

Prevalence of Nitrogen Fixation Genes in Dairy Cow Manure Across California



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Introduction

Manure from livestock is used as an organic fertilizer for crops, which changes the composition of the soil and its microbial communities. While ammonium is important for plant growth, nitrogen in other forms contributes to global warming through volatile emissions of nitric oxide and ammonia and eutrophication by leaching of nitrate into ground water. In addition, application of manure on the soil enhances the abundance and diversity of functional potential of the microbes in the soil. Notably, the microbial taxa contributing to nitrogen cycling in cow manure have yet to be identified. This study aims to determine the composition and functional capacity of the fecal microbiome of commercial dairy cattle on farms across California. Farms representing a variety of feeding and management systems were enrolled and fecal samples taken for metagenomic sequencing. Reads were queried against Hidden Markov Models from both the Functional Ontology Assignments for Metagenomics (FOAM) database and a custom set of genes involved in nitrogen cycling. All six genes used to predict for nitrogen fixation (*nifHDK* and *nifENB*) were found in all fecal samples. Farms showed variation in the abundance of genes involved in nitrogen cycling and abundance of families putatively involved in these processes. These findings will inform future methods to manage the fecal communities to improve the fertilization potential of manure.

Experimental Design

Housing Types



DNA Extraction

Metagenomic Sequencing
 Illumina HiSeq3000
 PE150bp

Quality Filtering:
 1. Trimming
 2. Decontamination

Six Frame Translation

Analysis:

1. Reference Based Taxonomy (Kaiju)
2. Phylogenetic Based Taxonomy (Phylosift)
3. Assembly of Genomes (MEGAHIT)
4. Binning (MetaBat)
5. Anvi'o

