Identification of Microbes Involved in Nitrogen Fixation in Dairy Cow Manure on Farms 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 microbial communities. While NH_4^+ is important for plant growth, nitrogen in other forms contributes to global warming through volatile emissions of NO and eutrophication by leaching of NO_3^- into ground water. In addition, application of manure on the soil shifts the abundance and diversity of functional potential of the microbes in the soil. Notably, the microbial taxa contributing to nitrogen fixation in cow manure have yet to be identified. This study aims 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 sequencing. Metagenomic reads were queried against Hidden Markov Models in a custom database 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 involved in these processes. Six species were identified that contained all genes necessary for nitrogen fixation. These findings will inform future methods to change the fecal communities to improve the fertilization potential of manure.

Experimental Design

Housing Types

Freestalls

2 Farms

Pastures

1 Farm

Drylot

1 Farm

DNA Extraction

Metagenomic Sequencing
Illumina HiSeq3000
PE150bp

Quality Filtering:
1. Trimming
2. Decontamination

Analysis:
1. Reference Based Taxonomy (Kaiju)
2. Assembly of Contigs (MEGAHIT)
3. Database Creation with Anvi'o
    - Custom Hmms
    - FOAM Database of Hmms
    - GhoastKOALA for KEGG assignment
    - NCBI COG Database
4. Binning contigs of interest (MetaBat)
5. Refined Taxonomy (SourMash)

Nitrogen Fixation Gene Count

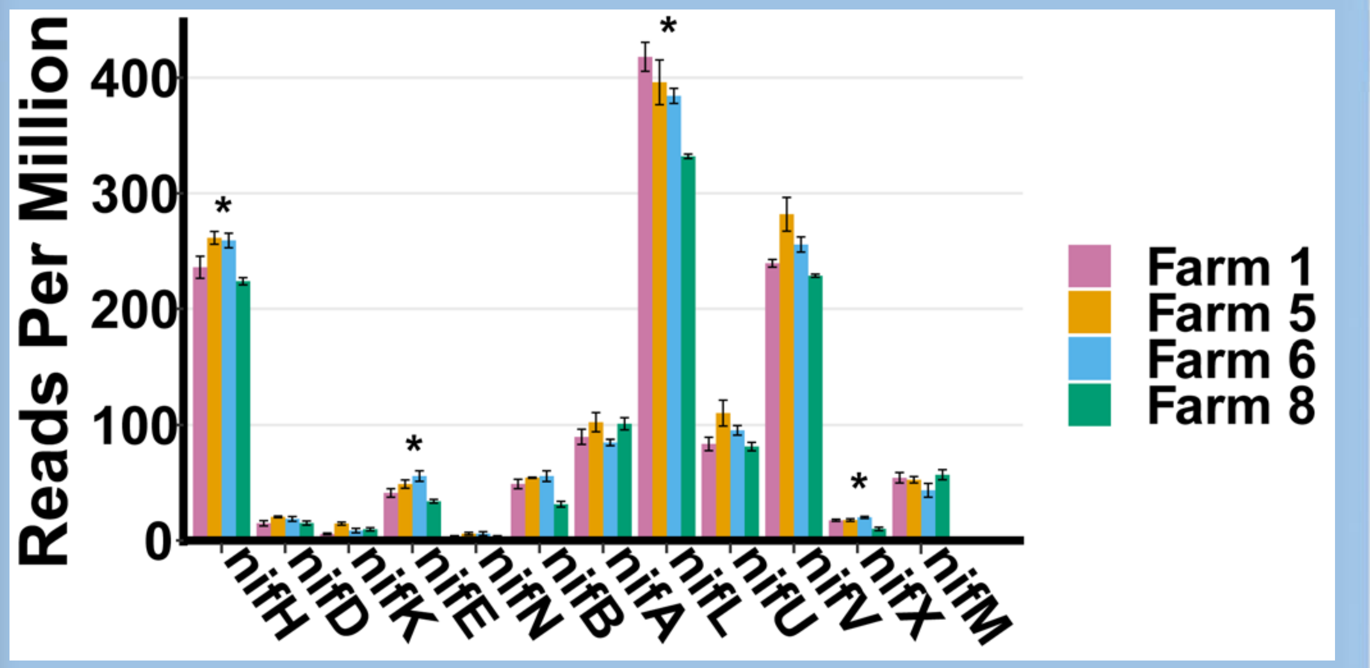


Figure 1: Metagenomic reads were trimmed for quality, Phix174 and hosts reads were removed. For each read all six frames were translated and searched with hmmscan (HMMERv3.1b2) against hidden markov models a custom set of nitrogen fixation genes. Data are reported as mean ± SEM. There was a significant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

