Britt Henke
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Temperature Patterns and the Effect on Microbial Populations during Composting of Human Wastes

Britt Henke
Office of Science, Community College Internship

College of Marin
Kentfield, California

Lawrence Berkeley National Laboratory
Berkeley, California

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ABSTRACT

How effective are static piles in treating human waste? If proven to be effective, thermopiles may provide a cheap alternative to water-intensive, infrastructure-based sewage systems and, as such, their application may help to reduce the impact of wastes on watersheds and to improve land use productivity. A pilot project found complete destruction of pathogens and fecal indicators in one to two weeks. In this phase of the study, samples collected from compost pile edges, where temperatures are lower and more variable, were analyzed and compared to samples collected from static pile centers to investigate microbial community structure and succession dynamics in relation to temperature during the composting process. We characterized bacterial community composition using a DNA microarray that probes for 16S rRNA genes of 59316 different bacterial taxa. Data showed near complete destruction of human gut microbes in the centers of compost piles after 17 days and variable destruction of human gut microbes at the edges after 36 days. Results indicate static piles are effective in treating human waste, provided that it is composted for at least 36 days. Further studies are recommended to determine optimal edge temperature for human gut microbe reduction.

INTRODUCTION

Clean water is a critical element for appropriate development of human populations. However, 50% of the world's population lacks safe drinking water, and pollution of water sources, often from poor sanitation, causes 25 million deaths per year [1]. Indeed, 37% of the world's population does not have adequate sanitation [2], and half of the world's diseases are transmitted by or through water [1], making clean water a major international public health priority. Both lack of proper sanitation systems and the use of water to treat human wastes contribute substantially to the international shortage of clean water. Thus, it is important to develop systems to treat human feces and urine that do not require massive amounts of water.

Human waste composting may be an economical and sustainable alternative to expensive, resource- and infrastructure-intensive sanitation methods. Composting human wastes converts them into a useful product, such as fertilizer, thereby meeting the needs of developed and developing countries by offering an affordable source-control sanitation method that ultimately pays for itself through the use of compost as fertilizer and or soil amendment. An additional benefit of composting human waste may be to bolster soil carbon sequestration, thus helping communities mitigate and adapt to climate change, while addressing international sanitation challenges [3].

Composting is often divided into three phases, characterized by parameters such as microbial successions, temperature and gas flow changes. The specific patterns of those parameters depend on the chemical and microbiological composition of the materials being composted, moisture levels and flow of various gases. In certain cases, during the first phase (which may last from one to two days), temperatures gradually rise to 40 to 50°C as microbes consume easily biodegradable carbon sources. In the second phase, thermophilic microorganisms predominate and temperatures may reach 55 to 70°C. During this phase of composting, pathogens as well as beneficial microorganism are killed and less biodegradable cellulosic substances are destroyed. Curing is the third phase, and it is marked by declines in temperature resulting from the fact that most readily biodegradable components of the compost have already been consumed. At this time, mesophilic microorganisms that grow at <40°C recolonize the compost [4], [5].

The Earth Sciences Division of Lawrence Berkeley National Laboratory (LBNL), in

cooperation with the Marin Carbon Project, and the Biology Department of College of Marin is conducting research to assess the potential of using the static compost pile process to treat human waste, so that the end product can be used as a fertilizer or soil amendment to enhance soil health and soil carbon sequestration. To achieve this, it is necessary to have a better understanding of the composition of microbial communities throughout the thermophilic process, to better correlate the relationship between the materials used in composting and temperature changes during the composting process. During the composting of human waste there is a succession of microbial communities [6]; the nature of this succession determines the microbial composition of the finished compost and ultimately its safety. Assuring the safety, from a public health perspective, of fully processed compost will increase the acceptance of composting as an alternative to water transport methods for waste disposal, removing the first barrier to widespread real-world application of composted human waste as a fertilizer.

Preliminary work by the Andersen lab was intended for initial assessment of the use of molecular biology techniques to determine microbial populations in human feces being composted. Nevertheless, this trial resulted in the complete destruction of pathogens and fecal indicators within one to two weeks. Data gathered from temperature probes at the center and edges of compost piles showed that the temperature of the compost piles was not uniform. Temperatures recorded at the edges of the compost piles were lower and more variable than those recorded in the center of the pile. This pattern of variation and its effect on the inactivation of human pathogens requires more study.

This phase of the research is intended to further probe the effectiveness of static piles in treating human waste. Although pathogen and fecal indicators were completely destroyed in the pilot project, that test said nothing about effectiveness of the composting process at the edges of thermopiles piles, where temperatures are more variable. By comparing the relative community structures of compost samples taken from the center of the compost pile with those from the edges, this study seeks to assess how the selective pressures of high temperatures and fluctuating oxygen concentrations in compost affect the total microbial community.

PROGRESS

Technical Approach

Compost Composition and Sample Collection

Human manure compost piles were constructed on November 9, 2012. Three replicate piles were lined up in a row, over top of a PVC ventilation pipe to aerate the compost with air supplied by a solar-powered fan. The 1.2 m^3 piles were encased with straw bail for insulation and labeled nine, eight, and seven, from left to right. The piles were covered with shade cloth to keep out the rain.

Three replicate compost mixtures were made to fill the piles. See Figure 1 for mixture contents and Figure 2 for analysis of pile composition. Each mixture was comprised of human waste, containing urine and feces, wood shavings, wood chips, and horse bedding. See Figure 1 for descriptions of each ingredient. Mixtures one and two are identical in their proportions. Mixture three contained half as much human waste.

Campbell Scientific data logger sensors were placed at the center and edges of each pile to record oxygen content and moisture levels hourly. Temperature was also monitored and recorded hourly at a top and bottom corner of each pile using Onset temperature sensors.

