Metagenomic Analysis of a Compost Microbiome

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Pingry Research

Introduction

Metagenomics is the study of genetic material taken from a population of organisms rather than from a single species or individual. It is used to research entire populations of microorganisms acting within an ecosystem rather than as single cells. The composition of microorganism populations change in response to factors in the environment such as temperature and pH level. By performing metagenomic analyses of genetic material from the Pingry composter, we hope to understand how the microbial community of the composter changes in response to these conditions. Since its inception in

2019, the Metagenomics Team has harvested, processed, and sequenced 24 samples from the Pingry composter, in addition to 2 from the composter at the North Country School. The sequencing results combine with information on the environmental conditions in the composter to form a robust body of data which we have only just begun to analyze in depth.
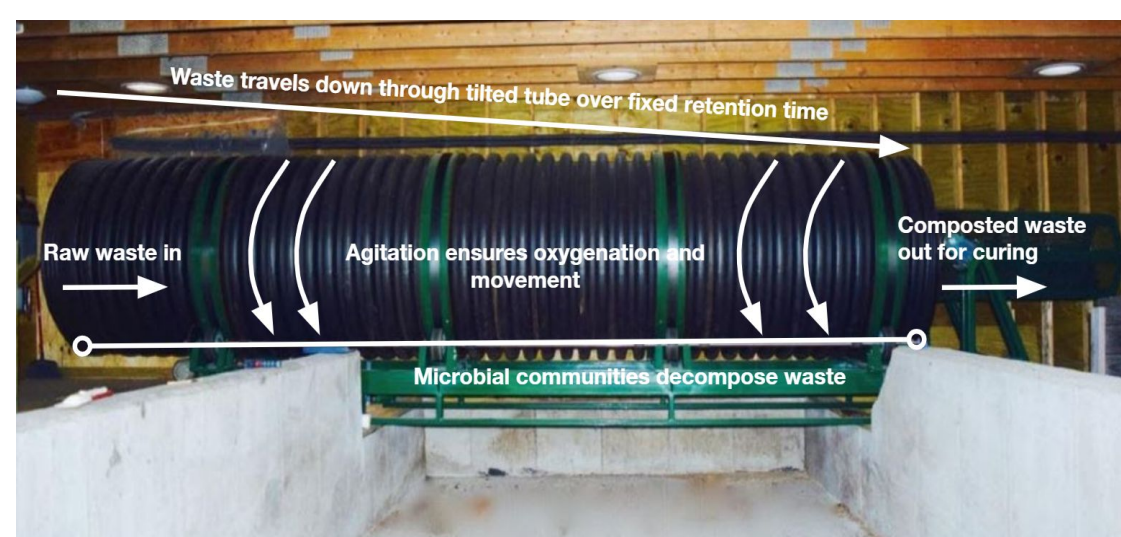

Figure 1 | Diagram of the Pingry composter which demonstrates how raw waste moves through the drum

Methods

Sample Collection & Purification

We sample soil from the composter and outsource the genetic sequencing to Azenta Life Sciences in South Plainfield. They perform next-generation sequencing, which uses genes exclusive to the kingdom being sequenced for. In bacteria, this gene is the 16S ribosomal RNA gene, and in fungi it is the ITS gene. We take 2 soil samples approximately every month from the distal (back) end of the composter and 10 ft into the middle, collecting pH readings with each sample. After soil samples are collected, their DNA is extracted using a Qiagen DNA Extraction kit. We also take temperature readings from the composter using two temperature dataloggers.

Post-Collection Quality Control

After the samples are purified, we use spectroscopy and gel electrophoresis to ensure that our DNA is of acceptable concentration and molecular weight respectively. Spectroscopy involves exposing the sample to electromagnetic radiation and reading how much of different frequencies were absorbed or reflected by the sample, indicating how much DNA is in the sample relative

