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

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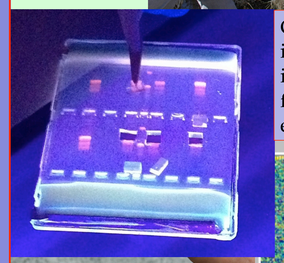
## ABSTRACT

How effective are static piles in treating human waste? If proven to be effective, thermopiles may provide a cheep 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 in 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.

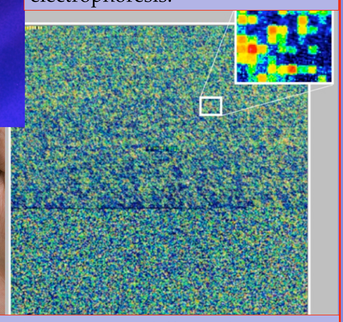
## METHODS

- Three replicate human manure compost piles containing human waste (urine and feces), wood shavings, wood chips, and horse bedding
- Hourly monitoring of temperature (center and edges), oxygen and moisture
- Periodic sampling for microbial DNA, carbon/nitrogen, greenhouse gases (CH4, N2O, CO2)
- PhyloChip characterization of microbial communities throughout process
- Human gut microbes used as conservative indicators of health risk

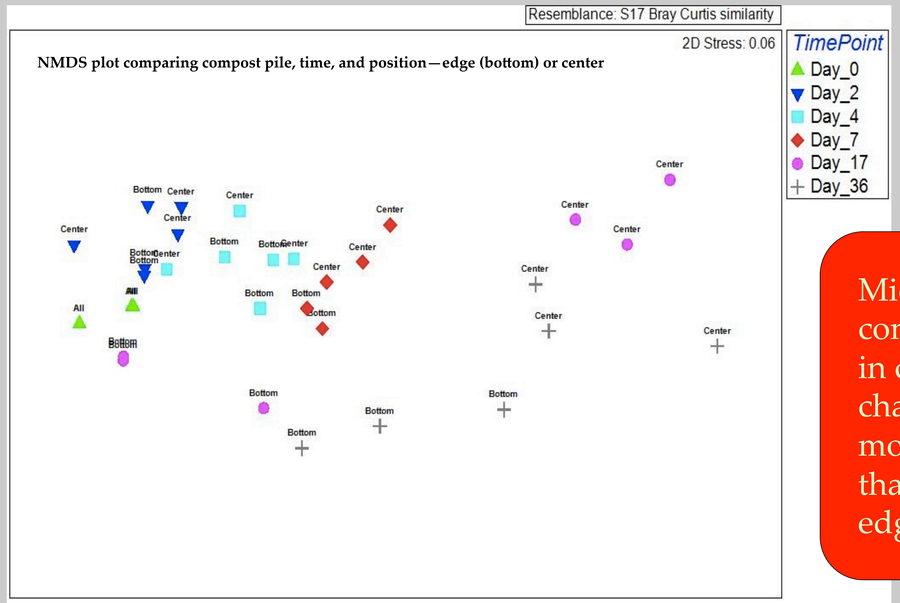
The straw bail encasing used to insulate compost piles



Gel extraction was required to isolate a Archaeal fragment of intact DNA from the agarose gel following agarose gel electrophoresis.

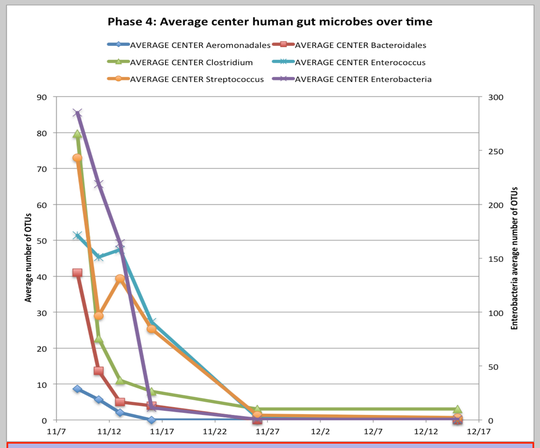



- Berkeley Lab PhyloChip detects 60,000 different bacteria and archaea in one test
- Measures occurrence and relative abundance of all organisms simultaneously
- Probes 16S rRNA gene composition of entire microbial community