On construction day (day zero), samples were collected from each compost pile, the horse bedding, and the raw human waste. After build day, the compost was sampled 2, 4, 7, 11,

17, 23, 30 and 38 days after the onset of the experiment, for a total of nine time-points. At each sampling after day zero, three biological samples were taken from each pile, one from the center, one from the bottom edge and one from the top edge, alternating between left and right corners. Samples of approximately twenty grams were collected using plastic gloves, one-use tweezers and glass jars. Samples were placed on dry ice immediately after collection and later put into a freezer at -80°C for storage.

Gas samples were collected from the center of the pile for CO2, CH4, and N2O measurement. The gas probes were fabricated from 26" lengths of 1" schedule 80 PVC pipe. The upper end of the pipe was closed with a pipe cap, which contained a 1/16" vent, with a 1/4" compression bulkhead fitting accommodating a septum for the sampling needle. The lower end of the probe had two slots, 1/2" wide and 4" long, and the end was closed with a second pipe cap. The lower end of the probe was wrapped with plastic screen, 1/16" mesh, wrapped around the pipe, and secured with stainless steel hose clamps to prevent the compost material from entering the tube and restricting gas diffusion into the probe. During sampling, the 1/16" vent was plugged. A 60 mL syringe was used to draw samples. To remove dead air, 60 mL were extracted two times (60 mL x 2), after which the 30 mL (30 mL x 1) sample was taken and inserted into a 20 mL sealed gas vial. Gas samples were stored at room temperature.

Total carbon and nitrogen (C:N) samples were also gathered. Before freezing, five to 10 g of each biological sample was separated out and placed into a drying tin. C:N samples were then dried in an oven at 100°C for two to four days. Dry weights were recorded; samples were sealed in bags and stored at room temperature.

Sample Homogenization and DNA Extraction

Four grams of sample that had been kept in a freezer at -80°C were weighed out and placed into sterile grinding jars—steel, screw top containers with a ball bearing. The jars were returned to the freezer for thirty minutes, after which they were dipped in liquid nitrogen for about a minute or until they stopped boiling. The jars were then put into the Tissue Lyser 2 machine and run at 30 s^{-1} for 30 seconds, after which the jars were submerged in liquid nitrogen again until they stopped boiling. Jars were placed back into the freezer at -80°C to warm them up before further processing.

DNA extractions were performed utilizing Zymo-ZR Fecal DNA MiniPrep, according to the manufacture's protocol (Zymo Research, Irvine, CA), and 0.15 grams of the homogenized sample. Extracted DNA was quantified using Quibit Fluorometer 2.0 (Invitrogen) according to the manufacture's protocol.

PCR 16S rRNA Amplification

Extracted gDNA concentrations were quantitated using Qubit® dsDNA HS Assay Kit (Invitrogen, Grand Island, NY, USA), following the method used by Hazen et al. (2010). Bacterial 16S rRNA were amplified, purified, and quantitated using gel electrophoresis (2% agarose E-gel; Invitrogen). The bacterial 16S rRNA were amplified with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Each PCR reaction contained 1× Ex Taq buffer (Takara Bio Inc., Japan), 0.025 units/µl Ex Taq polymerase, 200 uM dNTP mixture, 1.0 µg/µl BSA, and 200 nM each primer and 1 ng genomic DNA. Each sample was amplified in 8 replicate 25 µl reactions; the group were pooled together after amplification and before purification. The PCR conditions were 95°C (3 min), followed by

25 cycles 95°C (30 s), 48-58°C (30 s), 72°C (2 min) and a final extension 72°C (10 min). Amplicons were purified with the QIAquick PCR purification kit (Qiagen; Valencia, CA).

Preparation of Samples for PhyloChip Microarray

One-hundred ng of archaeal PCR product and 500 ng of bacterial PCR product from each sample were added to each G3 PhyloChip. Fragmentation, labeling, and hybridization wash and staining procedures were performed, as described in Hazen et al (2010).

PhyloChip Microarray Analysis

The arrays were scanned, and hybridization intensities were captured with GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA) and processed using methodologies previously described in Hazen et al. The hybridization score for an operational taxonomic unit (OTU) was calculated and the potential for cross-hybridization and background DNA were taken into account, as described in Hazen et al (2010). Data files were then processed using PhyCA software (LBNL), and data were analyzed as described in Hazen et al (2010).

Statistical Analysis

Statistical analysis, and distance matrices were performed using PRIMER-E [7]. PRIMER-E is a systems software commonly used for high-grade graphics and statistical computations. Inter-profile distances were calculated using Bray-Curtis distance matrix of similarity, and ordinations were performed with non-metric multidimensional scaling (NMDS). Analysis of Similarity (ANOSIM) was used to analyze the variance between sample groups when compared to relative compost age, and to determine which age groups were statistically similar or dissimilar to each other.

CONCLUSIONS/DISCUSSION

The results shown in the NMDS plot (Figure 3)—comparing pile number, location within pile, and time—indicate that, based on the OTU hybridization intensities, pile had less effect than either time or location within pile. This may indicate piles were true replicates, as it appears that similar processes occurred in all three piles and something other than pile drove change. Taking advantage of the PhyloChip's complete microbial characterization capabilities, we were able to cast a wide net by using all human gut microbes as biosolids indicators. This analysis of the effectiveness of static piles in treating human waste included the *Bacteroidales, Enterococcus, Streptococcus, Aeromonadales, Enterobacteria*, and *Clostridium* subphylums. Since the replicate piles yielded very similar microbial community data, the binary data indicating presence or absence was averaged so results could be more readily assimilated and correlated with temperature records. Using this information a "kill rate" was established for each human gut subphylum in the center and edge of the compost piles to help in comparing center and edge performance. As set out below, this information indicated near complete destruction of human gut microbes in compost pile centers after 17 days and variable destruction of human gut microbes in compost pile edges after 36 days.

