



# Composting Deactivation of CWD Prions



Amber Smith, Susanna Baker, Jonathan Girard, Robert Michitsch and Alex Thomas

## Introduction

Through hunting, slaughtering or ingestion of CWD infected cervids, exposure to specified risk materials is considered an exposure route to prions that might lead to infection. Prions cause transmissible spongiform encephalopathy (TSE) diseases in animals and humans. The composting process has proven effective for the biodegradation of recalcitrant organic contaminants, and the high number of microorganisms and high temperatures achieved during composting have prompted interest in this process for inactivating prions; however, literature on survival of prions in composting systems is limited. Since thermophilic temperatures do not definitively cause pathogen reduction, multi-barrier approaches are employed to improve pathogen inactivation. As such, primary-phase duration, C-substrate, anaerobic conditions, drying, storage, antagonistic microorganisms, ammonia evolution, microbial inoculants and other degradation methods have been used to establish an unstable habitat for pathogen survival. Compost piles offer or complement these different approaches, which may prove useful to degrade infectious prions.

## Objectives

- Compost CWD infected deer remains in a Summer and Winter Wisconsin climate.
- Inactivate *E. coli* NAR as an indicator of pathogen inactivation (taking place at University of Wisconsin-Stevens Point).
- Assess the composting process for CWD prion deactivation in soil, leachate and compost samples (taking place at University of Wisconsin-Madison).

