Investigation of the Interrelationship between Ruminal and Oral Microbiomes of Angus Bulls

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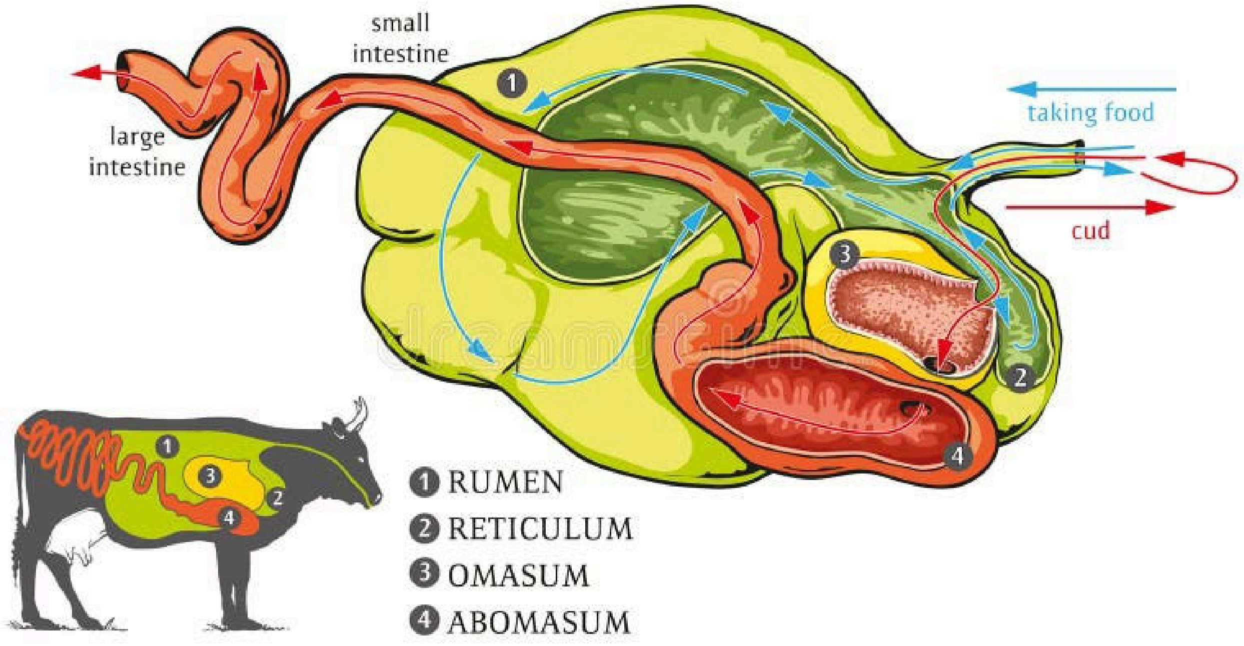
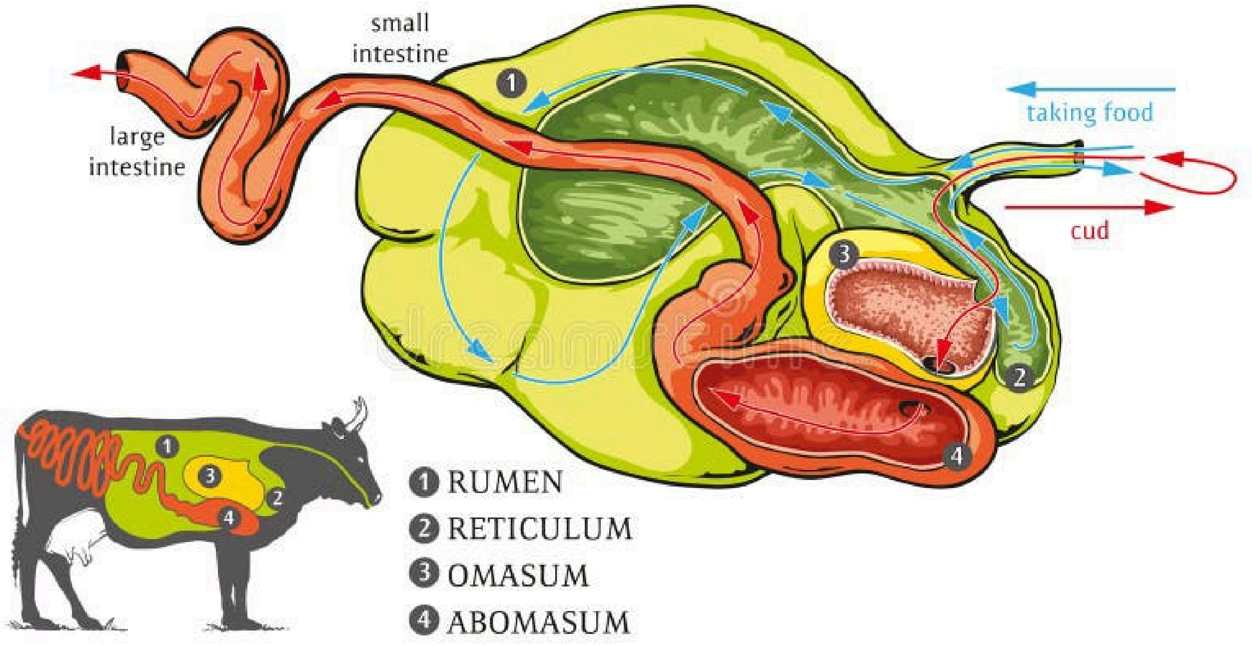
The Rumen



