



Introduction

- Warm-season grasses (WSG) can be incorporated into cool-season grass (CSG) rotational horse pasture systems to increase summer pasture yields.
- WSG are lower in non-structural carbohydrates than CSG.
- Fluctuations in bacterial community composition have been observed in pastured horses over time, but these alterations have not been evaluated for correlation with forage nutrient profiles (Fernandes et al., 2014, PLoS One; Salem et al., 2018, Sci. Reports).

Objectives and Hypothesis

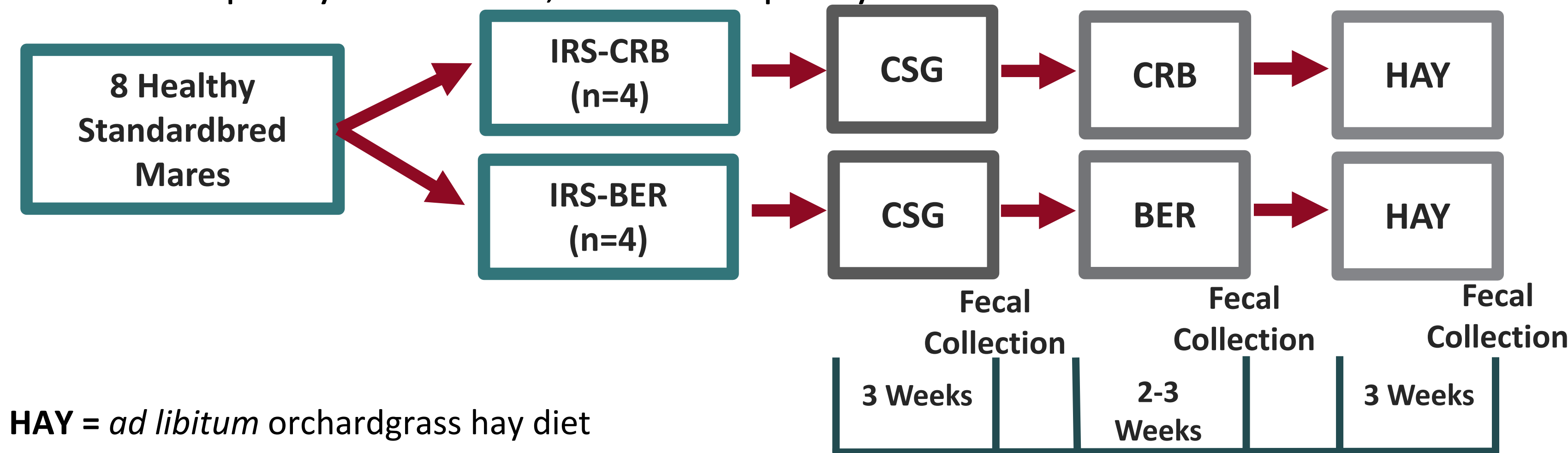
Objectives: Characterize shifts in the fecal microbiota of horses grazing different forage types within integrated CSG and WSG rotational pasture systems and explore relationships between forage nutrients and microbial composition.

Hypothesis: Microbial diversity and species composition would differ between forage types and would be influenced by fiber and soluble carbohydrate content of forages.

