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RESEARCH & INNOVATION CENTRE

Soil Applications for Flume Sediment

A Final Report prepared for the Ontario Processing Vegetable Growers - Ontario Tomato Research Institute

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Executive Summary

Vegetable processing companies produce vast amounts of soil sediment from washing field-grown vegetables. Due to this sediment being collected from multiple agricultural fields and farms, as well as being processed in an industrial facility (Highbury Canco and Conagra Brands), this sediment is classified as non-agricultural waste, which typically cannot be directly applied to field, and therefore incurs landfilling costs. Reusing it requires a Non-Agricultural Source Materials (NASM) application and approval from Ministry of Agriculture, Food and Agribusiness (hereafter OMAFA), which can be a lengthy process discouraging its reuse. With a single 150,000-square-meter facility generating over 2 million kilograms of flume annually, alternative reuse methods are needed to reduce costs and promote sustainability for the vegetable processing industry.

In 2023, the Vineland Research and Innovation Centre (hereafter Vineland) and the Ontario Processing Vegetable Growers (hereafter OPVG) evaluated reuse opportunities for flume sediment from processing. The study indicated potential benefits such as soil amendment in horticulture or arboriculture, but recommended additional analysis to characterize the flume properties that could enhance soil health and quality. This report builds upon the 2023 findings, as Vineland continued this research, exploring flume uses as a soil amendment and methods to measure or remove pathogens. The project's goal is to assess flume as a soil enhancer for field crop production, focusing on soil health.

Key findings from the project are as follows:

Key Findings

1	**Risks of Tomato Brown Rugose Fruit Virus Transmission** Tomato brown rugose fruit virus was observed in Conagra Brands flume, and further research is required on viral load. For reuse of flume sediment, crops that are not susceptible to rugose should be prioritized over those that are rugose-susceptible.
2	**Increased plant available nitrogen with incorporation of flume** Flume applications were observed to increase ammonia/ammonium nitrogen in the soil. This form of nitrogen is plant available and easily taken up by plant root systems.
3	**Increased soil respiration with incorporation of flume** Flume application was observed to increase soil respiration, which indicates that flume adds microbial activity to the soil, which can aid in soil organic matter breakdown and nutrient release.
4	**Flume has high electrical conductivity which was demonstrated to have high phytotoxic influence on seed germination** A clear relationship was observed across the data, with increasing flume concentrations resulting in progressively lower germination rates, shorter radicle lengths
5	**Caution should be exercised when working with and using both Highbury Canco and Conagra flume** Many animal and plant pathogenic species sequences were retrieved. The current analysis does not determine the incidence of live pathogens in the samples tested only whether their DNA was present at the time of study, and therefore while caution is warranted, the level of risk due to pathogens to health, crop quality and yield is unknown. It is not recommended to apply susceptible crops, or in areas near susceptible crops.
6	**Flume sediments from tomato production contain phytotoxic steroidal alkaloids as the most abundant chemical components.** These natural chemicals made by tomato plants are known phytotoxins and likely a major factor in the flume-dependent inhibition of seed germination and radicle growth.

Project Description

Background

Non-agricultural source materials provide organic matter that maintains soil productivity, reduces erosion, and supplies nutrients for crops, potentially reducing the need for growers to add fertilizer. Although vegetable processing sediment is classified as non-agricultural waste, making disposal costly, NASM approval can enable agricultural use of such waste, reducing processor costs and promoting sustainability. In 2023, Vineland explored reusing flume sediment from vegetable processing with OPVG. The aim was to assess flume properties for odor control and potential reuse, especially in horticulture and landscaping applications (eg. tree nurseries). Preliminary results suggest flume sediment could be a viable soil amendment, but thorough property assessment is necessary to ensure it benefits soil health and function. This study also identified the challenges of flume as a NASM, as the samples may not always meet the nutrient requirements of a NASM for field use. In the 2023 study, the flume samples analyzed from the Conagra Brands processing facility passed NASM testing, while the samples from Highbury Canco failed due to low nutrient levels in the samples.

Tomato flume qualifies for beneficial reuse under specific conditions of the Ontario Nutrient Management Act, 2002:

- Solid NASM materials: Total concentration of plant-available nitrogen, phosphate, and potassium must exceed **13,000 mg/kg**.

Despite showing optimal concentrations of magnesium, manganese, and iron, the flume sediment often lacks sufficient nitrogen, phosphorus, and potassium in its dry form to qualify as a NASM under the Nutrient Management Act. This presents the challenge of reusing flume sediment, as it relies on nutrients from fertilizers applied to a farm to help meet these requirements.

