

Archaea and Bacteria Community Dynamics During Thermophilic Composting of Cattle Manure Revealed using 16S rRNA PhyloChip Microarray Technology

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Abstract

Proper management of agricultural manure (solid biowaste) is important to protect water supplies, and mitigate green house gas emissions. Thermophilic composting of solid biowaste is a proposed alternative to current management practices. Compost and raw manure samples were collected from a field scale operation. To prepare these samples for PhyloChip microarray analysis, total DNA extractions were performed. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) from the extracted genomic DNA and quantified using gel electrophoresis. The PCR product was hybridized to the G3 PhyloChip microarray. The hybridization intensities were used to determine the taxa of archaea and bacteria present in each sample as well as compare corresponding relative population abundances. Archaea phyla Thaumarchaeota and Thermoprotei phyla Actinobacteria and Firmicutes dramatically increased the number of different detected subfamilies. Hybridization intensity values for ammonia oxidizing prokaryotes increased under thermophilic conditions and remained high in the endpoint, mesophilic compost samples suggesting potentially high levels of nitrification. Increased intensity values for methanogens in thermophilic conditions suggest a potential increase in methane production during composting. Conversely, the vast majority of detected methanotrophs had higher intensities in old mesophilic compost and sharply decreased with increasing temperature. More study is needed to definitively determine what taxa are dominant and which metabolic pathways are active relative to green house gas emissions.

Introduction

California dairies produce 70 billion pounds of solid waste per year, 80% more solid waste than states' residents. This study characterizes microbial community dynamics of archaea and bacteria, and community dynamics of functional groups involved in GHG emissions during thermophilic composting.

Land Application of Manure

- Current waste management practice -

Benefits

Supply nutrients to crops
Increase overall soil quality

Areas of Concern

Leaching of nutrients
Public health safety
Green House Gas emissions

Thermophilic Composting of Solid Waste

- Proposed alternative -

The biological process by which microbial activity decomposes raw organic material.

Main Phases

Psychrophilic
Mesophilic
Thermophilic

Temperature Ranges

< 0°C
= 10-50°C
> 50°C

PhyloChip Technology

PhyloChip, a high-density microarray, utilizes conserved regions of the 16S rRNA gene possessed by all bacteria and archaea.

Can detect relative abundance values on level below subfamily called Operational Taxonomic Unit (OTU).

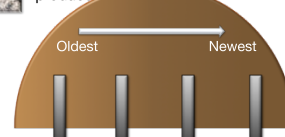
Sample Collection



Compost Pile

8 Compost Samples

Field scale compost pile comprised of organic dairy cattle manure, wood chips and raw, pre-consumer produce



Relative Age (weeks)	19	15	9	3
Temperature (°C)	41.7	58.9	71.1	54.4
Sample Names (duplicates)	A4_41.7 B4_41.7	A3_58.9 B3_58.9	A2_71.1 B2_71.1	A1_54.4 B1_54.4

3 Manure Samples

Organic dairy cow manure used for compost piles. Served as "time-zero". No manure temperatures recorded.



Manure Pile

Sample Processing

- Mortar and pestle used to homogenize samples.
- DNA extractions performed using Zymo-ZR Fecal DNA MiniPrep according to manufacturers' protocol.
- 16S rRNA PCR amplification and quantification using universal primers.
- PhyloChip Processing:



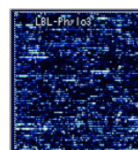
PhyloChip



Fluidics Station



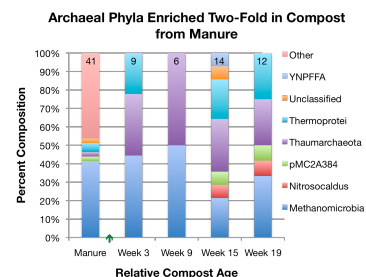
PhyloChip Scanner



PhyloChip Array Image

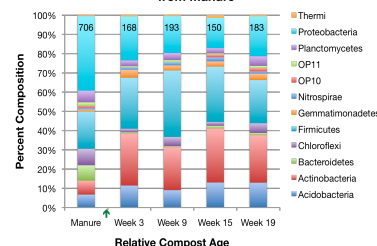
Results

Richness of Archaea and Bacteria Phyla



Archaeal Phyla Enriched Two-Fold in Compost from Manure: Composition reflects those subfamilies, which had an OTU whose average relative abundance increased two-fold to that of the manure samples, and were detected in at least one of the sample replicates, based on subfamily binary present/absent calls. The Manure samples served as baseline samples, and are representative of all the subfamilies within each phylum originally detected prior to compost treatment. Graph is scaled to 100%. The numbers at the top of each bar represent the total number of different subfamilies within that age group. The green arrow between Manure and Week 3 indicates the addition of food waste and wood chips.

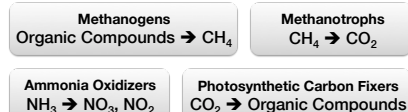
Bacterial Phyla Enriched Two-Fold in Compost from Manure



Bacterial Phyla Enriched Two-Fold in Compost from Manure: Composition reflects those subfamilies, which had an OTU whose average relative abundance increased two-fold to that of the manure samples, and were detected in at least one of the sample replicates, based on subfamily binary present/absent calls. The Manure samples served as baseline samples, and are representative of all the subfamilies within each phylum originally detected prior to compost treatment. Graph is scaled to 100%. Number at the top of each bar represents the total amount of subfamilies in the age group. The green arrow between Manure and Week 3 indicates the addition of food waste and woodchips.

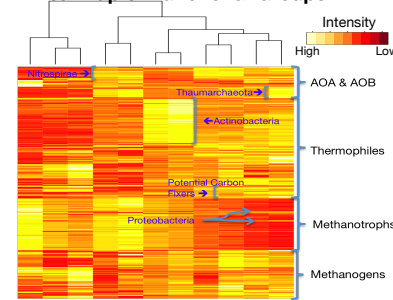
Green House Gas (GHG) Implications

Based on community dynamics of functional groups



Results

Heatmap of Functional Groups



Heatmap of Functional Groups: Displays shifts in relative abundance values for those OTUs involved in ammonia oxidation (AOA & AOB), methane formation (methanogens), methane consumption (methanotrophs) as well as thermophilic organisms including those with carbon fixation pathways. Samples M1-3 are manure samples which all clustered similarly. All compost samples clustered separately from manure samples. A4_41.7 and B4_41.7 are mesophilic samples from week 19 and clustered separately from thermophilic samples. Samples A2_71.1 and B2_71.1 are peak thermophilic, whilst A1_54.4 and B1_54.4 are pre-peak thermophilic and A3_58.9 and B3_58.9 are post peak thermophilic.

Conclusions

- Phyla Actinobacteria, Firmicutes, Thaumarchaeota and Thermoprotei dramatically increase in richness with composting.
- Nitroscaldus subfamilies were only detected in 15 and 19 week old samples.
- Bacteroidetes and Proteobacteria decrease in richness with composting.
- Ammonia oxidizing organisms increased in hybridization intensities under thermophilic conditions, suggesting high levels of nitrification.
- Thermophilic organisms increased in intensity values with increased temperatures, including organisms with phototrophic carbon fixation pathways.

Methanogen and methanotroph intensities were inversely related, suggesting increased methane emissions during the thermophilic phase.

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