

Abstract

- Fungi provide crucial ecosystem services in multiple ecosystems.
- Most thrive as free-living organisms, but can sometimes form commensal relationships other organisms
- Anaerobic gut fungi (AF, phylum Neocallimastigomycota) reside in the alimentary tract of herbivores.
- Little is known regarding the scope of diversity of these elusive, anoxic microorganisms.
- We aim to characterize the diversity of AF in the herbivorous gut on a global scale using culture-independent approaches.
- We have collected >1,000 samples, from >50 type of animals, across 5 continents.
- My focus is to examine patterns and determinants of the diversity of AF in the equine alimentary tract.
- To this end, I extracted DNA from >100 fecal horse samples, and used polymerase chain reaction (PCR) to amplify a specific marker gene (D1/D2 LSU).
- High throughput sequencing was conducted, sequence analysis is currently underway.
- Preliminary analysis revealed a high level of AF diversity within the equine alimentary tract.
- Currently, I am attempting to identify and quantify the impact of various factors (animal feed, age, sex, location) in shaping the AF community in horses via implementing a wide range of statistical and phylogenetic approaches.

Introduction

Equine Digestive Tract

- A unique symbiotic relationship exists in the equine gut between the host species and microbes that colonize it.
- The equine alimentary tract is home to trillions of microbial cells consisting of bacteria, anaerobic fungi, protozoa, methanogenic archaea and bacteriophages.
- Without these microscopic tenants, horses would not be able to obtain essential nutrients from the plant material they consume

Anaerobic Gut Fungi

- Produce powerful plant-degrading enzymes that play a key role in the conversion of feed to sugars and fatty acids