Differences in Rumen Microbiota Have Been Linked With Animal Performance:

- Shabat et al., 2016: Microbiome-dependent mechanisms are associated with ruminants’ ability to extract energy from their feed
- Myer et al., 2015; Carmichael et al., 2024: Ruminal microbial profile of beef cattle has been associated with feed efficiency
- Carcass composition (Krause et al., 2020)
- Methane emissions (Hook et al., 2010; Tapio et al., 2017)

The Rumen



Therefore, studying the ruminal microbiome can provide valuable information!

But how can we obtain ruminal samples?

Methods of Rumen Sample Collection

- Orogastric Intubation
- Rumenocentesis
- Rumen Fistulation

Methods of Rumen Sample Collection

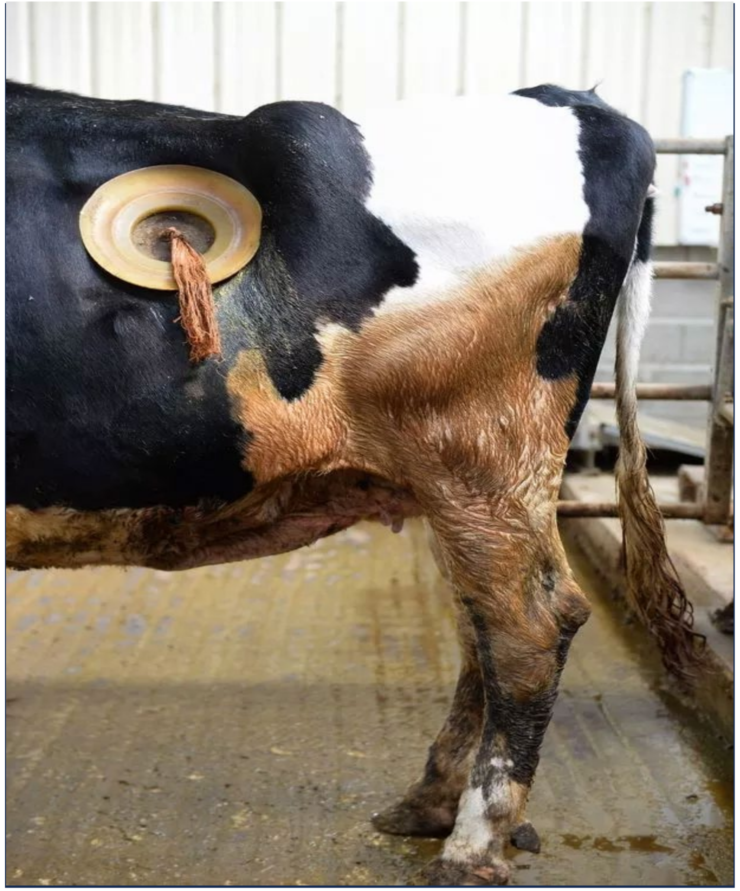
Rumenocentesis

Rumen
Fistulation

Methods of Rumen Sample Collection

Rumen Fistulation

Methods of Rumen Sample Collection



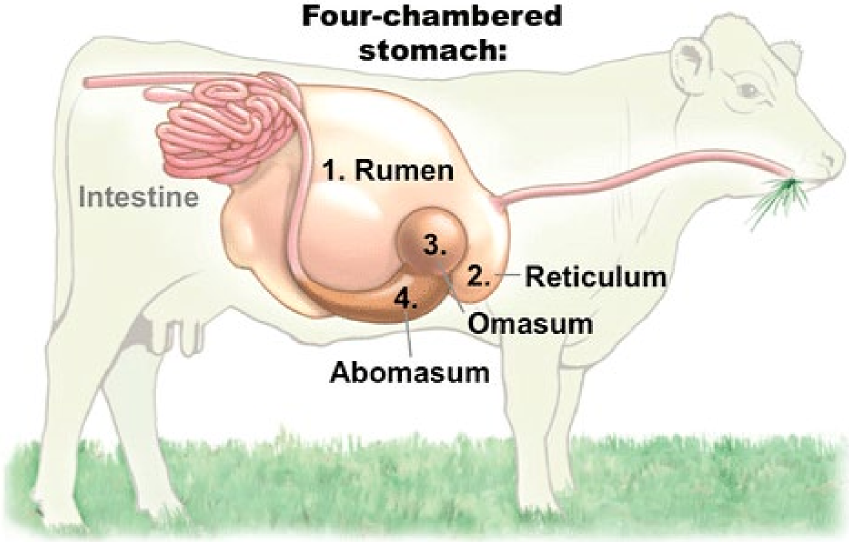
Challenges in Rumen Sample Collection Methods

