Survey of Microbial Fecal Populations Across California Dairies

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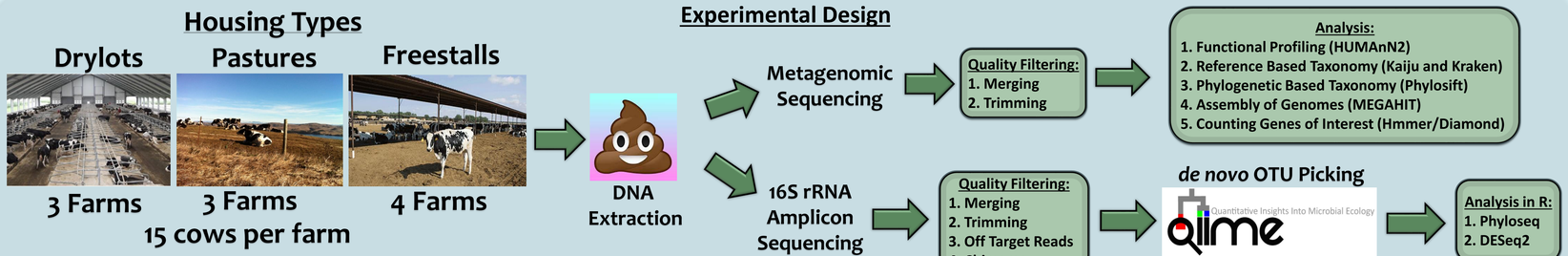
Introduction

Nitrogen waste management has become crucial with intensification of livestock production as it is of environmental and economic interest. Nitrogen is the most expensive element to balance in rations and high levels are required for peak performance. However, excess nitrogen is secreted in urine and feces contributing to global warming through volatile emissions of nitric oxide (N_2O) and ammonia (NH_3) and eutrophication by leaching of nitrate into ground water (NO_3^-). The microbiome plays a critical role in nitrogen cycling in ruminants. Increased access to next generation sequencing has allowed better resolution of these communities, however, the field lacks a robust analysis surveying common microbial populations and their metabolic functions in cattle. This surveys is one of the first to determine the composition and functional capacity of the microbiome of dairy cattle on commercial operations across northern/central California. Ten farms representing a variety of feeding and management systems were enrolled. Metagenomic and 16S amplicon data was analyzed from fecal samples that were collected from 15 cows from each farm over a seven month period. The goal of this survey is to clarify normal microbial communities and their contributions to nitrogen cycling in dairy cattle. This data will further generate hypothesis for strategies that target the microbiota to increase nitrogen mineralization and reduce environmental impact of dairies. The first step of this survey was determining the make-up of species and their variation across farm.

Experimental Design



Species Richness Significantly Different Across Farms

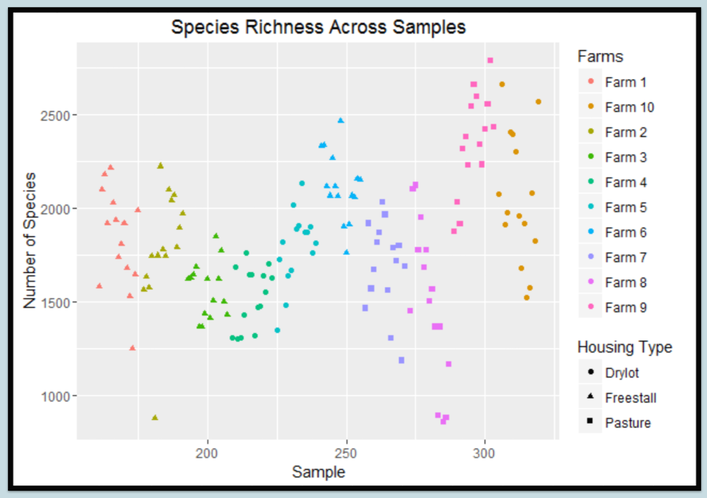


Figure 1: 16S rRNA amplicon paired-end sequences were trimmed for quality and length, Phix174 and chimeric reads removed and QIIME was used for *de novo* OTU picking and analysis of species richness (SR) across samples. There was no effect of housing type on SR, but there was a significant effect of farm as measured by Kruskal-Wallis rank sum test. P-value of <0.01 was considered significant.