Outside of the nutrient content of flume, the 2023 analysis of Highbury Canco's flume sample revealed its potential benefits when used as a soil amendment. Soil respiration tests showed high microbial activity, enhancing biological function. The flume sediment displayed optimal saturated hydraulic conductivity, suggesting improved infiltration and reduced stormwater runoff. No toluene or methyl phenol were detected in the chemical analysis. The 2023 Conagra flume sample showed limited biological and nutritive function but had beneficial hydrological properties for horticultural and landscaping uses. Low microbial activity indicated limited soil microbial support. Nutrient analysis revealed optimal total nitrogen and total potassium, suggesting potential long-term plant growth benefits. The primary volatile organic compound was toluene (>75%), with methyl phenol (cresol) also present (~20%). Toluene's presence likely results from anaerobic fermentation during flume storage. Mitigation strategies might reduce toluene and its malodorous byproducts. Vineland’s 2023 results indicated that the flume sediment might be applicable to build microbial soil communities, reduce compaction and improve the overall infiltration and water holding capacity of field soils used to produce non-rugose-susceptible crops. Although they are key soil properties to build soil health, these properties are not a focus of the NASM requirements for beneficial reuse.

The current (2024) study focuses on the assessment of nutrient requirements for new batches of flume and potential soil applications for flume, with a focus on the four following activities:

1) Soil incubation trials and NASM-related analyses to define recommended application rates for agricultural use. A lab-based incubation trial was conducted to evaluate carbon mineralization/ breakdown of the flume amendments. Soil respiration, pH, organic matter, electrical conductivity, texture and nutrient release were also evaluated.
2) Plant assays based on rate recommendations from soil incubation trials to assess phytotoxicity. A seed germination trial was conducted with three crops (radish, cucumber and tomato) treated with aqueous flume extracts.
3) Microbial screening of flume via metagenomic analysis. Metagenomic profiling of the flume was conducted to reveal microbial composition.
4) Metabolomic chemical analysis. Liquid chromatography coupled with mass spectrometry was used to create high-resolution chemical fingerprints for the flume samples to compare and correlate microbiome profiles and phytotoxicity assays. Gas-chromatography coupled with mass spectrometry was used to assess volatile odorant content, with particular focus on toluene and its malodorous metabolites present in flume, allowing for a comparison between results in 2023 and 2024 samples.

Risks of ToBRFV Transmission

Tomato brown rugose fruit virus (ToBRFV) is a Tobamavirus which affects solanaceous crops including tomatoes and peppers. ToBRFV causes significant losses in greenhouse tomatoes, however, it has a limited impact on field tomatoes. ToBRFV represents the most significant threat to greenhouse tomato production globally. Tomatoes have resistance to Tobamaviruses through resistance genes Tm-1, Tm-2 and Tm-2^2, however ToBRFV (a novel Tobamavirus) has overcome this resistance. ToBRFV is a single-stranded positive-sense RNA virus. It can cause disease symptoms in greenhouse peppers, while plants such as dandelions, nightshades and petunias can act as reservoirs. On infected plants, leaves experience leaf curling, narrowing and mottling; they express stunted growth and tomato fruit is typically smaller, misshapen and with visible mottling and brown rugose. ToBRFV contamination can occur at any point through the supply chain. Rugose is mainly spread through mechanical contacts e.g. agricultural tools, workers hands and clothing, as well as during crop maintenance. Rugose can also spread through infected seeds, hydroponic transmission as the virus is shed from infected plant roots, and buzz pollination using bumblebees. There is evidence of wastewater transmission in areas such as Markham and Mississauga, where there is no tomato production, suggesting ToBRFV is widespread in the food chain (Nash et al., 2024). There is no cure for ToBRFV, meaning an integrated management approach must be taken.

Keeping this in mind, the 2024 samples from Highbury Canco and Conagra were tested for ToBRFV. The Conagra sample tested positive. Due to the viruses' highly infectious nature, this positive rugose result limited our ability to study the sample. In line with this, any future flume sediment being considered for use must be independently evaluated to characterize its functional properties, as well as microbial and viral load.

Activity 1: Soil incubation trials and NASM-related analyses to define rate of application for agricultural use

Background

Application of an organic or soil amendment to agricultural soil can improve its physical (porosity, bulk density, saturated hydraulic conductivity, etc.), chemical (macro- and micro-nutrients) and biological (organic matter, soil respiration/ carbon mineralization, nitrogen mineralization, etc.) properties (Figure 1; Wuest and Gollany 2012; Moebius-Clune et al. 2016). Depending on the amendment used (i.e. flume, compost, manure, straw, biosolids), modifications to a soil's physical, chemical and biological properties will vary, where some amendments will predominantly impact the physical properties, while others will improve the chemical and/or biological properties (Wuest and Gollany 2012). Changes to a soil's properties, as influenced by the type of amendment used, can result in short- (one to less than one growing season) or long-lived (multiple growing seasons) modifications. Therefore, end users must fully understand how an amendment will perform *in situ* prior to horticultural application and use. In-lab testing facilitates can define these characteristics.