- Require skilled people
- Require specialized equipment and tools
- Invasive

Potential Alternative:

Oral Swab

Oral Swab as Proxy



Rumination involves regurgitating material from the rumen to the mouth, where it is re-chewed and re-salivated, then swallowed and returned to the rumen (Welch et. al., 1982).

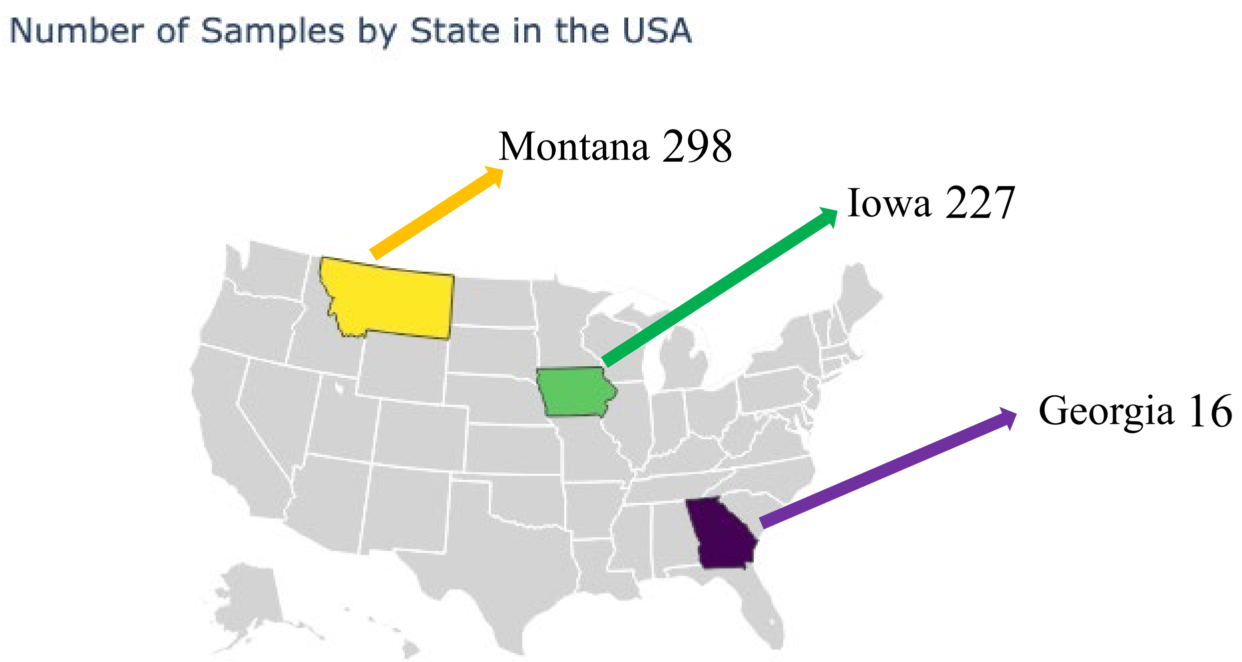
- Easier
- Quicker
- Less invasive

Oral Swab as Proxy

- Tapio et al. (2016): Showed similar relative abundance of microbial taxa in ruminal and oral samples
- Young et al. (2020): Found high degree of similarity between ruminal and oral microbiomes of dairy cows, depending on the time swabs were collected
- Amin et al. (2021): Found significant correlation between oral and rumen microbiomes

<u>Our hypothesis</u>: Non-invasive buccal swabbing can reliably serve as a proxy for assessing the rumen microbiome, providing an efficient and less invasive method for studying the rumen microbiome.