Experimental Design

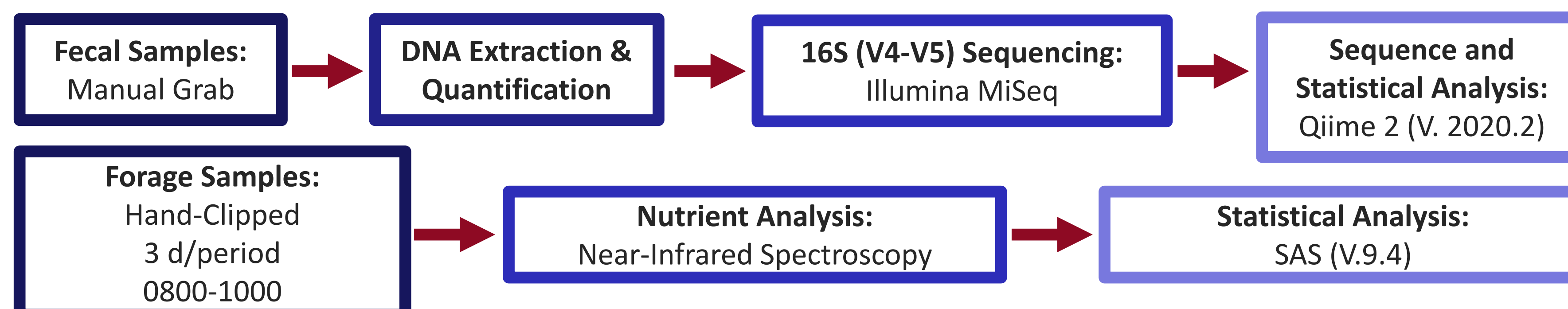
Integrated Rotational Systems (IRS)

- 2 grazing systems
 1. **IRS-CRB:**
WSG = "Quick N Big" Crabgrass (CRB)
 2. **IRS-BER:**
WSG = "Wrangler" Bermudagrass (BER)
- 6 sections per system
- 3 sections per system = CSG; 3 sections per system = WSG



* HAY = *ad libitum* orchardgrass hay diet

Materials and Methods



- Microbiome results did not differ by system and combined data are presented (n=8).

Results

Nutrient	Forage				SEM
	CSG		WSG		
	IRS-CRB	IRS-BER	IRS-CRB	IRS-BER	
Digestible Energy (Mcal/kg)	2.22 ^a	2.34 ^b	2.03 ^c	2.15 ^{abc}	0.03
Crude Protein (%)	21.47 ^a	23.67 ^a	22.67 ^a	21.40 ^a	1.29
Acid Detergent Fiber (%)	29.97 ^a	30.53 ^a	36.93 ^a	31.43 ^a	1.06
Neutral Detergent Fiber (%)	55.43 ^{ab}	50.47 ^{ac}	61.80 ^{bd}	58.70 ^a	1.46
Non-Structural Carbohydrate (%) [§]	11.5 ^a	11.3 ^a	2.63 ^b	7.10 ^a	0.48
Water Soluble Carbohydrate (%)	10.23 ^a	10.16 ^a	2.27 ^b	4.80 ^{bc}	0.63
Ethanol Soluble Carbohydrate (%)	6.77 ^a	8.27 ^a	1.93 ^b	4.03 ^a	0.70
Starch (%)	1.23 ^{ab}	1.13 ^{ab}	0.37 ^a	2.30 ^b	0.19

Table 1. Nutrient composition of forages in integrated rotational grazing systems.

[§] Calculated as the sum of water-soluble carbohydrates and starch fractions.

^{abcd} Denotes significant differences within rows (p<0.05). Data was analyzed using the MIXED procedure with repeated measures (Tukey's post-hoc adjustment).