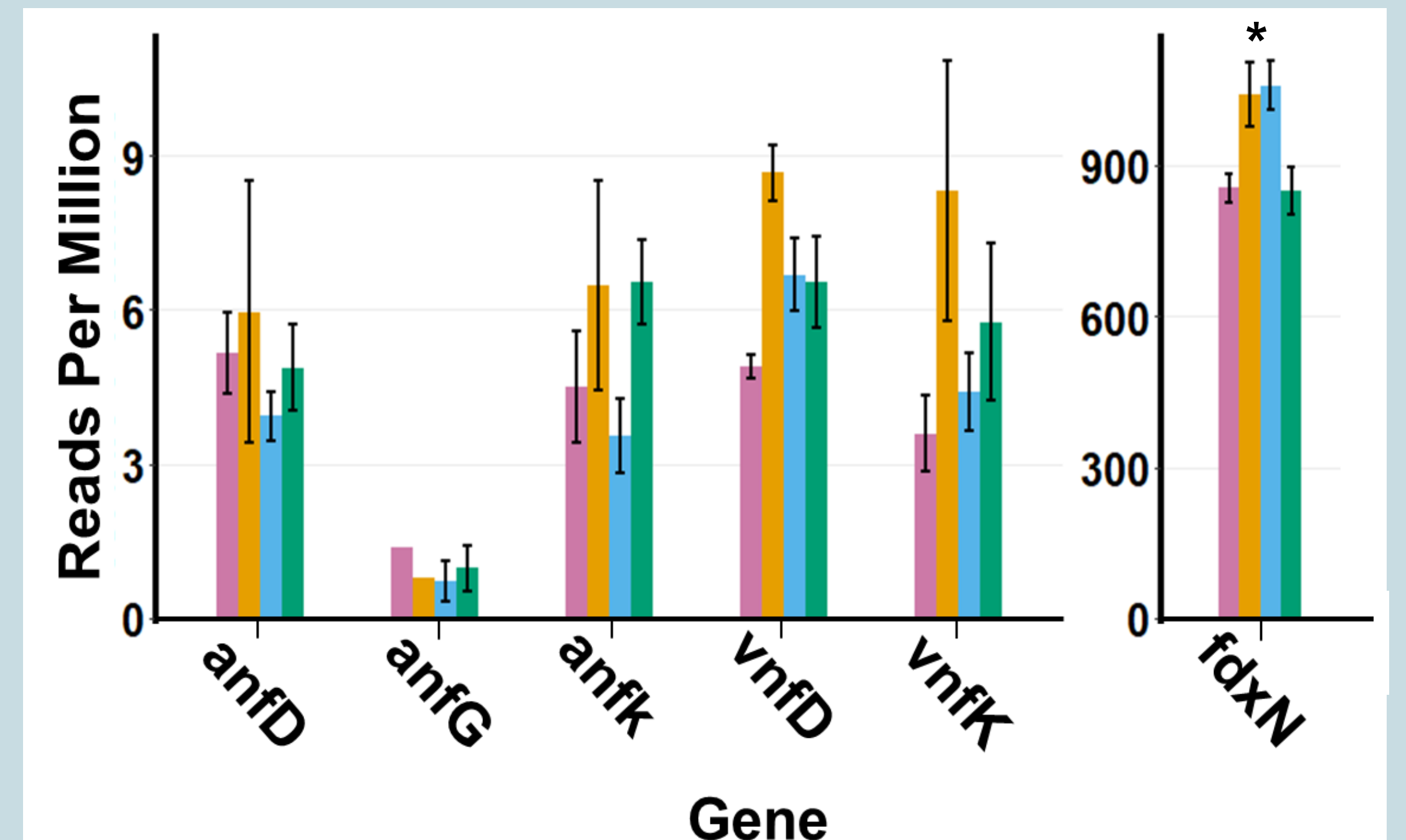
