

Evaluating the impact of a high-starch and high-forage diet on the ruminal solid, liquid, and epimural microbiota of dairy cows

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Background

- Dairy cattle are important agricultural animals which house an essential community of microbes within their rumen.
- This rumen microbiome is crucial for providing nutrition, generally in the form of volatile fatty-acids (VFAs), from otherwise host-inaccessible dietary components.
- The total rumen microbial community can be broken into three principle components: the luminal solids, luminal liquids, and epithelial, or epimural, microbiota.
- Relative to luminally associated communities, little is known about the epimural community.
- To address this gap in knowledge, we decided to explore how diet impacts both the luminal and epimural bacterial communities.
- Here, we characterized the bacterial communities associated with ruminal solids (RS), liquids (RL), and ventral-sac (VS) epithelial tissue of 13 lactating Holstein dairy cows fed high forage and high starch diets.
- We then pair this microbial data with VFA data to explore what environmental factors may be shaping the VS epimural bacterial community.

Diet impacts the molar-proportion of several VFAs

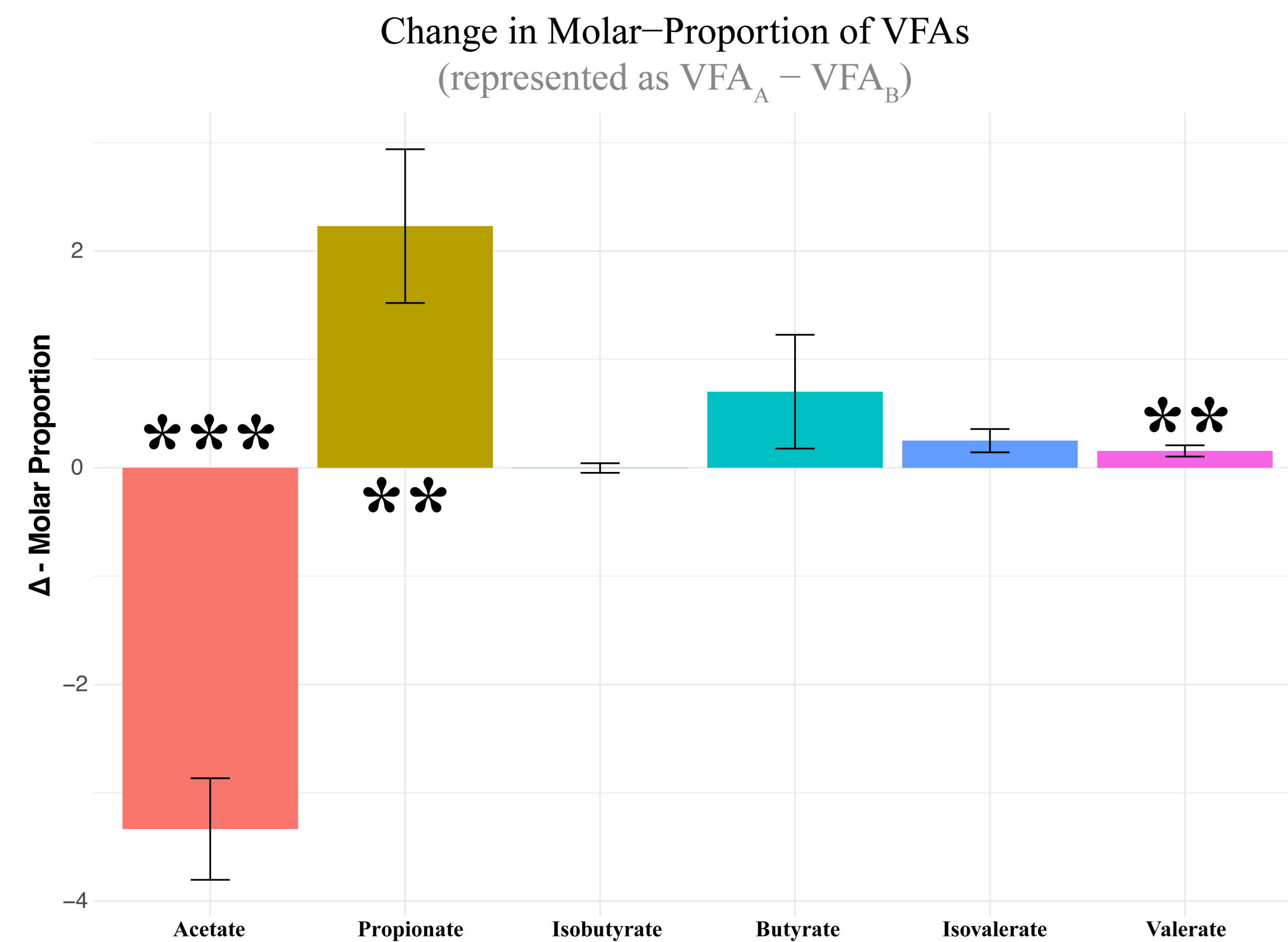


Figure 1: Bar plot displaying the change in molar-proportion of different volatile-fatty acids (VFAs) in response to diet. Bars represent the distributions obtained by taking each animal's VFA molar-proportion under **diet-A** (high-starch) and subtracting that animal's VFA molar-proportion under **diet-B** (high-forage). Asterisks represent the level of significance obtained by testing these results with either paired T-tests or non-parametric paired Wilcoxon-tests (* = <0.05; ** = < 0.01; *** = < 0.001). Acetate was highest in **diet-B**, while propionate and valerate were highest in **diet-A**.

Diversity of RS communities affected by diet-treatment but VS is always the most diverse and rich

