Emma Trotta
Supervisor: Naowarat Cheeptham, Lisa Forth
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Directed Studies Final Report:

A microbial perspective on cardboard waste reduction through vermicomposting

Intro/Background: objective

Our world is a precious and complex system. The way we treat the environment can have and has already had catastrophic impacts. Fortunately, our world is forgiving and able to adapt to changes. If the entire planet is able to change, why can't we as a society change as well? Taking care of the environment will ultimately benefit the world we live in. Specific to agricultural systems, there are many aspects that can be improved upon. For example, composting is a great way that every individual person can make a positive impact on the environment and improve agricultural systems as well as reduce waste that would otherwise end up in landfills.

Vermicomposting is a specialized method of composting that involves earthworms to break down organic matter. In the digestive tract of earthworms, digestive enzymes, hormones and microorganisms break down organic material. The organic material that passes through the digestive tract of the earthworm gets excreted in the form of "castings", which can be used as compost for plant agriculture (Pathma and Sakthivel 2012).

The conditions for vermicomposting to work successfully requires a "bedding" for the earthworms to live in. The bedding helps control moisture and provide protection for the earthworms. The object of this study is to determine if the difference in bedding type used in vermicomposting, either a mixture of leaf mold and cardboards or just leaf mold, will impact the microbial diversity of the compost product. This project focuses on the effects cardboard bedding can have on microbial diversity in compost. Compost with an increased microbial community composition balances the soil ecosystem and improves the health of the soil and plants (Pathma and Shakthivel 2012). Many microbes share a symbiotic relationship with plants and aid in plant growth and yield, nutrient uptake and nutrient cycling. Therefore, having more bacterial and fungal diversity within the compost results in a preferable compost for agricultural use (Pathma and Shakthivel 2012).

This project additionally aims to tackle the issue of cardboard recycling. In Canada, 85% of old corrugated boxes are recycled, however this still results in a high percentage that ends up in Canadian landfills (PPEC. 2023). Cardboard that ends up in landfills contributes to the large amount of greenhouse gas emissions released into the atmosphere. In Canada, methane gas from landfill contributes to 20% of the national methane emissions (Malmir et al. 2023). The

cardboard that is recycled goes through the recycling process which requires large amounts of water by using a hydrapulper to break down the material (Recycle BC. 2023). Environment Canada stated that it takes about 324L of water to produce only 1kg of paper (BPR 2023). Using cardboard as bedding for vermicomposting can help reduce the cardboard that ends up in landfills and reduce the water consumption required in the recycling process.

With the help of the City of Kamloops our goal of reducing cardboard waste and creating a compost produced that is beneficial for agriculture use can be achieved for this project. The City of Kamloops is funding this project through the 2024 Climate Action Grant. Through the City of Kamloops Residential Waste Collection Program, they are trying to shift the behavior of local residents and encourage them to compost their food waste but also their cardboard. This partnership with the community allows this research to be funded for laboratory supplies, DNA and RNA metagenomic sequencing as well as an opportunity to spread the word about the benefits of composting.

Methods:

To conduct this experiment, vermicompost was created by Lisa Forth. To prepare the compost for this project, pre-composted food and yard waste began to decompose in large green composting bins in September of 2023 as initial food for the earthworms. Leaf and leaf mold was collected from the Kamloops area to be used for the project. For this experiment small plastic bins were used to hold the compost. Ten bins in total were used, five bins of the 100% pre-composted yard waste bedding blend and five bins of 50% pre-composted yard waste bedding blend and 50% cardboard.

1 lb of Red Wiggler worms (approximately 1000 worms) ordered from Pacific Composting were used in this experiment. Once the worms arrived, they remained in their box for an additional 24 hours before being moved to their new bedding to reduce the stress on the worms. 100 worms were placed into each bin containing its specific type of bedding. The bins were separated into non-cardboard and cardboard containing compost bedding. Each tray was covered with bubble wrap to retain moisture and allow for air circulation and additional covered with loose black plastic to keep light out.

To maintain the compost, food waste was added based on how much food is already present for the worms to eat. Additionally, to keep the desired moisture, each bin was sprayed with DE chlorinated water mixed with molasses and Sea Storm and sprayed in the bins, to lightly re-moisten the bedding.

For each tray the bedding was pushed to the right side of the tray and new food waste was added to the left side of the tray once the thermophilic stage or active composting stage finishes

and cools down. This method of feeding causes the worms to move to one area which should allow for optimal breeding.

Table 1. Components of pre-composted yard waste and cardboard bedding for composting bins.