Especially in terms of pile centers, there was a marked difference in microbial populations from the beginning to the end of the 36-day composting process. This is illustrated in the NMDS plot comparing time and location (Figure 4). This finding is supported by the Simper analysis, which shows an average dissimilarity of 35.31 between the sample contents in the center from the beginning to the end of the five-week trial. Data obtained from samples taken

from the centers of the compost piles indicate near complete destruction of human microbes within 17 days (Figure 5). On 11/26/12, after 17 days, the human gut microbe center kill rate was 100% for *Bacteroidales, Enterococcus, Aeromonadales,* and *Enterobacteria*, with 99% for *Streptococcus* and 96% for *Clostridium*. After 11/26/12, human gut microbes remained nearly constant for the final 19 days of the study (Figure 6).

Based on OTU hybridization intensities, binary data reveal a marked distinction between microbial populations on pile edges from day zero to the trial's completion on day 36. As the NMDS plot comparing time and position indicates, the final edge microbial community more closely resembled the initial community than did the final center microbial community. With an average dissimilarity of 20.96 between the finished compost and raw material in the edge zones, the Simper analysis also indicates the final compost at the edge was more dissimilar to the finished compost at the center—with an average of 22.22—than it was to the initial raw material.

Data obtained from samples taken from the edges of the compost piles indicate variable destruction of human gut microbes after 36 days (Figure 7). On day 17, when center kill rates were 96-100%, edge kill rates were only 71-89%. Thus reduction of human gut microbes was considerably slower in compost pile edges than it was in the centers. On day 36, the human gut microbe edge kill rate was 100% for *Aeromonadales,* 99% for *Streptococcus* and *Enterobacteria,* 98% for *Enterococcus,* 89% for *Clostridium,* and 83% for *Bacteroidales.* A 17% and 11% edge survival rate for *Bacteroidales* and *Clostridium* may seem worrisome, but this information should be put in context. Using the entire human gut biome to indicate human health risk is a conservative approach. Fecal coliforms, which are often used as indicators of pathogenic bacteria, were completely reduced throughout the piles, as denoted by the 99-100% kill rate of *Enterobacteria* in center and edge zones. It is not clear whether human gut microbes would have continued to decline in the edge zones of compost piles, as trend lines suggest. A future test is recommended to observe the fate of human gut microbes over a longer period of time.

Results indicate *Bacteroidales* and *Clostridium* require higher temperatures in order to be eliminated. This makes sense because these are hardy microbes that are adapted to the warm acidic environment of the human gut. This is particularly true of organisms from the phylum *Clostridium,* which are spore forming and typically have thick cell walls. *Bacteroidales* that persisted are part of the *Rikenellaceaell* family. *Clostridium* that remained are either of the genus *Clostridium* or the family *Ruminococcaceae.* The *Bacteroidales* or *Clostridium* that endured in compost pile centers also persisted in compost pile edges. If compost does not reach high enough temperatures, it may provide the perfect environment for these organisms to thrive. Judging from the higher kill rates achieved in the center, where temperatures were higher (Figure 9 and 10), it seems imperative that these microbes be forced out of their comfort zone by attaining high temperatures throughout the compost pile. Similarly, the lower edge temperatures of pile 7, which maxed out around 40°C, correspond to lower edge kill rates when compared with the edge of pile 8, which attained 60°C during the thermophilic phase. However, pile 9 was somewhat of an anomaly, as its edge temperatures were very similar to those of pile 8, but its edge kill rate was substantially lower than the edge kill rate of pile 8 or pile 7 (Figure 11). This may be due to the fact that, in order to avoid disrupting temperature sensors, biological samples were taken from the corner of compost piles opposite the sensors.

Our results indicate that composting is an effective method of treating human waste, so long as certain parameters are met. Thirty-six days appears to be the minimum amount of time required for substantial reduction of human gut microbes. Compost pile temperature is important and, as there is typically less variation in pile centers, it is recommended that pile edges be

monitored to ensure thorough pathogen reduction. Thus our findings are consistent with earlier studies indicating that "lower pile corners are the most logical parts of the pile to monitor because these are the portions of the pile undergoing the least heating" [9]. Insulating compost piles may help achieve a constant temperature throughout the pile, possibly facilitating a quicker and more complete reduction of human gut microbes in compost pile edges. Further studies are recommended to determine optimal edge temperature for human gut microbe reduction. Although temperature is important, it is not the only factor; time is another critical element involved in human gut microbe reduction via static piles.

FUTURE WORK

Our current thermophilic composting project examines how the microbial community in the source material changes during the life cycle of the composting process. Understanding this process will be essential to optimize not only the process, but also the overall safety of such practices. Assuring complete pathogen destruction in fully processed compost will increase the acceptance of this practice as an alternative to water transport methods for waste disposal.

Equally important, composted wastes have the potential to offset a significant portion of CO2 production and to uphold the sustainability and productivity of grassland ecosystems managed for grazing and livestock production. Further, such processes will recover critical nutrients that are rapidly being depleted, such as phosphate fertilizer, which is already experiencing rising costs and is estimated to run out in only a few decades. To this end, the Andersen Group is collaborating with the Silver Lab at U.C. Berkeley and the Rathmann Foundation to examine the use of rangeland management practices to increase carbon sequestration. Studies in the application of compost to rangeland have shown that net ecosystem carbon storage can be increased by 25-70%, while simultaneously increasing net primary productivity and water retention in the soil.

The Thermopile Project also has two subsidiary projects that are international in scope: DNA Everywhere and SOIL (Sustainable Organic Integrated Livelihoods). The concept behind DNA Everywhere is to bring the Thermopile Project to communities around the globe in need of an affordable procedure to reliably sanitize human waste in a sustainable manner. Composting meets the challenge, not only offering an affordable source-control sanitation method, but one that ultimately pays for itself through the sale of compost as a nitrogen-rich soil amendment. SOIL is a pilot project that will be taking place in Haiti this fall. In conjunction with Phase I of DNA Everywhere, the purpose of the Haiti SOIL project is to produce a complete set of protocols for DNA extraction and analysis in the field as well as training and equipment for the operation. Results will indicate whether the finished compost is safe for land application, while also producing genetic information that will be used to populate the GreenGenes database.