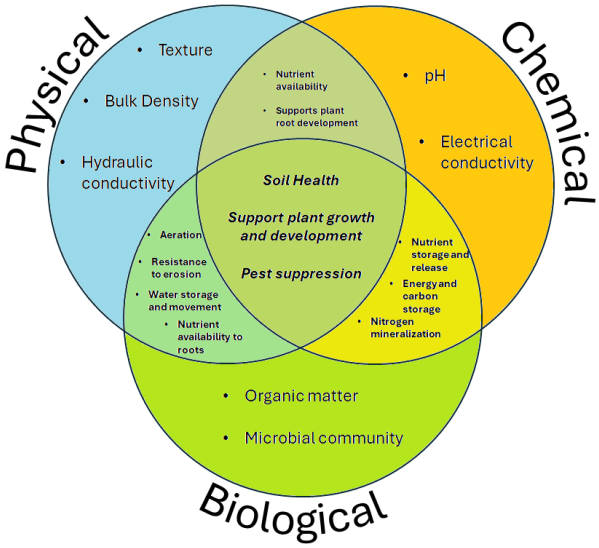
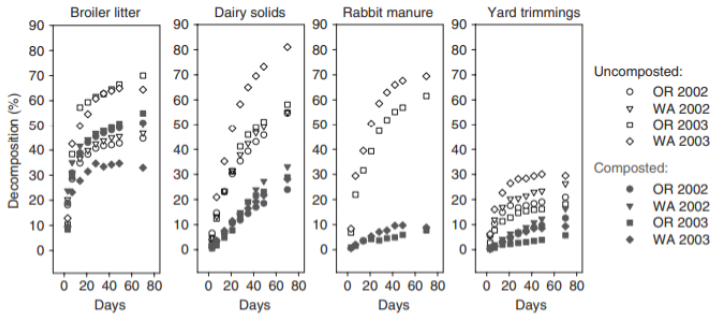


Figure 1 *Physical, chemical and biological growing media properties. As demonstrated in this figure, each property overlaps and influences one another. Image adapted from* https://extension.umaine.edu/cranberries/grower-services/workshops-and-meetings/berry-soil-



from Washington (WA) and Oregon (OR) field sites in 2002 and 2003. Open symbols represent uncomposted organic amendments, whereas filled symbols represent amendments that were 'composted'

In-Lab Incubation Trials at Room Temperature (~21℃)

Depending on the longevity or breakdown rate of an amendment, the corresponding physical, chemical and biological properties of soil could change rapidly and last for short periods, or slowly and gradually change soil properties over time. Therefore, it is important to track these changes to evaluate an amendment's overall performance in a soil overtime.

Previous research has focused on organic amendments such as manure and compost, as these materials contain high amounts of organic matter (>15%). As demonstrated in Vineland's 2023 OPVG Final Report, limited research exists on flume sediment from vegetable processing. Based on research on organic amendments with high organic matter, the longevity of an organic amendment will vary depending on its initial make-up and physical, chemical, and biological composition. In general, organic amendments with a lower C:N ratio will have greater decomposition and breakdown rates, which, depending on its application and use over a growing season, may require additional applications, or 'top-up'. Gale et al. (2006) evaluated different manures, composts and specialty products and observed that decomposition rates varied greatly by product. After 70 Days of room temperature incubation (22°C), decomposition of organic amendments ranged between 8 to 92% (Figure 2, Gale et al. 2006). Organic amendments that were observed to have lower C:N ratios (un-composted material), had significantly greater decomposition rates as compared to composted materials. Decomposition rates were especially high for manure products such as dairy manure solids and rabbit manure.

This breakdown process of organic amendments performed by microbes is vital for nutrient release and, subsequently, nutrient availability to plants. Organic matter contains macro- and micro-nutrients that are primarily released through decomposition (also referred to as the mineralization process). Decomposition allows nutrients to become available to plant root systems. This process is vital for the release of nitrogen, phosphorus, and sulfur, and it has also been observed as a source of calcium, magnesium, sodium, and potassium in certain amendments. Where it is known that the biological properties of a given organic amendment impact on its associated chemical properties, it is important to note that physical properties also change. Microbial communities provide structure to the soil by creating aggregation between different particles. Therefore, when adding organic amendments to a growing media, it is important to understand how these amendments will alter the biological, chemical and physical properties of the growing media over a period to predict how plants growing within this media will be affected with respect to their growth and productivity.

In-lab incubation trials are centered on the characterization of an organic amendment's (in this case flume) physical, chemical and biological properties, by evaluating the breakdown of the material at room temperature over the course of a three-week period, while quantifying the associated impact on growing media and plant performance.