Phylum Variation Across Farms

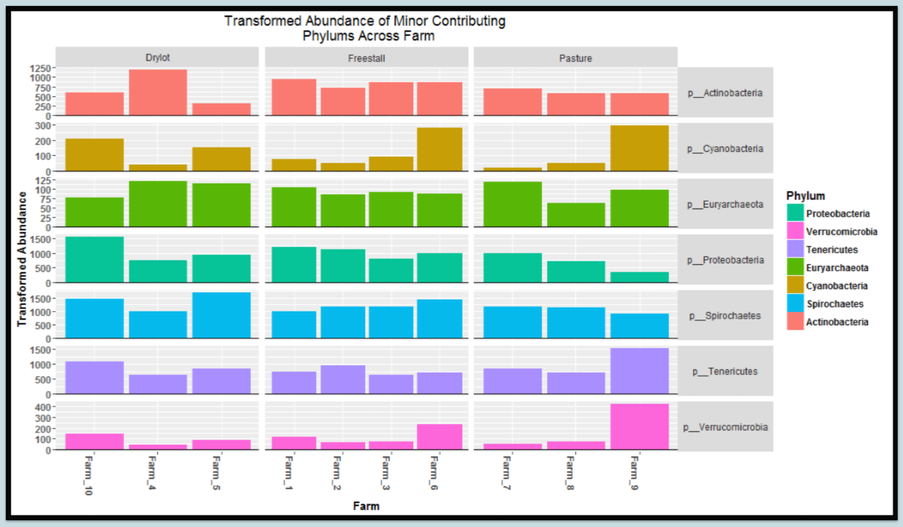


Figure 2: Firmicutes and Bacteroidetes were the dominant phylum across farms with average relative abundances of 61.28% ± 0.03 and 27.57% ± 0.04 respectively. Proteobacteria, Actinobacteria, Fibrobacteres, Tenericutes, Spirochaetes. Verrucomicrobia, Cyanobacteria and Euryarchaeota were all minor contributors with average relative abundances below 0.002%. Planctomycetes, Lentisphaerae, Elusimicrobia and Fibrobacteres were all low abundance phylums not present on all farms.

Coriobacteriaceae Significantly Decreased on Non-Pasture

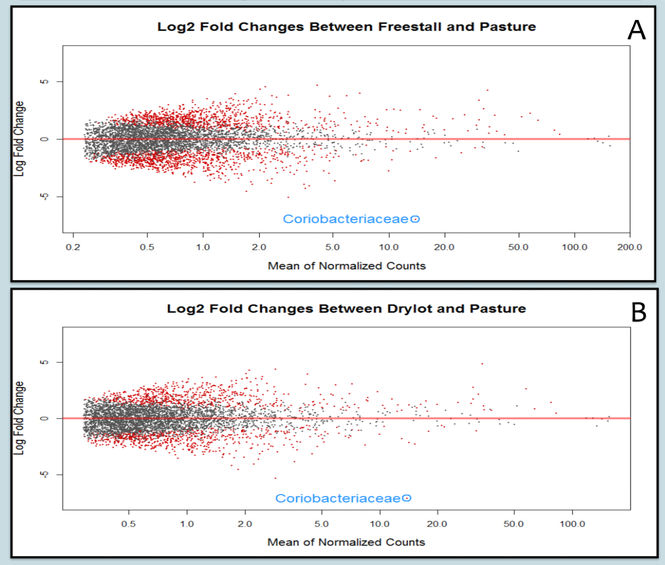


Figure 3: Transformed OTU counts were determined to account for differences in library size using a negative binomial generalized linear model in the DEseq2 Package in R. Log fold changes of bacteria counts between (A) Pasture compared to Freestall systems and (B) Pasture compared to Drylot housing. Significantly changed OTU counts are shown in red determined by $p \leq 0.01$. The most significantly changed OTU showed a decrease in *Coriobacteriaceae* for both non-pasture systems compared to pasture.

Fold Changes in Abundance of Bacterial Families When Comparing Housing Types

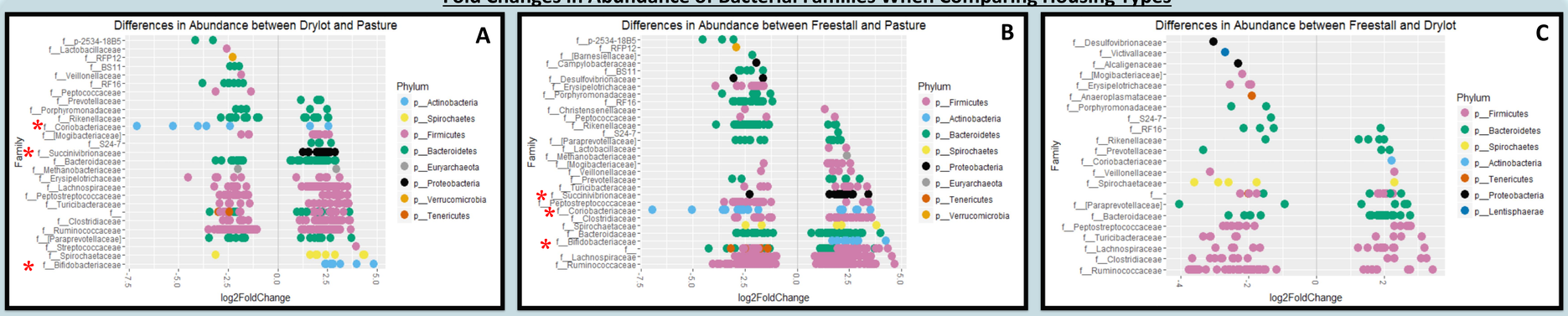


Figure 4: 16S amplicon paired-end reads were merged, trimmed for quality and length, Phix174 and chimeric reads removed. QIIME was used for *de novo* OTU picking. Transformed OTU counts were determined to account for differences in library size using a negative binomial generalized linear model in the DEseq2 Package in R. Significant log fold-changes of bacteria family counts between (A) Drylot and Pasture (B) Freestall and Pasture and (C) Freestall and Drylot are presented. Notable, common fold-changes for both Freestall and Drylot compared to Pasture denoted with *. Adjusted P <0. 01 considered significant.