Relative Abundance of Families Potentially Involved in Nitrogen Fixing

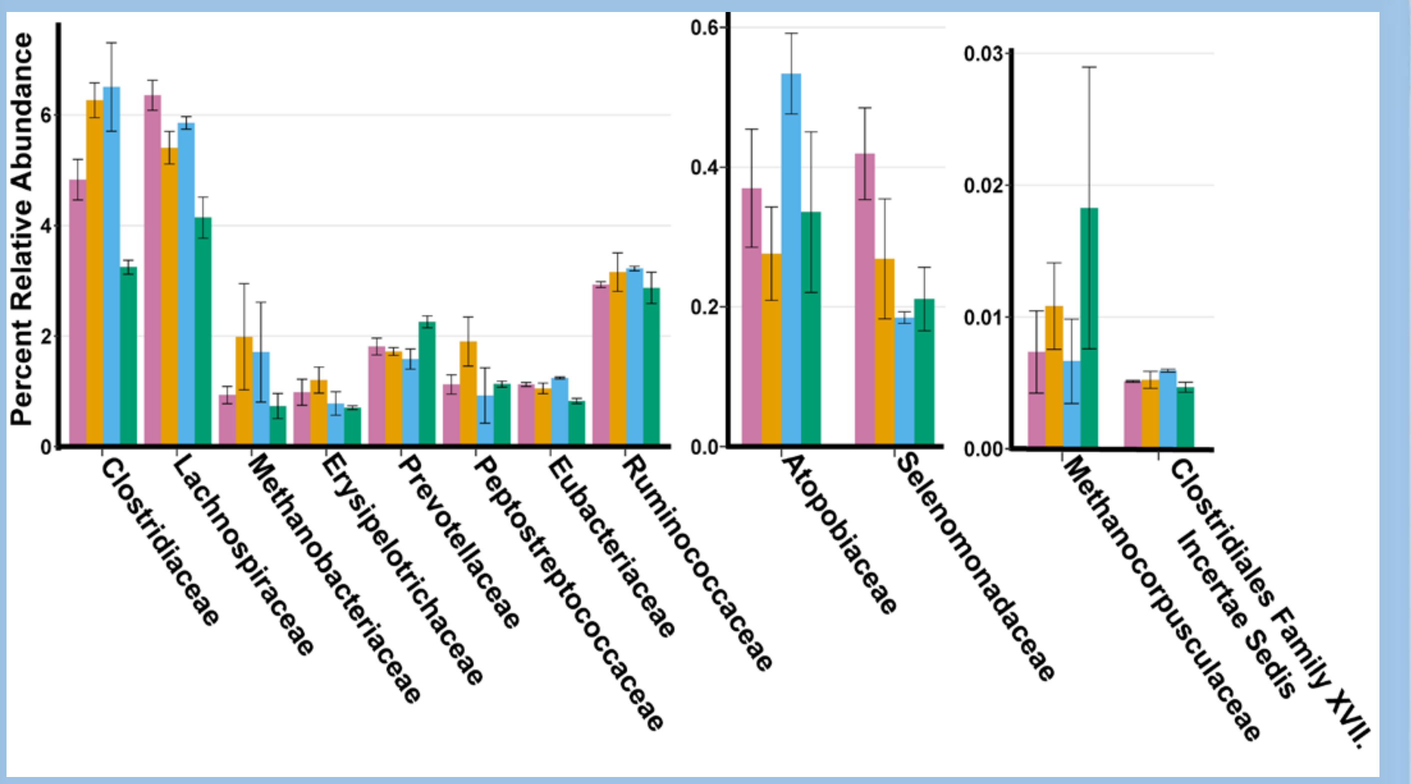
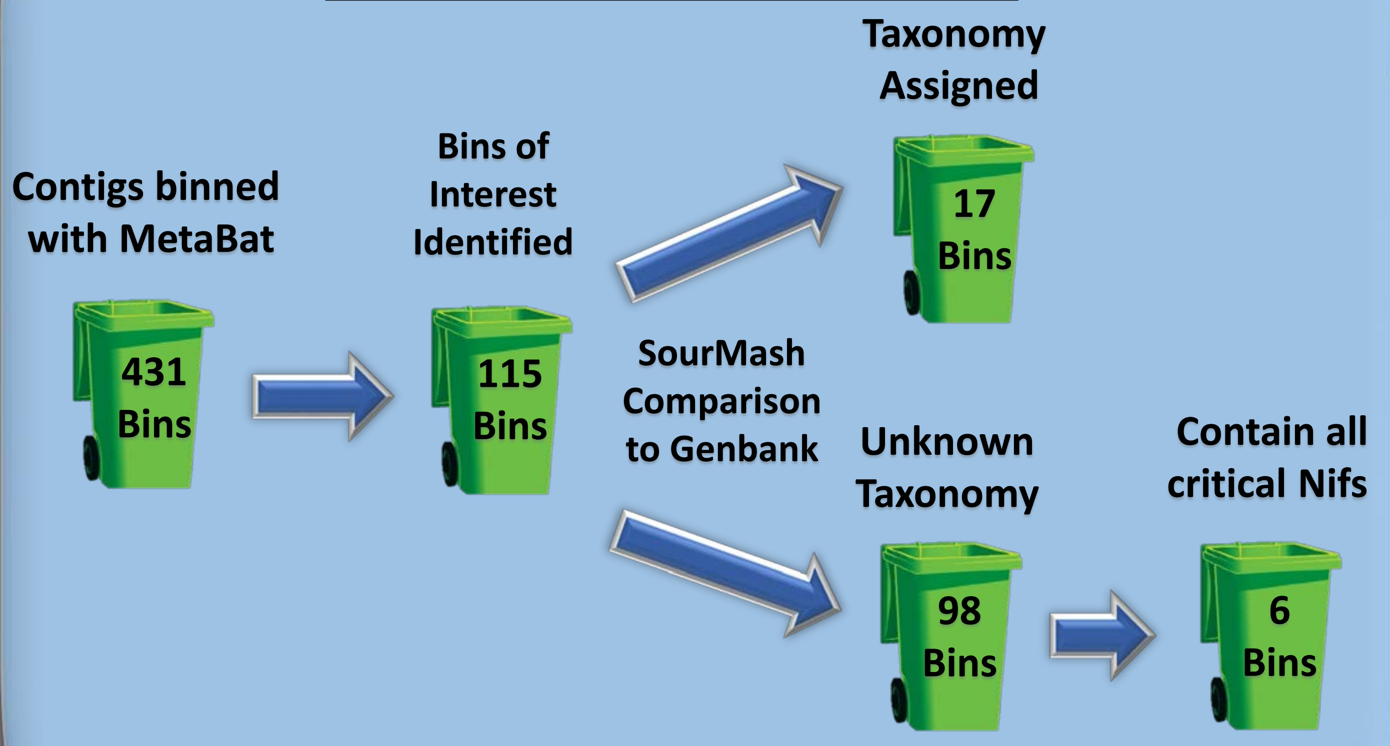