Experimental Design

In October 2024 Vineland conducted a soil laboratory-based incubation trial at room temperature (~21°C) on loam agricultural soil collected from one of Vineland's agricultural fields. Flume samples from Highbury Canco were sent to A&L Laboratories for NASM chemical analysis to identify nutrient composition, allowing us to determine recommended application rates to incorporate in the agricultural soil (as described in further details below). Based on the NASM analysis, low, moderate and high application rates of the Highbury flume sample were established. High application rates (50 tonnes/ha) were based on OMAFA Ontario's Agricultural Planning Tools Suite (AgriSuite) for crop requirements and NASM approval, moderate rates (25 tonnes/ha) were based on using half the applicate rate

recommended by AgriSuite, and low rates (12.5 tonnes/ha) were based on evaluating a 75% reduction compared to the AgriSuite Tool. The main objective was to refine flume rates to ensure a controlled release of nutrients from this organic material that does not impact on the surrounding environment, particularly with respect to source water protection, while still providing enough nutrients for crops to use.

Flume NASM Evaluation

AgriSuite is the Ontario Government's agricultural and environmental suite of tools related to crop management, nutrient management and minimum distance separation. This suite of tools provides an excellent indication for nutrient management using organic amendments and/or fertilizers, particularly with respect to field applications. The AgriSuite tools are designed to:

- Save money by potentially optimizing the amount of fertilizer and other materials used
- Reduce the environmental impact of on-farm generated nutrients
- Protect drinking water sources
- Reduce soil erosion and maintain soil productivity
- Manage the beneficial use of off-farm waste on agricultural land

Included within AgriSuite is the Non-Agricultural Source Material (NASM) Plan tool that can be used by qualified individuals to determine beneficial reuse of material that is sourced from non-agricultural systems, which includes flume. The OMAFA AgriSuite Tools were used to assess the flume incorporation rates selected in the laboratory incubation trial, based on laboratory analysis. Prior to applying the AgriSuite Tools, the flume was sent to a third-party laboratory (A&L Laboratories) for chemical analysis which included dry matter, total nitrogen, phosphorus and potassium, ammonium-nitrogen, and heavy metals. These results were then analyzed/compared with the AgriSuite Tools to determine whether the flume materials comply with the government standards and are well suited for application in agricultural fields. Based on these laboratory results from A&L, high, moderate and low incorporation rates were identified, to evaluate a wide range of hypothetical application rates to soil for crops ranging from low nutrient requirements like radish to high nutrient requirements like cucumbers.

Incubation Trial

Temporal based incubation trials are centered on evaluating the soil’s physical, chemical, and biological properties over a set period, and how the soil amendment alters these soils’ properties. In addition, microbial activity can be used to estimate how an amendment can alter the microbial activity of a soil, allowing for breakdown of organic matter and nutrient release. Carbon mineralization (also understood as ‘soil respiration’), which is the carbon dioxide released when the organic matter in soil is broken down by microorganisms. As demonstrated in Vineland’s 2023 OPVG Final Report, flume sediment has the potential to increase microbial activity in soil, aiding in nutrient release.

To understand the temporal influence of flume on agricultural soil, an incubation trial was conducted over a 21-day period at room temperature. Flume was applied to agricultural soil

in lab and soil health analysis was evaluated throughout the incubation trial. An Alkali-Trap Method (modified Cornell Soil Health Assessment Protocol) was used to evaluate carbon mineralization/breakdown of the flume amendments. To understand the release of nutrients such as phosphorus, potassium, magnesium, etc., subsamples were collected at 4 time points: 1, 7, 14 and 21 days after incorporation from the incubation trial, and were evaluated for nutrient analysis by A&L Laboratories. The analysis package selected was S1B+S7+NO_3-N and NH_4-N, which includes the following parameters:

- Organic Matter
- Soil pH
- Micro and macro nutrients - Phosphorus, Potassium, Magnesium, Calcium, Sodium, Aluminum, Zinc, Manganese, Iron, Copper, Boron, Sulphur
- Calculated cation exchange capacity (CEC)

To account for different application rates that could be used for different crops, three flume application rates as well as a control (no flume added) were evaluated. Application rates of flume included 50 tonnes/ha, 25 tonnes/ha and 12.5 tonnes/ha (high, medium, low; wet weight).

Results and Discussion

Flume NASM Evaluation

NASM requirements for solid materials must meet a total concentration of plant-available nitrogen, phosphate, and potassium exceeding 13,000 mg/kg (or ppm). A&L laboratories' chemical analysis showed both flume samples from Highbury (from years 2023 and 2024) and from Conagra in 2023 (as 2024 samples tested positive for rugose and were unable to undergo further testing); Table 1). Heavy metal levels in all samples were well below the NASM standards (Table 2).

NASM Plant available nutrient requirements:

$$Plant\ Available\ Nitrogen = (NH3, NH4 - N) + (NO3, NO2 - N) + (0.3\ X\ Oraganic - N)$$

$$Plant\ Available\ Phosphorus = 0.4\ X\ Total\ Phosphorus\ (as\ P2O5)$$

$$Plant\ Available\ Potassium = 0.9\ X\ Total\ Potassium\ (as\ K2O)$$

Note – these regulatory calculations for plant available nutrients may not be suitable for soil based NASM's.