## Study Area

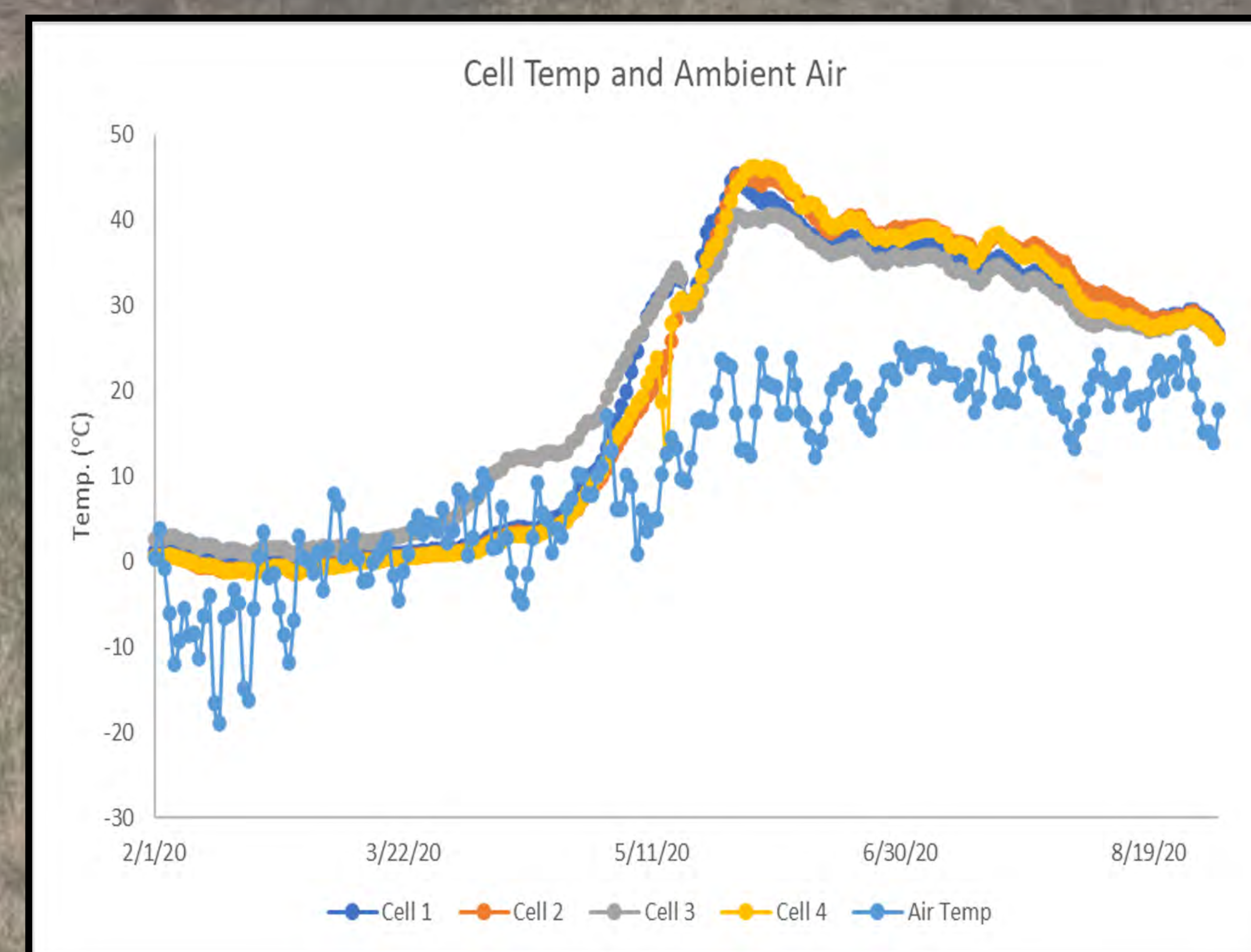


Map of the Almond Farm Site in Almond, WI, where composting took place. The site, a deer farm that was infected with CWD, is now restricted and double-fenced.



## Methods

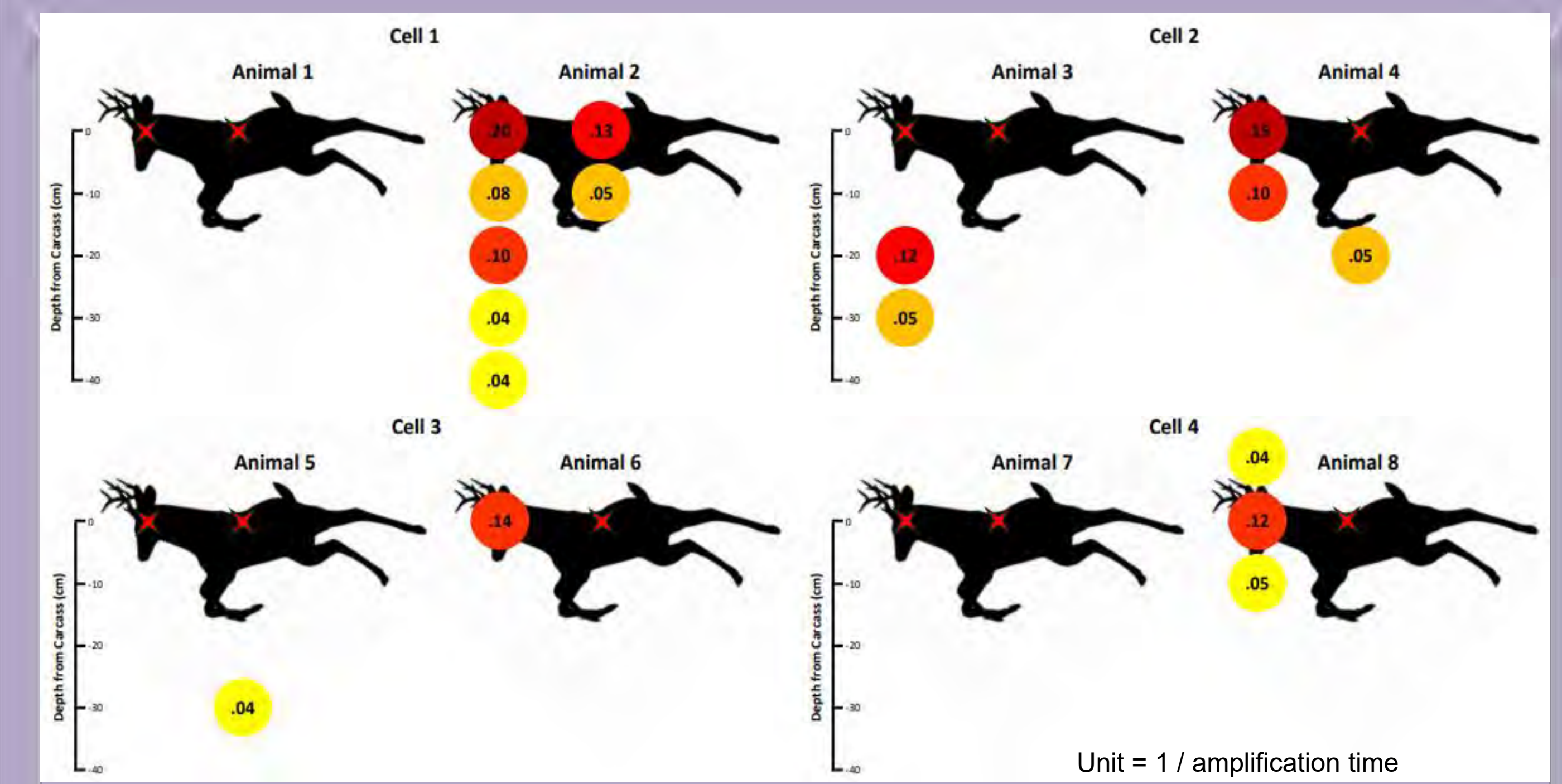
- Five 8 x 16' composting cells constructed.
- Four variable cells and one control cell.
- In each cell: 0.5" layer of pea gravel, 12" layer of locally sourced subsoil; 24" layer of locally sourced sawdust; CWD infected cervid butchery waste with CWD infected brain matter; 12" of sawdust, shaped to create typical composting windrow.
- Composting process monitored continuously in real-time by thermocouple temperature probes.
- Soil moisture and soil temperature sensors placed in soil layer of each cell.
- Compost temperature sensors placed below and above the deer carcasses in each cell.
- Each cell has 3 dialysis bags containing non-pathogenic strain of *E. coli* NAR and homogenized CWD-infected brain material. *E. coli* NAR inoculated into compost matrix in addition to dialysis bags.
- Effluent samples taken periodically to determine presence of *E. coli* NAR and prion. RT-QuIC (real-time quaking-induced conversion) used to detect presence of CWD at University of Wisconsin-Madison.
- Active composting phase for 4-6 months, followed by secondary composting phase of 3-6 months after samples are taken.
- Emptied CWD infected material and sawdust out of the cells and onto tarps that are also monitored for temperature and additional breakdown.
- Final compost samples analyzed according to United States Composting Council Seal of Testing Assurance parameters, and for *E. coli* NAR and prion inactivation.