Sample collection



- Five Commercial Farms in 3 states (total of 541 Registered Angus Bulls)
- Average weight: 568 ± 32 Kg
- Average age: 19 ± 6 Months
- At least 3 weeks on feed before sample collection
- Samples collected early in the morning (no prior information on rumination)

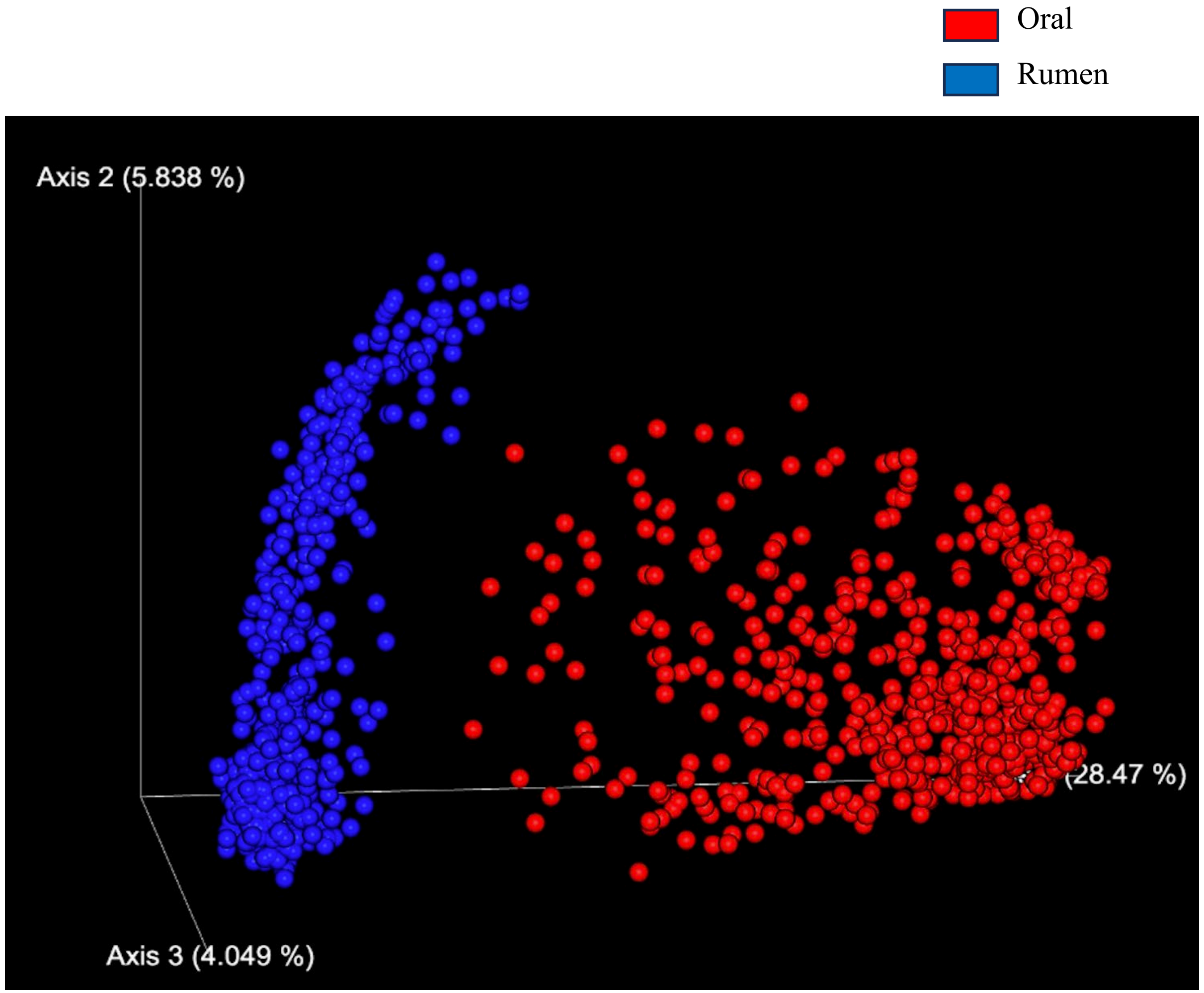
Sample & Data Processing

- DNA was extracted, amplified, and sequenced (V3-V4 regions of the 16S rRNA gene)
- Sequencing was carried out on an Illumina MiSeq platform using a MiSeq v3 reagent kit