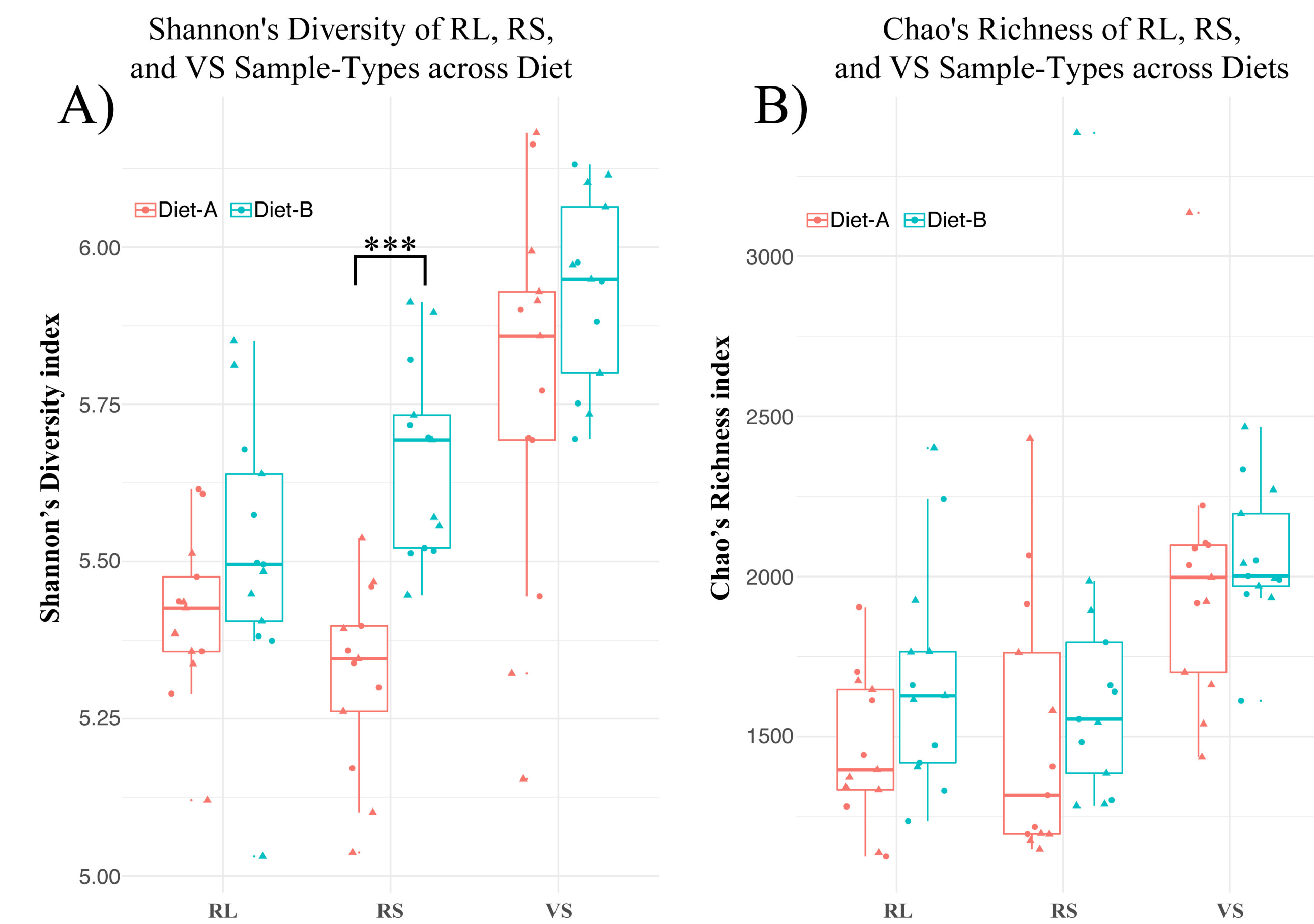


Figure 2: Boxplots presenting A) the Shannon's diversity, and B) the Chao's richness of RL, RS, and VS communities under each diet. **Diet-A = High-Starch; Diet-B = High-Forage**. Asterisks represent the level of significance obtained by paired T-tests tests (* = <0.05; ** = < 0.01; *** = < 0.001). Independent of diet, epimural communities generally observe higher diversity and richness, relative to both luminal communities.