Pre-composted yard waste bedding blend 100%	**Pre-composted yard waste bedding blend 50% and cardboard 50%**
City of Kamloops pre-composted yard waste	City of Kamloops pre-composted yard waste
Egg shell dust	Egg shell dust
Sea Storm (combination of seaweed, kelp and humic acid)	Sea Storm (combination of seaweed, kelp and humic acid)
Black Strap Molasses	Black Strap Molasses
River bottom mineral rock dust	River bottom mineral rock dust
Leaf mold	Leaf mold
	Shredded cardboard

Samples of the non-cardboard and cardboard containing compost were collected about every month in the premature composting phases. Once samples were obtained they were taken to the Thompson Rivers University CL-2 Cave Microbiology Laboratory.

To determine the microbial growth within the two compost bedding types, serial dilutions were made and plated on both Nutrient agar and Potato dextrose agar plates. To create the serial dilutions, the compost samples were mixed with sterile water to create a slurry. 100µl of the stock slurry solution was transferred to an eppendorf tube containing 900µl of sterile water. From this, 100µl of the diluted samples was added to the next eppendorf tube and was repeated up until a x10-6 dilution was obtained. Dilutions x10-1, x10-3, x10-4, and x10-5 for both the cardboard and non-cadboard samples were plated on Nutrient agar and Potato dextrose agar and used the hockey stick method to spread plate the bacteria. The agar plates were placed in the incubator at 30°C for 24 hours. The x10-4 plated dilution was incubated for 72 hours.

Colony Forming Units (CFU) from the initially plated bacteria and fungi were counted for the six different plated dilutions. Not every plate was valid in these calculations and therefore was not used because the number of colonies exceeded 300. Only plates that lay within the range of 30-300 colonies per-plate were used to calculate the CFU.

From the initially plated bacteria and fungi, macroscopic morphology observations like colour, size, shape, margins and elevation was determined in order to separate bacteria and fungi

into different “species”. Different species were isolated on the same agar and incubated at 30°C. The bacteria grown on Nutrient agar was intubated for 144 hours while the fungi present on the Potato dextrose agar was isolated and intubated for 48 hours.

From these isolates on the Nutrient agar, the different bacteria that grew was subjected to a Gram stain test to determine its microscopic morphology. The bacteria was visulated under 100x power and oil for the best results. The fungi grown on Potato dextrose agar will also be stained to observe the microscopic morphology using Methyl Blue, later in the project.

The results of the macroscopic and microscopic morphology observations were used for statistical tests, Simpson's Diversity Index, Shannon Diversity Index and Bray Curtis Dissimilarity to determine the microbial diversity between the two composting types.

Results:

When comparing the microbes present in vermicompost that contains cardboard in its bedding and compost that does not, it is clear that there is a difference between the two bedding types. Based on the microscopic and macroscopic morphology of the bacteria colonies grown on Nutrient agar, it was found that both bedding types did contain some similar bacteria but also varied. In the non-cardboard containing samples seven different bacterial species were identified (R=7) and for the compost samples containing cardboard, ten different species were identified (R=10). From these identified bacteria three of them are present in both the cardboard and non-cardboard samples while the others are different. The three similar bacteria based on macroscopic morphology are the colonies that are white and then turn gray over time, white elevated colonies and light tan glossy colonies.

For each of the bacteria identified, the colonies from all the plated dilutions were counted. From these counted colonies, the ratios needed for Simpson's Diversity, Shannon’s Diversity and Bray Curtis Dissimilarity were calculated. The results of the statistical test show that vermicompost that does not contain cardboard has a Simpson’s diversity index of 0.59 (D=0.59) and Shannon Diversity of 1.2 (H’=1.2), while the samples that does contain cardboard have a Simpon’s diversity index of 0.68 (D=0.68) and a Shannon Diversity of 33.6 (H’=33.6). When the two bedding types are compared to each other it gives a Bray Curtis Dissimilarity of 0.76 (BCd=0.76).

Table 2. Statistical analysis of non-cardboard and cardboard vermicompost samples to determine microbial diversity based on Simpson's and Shannon's Diversity Index and species richness.

	Simpson's Diversity Index (D)	Shannon's Diversity Index (H')	Richness
Non-cardboard	0.59	1.2	7
Cardboard	0.68	33.6	10

The results of the microscopic observation based off of the Gram staining, revealed that Gram positive bacteria are the most abundant in both bedding types. Gram positive bacteria found in cardboard containing compost took up 63% and the non-cardboard compost showed 83% Gram positive bacteria as seen in Figure 1. From the Gram staining results it was interesting to find that most Gram positive rod shaped bacteria in both the bedding types contained endospore bacteria. As seen in Figure 2, the pink circles within the purples rod bacteria is the endospore found in a non-cardboard sample.