- Sequencing data were analyzed using QIIME2, and microbial function was predicted using PICRUSt2
- Microbial reference database: Greengenes2 (McDonald et al., 2023)
- Differences between groups for taxa abundance and alpha diversity indexes were assessed by Kruskal-Wallis tests
- Differences in beta diversity were assessed by Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations

Microbial Richness, Evenness and Diversity

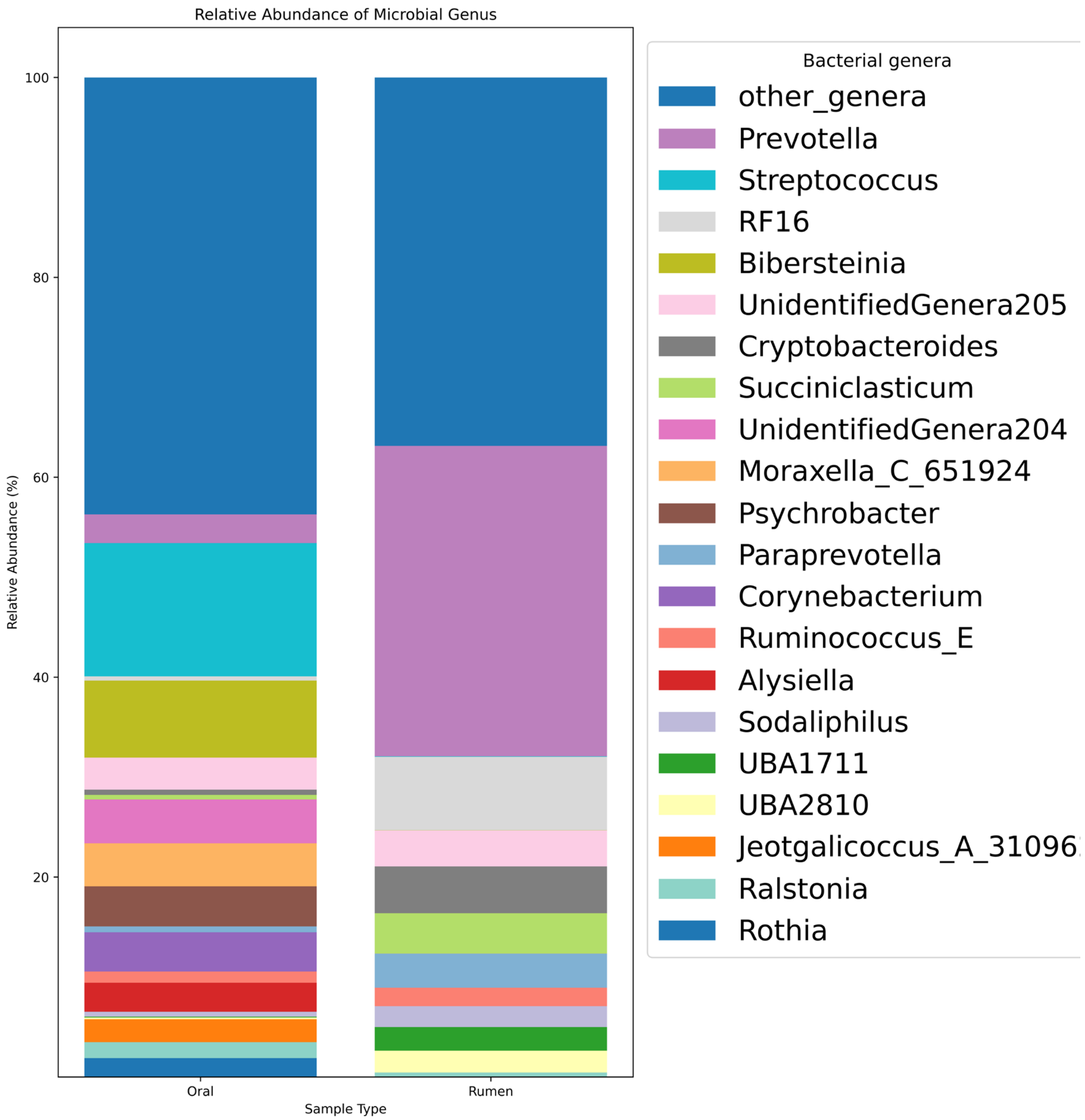
(P < 0.01 for all 3 metrics)