Community composition and structure are impacted by diet and sample-type

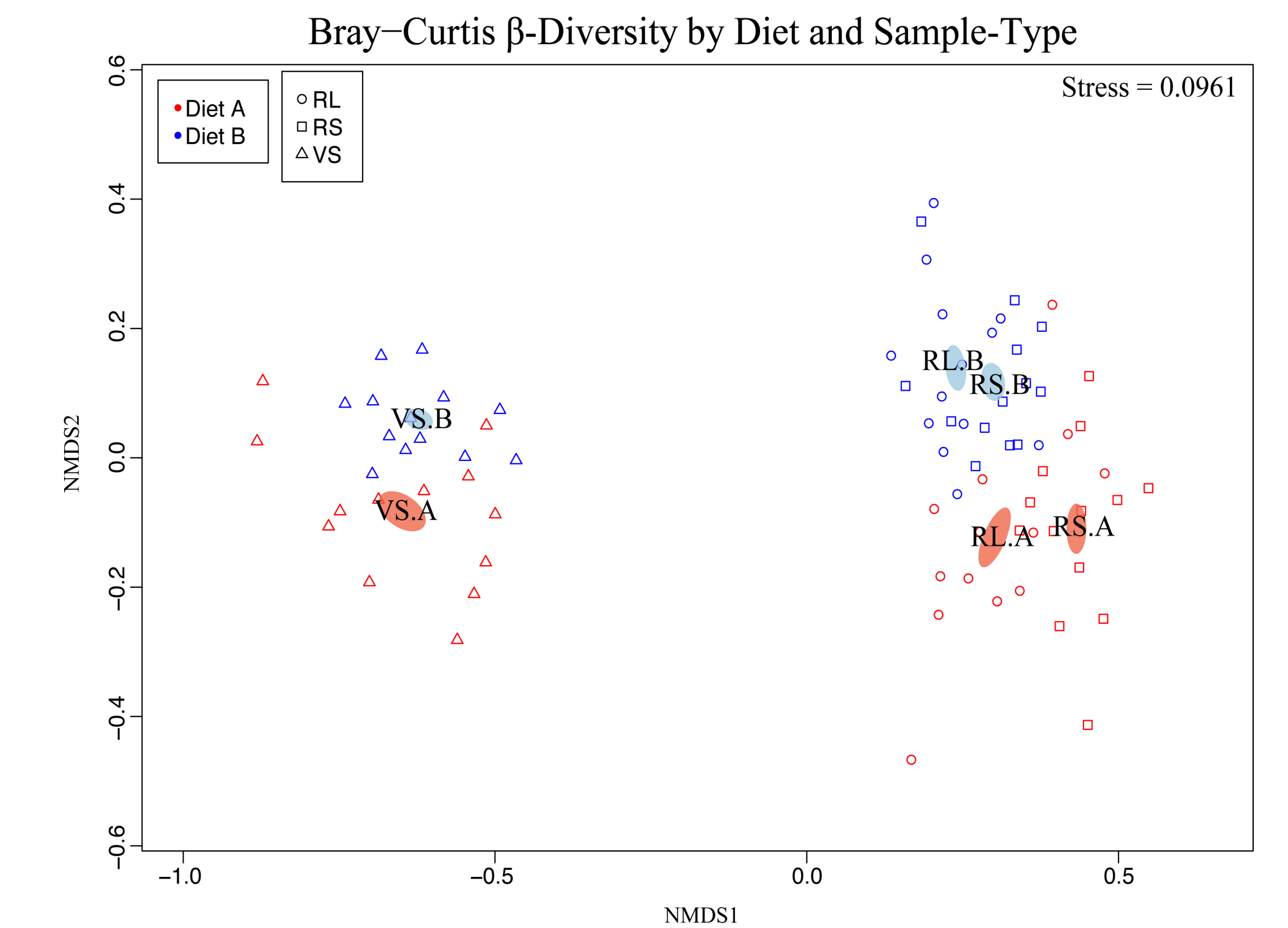
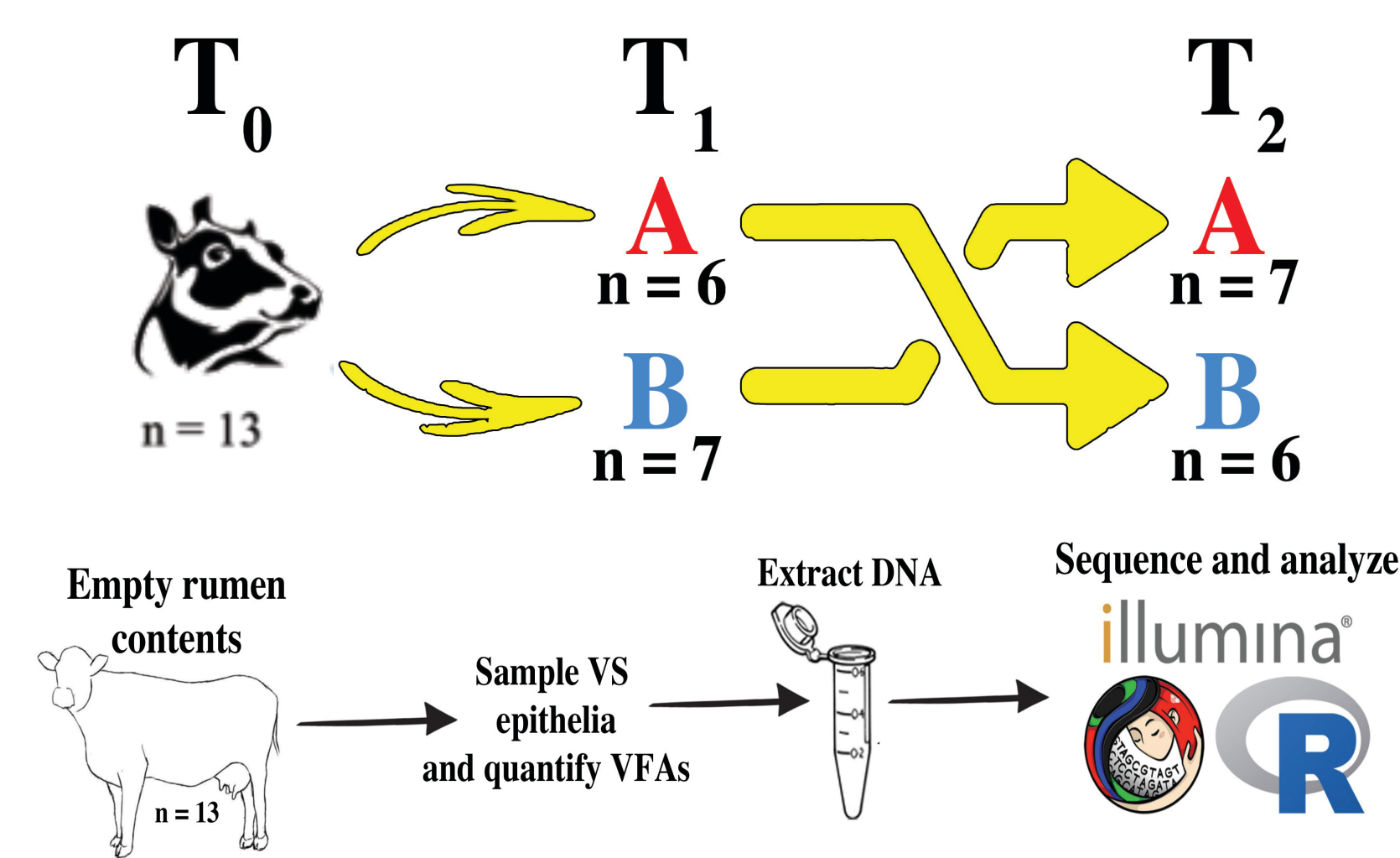