## Results

Sample	pH	% Moisture	C/N	Ext Ammonium (mg N/g)
Soil	6.18	12.92%	6.02	5.75E-02
Initial Soil	6.87		8.44	2.51E-03
Composts	6.89	69.47%	42.78	8.27E-01
Initial Sawdust	4.89		99.73	2.05E-04
Ref. Soil	5.42		9.08	5.89E-03
Ref. Compost	7.13		13.34	7.97E-03

Sample	Ext Nitrate (mg N/g)	Ext P (mg P/g)	Ext K (mg K/g)
Soil	1.68E-01	2.45E-02	1.11E-01
Initial Soil	1.47E-01	1.98E-03	7.98E-02
Composts	3.41E-01	2.86E-01	4.83E-01
Initial Sawdust	0	2.47E-02	2.76E-01
Ref. Soil	6.94E-05	3.85E-03	3.47E-02
Ref. Compost	9.91E-02	2.38E-01	1.2



## Future Work

- Explore variables in the composting process, such as inoculated microorganisms, carbon source and additional feedstocks, soil type and textural differences underneath a pile, source of waste (carcass vs. butchery waste), or other primary phase composting parameters.
- Further explore the impact of various soil types and factors on the infectivity of the prion.
- Investigate potential vectors for the spread of the prion, such as insects and birds.



## Acknowledgements

This is a collaboration between the Michigan and Wisconsin Departments of Natural Resources; the Wisconsin Department of Agriculture, Trade and Consumer Protection; Michigan State University; University of Wisconsin-Madison; University of Wisconsin-Stevens Point. The adaptation of RT-QuIC for detection of CWD prions in compost samples will be conducted at UW-Madison by Dr. Joel Pedersen and team.



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