Table 1. A&L Laboratories nutrient analysis values and NASM requirement standards for Highbury Canco (Highbury) and Conagra flume samples tested in 2023 and 2024.

Nutrient	Unit	Highbury *2023*	Highbury *2024*	Conagra *2023*
Total Potassium (as K_2O)	%	0.2	0.3	0.5
Total Phosphorus (as P_2O_5)	%	0.3	0.4	0.5
Total Nitrogen	%	0.2	0.5	0.8
Ammonia (NH_3/NH_4-N)	*ppm*	*589.0*	*936.6*	*886.4*
Nitrite ($N0_3$/NO_2-N)	*ppm*	*114.4*	*3.9*	*7.4*

Heavy Metal	Standards in ppm for Regulated Metals in NASM (Source: Schedule 5 of the Regulation)	Highbury *2023 (ppm)*	Highbury *2024 (ppm)*	Conagra *2023 (ppm)*
Arsenic	13	2.7	4.0	4.3
Cadmium	3	BDL[1]	BDL	BDL
Chromium	210	19.4	31.0	33.7
Cobalt	34	3.6	4.8	6.4
Copper	100	16.2	19.5	39.4
Lead	150	9.0	10.0	15.0
Mercury	0.8	BDL	BDL	BDL
Molybdenum	5	1.7	2.9	2.2
Nickel	62	12.0	13.6	28.5
Selenium	2	BDL	BDL	BDL
Zinc	500	53.6	64.4	133.5

[1] BDL - below the limit of detection

The challenge of the flume samples is this is a soil based NASM, where the NPK (total) is relatively low compared to a higher organic material such as a Grade B Compost with nitrogen typically above 1% and sometimes closer to 2%. In addition, total potassium and phosphorus are usually greater than 1.0% and 0.5% respectively. Although these samples failed to meet the NASM nutrient requirements, Vineland's incubation trial was still conducted to evaluate the nutrient benefits as a soil amendment.

Incubation Trial

Organic matter percentage remained constant, ranging from 1.8 to 2.0% from days 1 to 21 across all treatments (Figure 3A), indicating that flume does not modify soil organic matter at application rates of 12.5, 25 and 50 tonnes/ha (low, medium, high respectively; wet weight) compared to the control. Although organic matter did not change across the treatments, the incorporation of flume to the soil increased some macronutrients, which is a requirement for the beneficial reuse of a NASM soil amendment.

Similar to the results of the organic matter, phosphorus remained relatively stable for the three application rates of flume: high, medium and low and the control (range 15.0 to 19.7ppm). Slight fluctuations were observed within the flume application, but these were relatively consistent between treatments and the control over time (Figure 3B). The low incorporation rate showed the greatest phosphorus variation, with levels dropping by 22% from days 1 to 7, then increasing by 24% from days 7 to 21. The phosphorous content in the samples with a moderate incorporation rate remained steady until day 14, then increased by 15% on day 21, suggesting P release between days 14 to 21. The high incorporation rate samples saw a 13% decrease in phosphorus over the 21-day period. Low and moderate flume incorporation rates released more phosphorus than the control, while high rates showed a notable decrease (Figure 3B). In contrast, potassium increased notably in all flume treatments (Figure 3C). The potassium content in the control remained constant throughout the trial, compared to increasing application rates of flume which increased soil potassium content in the soi.

Vineland determined the amount of nitrate and ammonia/ammonium present in the flume. These forms of nitrogen are plant-available and represent the source of nitrogen that can be taken up and used by the plant relatively quickly, supporting plant structure and growth. Compared to nitrate, ammonia/ammonium is often preferred by some plants because it requires less energy to assimilate. However, high concentrations can be toxic, leading to imbalances in soil pH and can affect plant nutrient uptake. Nitrate levels differed between the control and flume NASM treatments (Figure 3D). Nitrate in the control soils (no flume) increased at a rate of 48% between days 1 to 14, after which levels did not change significantly. In all flume treatments, nitrate dropped from day 1 to 7 before increasing steadily from days 7 to 21 by percent rates of 40%, 72%, 106% for low, moderate and high incorporations respectively. On day 1, low and moderate incorporations had comparable levels of nitrate to the control, but the high incorporation rate had lower nitrate levels in comparison and remained lowest across all timepoints and between all treatments. Overall, control had the highest ppm of nitrogen over the incubation period.