to other proteins and organic molecules. The gel electrophoresis step involves running an electric current through DNA placed at the end of an agarose gel. This measures the size of the DNA. We want all of our DNA condensed at the front (top) of the gel as it has to be long enough to be able to be sequenced.
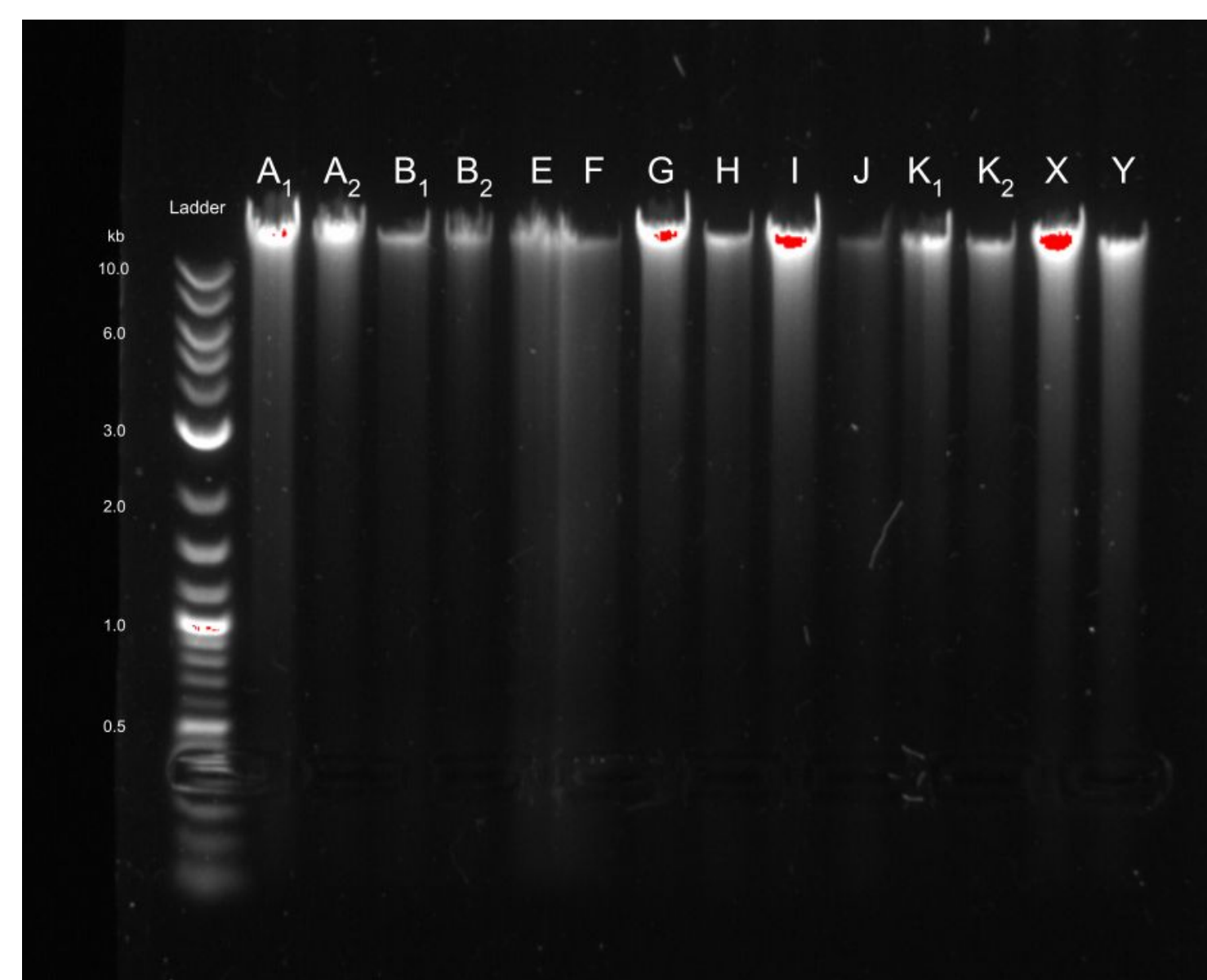

Figure 2 | This gel was used to confirm high molecular weight in most of the purified samples. The results were consistent with measurements of DNA concentration through spectroscopy