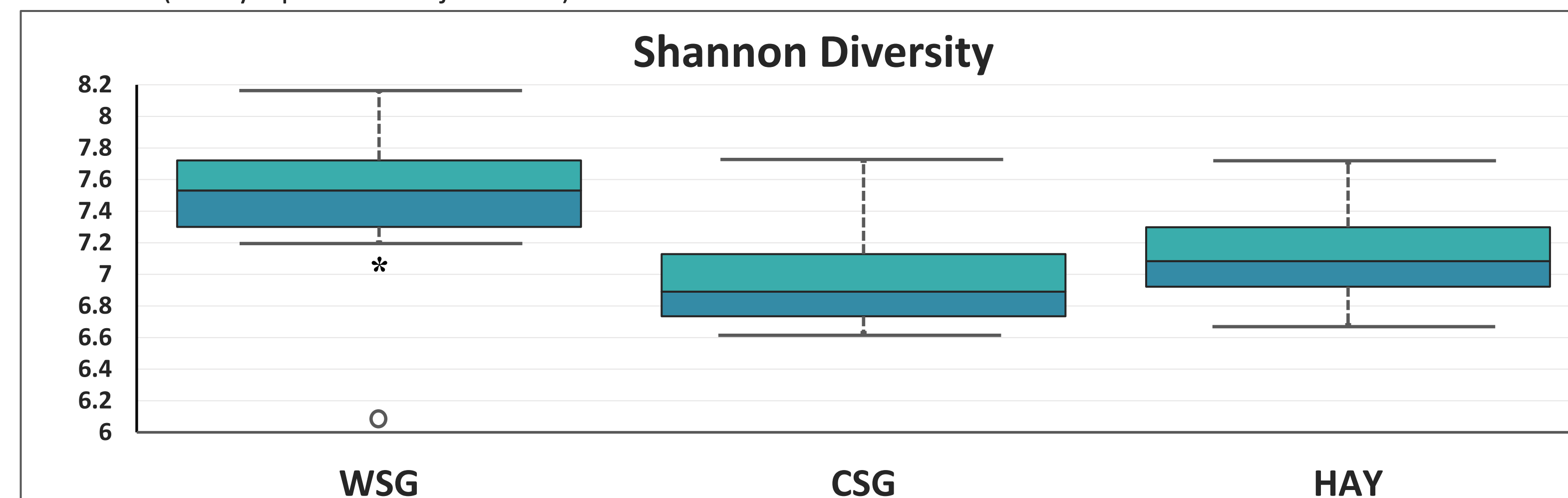


Figure 1. Shannon diversity index of fecal microbiota across forage types.

Data was analyzed by Kruskal-Wallis Test with an FDR correction for multiple pairwise comparisons.

* Denotes significance (p<0.05).

○ Indicates an outlier in the data.

Nutrient [§]	Alpha Diversity Correlations (R-value)				Random Forest Regressor Prediction Model Accuracy (R-value)
	Pielou's Evenness	Observed OTUs	Shannon	Faith's Phylogenetic	
Non-Structural Carbohydrate	-0.53	----	-0.49	----	0.92
Water Soluble Carbohydrate	-0.53	----	-0.49	----	0.98
Ethanol Soluble Carbohydrate	-0.60	-0.48	-0.60	-0.53	0.91

Table 2. Forage nutrients with significant (p<0.05) Spearman correlations with alpha diversity and predictive random forest regression models.

[§] Nutrients not listed had no significant correlations with diversity metrics nor could be predicted from microbial composition with a random forest regressor.



Figure 2 (right). Top 30 features predictive of forage type.