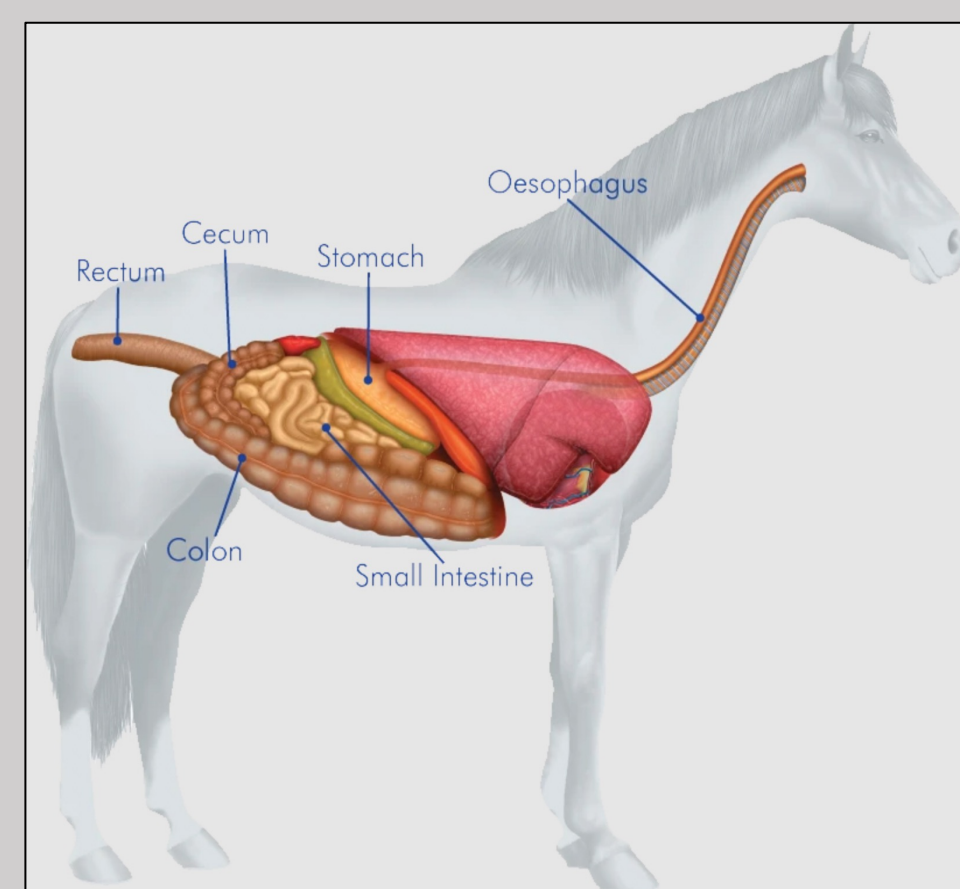


Figure 1
Equine Digestive Tract

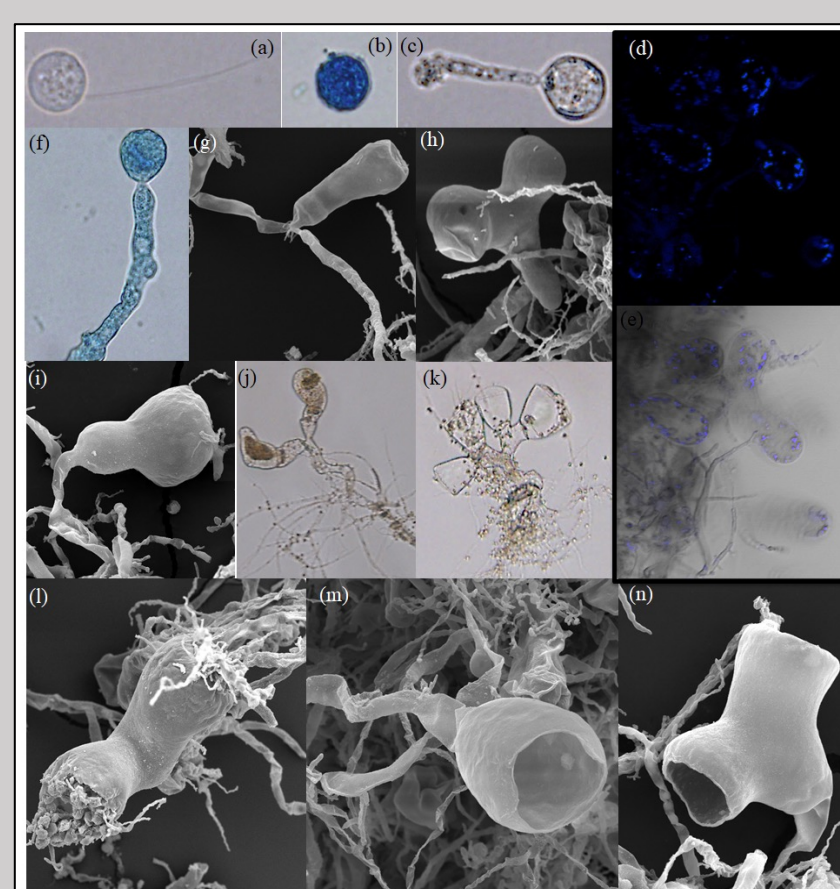


Figure 2
Anaerobic gut fungal strains

Goals and hypotheses

- Goal 1:** Discover and characterize novel groups of AF in the equine alimentary tract.
- Hypothesis 1:** Sampling a large number of equine fecal samples will lead to discovery of novel AGF groups.
- Goal 2:** Characterize patterns of fungal diversity in the equine alimentary tract.
- Hypothesis 2:** Level of diversity will vary greatly between horses and will be dependent on specific measurable factors.
- Goal 3:** Elucidate factors shaping community structure in the equine alimentary tract
- Hypothesis 3:** Community structure will vary greatly between horses and will be dependent on specific measurable factors.

Materials and Methods

Sampling:

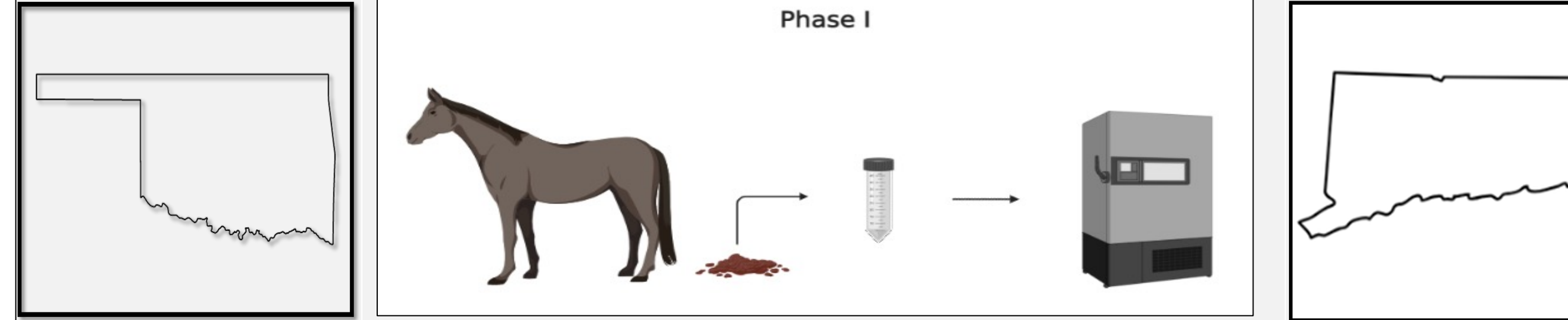


Figure 3: Sampling Method

- Collected >100 Samples from across Oklahoma and Connecticut.
 - Samples stored in Falcon Tube at -20^o C until Experimental analysis conducted.
- Experimental Methods:**
- DNA extracted from sample using a commercial kit.
 - Amplification of D1/D2 Large ribosomal subunit gene marker using Single Molecule Real Time (SMRT) PCR.
 - Amplicon clean up.

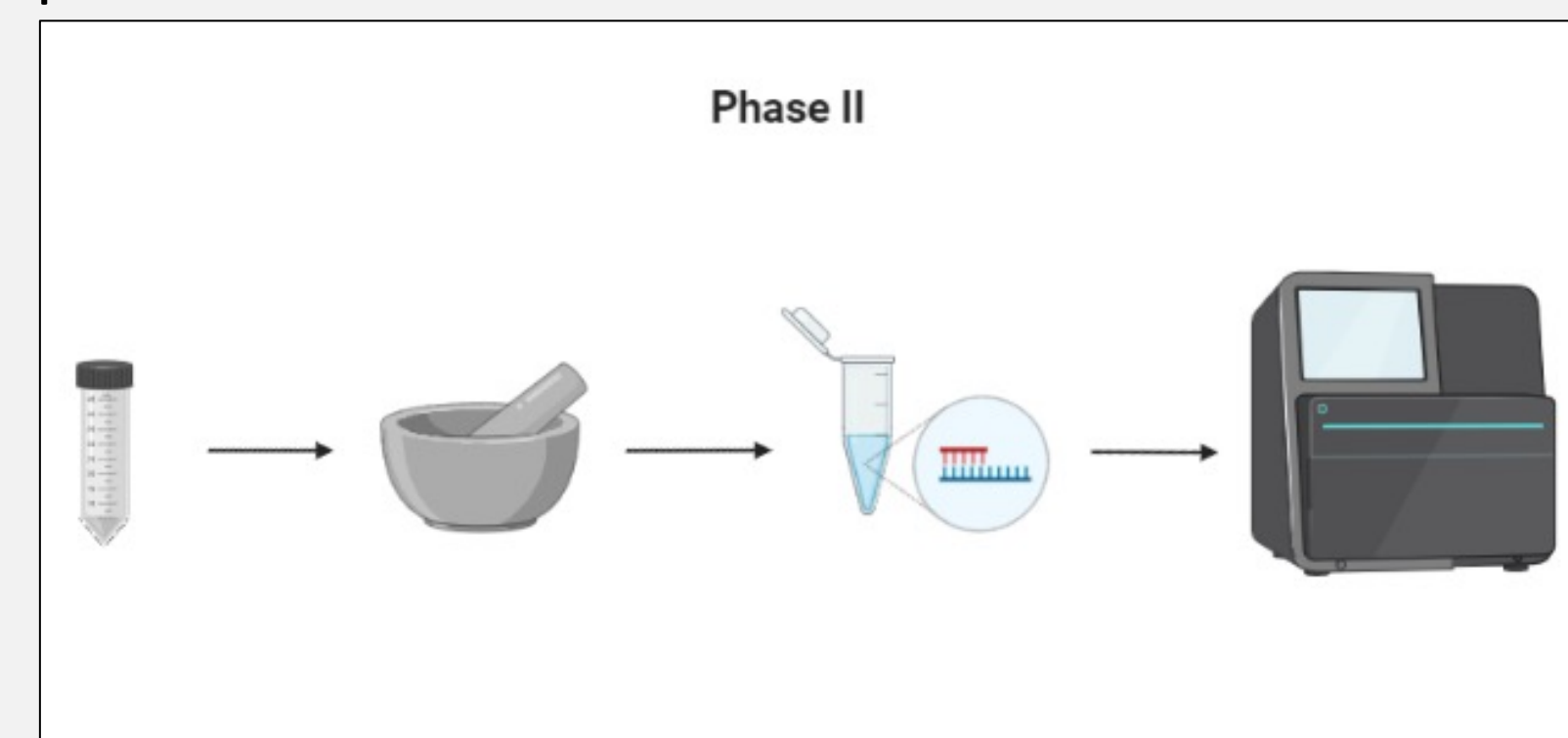


Figure 4: Experimental Approach

Data Analysis: Phylogenetic Analysis, α and β diversity analysis, and ordination methods conducted using Mothur, and R