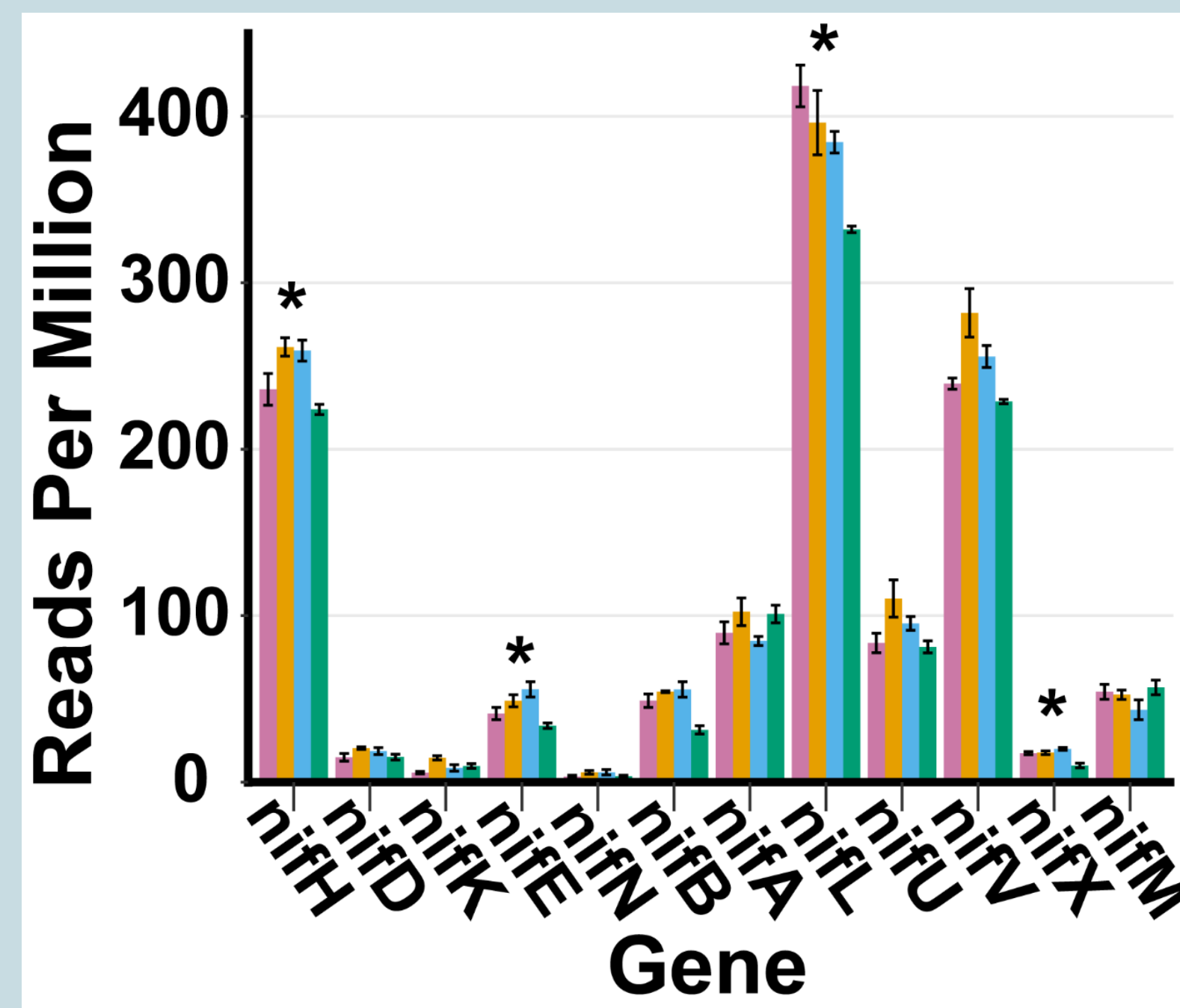
Gene Count:

1. Custom Hmms
2. FOAM Database Hmms

Nitrogen Fixation Gene Count

Farm 1 (pink), Farm 5 (yellow), Farm 6 (blue), Farm 8 (green)

Figure 1: Metagenomic reads were trimmed for quality, Phix174 and host reads were removed. For each read all six frames were translated and searched with hmmscan (HMMERv3.1b2) against hidden markov models (HMMs) of a custom set of nitrogen fixation genes. Data are reported as mean \pm SEM. There was a significant effect of farm (*) for some *nif* genes and *fdxN* as measured by Kruskal-Wallis rank sum test. P-value of <0.05 was considered significant.



Nitrogen Fixation Genes in Assembled Contigs

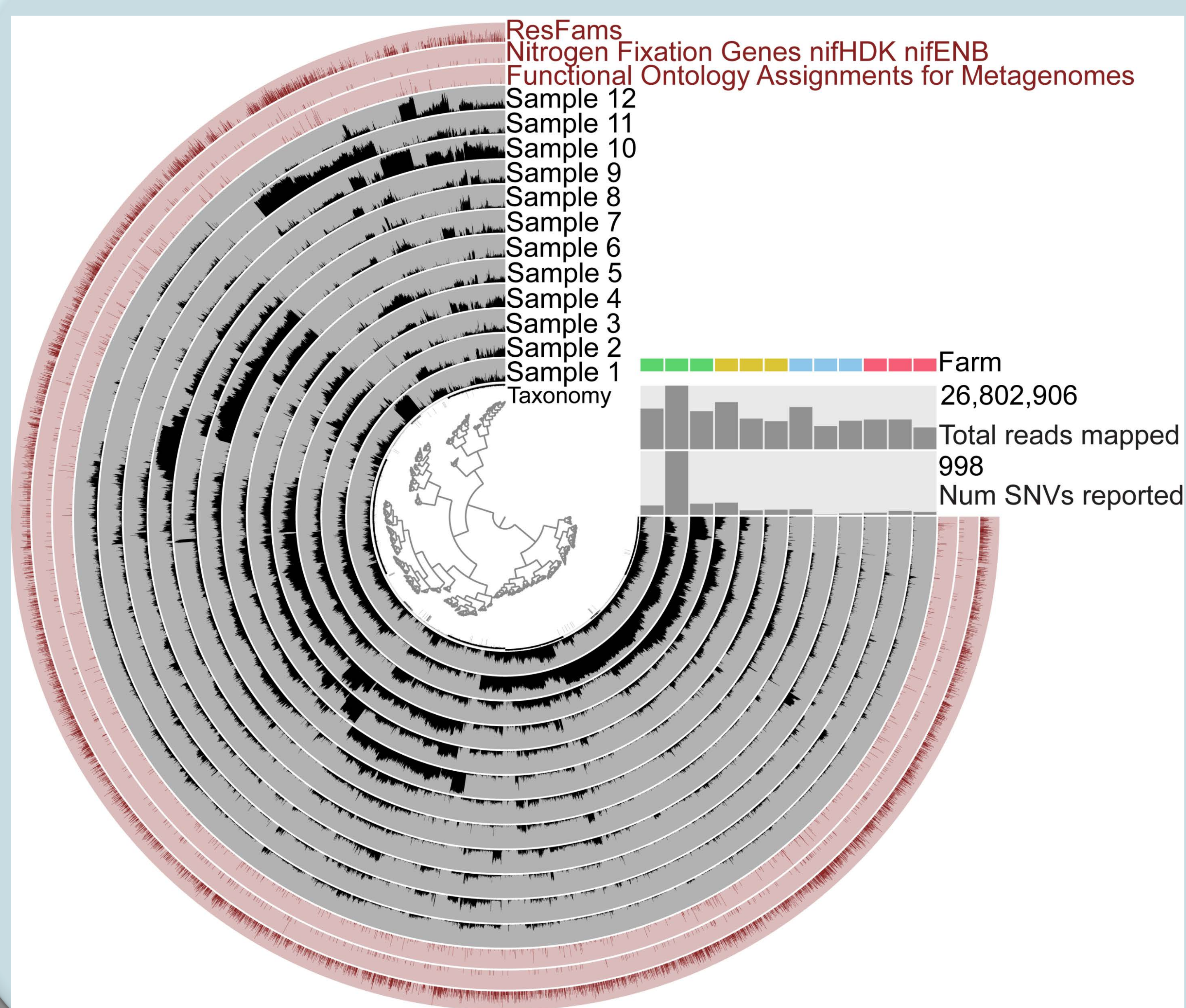


Figure 2: Cleaned metagenomic reads were normalized to 5x coverage, co-assembled into contigs with MEGAHIT and compiled into a database with Anvi'o. The Contig database was searched with HMMs from the FOAM database and a set of custom HMMs for matches to proteins involved in nitrogen cycling. Taxonomy was assigned to contigs with Kaiju.

Relative Abundance of Bacterial Families Potentially Involved in Nitrogen Cycling