Ammonia/ammonium was notably different across all treatments with the high incorporation rate exhibiting the highest amount of ammonia/ammonium in comparison to all other treatments at all timepoints (Figure 3E). Ammonia/ammonium decreased in control, and moderate rate treatments over time by 50% and 32% respectively. The low incorporation rate treatment remained consistent at about 10 ppm across all 21 days (Figure 3E). Ammonia/ammonium increased in the high incorporation treatment by 38.5% from day 1 to 7 after which it remained relatively stable at an average ppm of 24.6 from days 7 to 21 with an overall increase from day 1 to 21 of about 23%. All NASM treatments had significantly higher levels of ammonia/ammonium than the control throughout the trial. In the low and moderate treatments, ammonia/ammonium was approximately 122% and 169% higher than the control while, ammonia in the high incorporation rate treatment was about 433% higher than the control throughout the incubation period. This needs to be monitored in the field when high application rates are used, especially in fields with poor drainage.

Additional soil analysis for the incubation trial can be found in the Appendix Table’s 1 to 4.

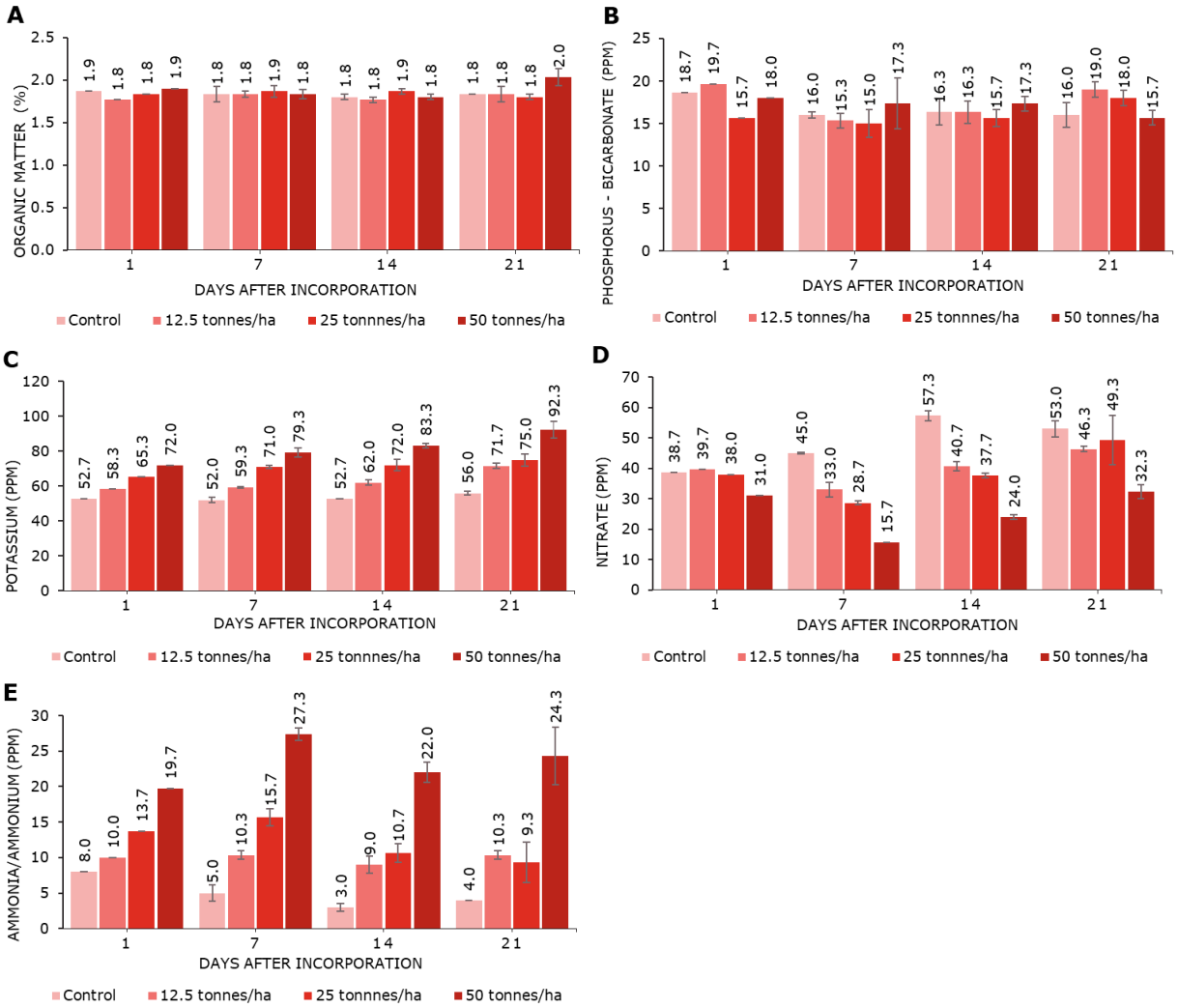


Figure 3. *Flume incubation trial nutrient release details at 1, 7, 14 and 21 days after incorporation for all treatments (control, low = 12.5 tonnes/ha, moderate = 25 tonnes/ha, and high = 50 tonnes/h) for A. organic matter, B. phosphorus (bicarbonate), C. potassium, D. nitrate, and E. ammonia/ammonium. Error bars represent standard error.*

Nutrient Release

To estimate the microbial activity for each application rate, the amount of carbon dioxide released (carbon mineralization/ respiration) was determined over a 21-day period at room temperature. The amount of carbon dioxide released serves as an indicator of the soil's "breathing," with higher levels of carbon dioxide released signaling increased microbial activity. The control soil released ~100mg of carbon dioxide over the 21-day period, compared to the 50 tonnes/ha application rate at ~800mg, 8 times increase in apparent microbial activity. Based on these results, flume is demonstrated to increase soil respiration and the breakdown of organic matter in the soil, which has the potential to increase nutrients over time through mineralization processes.