Results

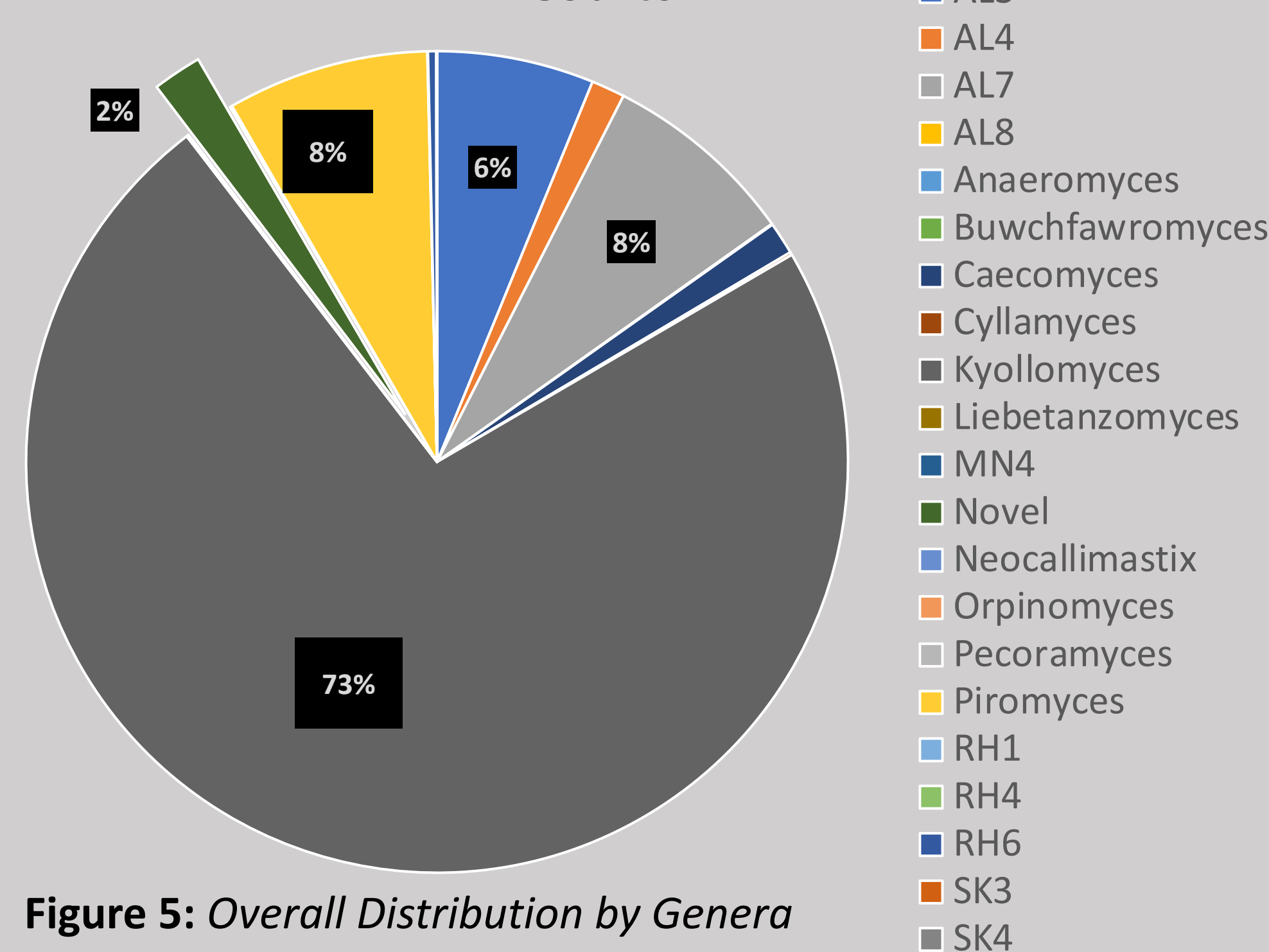


Figure 5: Overall Distribution by Genera

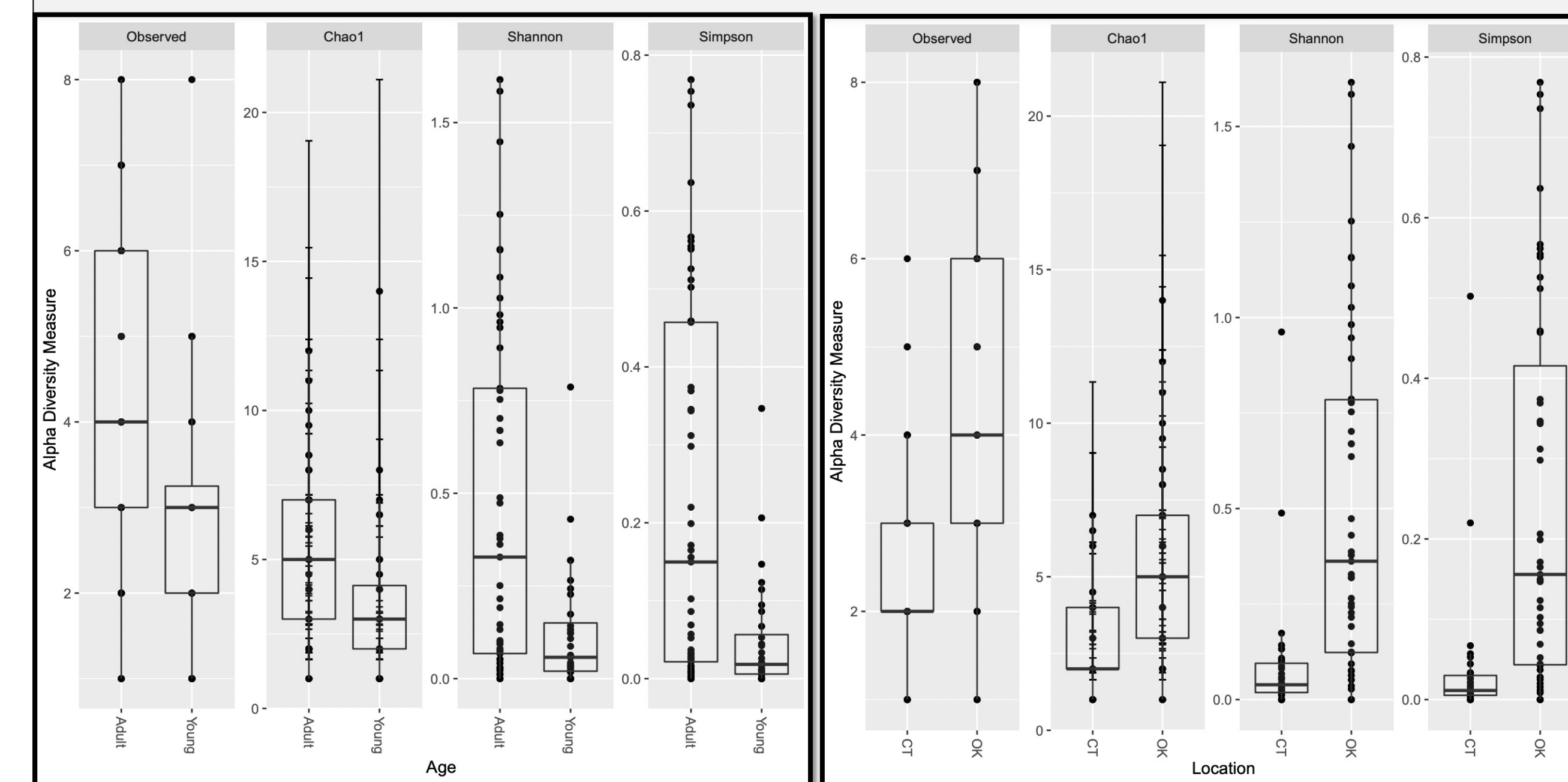
- Representatives of 20 different Genera were identified
- 2% of which belong to novel yet uncharacterized Genera
- Majority of sequences belong to the Genus Khyollomyces

Acknowledgments

- National Science Foundation (NSF)
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- The Lew Wentz Foundation

Results (Cont.)