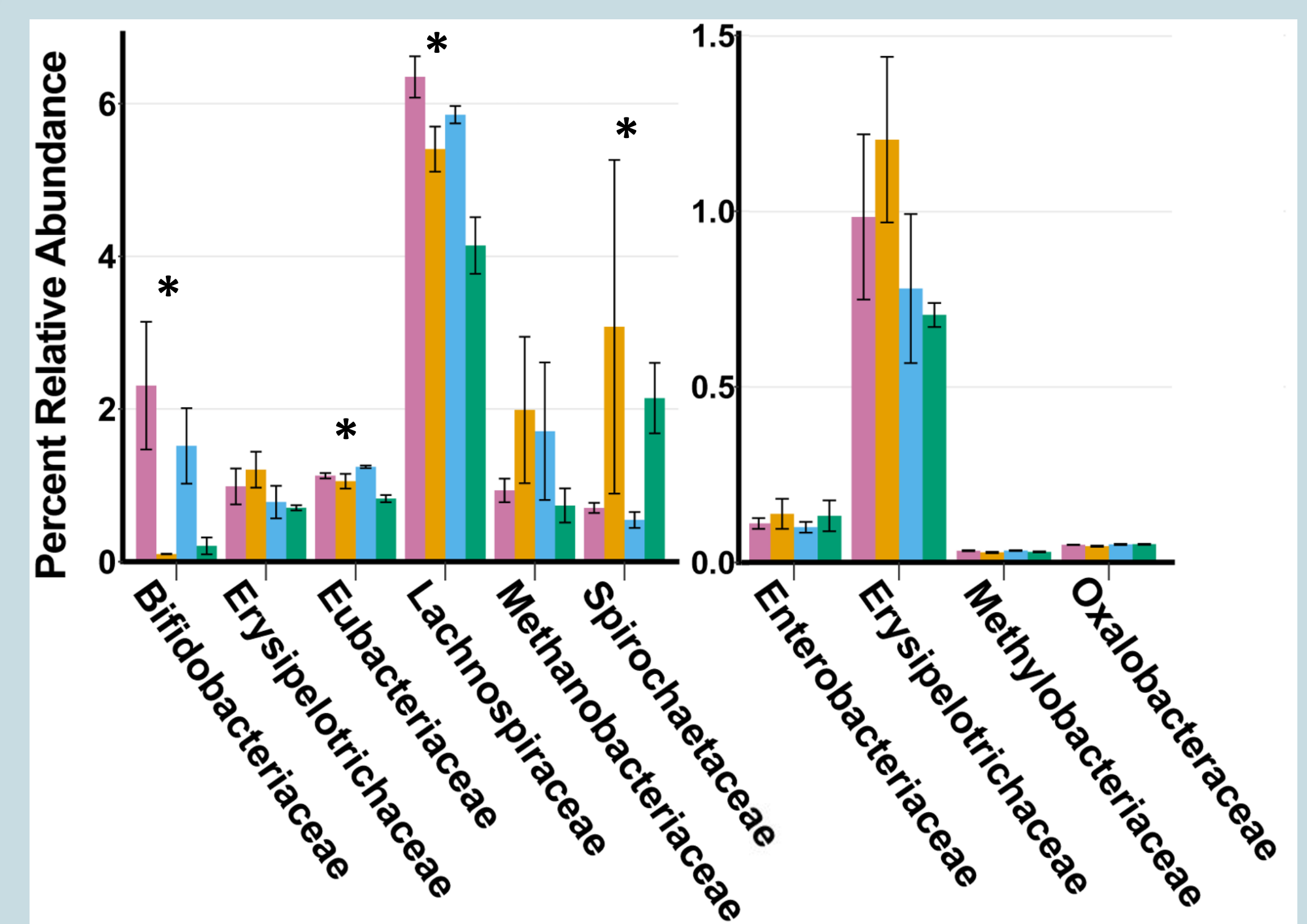


Figure 3: Cleaned metagenomic reads were translated and assigned taxonomy using protein level classification with Kaiju. The relative abundance of reads assigned to families whose contigs contain significant hits ($E < 1 \times 10^{-15}$) against HMMs from the FOAM database of proteins involved in nitrogen cycling are presented as mean \pm SEM. Significant differences measured by Kruskal-Wallis rank sum test are shown with an asterisk. P-value of <0.05 was considered significant.

Conclusions

- All six genes used to predict for nitrogen fixation (*nifHDK* and *nifENB*) were present in all fecal samples.
- Abundance of *nifH*, *nifE*, *nifL* and *nifX*, which are involved in nitrogen fixation, significantly varied by farm.
- Hits against the FOAM database were commonly found in contigs assigned to the families *Lachnospiraceae* and *Methanobacteriaceae* suggesting these may be involved in the nitrogen cycle in feces from cattle.
- The relative abundance of *Lachnospiraceae* was significantly different across farms.