IMPACT ON LABORATORY MISSION

The Thermopile Project is project is fully consistent with Lawrence Berkeley National Laboratory's mission to bring science solutions to the world by addressing the international need for alternative means of sanitizing human waste, thereby reducing water contamination and associated disease. The Thermopile Project also addresses the Department of Energy's overarching mission to advance National Security by improving and systematizing a method of sanitizing human waste that can be employed in disaster scenarios when sewer systems are rendered defunct. In such situations, knowledge gained from the Thermopile Project may allow communities to avoid disease transmitted through water contamination.

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FIGURES

Figure 1. Compost mixture composition and ingredients

MIXTURES:

	Composition	Amount	"Units"	Total Measure	Volume (Gal)	NOTES:	
M1	Human Waste	110	Gal	110 Gal	110	The wood shavings, wood chips and horse bedding were wet when added; these components were mixed the day prior and left out overnight in the rain.*	* NOAA captured the following about the weather on 11/2/12: "THE FOLLOWING WEATHER WAS RECORDED YESTERDAY." LIGHT RAIN, FOG, HAZE. RELATIVE HUMIDITY (PERCENT) HIGHEST 100 300 AM LOWEST 52 400 PM AVERAGE 76
	Wood Shavings	2	Buckets	60 Gal	60		
	Wood Chips	2	Scoops	2 Cubic Yards	404		
	Horse Bedding	2	Scoops	2 Cubic Yards	404		
M2	Human Waste	110	Gal	110 Gal	110		
	Wood Shavings	2	Buckets	60 Gal	60		
	Wood Chips	2	Scoops	2 Cubic Yards	404		
	Horse Bedding	2	Scoops	2 Cubic Yards	404		
M3	Human Waste	54	Gal	55 Gal	55		
	Wood Shavings	2	Buckets	60 Gal	60		
	Wood Chips	2	Scoops	2 Cubic Yards	404		
	Horse Bedding	2	Scoops	2 Cubic Yards	404		

MIXTURE INGREDIENT DESCRIPTIONS:

Human Waste	From 3-275 Gal containers. Each mixture was made with the waste from a different container, so we would be able to calculate volume based on remaining liquid level.
Wood Shavings	Clean/dry, from the gray plastic buckets.
Wood Chips	From a large pile at the site. Mildly composted in some areas.
Horse Bedding	Fresh, fine grade, fluffy, saw-dust/hay mixture with horse droppings.

Figure 2. Compost pile contents

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PILES:							Total Composition (L)		
	Mixture	Mixture	Composition	Amount	Measure	Volume (Gal)	Composition	Volumes (Gal)	Percents (%)
P9	66.66%	M1	Human Waste	73.34	Gal	73.34	Human Waste	91.68	9.59%
			Wood Shavings	40.00	Gal	40.00	Wood Shavings	60.00	6.28%
			Wood Chips	1.33	Cubic Yards	268.63	Wood Chips	401.93	42.06%
			Horse Bedding	1.33	Cubic Yards	268.63	Horse Bedding	401.93	42.06%
								955.54	
	33.33%	M3	Human Waste	18.34	Gal	18.34			
			Wood Shavings	20.00	Gal	20.00			
			Wood Chips	0.66	Cubic Yards	133.30			
			Horse Bedding	0.66	Cubic Yards	133.30			
P8	66.67%	M2	Human Waste	73.34	Gal	73.34	Human Waste	91.68	9.59%
			Wood Shavings	40.00	Gal	40.00	Wood Shavings	60.00	6.28%
			Wood Chips	1.33	Cubic Yards	268.63	Wood Chips	401.93	42.06%
			Horse Bedding	1.33	Cubic Yards	268.63	Horse Bedding	401.93	42.06%
								955.54	
	33.33%	M3	Human Waste	18.34	Gal	18.34			
			Wood Shavings	20.00	Gal	20.00			
			Wood Chips	0.66	Cubic Yards	133.30			
			Horse Bedding	0.66	Cubic Yards	133.30			
P7	66.67%	M2	Human Waste	73.34	Gal	73.34	Human Waste	91.68	9.59%
			Wood Shavings	40.00	Gal	40.00	Wood Shavings	60.00	6.28%
			Wood Chips	1.33	Cubic Yards	268.63	Wood Chips	401.93	42.06%
			Horse Bedding	1.33	Cubic Yards	268.63	Horse Bedding	401.93	42.06%
								955.54	
	33.33%	M3	Human Waste	18.34	Gal	18.34			
			Wood Shavings	20.00	Gal	20.00			
			Wood Chips	0.66	Cubic Yards	133.30			
			Horse Bedding	0.66	Cubic Yards	133.30			

Figure 3. Based on OTU hybridization intensities, this NMDS plot compares compost pile number, location within pile, and time, and indicates that pile number had less effect than either time or location within pile, which may be an indication that piles were true replicates.