PCoA of Phylogentic Taxonomic Assignment

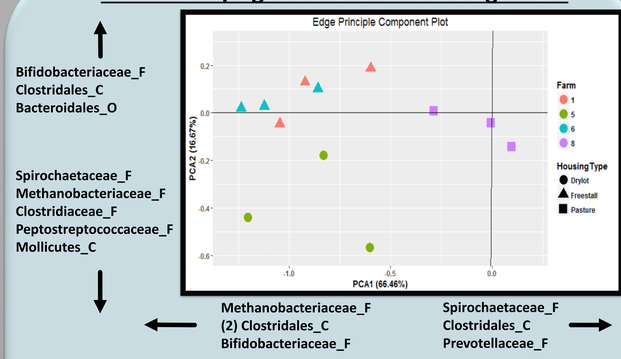


Figure 5: Quality filtered and merged pair metagenomic reads were assigned phylogenic assignments with PhyloSift and ordination taking into account phylogenetic structure was calculated with Guppy and plotted in a PCA plot in R. Specific taxa contributing to PCA1 and 2 are present at highest level of classification.

Relative Abundance of *Bifidobacteriaceae* and *Coriobacteriaceae* Varies by Farm

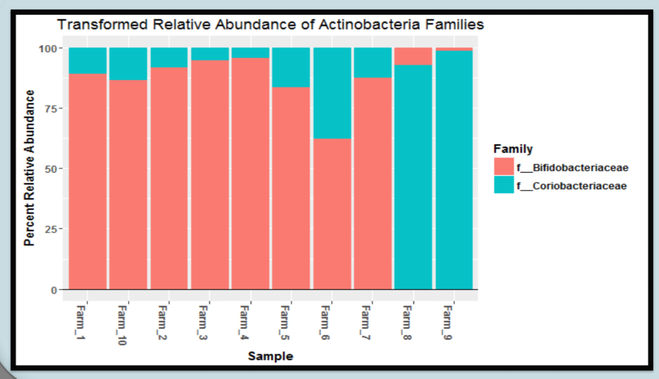


Figure 6: OTU counts were transformed to account for differences in library size with a negative binomial generalized linear model in the R package DEseq2. Bar graph made with the Phyloseq R package. Two out of three pasture-based farms had a decreased abundance of *Bifidobacteriaceae* compared to *Coriobacteriaceae* while the other had the reverse mirroring a majority of the other farms. Farm 6 had a relatively even split of both families.

Relative Abundance of *Proteobacteria* Across Farms

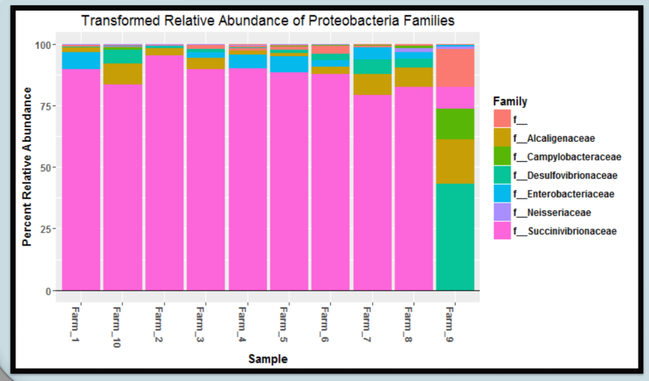


Figure 7: Transformed OTU counts to account for differences in library size were determined with a negative binomial generalized linear model in the R package DEseq2. Bar graph made with the Phyloseq R package. Farm 9 that was strictly pasture based had the lowest abundance of *Succinivibrionaceae* and the highest amount *Camplylobacteraceae* and *Desulfovibrionaceae*. These changes were not seen on Farm 7 and 8 which are pasture based as well, but do receive grain supplementation.

Conclusions

- Based on 16S data, abundances of Firmicutes and Bacteroidetes were consistent across farms and minor contributing phyla showed variation.
- Strictly Pasture fed animals had greater diversity of Proteobacteria families characterized by a decrease in *Succinivibrionaceae* and increases in *Camplylobacteraceae* and *Desulfovibrionaceae.*
- The data shows a difference in the ratio *Bifidobacteriaceae* compared to *Coriobacteriaceae* across farms.

Future Directions

- Determine functional differences using metagenomics analysis
- Determine major bacterial contributors to nitrogen cycling
- Investigate variations in antibiotic resistance across farms
- Investigate metabolic consequences of a difference in the ratio of *Bifidobacteriaceae to Coriobacteriaceae*
- Determine metabolic outcomes of increases of *Desulfovibrionaceae* and decreases in *Succinivibrionaceae*