Figure 3: Non-metric multidimensional scaling plot (NMSD) displaying Bray-Curtis dissimilarity of bacterial communities. **Diet-A = High-Starch; Diet-B = High-Forage**. Each point represents a single sample, and distance between points represent dissimilarity between communities. Standard error ellipses show grouping and variance within sample groups. Pairwise PERMANOVA and dispersion tests, blocking by individual cow, suggests each sample-type is associated with its own community and that diet significantly impacts community composition and structure of all sample types. Additionally, epimural communities under **diet-B** are associated with significantly lower variance compared to **diet-A**.

Experimental Design



High-starch diet increases number of unique VS OTUs

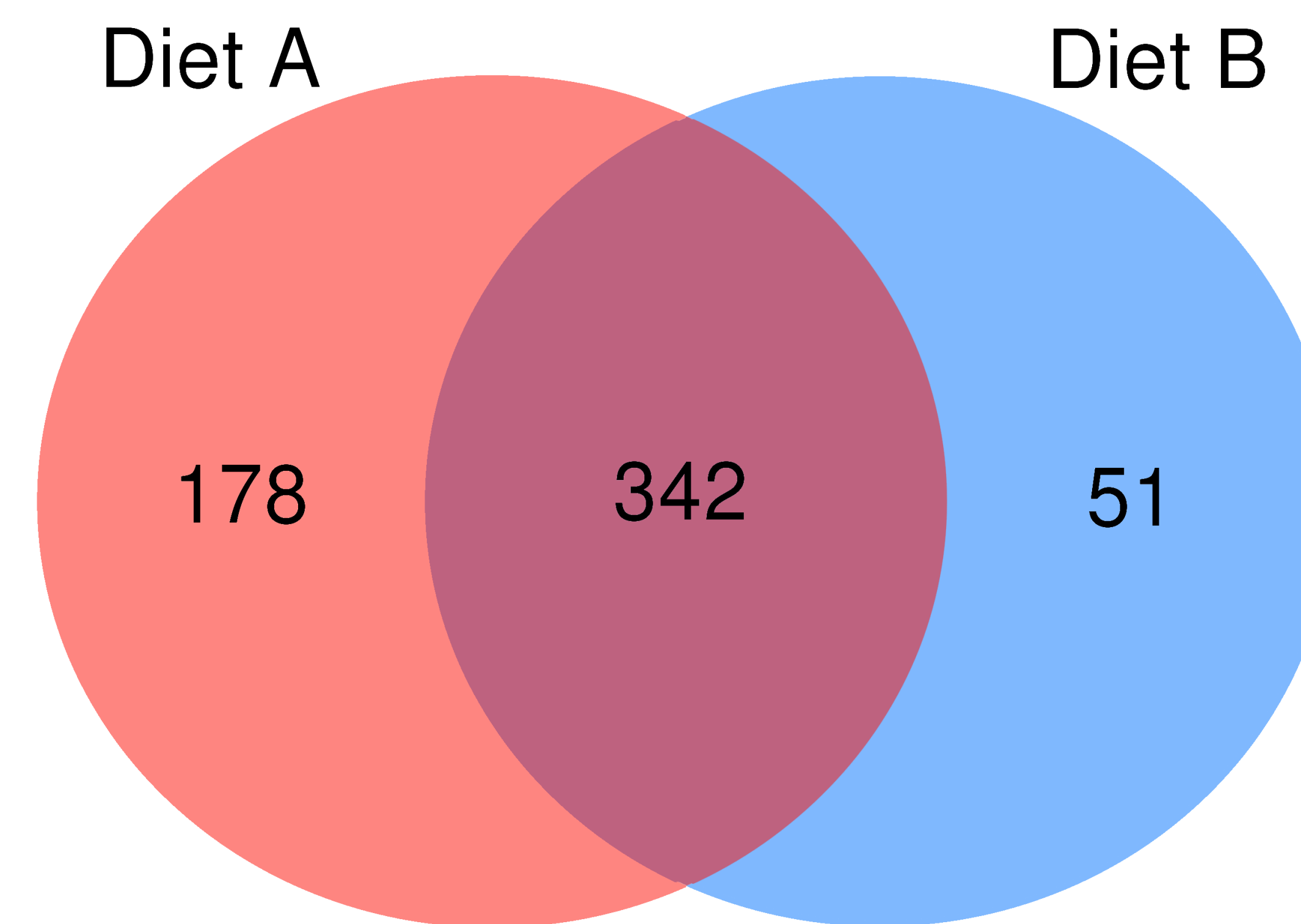


Figure 4: Venn diagram showing shared and distinct operational taxonomic units (OTUs) found in the VS samples under different diets. **Diet-A = High-Starch; Diet-B = High-Forage**. Only those OTUs found at $\geq 0.1\%$ relative abundance, in at least one sample, were considered. **Diet-A** observes a higher number of unique OTUs compared to **diet-B**.