Results

We receive the sequencing data a couple weeks after samples are sent to Azenta. One of the most important pieces of data is the relative abundance of different taxa in each sample. This relative abundance data allows us to evaluate how bacterial populations change in response to shifting environmental conditions in the composter, as well as compare the bacterial populations present at different stages of the decomposition process.
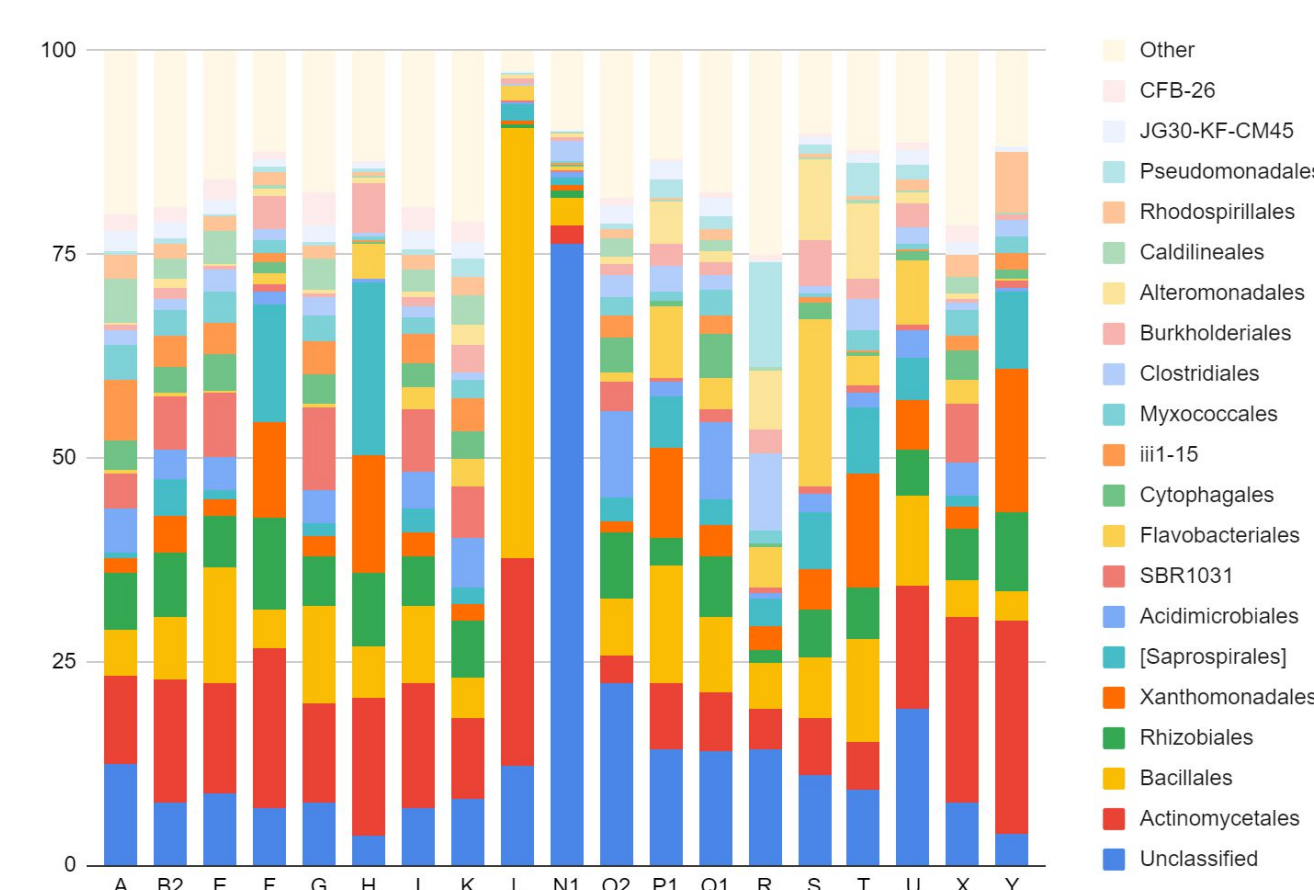

Figure 3 | This metabarcode chart shows the relative abundances of the top 20 bacterial families found in the compost samples. Samples X and Y are from the North Country School composter.

We utilize several statistical tests to analyze the data and detect correlations between bacteria abundances and other factors, such as pH.

Taxon	Region P-Value
Cytophagales	0.001
Acidimicrobiales	0.005
JG30-KF-CM45	0.014
Caldilineales	0.017
iii1-15	0.026
Rhodospirillales	0.028
CFB-26	0.033
SBR1031	0.047
Xanthomonadales	0.085
Myxococcales	0.102
[Saprospirales]	0.113
Pseudomonadales	0.286
Rhizobiales	0.354
Burkholderiales	0.375
Clostridiales	0.481
Alteromonadales	0.486
Bacillales	0.529
Unclassified	0.561
Flavobacteriales	0.595
Actinomycetales	0.605

Figure 4 (left) | This graph shows p-values for the bacterial families in the middle and distal regions of the composter. Lower p-values indicate that there may be an underlying difference between the populations of bacteria.

Figure 5 (right) | Linear regression was used to crudely determine the presence of correlations between sample pH and the abundance of different bacterial families. No strong correlations emerged from the data.

Taxon	pH R-Value
Bacillales	0.347
Unclassified	0.239
[Saprospirales]	0.126
Burkholderiales	0.104
Clostridiales	0.095
Pseudomonadales	0.074
Alteromonadales	0.045
Flavobacteriales	-0.008
CFB-26	-0.078
Xanthomonadales	-0.103
Actinomycetales	-0.118
SBR1031	-0.137
Caldilineales	-0.234
Myxococcales	-0.322
Cytophagales	-0.364
iii1-15	-0.374
Acidimicrobiales	-0.382
Rhizobiales	-0.421
JG30-KF-CM45	-0.427
Rhodospirillales	-0.517

Future Directions

We are planning on focusing our research on analysis of the data we have received while continuing to sample. We also plan to evaluate the data by comparing it with data from literature on composter microbiomes in more tropical climates, as research on composters in temperate climates, like that of New Jersey, is less available. We aim to create a spread of how the bacteria and fungi change throughout the composter by increasing the amount of samples we take across the composter. We hope to analyze a greater number of samples across a large spread of time to gauge how conditions change the microbial communities. Additionally, we hope to specifically map the temperature throughout the composter, narrowing down regions of high or low heat. We will also continue to collect monthly soil samples and continue to monitor datalogger temperature runs and reads.

References

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2. Gilmore, S. P., Henske, J. K., Sexton, J. A., Solomon, K. V., Seppälä, S., Yoo, J. I., Huyett, L. M., Pressman, A., Cogan, J. Z., Kivenson, V., Peng, X., Tan, Y., Valentine, D. L., & O'Malley, M. A. (2017). Genomic analysis of methanogenic archaea reveals a shift towards energy conservation. *BMC Genomics*, *18*(1). https://doi.org/10.1186/s12864-017-4036-4