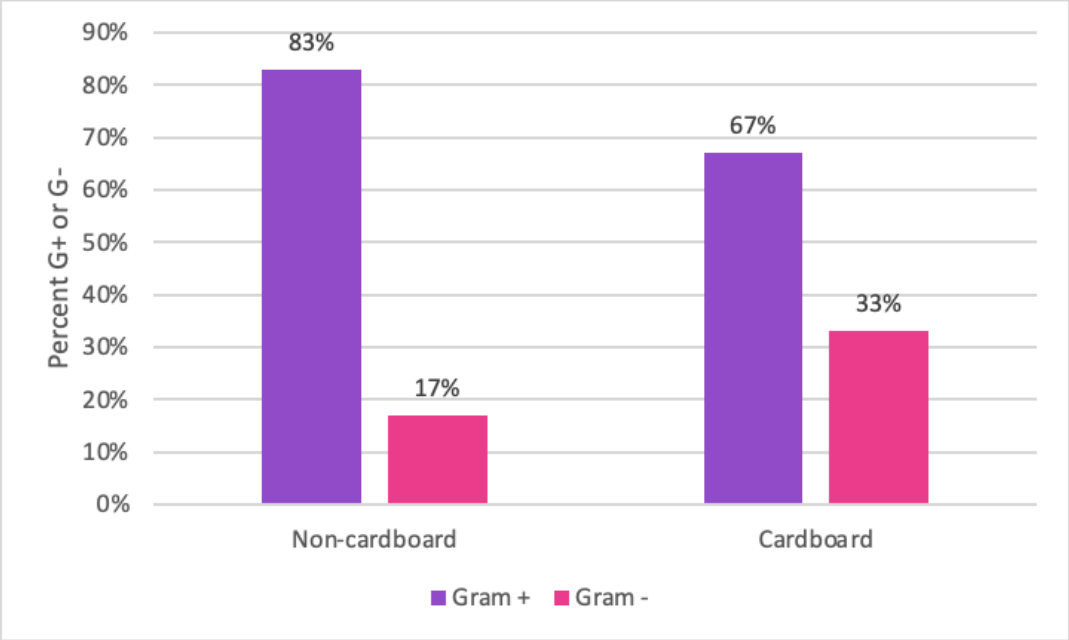

Figure 1. Gram staining results of the bacteria grown on Nutrient agar for both non-cardboard and cardboard vermicompost samples.

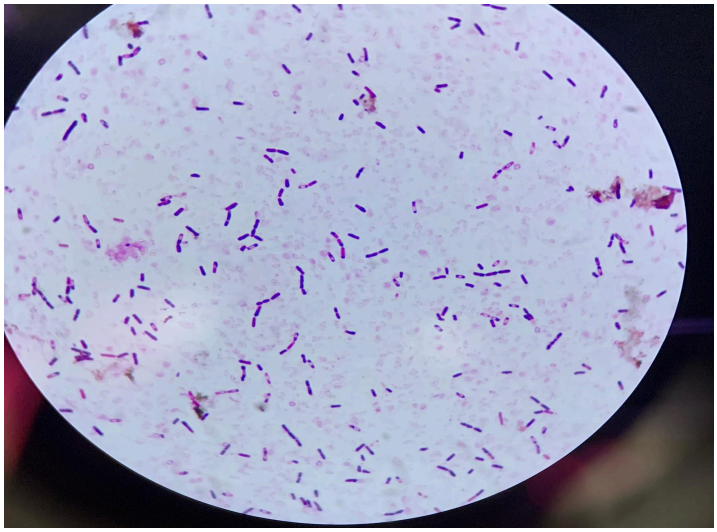

Figure 2. Gram positive rod shaped endospore bacteria found in non-cardboard vermicompost.

Discussion/ Future work:

Based on the results of this experiment it is found that the microbial community does differ by changing the bedding type in vermicompost from having no cardboard to adding cardboard. The Bray Curtis Dissimilarity of 0.76 which is close to 1 implies that the two communities are different from one another. It is also found that vermicompost containing cardboard has a greater species richness and diversity within the community (R=10, D=0.68). Having a more diverse microbial community means that there is a greater variety of bacteria to perform different roles within the community. As mentioned before, the relationships bacteria share with plants is important for plant growth and nutrient cycling. Having a more diverse array of bacteria present in the cardboard compost could potentially allow for better plant health and yield.

As society tackles to fight Climate Change, being able to reduce and reuse items has now more than ever been one of the top priorities. Not only does cardboard improve the compost that can be used in agricultural systems, it also helps reduce cardboard waste that would end up in landfills and as well as help reduce waste consumption in order to recycle the cardboard. Composting cardboard is a less destructive way to dispose of it without causing an immense increase in greenhouse gasses or over consumption of water.

To further confirm the results of this experiment, metagenomic sequencing of the 16S rRNA gene will be done. The sequencing results will provide a more accurate interpretation of the similarities and differences between the two microbial communities. With sequencing confirmation that could support the results of this experiment, will lead to other research questions. Further research into the cardboard bedding type can be done to explain why the

microbial community and earthworms are reacting differently to the presence of cardboard and if the bacteria present will aid in plant health and yield.

Further research partnering with the City of Kamloops will be looking at different types of cardboard that contains heavy inks, dyes and glue to see if these toxins have an impact on the microbial community.

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