Genera which differ among groups illustrate roles of different communities

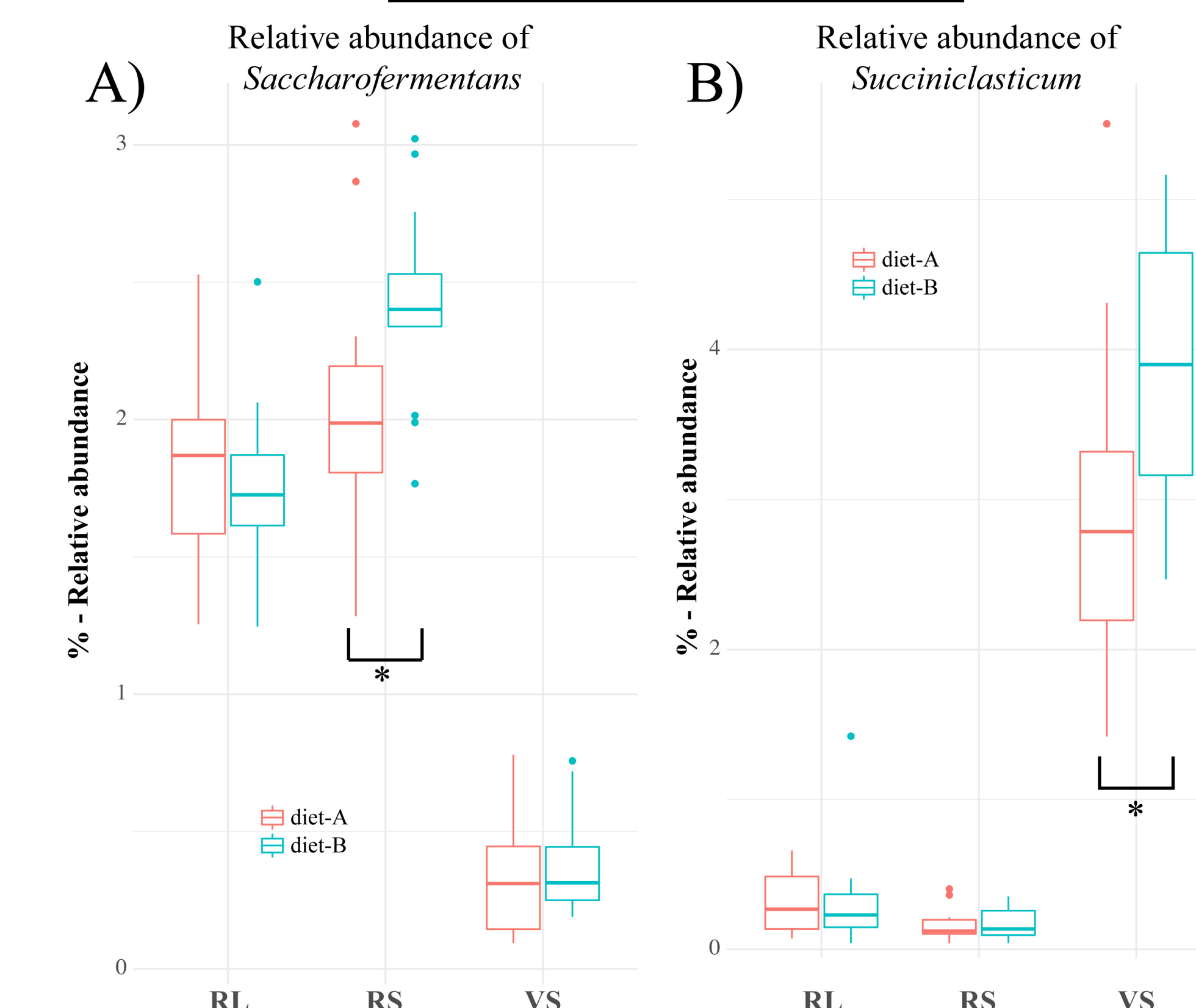


Figure 5: Boxplots displaying two genera implicated by simpler as contributing to the dissimilarity between diet-treatment and sample-type groups. **Diet-A = High-Starch; Diet-B = High-Forage**. Data presented as relative abundance. Asterisks represent the level of significance obtained by non-parametric paired Wilcoxon-tests (* = <0.05; ** = < 0.01; *** = < 0.001).

Preliminary Hypotheses

- High starch and high forage diets will lead to different VFA profiles**
- High starch will lead to increased molar-proportions of propionate
 - High forage will lead to increased molar-proportions of acetate
- Different diets will lead to changes in epimural bacterial communities**
- Diet will impact all three major rumen microbiota
 - Both community structure and composition will change
- Diet-dependent changes in bacterial communities may reflect the functions and selective pressures of each rumen community**
- Luminal bacteria will be shaped directly by diet-treatment
 - Epimural bacteria will be shaped indirectly by diet-treatment via differences in luminal fermentation and subsequent VFAs profiles

Conclusions

- Diet significantly impacts the molar proportions of several VFAs**
 - Acetate is higher under **diet B** while Propionate and Valerate are higher under **diet A**