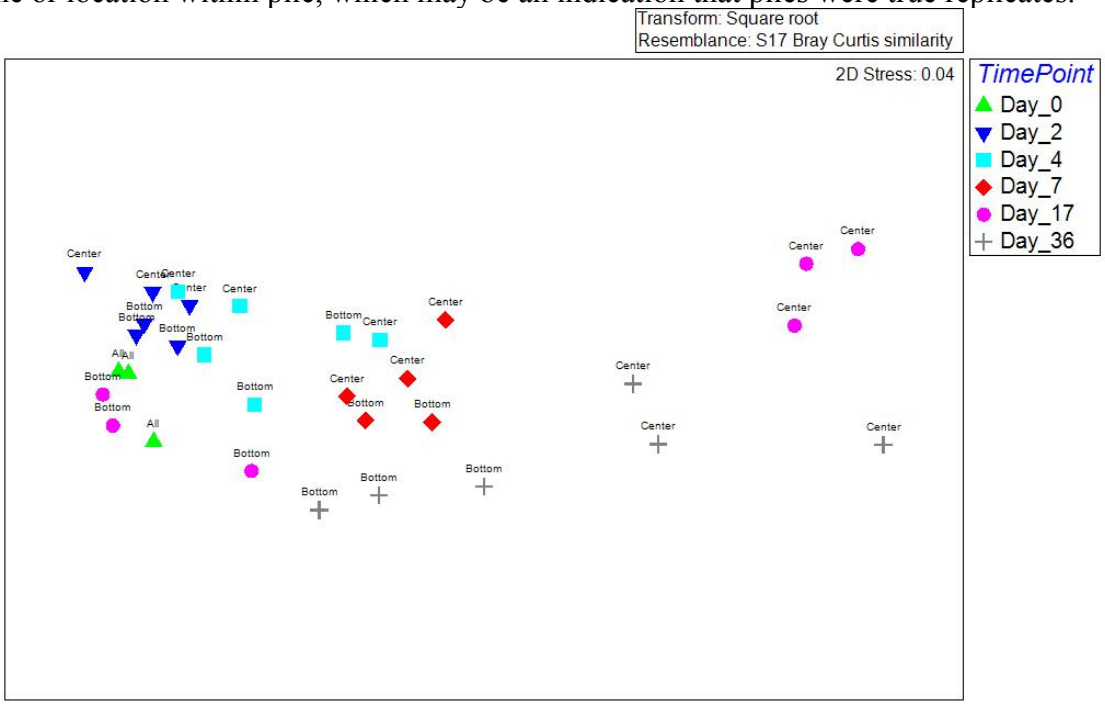

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Figure 4. Based on OTU hybridization intensities, this NMDS plot compares time and location. Especially in terms of compost pile centers, there was a marked difference in microbial populations from the beginning to the end of the 36-day composting process.

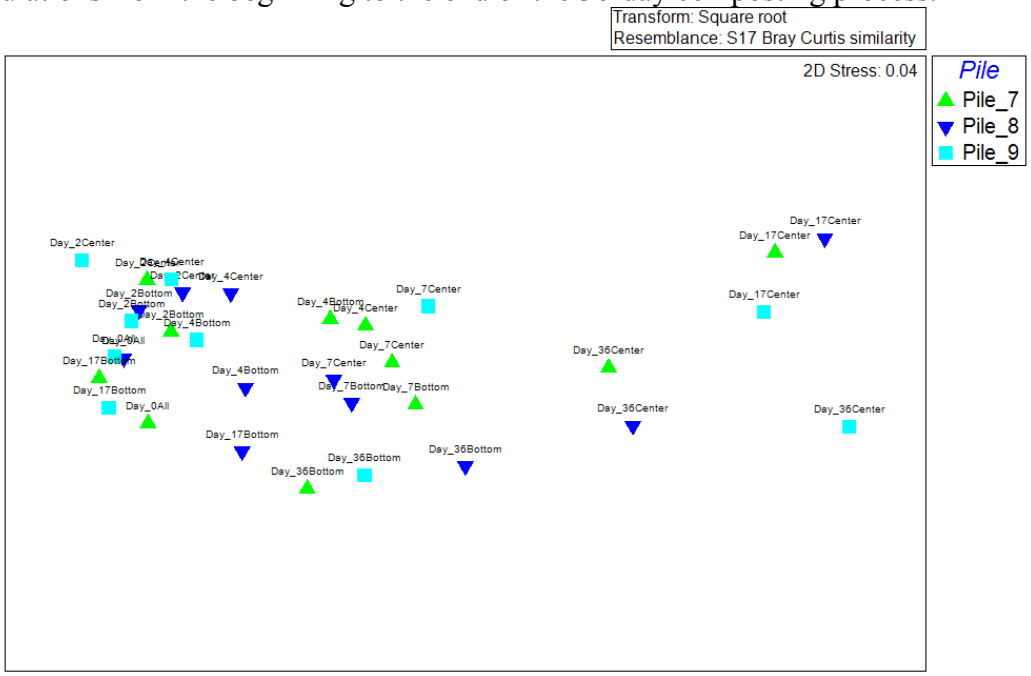

Figure 5. Average human gut microbes in compost pile center over time. Near complete destruction of human microbes occurred in 17 days.