Figure 2: Cleaned metagenomic reads were translated and assigned taxonomy using Kaiju. The relative abundance of reads assigned to families whose contigs contains significant hits ($E<1x10^{-15}$) against HMMS from the FOAM database of proteins involved in nitrogen cycling are presented as mean ± SEM.

Identification of Species of Interest



Contigs binned with MetaBat
431 Bins

Bins of Interest Identified
115 Bins

SourMash Comparison to Genbank

Taxonomy Assigned
17 Bins

Unknown Taxonomy
98 Bins

Contain all critical Nifs
6 Bins

Identification of Species with Nitrogen Fixing Genes

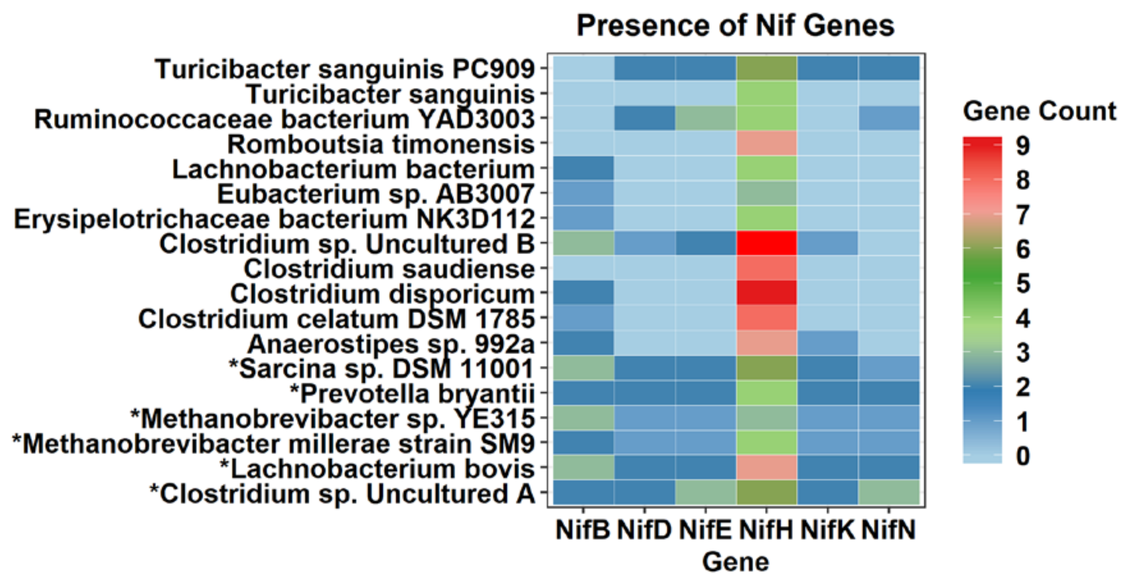


Figure 3: Bins that containing contigs with significant hits ($E<1x10^{-05}$) against HMMs of critical nitrogen fixation genes were identified and taxonomy was assigned with SourMash. Full genomes were downloaded from genebank and searched with the same HMMs. Species with * contain all critical Nif genes.

Conclusions

- Abundance of some genes involved in nitrogen fixation significantly varied by farm.
- Hits against the FOAM database were commonly found in contigs assigned to the families *Clostridiaceae* and *Methanobactericaceae.*
- Many hits in contigs containing nif genes and bins with these contigs were unable to be assigned below kingdom or phylum.

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