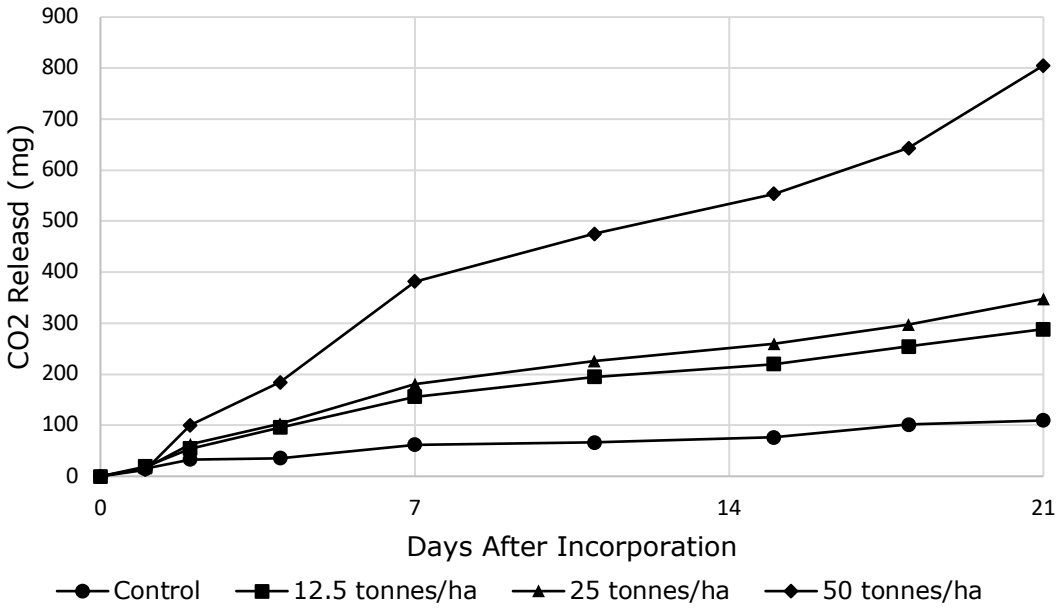


Figure 4. Mean cumulative carbon dioxide release of the three *flume application rates.*

Activity 2: Plant assays based on rate recommendations from soil incubation trials to assess phytotoxicity

Background

A seed germination bioassay was conducted to determine the optimal percentage of Highbury Canco flume which can be applied to seeds/seedlings by monitoring for phytotoxicity. A lab-based trial was conducted in petri dishes to determine thresholds at which flume could be applied. We assessed application rates on three different crop seeds: radish, cucumber and lettuce. Phytotoxicity refers to the potentially toxic effects that substances such as chemicals, fertilizers and pesticides can have on plants. Even natural plant derived compounds can have phytotoxic effects. In the context of seed germination, phytotoxicity describes adverse effects on the process of seeds sprouting and growing into seedlings, such as delayed or inhibited germination, poor seedling growth, damage to seed tissue, and reduced radicle growth. By understanding the effect of flume on seed germination, this will inform potential uses of flume as a soil amendment during the sowing of new crops.

Experimental Design

Highbury Canco Flume was maintained in its aqueous form by allowing the Flume to settle for 24 hours. Flume water was extracted and diluted with reverse osmosis (RO) water to create four dilution rates (25, 50, 75 and 100% v/v flume:water) with 0% serving as a positive control (Table 3). These four dilution rates, along with a control, were evaluated for pH and electrical conductivity, before use in the germination assay. Radish, cucumber, and lettuce seeds (10 seeds per petri dish, 3 replicates per treatment) were placed on sterile filter paper and then wetted with their respective flume dose rate. The seeds were allowed to incubate at room temperature for 7 days, after which the germination rate (GR), radicle length (RL), and germination index (GI) were assessed.

Phytotoxicity parameters were calculated according to [1-3] adapted from Luo et al., 2018.