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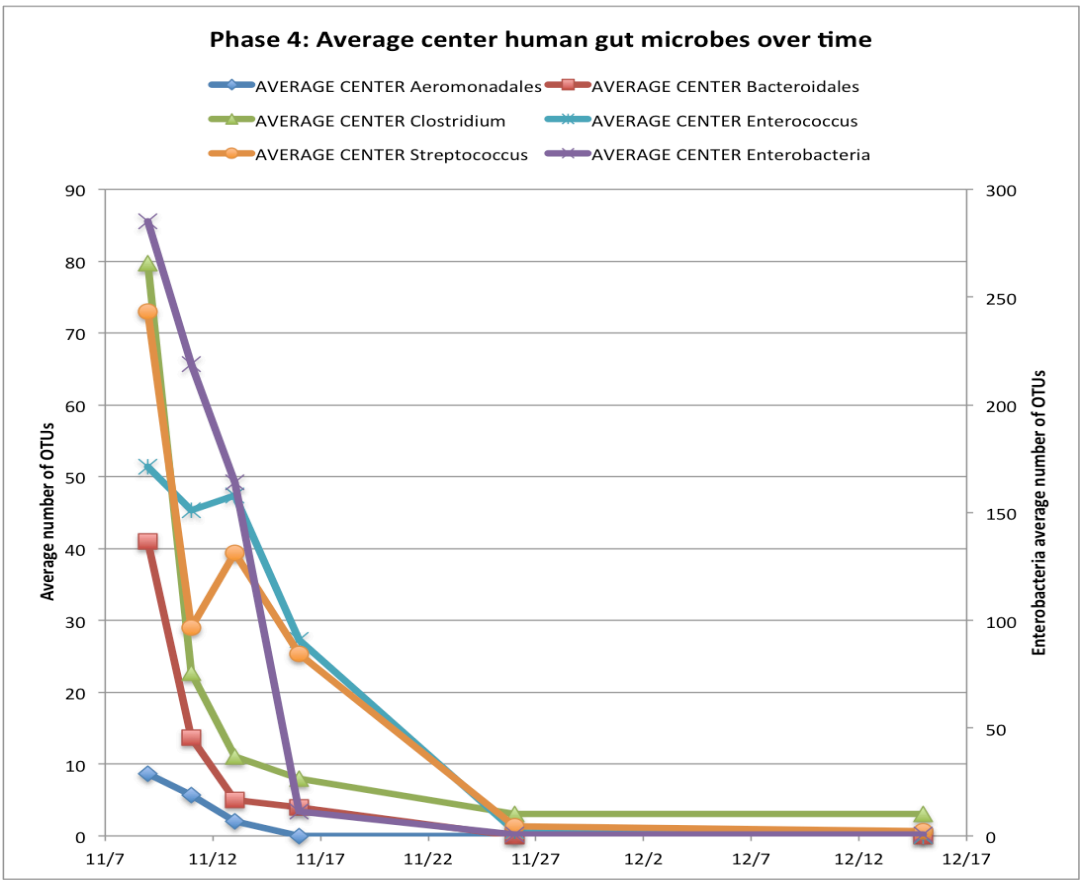

Figure 6. Average human gut microbes over time in compost pile center and center kill rate

Average human gut microbes over time in pile center							
	11/9/12	11/11/12	11/13/12	11/16/12	11/26/12	12/15/12	% Killed
AVERAGE CENTER Aeromonadales	9	6	2	0	0	0	100
AVERAGE CENTER Bacteroidales	41	14	5	4	0	0	100
AVERAGE CENTER Clostridium	80	23	11	8	3	3	96
AVERAGE CENTER Enterobacteria	285	219	164	11	1	1	100
AVERAGE CENTER Enterococcus	51	45	47	27	1	0	100
AVERAGE CENTER Streptococcus	73	29	39	25	1	1	99

Figure 7. Average human gut microbes in compost pile edge over time. Variable destruction of edge human microbes occurred in 36 days.

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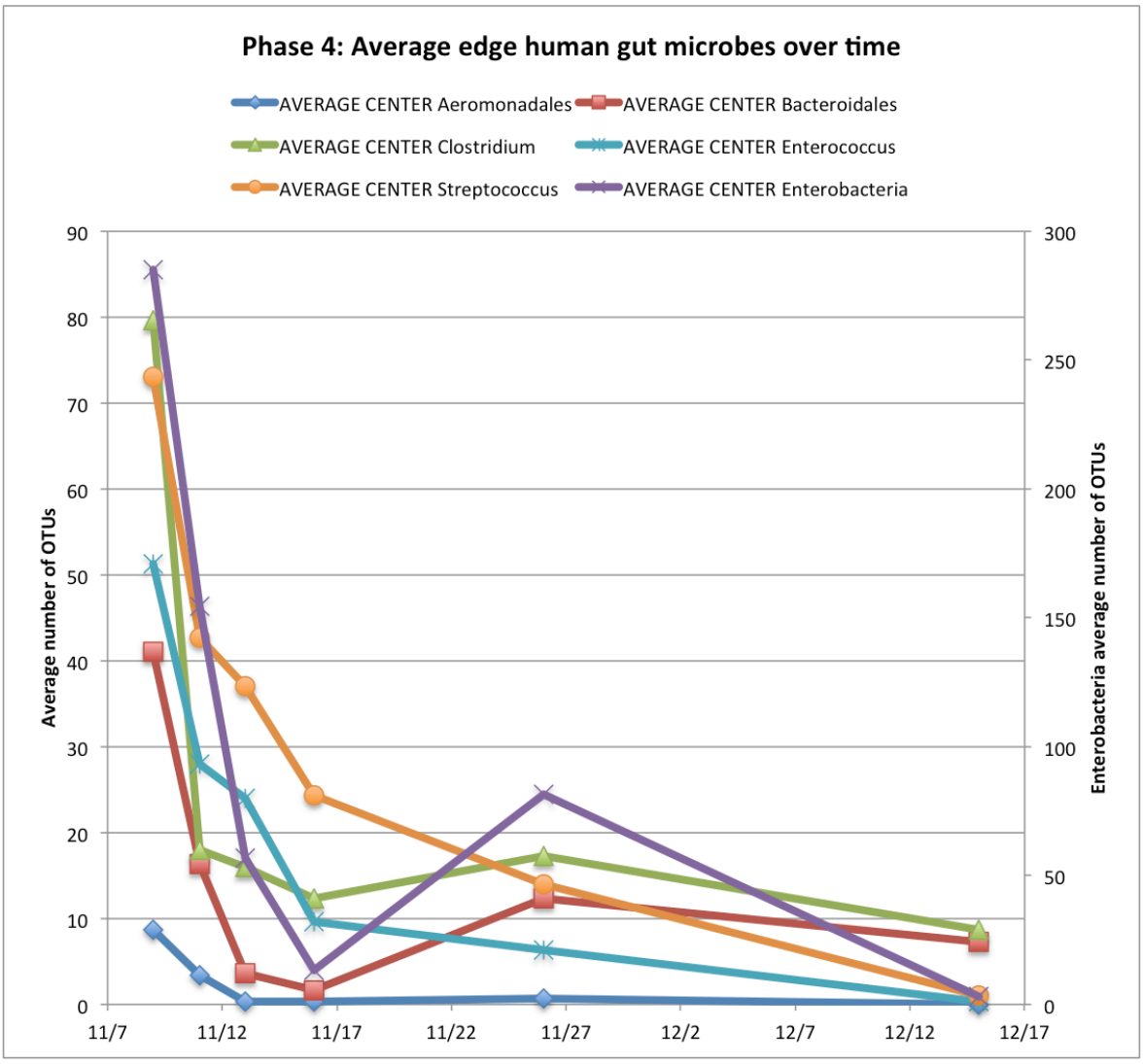