- Only diversity of RS communities affected by diet-treatment**
 - Epimural communities consistently more diverse and richer
- Bacterial composition and structure are impacted by diet and sample-type**
 - The VS community under **diet B** is significantly less variable relative to **diet A**
 - **Diet A** observes a larger number of unique OTUs
- Genera which differ among groups illustrate roles of different communities**

Acknowledgments

We thank D. Amundson, G. Brink, A. Edwards, M.B. Hall, A. Holub, S. Moore, K. Ostendorf, W. Radloff, K. Reed, S.S. Tumuluru, and G. Zanton for help with rumen emptying and epimural sampling. I would especially like to thank Mike Maroney for his assistance in facilitating epimural sampling. This research was supported in part by an appointment to A. Fischer from the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). The ORISE is managed by Oak Ridge Associated Universities (ORAU) under DOE contract number DE-SC0014664. This research was also supported by a USDA Agricultural Research Service (Washington, DC) CRIS project 5090-31000-026-00-D to W. Li, a USDA Agricultural Research Service (Washington, DC) CRIS project 5090-31000-025-00D to K. Kalscheur and a USDA National Institute of Food and Agriculture HATCH grant WIS02007 to G. Suen. All opinions expressed in this paper are the author's and do not necessarily reflect the policies and views of USDA, ARS, DOE, or ORAU/ORISE.