carbohydrate to produce succinate and acetate (44) and were also discriminant of pack-associated communities. Interestingly, this family has been linked to lower methane emissions (45). Contrastingly, the family RFP12 from the phylum Verrucomicrobia was discriminant of pack-associated communities but has been negatively correlated with lower methane emissions (46) and Methanobacteriaceae, known methane producers in the rumen

(47, 48), were over-represented in fluid-associated communities. Our results suggest that efforts to promote or negate the growth of these families implicated in methane emissions should target the flora that are abundant in the ruminal fibrous pack and fluid.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/BioProject/PRJNA749669>.

ETHICS STATEMENT

The animal study was reviewed and approved by Colorado State University's (CSU) Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

KB, PM, TE, JM, RD, MW, and AR participated in and provided oversight of all aspects of the study including securing funding, design, laboratory procedures, data analysis, and report preparation. CW participated in study conduct and laboratory analyses. LP performed the data analysis and wrote the manuscript. All authors read, edited, and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2022.897996/full#supplementary-material>

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