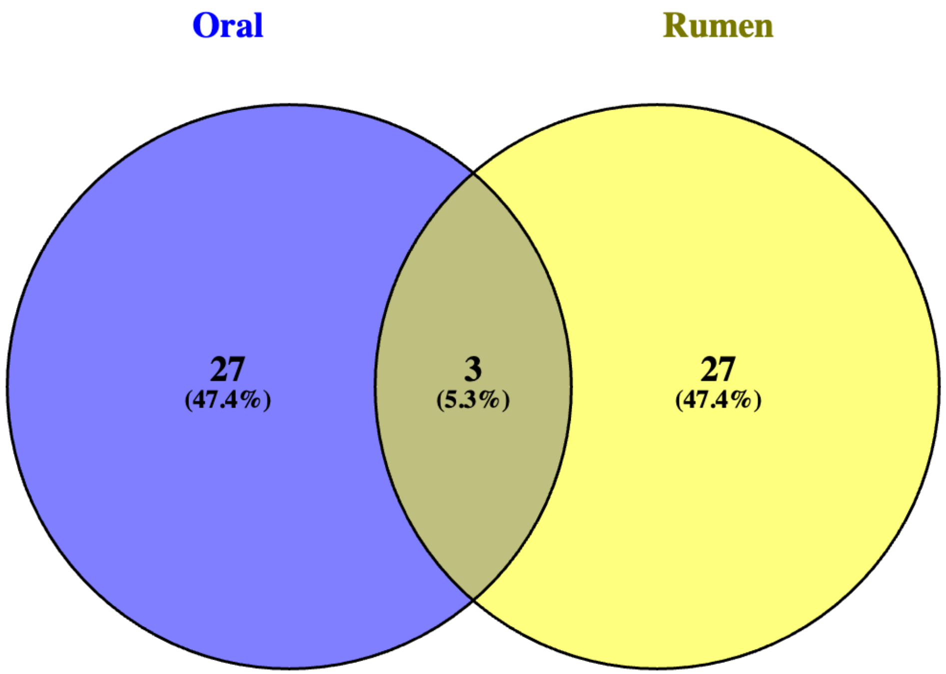
Beta Diversity (Unweighted UniFrac Distances)



- Permutational Multivariate Analysis of Variance (PERMANOVA) $P = 0.001$
- Clear separation of oral and rumen samples along Axis 1
- Rumen samples (Blue) cluster distinctly from Oral samples (Red)
- Microbial community is different between sample types