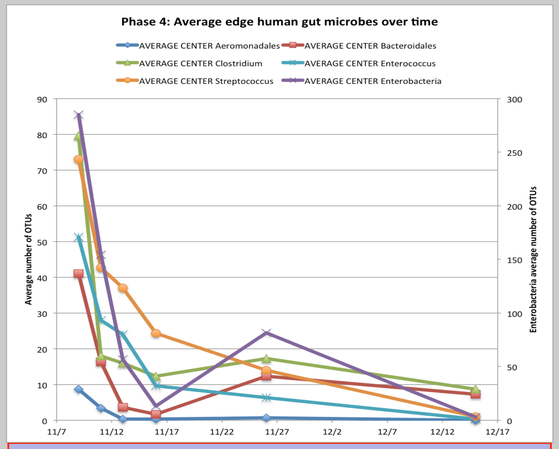



Sample clustering is primarily dependent on time and location, not pile. It is not until day 17 that microbial communities really began to differentiate. (NMDS plots are based on OTU hybridization intensities.)

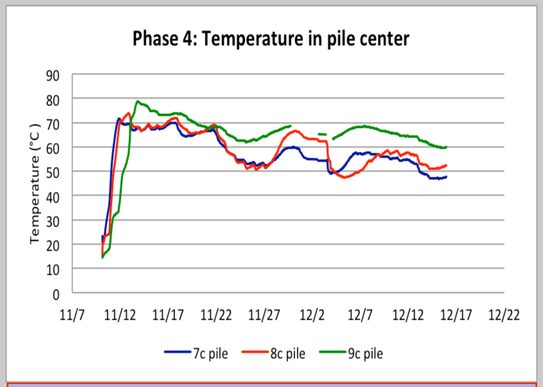
Microbial communities in centers changed more rapidly than those in edges



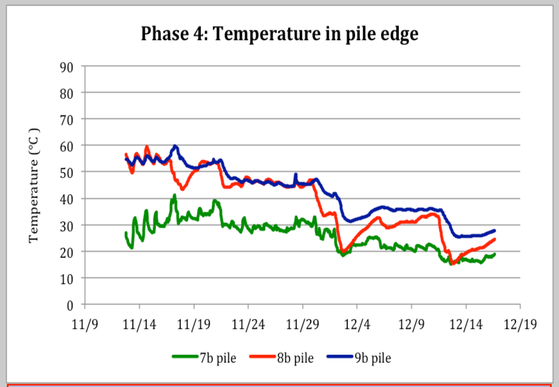

After 17 days, the center human gut microbe kill rate was 96-100%.




After 36 days, the edge kill rate was 98-100% for *Aeromonadales*, *Streptococcus*, *Enterobacteria*, and *Enterococcus*. For *Bacteroidales* and *Clostridium*, edge kill rates were lower, between 83-89%.




Compost pile temperature in pile center over a 36-day monitoring period. All three pile centers reached 68°C within the first 3 days.




Compost pile temperature in pile edge over a 36-day monitoring period. The edge of pile 7 does not exceed 41°C, while the edges of pile 8 and 9 hit 60°C.

## RESULTS

**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 | Kill Rate |
|---|---|---|---|---|---|---|---|
| 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 % |

**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 | Kill Rate |
|---|---|---|---|---|---|---|---|
| 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 EDGE **Enterococcus** | 51 | 28 | 24 | 10 | 6 | 0 | 98 % |
| AVERAGE EDGE **Streptococcus** | 73 | 43 | 37 | 24 | 14 | 1 | 99 % |

## CONCLUSION

**Summary**

- Near complete reduction of human gut microbes occurred in compost pile center after 17 days.
- After 36 days, compost pile edges achieved variable reduction of human gut microbes. At this time, the the edge kill rate was 98-100% for *Aeromonadales*, *Streptococcus*, *Enterobacteria,* and *Enterococcus*. The edge kill rate for *Bacteroidales* and *Clostridium* was 83% and 89% respectively.

**Conclusions**

- Based on our results, human waste needs to be composted for at least 36 days, to render it safe for land application.
- The persistence of 17% of *Bacteroidales* and 11% of *Clostridium* may be 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.
- 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.
- Results indicate static piles are effective in treating human waste; however, further studies are recommended to determine optimal edge temperature.

## ACKNOWLEDGMENTS

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