Figure 8. Average human gut microbes over time in compost pile edge and edge kill rate

Average human gut microbes over time in pile edge							
	11/9/12	11/11/12	11/13/12	11/16/12	11/26/12	12/15/12	% killed
AVERAGE EDGE Aeromonadales	9	3	0	0	1	0	100
AVERAGE EDGE Bacteroidales	41	16	4	2	12	7	83
AVERAGE EDGE Clostridium	80	18	16	12	17	9	89
AVERAGE EDGE Enterobacteria	285	154	57	13	82	3	99
AVERAGE EDGER Enterococcus	51	28	24	10	6	0	98
AVERAGE EDGE Streptococcus	73	43	37	24	14	1	99

Figure 9. Temperature in compost pile center over time

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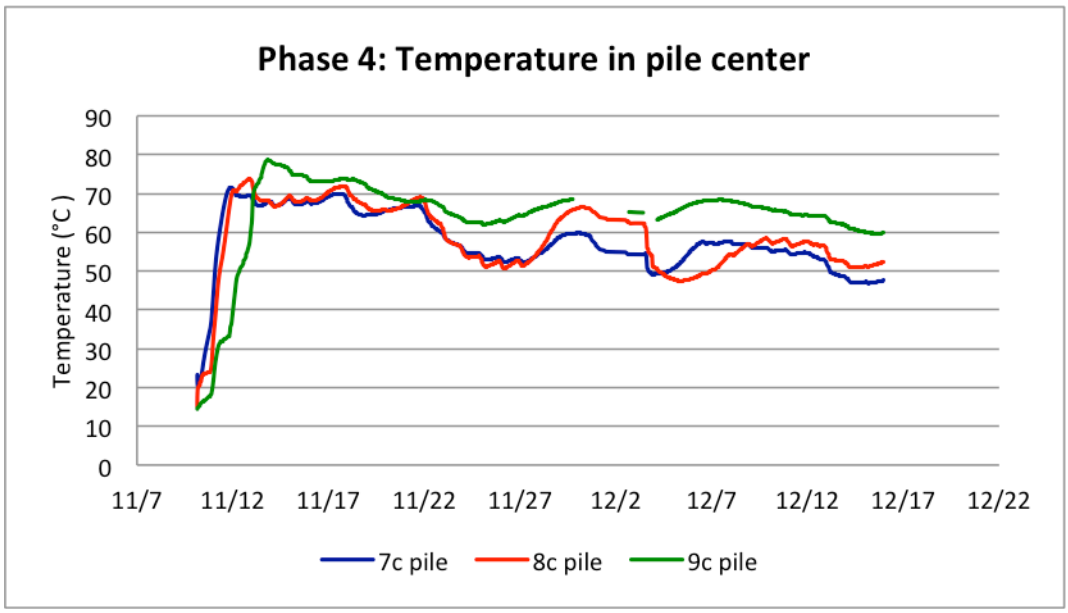