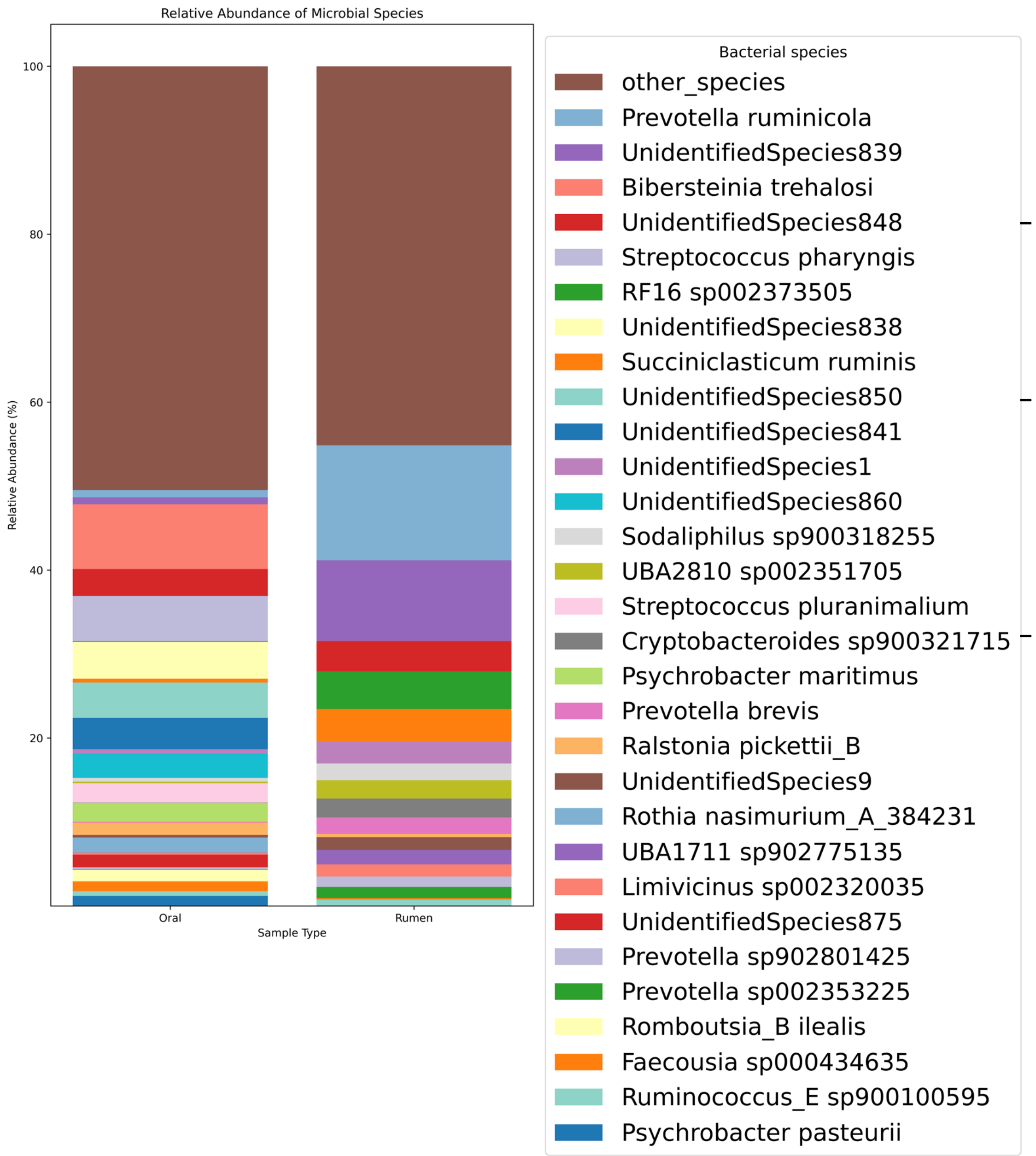
Relative Abundance – Genus level





- *Prevotella*
- *Ruminococcus*
- unidentified genus of family *Lachnospiraceae*

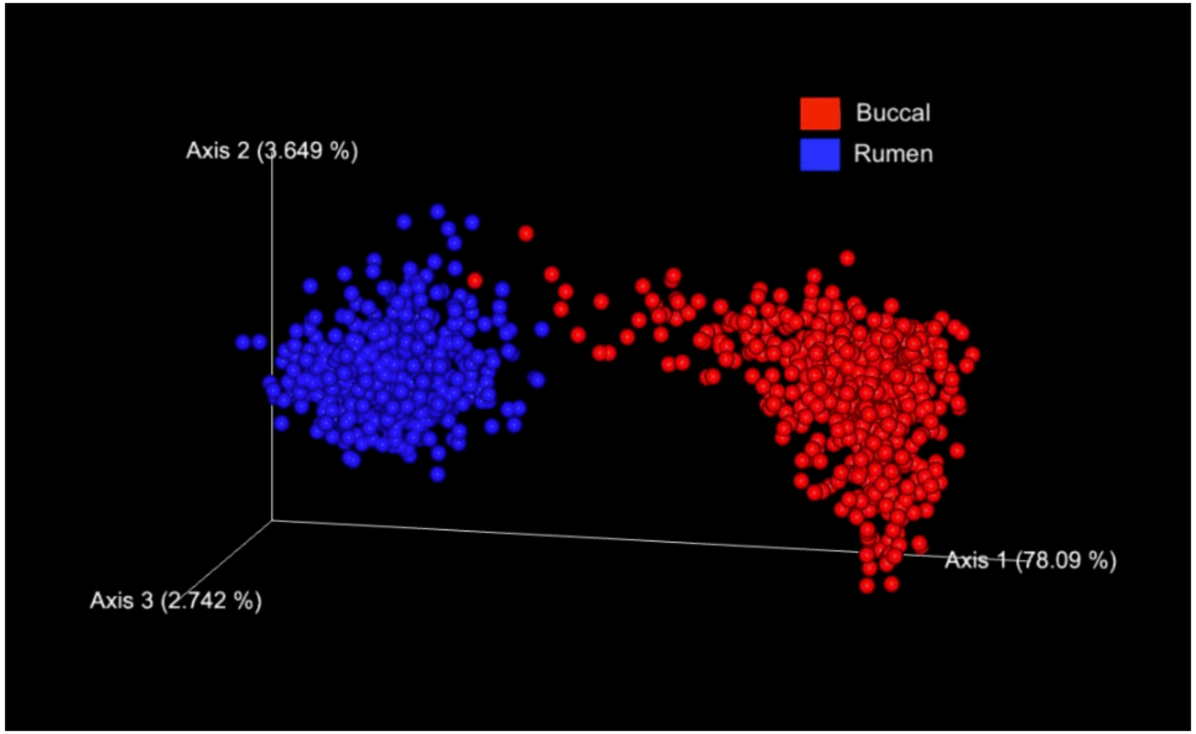
Relative Abundance – Species Level



- Rumen samples were dominated by *Prevotella ruminicola* (13.68%), which was only 0.86% in Oral samples ($P < 0.001$)
- *Prevotella ruminicola* is an anaerobic bacterium
- Oral samples were dominated by *Bibersteinia trehalosi* (7.71%), which was only 0.02% on Rumen samples ($P < 0.001$)

Bibersteinia trehalosi is a facultative anaerobe bacterium

Bray Curtis Distances on the Occurrences of Metabolic Pathways (microbial function)



- PCoA plot shows distinct separation between rumen and oral samples
- (PERMANOVA) P = 0.001, highlighting significant functional differences
- Clustering patterns suggested unique enzyme activity and metabolic functions in each environment

Top 15 Enzymes Expressed in the Rumen and Oral Samples

Enzyme	Rumen Frequency	Buccal Frequency	Metabolic Function
DNA polymerase I (EC:2.7.7.7)	120459410	112631813	No
Holliday junction DNA helicase (EC:3.6.4.12)	116701843	103632881	No
Peptidylprolyl isomerase (EC:5.2.1.8)	80554161	55552641	No
Histidine kinase (EC:2.7.13.3)	43871348	69963752	No
DNA-directed RNA polymerase (EC:2.7.7.6)	43632514	47334327	No
Oxidoreductases (EC:1.6.5.3)	85499402	0	Yes
Site-specific DNA-methyltransferase (EC:2.1.1.72)	41106026	37143714	No
2-oxoglutarate synthase (EC:1.2.7.3)	53779748	0	Yes