Figure 6: α Diversity



- Diversity indices suggest a slightly higher level of diversity in adult horses as compared to young ones
- Preliminary analysis suggest Oklahoma samples have higher level of diversity than Connecticut samples

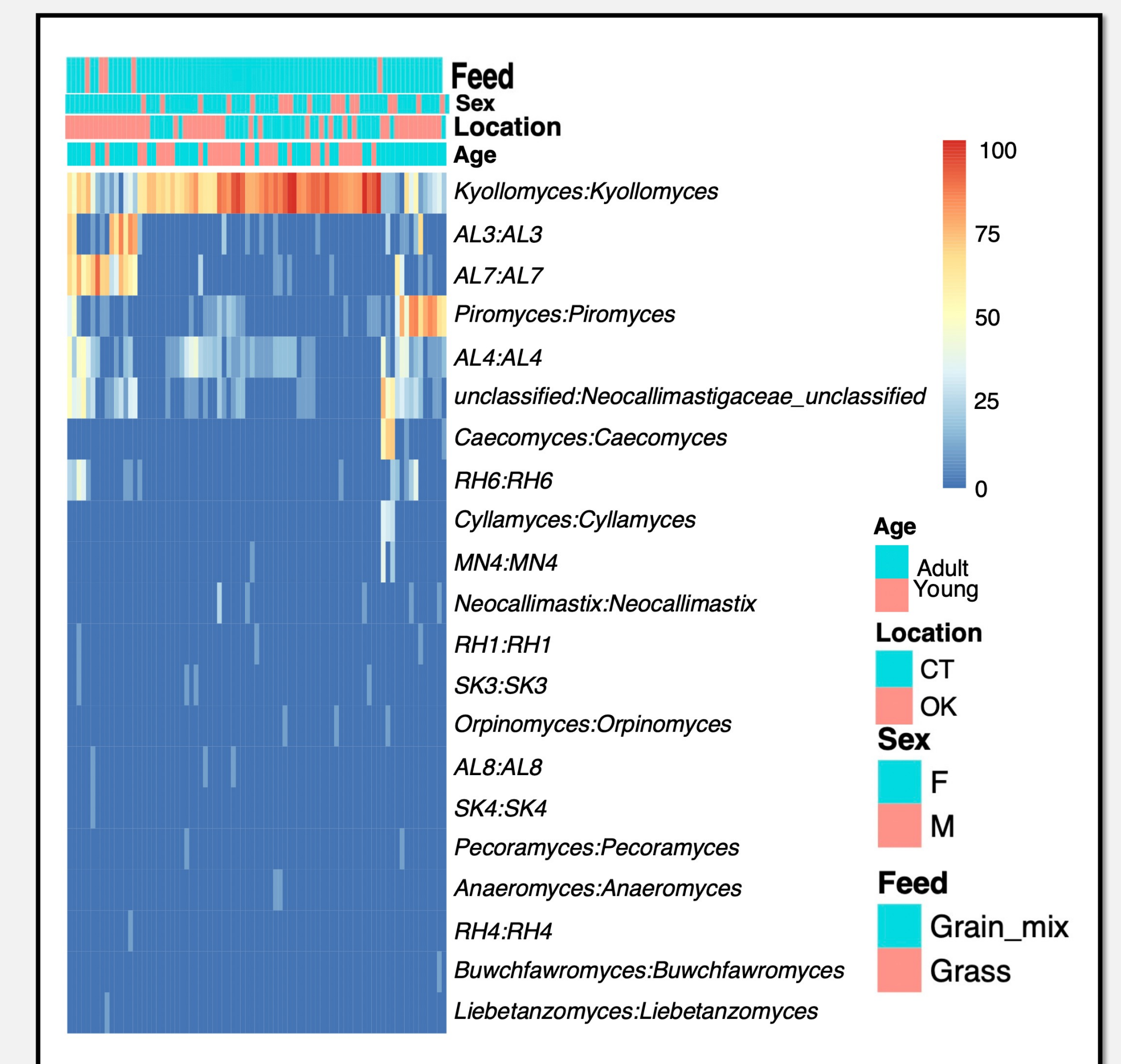


Figure 7: β Diversity

- Age and location plays the most important role in shaping AF community structure (P-Value = .006988 & .00013 respectively)

Conclusions

- Sequencing of initial samples has led to discovery of 323 unclassified sequences (H1).
- Level of diversity and community structure varies between horses with Age and Location being the most significant factors (H2 & H3).

Future Work

- Retrieve sequences on remaining samples and continue to conduct various analyses.
- Investigate unclassified (novel) sequences.