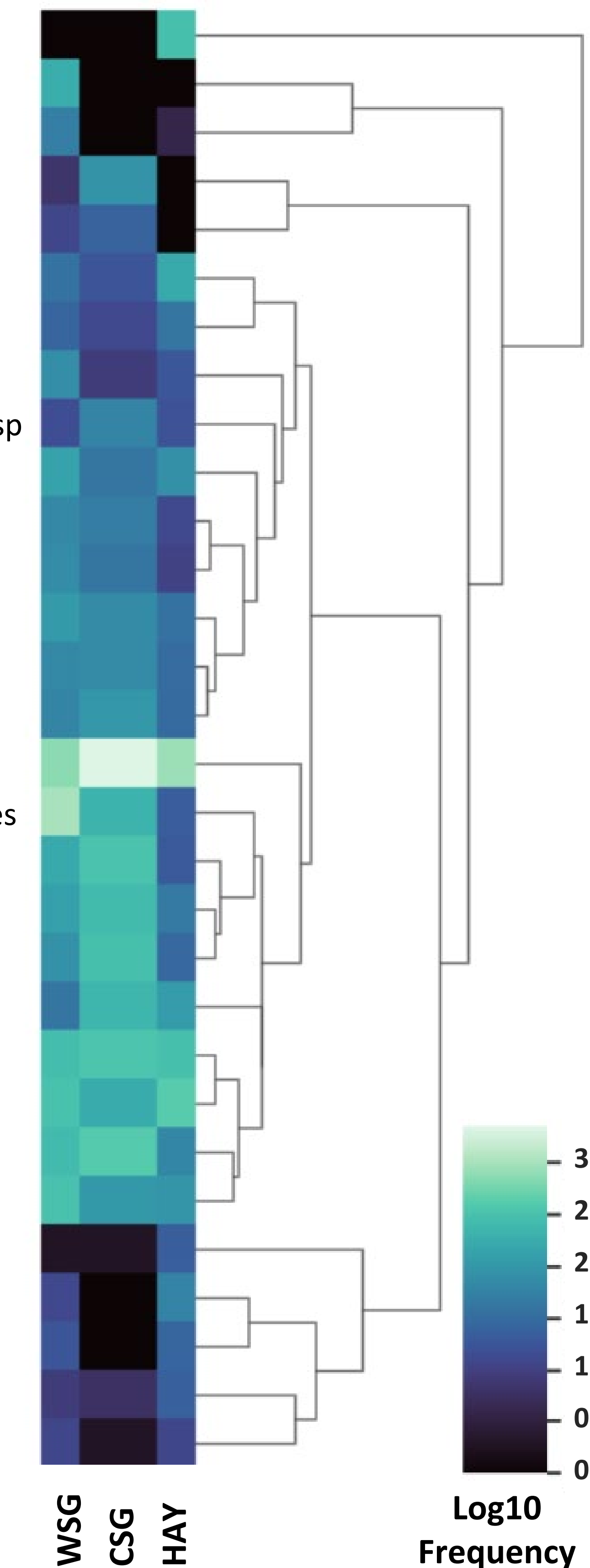
Important features were identified by recursive feature elimination with a random forest classifier. Model accuracy for prediction of forage type based on microbial composition was 1.0 (p<0.05).

[§] Taxonomic classification of each feature is shown. Taxonomy was assigned against the Greengenes database (13_8).

* This feature within the genus *Coprococcus* was identified as the most important feature for prediction of forage type as well as for prediction of forage soluble carbohydrate content (regression model accuracy shown in Table 2 [below]).

Taxon[§]

- Streptococcus luteciae
- Coprococcus sp. *
- Clostridiales sp.
- Coprococcus sp.
- Prevotella sp.
- Ruminococcaceae sp.
- Clostridiales sp.
- Clostridiales sp.
- Phascolarctobacterium sp.
- Treponema sp.
- RFP12 sp.
- Veillonellaceae sp.
- Prevotella sp.
- Lacnospiraceae sp.
- Clostridiales sp.
- Ruminococcaceae sp.
- Fibrobacter succinogenes
- Ruminococcaceae sp.
- Bacteriodales sp.
- RF16 sp.
- Bacteriodales sp.
- Bacteriodales sp.
- Bacteriodales sp.
- Bacteria sp.
- Bacteriodales sp.
- Clostridiales sp.
- CR231 sp.
- Ruminococcaceae sp.
- Ruminococcaceae sp.
- Lachnospiraceae sp.



Conclusions and Future Directions

- Forage type impacted microbial community structure and composition in the grazing horse.
 - Greater species richness and evenness in horses adapted to WSG compared to CSG or HAY.
 - Random forest modeling identified key features positively or negatively responding to changes in dietary forage. Further analysis is needed to identify features characteristic of horses consuming each forage type.
- Results indicate that soluble carbohydrates, particularly dietary sugars, were more influential than fiber in driving shifts in the gut microbiota.
 - In humans and mice, dietary fiber influences health outcomes in a microbiome-dependent manner (Zhang et al., 2016, EBioMedicine; Zhao et al., 2018, Science).
 - Effects of dietary starch and fiber in mixed forage and concentrate diets on the equine hindgut microbiome have been extensively reported (Julliard and Grimm, 2017, J. Equine Vet. Sci.).
- Additional research is needed to determine implications of these microbial community differences (if any) in equine physiology and health.