Figure 10. Temperature in compost pile edge over time

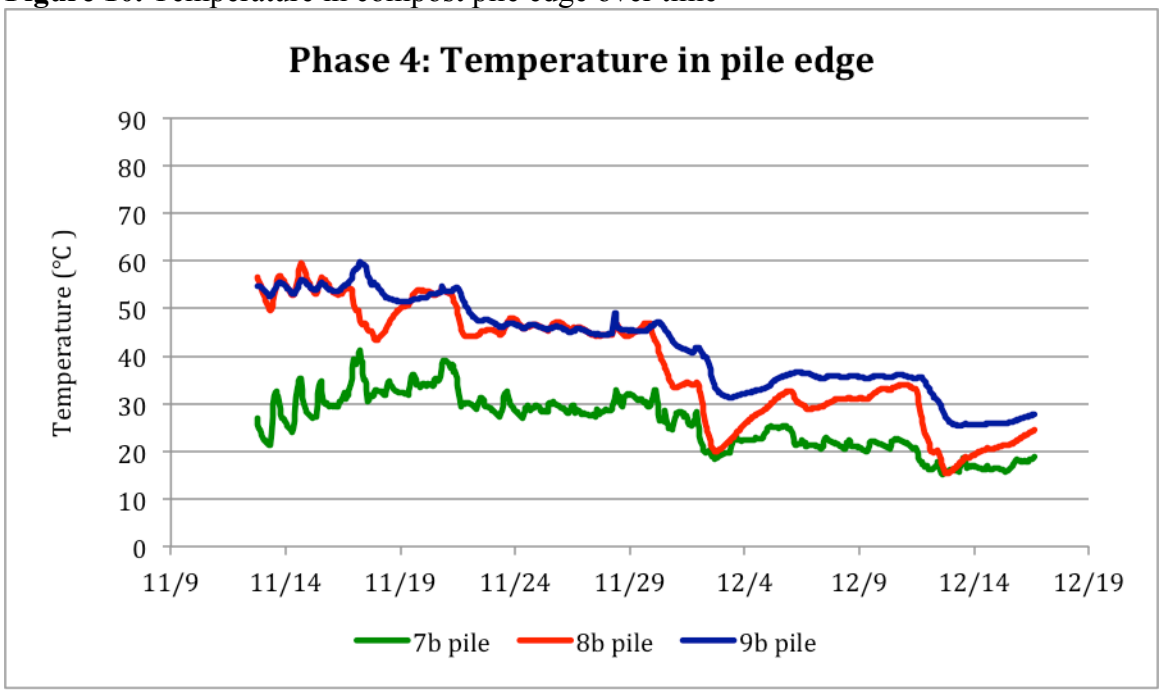

Figure 11. Human gut microbes over time in compost pile edges and kill rates

Human gut microbes over time in pile 7 edge							
	11/9/12	11/11/12	11/13/12	11/16/12	11/26/12	12/15/12	kill rate %
Pile 7 EDGE Aeromonadales	14	1	0	0	1	0	100
Pile 7 EDGE Bacteroidales	55	20	2	2	16	8	85
Pile 7 EDGE Clostridium	96	17	11	11	13	11	89
Pile 7 EDGE Enterobacteria	182	71	30	30	69	2	99
Pile 7 EDGE Enterococcus	45	30	18	18	8	0	100
Pile 7 EDGE Streptococcus	94	48	37	37	23	2	98

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Human gut microbes over time in pile 8 edge	11/9/12	11/11/12	11/13/12	11/16/12	11/26/12	12/15/12	Kill Rate %
Pile 8 EDGE Aeromonadales	7	5	1	1	1	0	100
Pile 8 EDGE Bacteroidales	31	15	3	3	6	4	87
Pile 8 EDGE Clostridium	75	20	26	26	25	6	92
Pile 8 EDGE Enterobacteria	332	135	32	10	32	2	99
Pile 8 EDGE Enterococcus	46	0	12	11	0	0	100
Pile 8 EDGE Streptococcus	62	43	43	36	7	0	100

Human gut microbes over time in pile 9 edge	11/9/12	11/11/12	11/13/12	11/16/12	11/26/12	12/15/12	kill rate %
Pile 9 EDGE Aeromonadales	5	4	0	0	0	0	100
Pile 9 EDGE Bacteroidales	37	14	6	0	15	10	73
Pile 9 EDGE Clostridium	68	17	11	0	14	9	87
Pile 9 EDGE Enterobacteria	341	257	109	0	144	5	99
Pile 9 EDGE Enterococcus	63	54	42	0	11	1	98
Pile 9 EDGE Streptococcus	63	37	31	0	12	1	89

APPENDIX

Additional Background Information about Composting

The main objective of composting is to produce a product free of pathogenic microorganisms that can be used as soil fertilizer and or amendments. Raw wastewater sludges are known to contain organisms pathogenic to humans—including viruses, protozoa, parasitic worms or their eggs, and bacteria [7] such as *Salmonella typhi* [9]. It is anticipated that composting is effective in reducing the population of human pathogens via multiple mechanisms, including heat exposure [9], [10], microbial antagonism (including antibiotic production and direct parasitism) [12], [13], organic acid and ammonia production [14], and nutrient competition [11], [13].

It is necessary to obtain more information concerning the relationships between microbial populations in composting piles and temperature. Burge et al. (1981) showed that "the degree of pathogen destruction may be determined by monitoring pile temperature" [9] and further, "due to the variability of the concentrations and effects" [9] of pathogens, it is necessary to rely on heat as the destructive agent in the composting process [9], [15]. Nevertheless, there is conflicting information regarding the critical temperature for pathogen reduction. It has been stated that pathogen reduction occurs when the compost reaches 55°C [10]; however another study recommended a threshold of 60°C to "maximize biological activity and minimize ineffective waste treatment" [16]. High temperatures (60-70°C) during the thermophilic stage of composting have been shown to reduce salmonella regrowth when compared with lower temperatures (38-55°C) [12], [4] because high temperatures decreased microbial activity [4], inhibiting the beneficial work of biological control agents to ward off reinoculation of pathogens during composting. Thus, although thermophilic composting has been employed for centuries, there is still debate over how best to achieve a safe final product. Some research suggests that it may be possible to compost human waste at lower temperatures by utilizing populations of antagonistic microbes that thrive at those temperatures to reduce human pathogen populations to acceptable levels [17].

To safely use composting as a practical method to treat human waste, such that the resulting product can be used, it is necessary to develop practical and reliable tests to assess the safety of the composted material. Although testing for the presence of fecal coliforms has been used to assess quality control, this method is no longer considered reliable due to the fact that fecal coliforms grow well in finished compost, making it impossible to prevent the reinoculation

of the product at outdoor compost sites [9].Finding a test to reliably assess compost safety requires a better understanding of the dynamics of microbial succession implicit in the composting process as well as the way in which temperature fluctuations relate to compost mixture composition.

Additional Information about Results

The 16S rRNA gene was chosen for probe analysis to identify entire microbial communities and their dynamics because it is highly conserved evolutionarily. Nevertheless, this high degree of conservation also leads to resolution issues when looking at detailed levels of genera, species, or pathogenic strains such as *Escherichia coli* O157:H7, due to the fact that multiple species can fall into a single OTU. This means that while the PhyloChip may detect a certain OTU as present in the samples, it could be a case in which the 16S rRNA gene sequence of one species is too similar to differentiate it from a number of related species. While it is unlikely that the species that fall into the same OTU as a pathogen will behave in the same or similar manner across thermophilic composting, it has been shown that microbial reactions to environmental changes can differ drastically between strains, species, and, especially genera [8]. Another criticism of the PhyloChip is that it does not distinguish living from non-living, so even after a microorganism dies, the PhyloChip will indicate its presence as long as that organism's DNA persists. Finally, much of the analysis presented in this paper is based on PhyloChip binary data that is dependent on predetermined cutoff values. Any species that misses even one of the cutoff values will be given a '0', indicating absent. Thus, although this examination of pathogen destruction is comprehensive, it is not complete. Ideally, this study would be complemented with additional work.