Beta-glucosidase (EC:3.2.1.21)	46359255	0	Yes
Electron transfer complex I (EC:1.6.5.3)	0	44933028	Yes
Beta-galactosidase (EC:3.2.1.23)	44577909	0	Yes
L-sorbose PTS permease (EC:2.7.1.69)	0	41750992	Yes
Beta-ketoacyl-ACP reductase (EC:1.1.1.100)	0	32172969	Yes
Acetyl coenzyme A carboxylase (EC:6.4.1.2)	0	31720923	Yes
DD-peptidase (EC:3.4.16.4)	25629129	25629129	No

- Shared enzymes are primarily related to DNA processing rather than metabolic functions.

- Distinct enzymatic activities.

Expression of Beta-glucosidase was only detected in the rumen, not in the oral cavity

Beta-glucosidase is the key enzyme involved in the breakdown of cellulose, a major component of plant cell walls.

Beta-glucosidase specifically acts on cellobiose, a disaccharide produced by the partial breakdown of cellulose, converting it into two glucose molecules.

Conclusions

- ❖ At all levels evaluated, the ruminal and oral microbiomes had marked differences:
  - ◉ Rumen had greater richness, evenness, and diversity
  - ◉ Microbial profiles differed (of the top 30 genera in each location only 3 overlapped)
  - ◉ Predominant species in Rumen: *Prevotella ruminicola*; Oral: *Bibersteinia trehalose*
  - ◉ Microbial function was also markedly different

- ❖ For samples randomly collected (no information about rumination times), oral samples are not a good proxy for the rumen

- ❖ **To study the ruminal microbiome, we must sample the rumen directly**

Thank You!

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