> ***[1] Germination rate (GR)*** *= (# germinated seeds in aqueous extracts ÷ # germinated seeds in Control) * 100*
>
> ***[2] Radicle length (RL)*** *= (Radicle length of all seeds in aqueous extracts ÷ Radicle length of all seeds in Control) * 100*
>
> ***[3] Germination Index (GI)*** *= (Germination rate * Radicle length) ÷ (Germination rate in Control * Radicle length in Control)*

A GI greater than 80% indicates no phytotoxicity, while a GI below 80% suggests moderate phytotoxicity, and a GI below 50% indicates high phytotoxicity (Luo et al., 2018).

Results and Discussion

Dilution Rate, pH and Electrical Conductivity

The pH and electrical conductivity of each dilution rate was recorded (Table 3). The pH of the control sample is around 7.2. When the flume is added, the pH decreases to 5.7 and

remains consistent in all flume dose rates. As the percentage of flume increases, so too does electrical conductivity, with a maximum EC of 9.05 mS/cm in 100% flume. Electrical conductivity, a measure of solution salinity, impacts plant water uptake, as high salinity restricts water availability to roots. The optimal EC ranges for lettuce, radish and cucumbers include 0.8-1.2, 1.6 – 2.2 and 1.8 – 3.0 mS/cm, respectively. Seed germination is particularly sensitive to salinity, with an electrical conductivity threshold of approximately 2 mS/cm. When the electrical conductivity is higher than this threshold, we typically observe reductions in germination.

Table 3. pH and electrical conductivity of 4 flume dilutions in reverse osmosis water

Flume Dose Rate (% v/v)	pH	EC (mS/cm)
100	5.7	9.05
75	5.7	7.04
50	5.7	4.9
25	5.7	3.22
0	7.2	0

Lettuce

Table 4. Phytotoxicity indicators in lettuce at various flume concentrations

Flume Dose Rate (% v/v)	Mean Germination Rate (%)	Mean Radicle Length (mm)	Mean Germination Index (%)
100	0.00	0.00	0.00
75	0.00	0.00	0.00
50	0.00	0.00	0.00
25	0.00	0.00	0.00
0	76.67	12.63	100.00

The control group had the highest mean GR, RL and GI, with no germination observed for the treatments of flume (Table 4). The mean RL for lettuce was 12.63 ± 2.5 mm for the control, while there was no radicle growth for the treatment doses. Likewise, the GI remained below 0% for all doses except the control, highlighting the severe phytotoxic effects of the treatments on lettuce seedlings (Table 4, Figure 5).

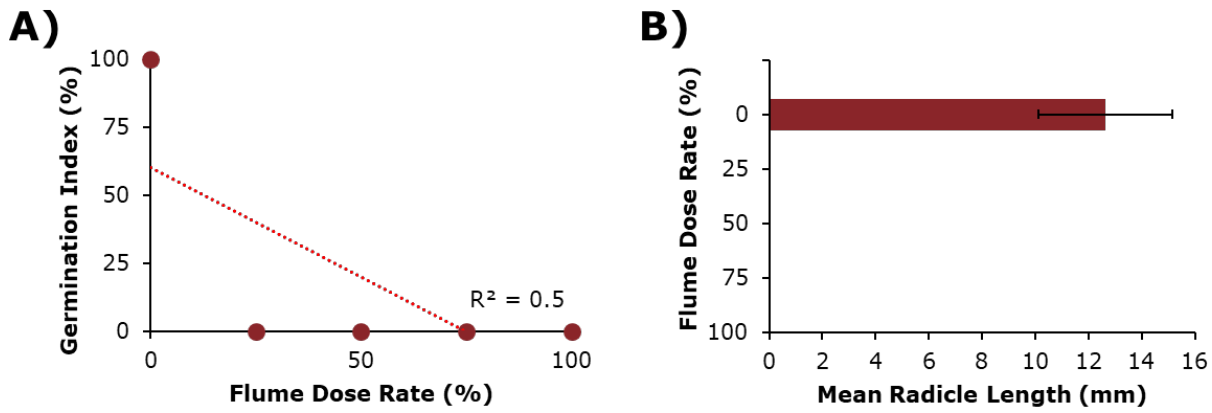


Figure 5. *Lettuce germination index (A) and mean radicle length (B) at varying flume dose rates (%). Error bars represent standard error.*

Radish

Table 5. Phytotoxicity indicators in radishes at various flume concentrations.

Flume Dose Rate (% v/v)	Mean Germination Rate (%)	Mean Radicle Length (mm)	Mean Germination Index (%)
100	0.00	0.00	0.00
75	0.00	0.00	0.00
50	13.33	0.33	0.19
25	6.67	0.17	0.08
0	96.67	48.70	100.00

The control group had the highest mean GR, RL and GI, with sharp declines observed across all treatment doses, with 75% flume leading to no germination (Table 5). As the flume dose increased, there was a concomitant decrease in mean germination rate.

Radicle length measurements could not be effectively compared between treatments due to the extremely low germination rates (Table 5, Figure 6). The germination index (GI) remained below 1% for all doses except the control, underscoring the major phytotoxic effects of the treatments on radish seedlings.

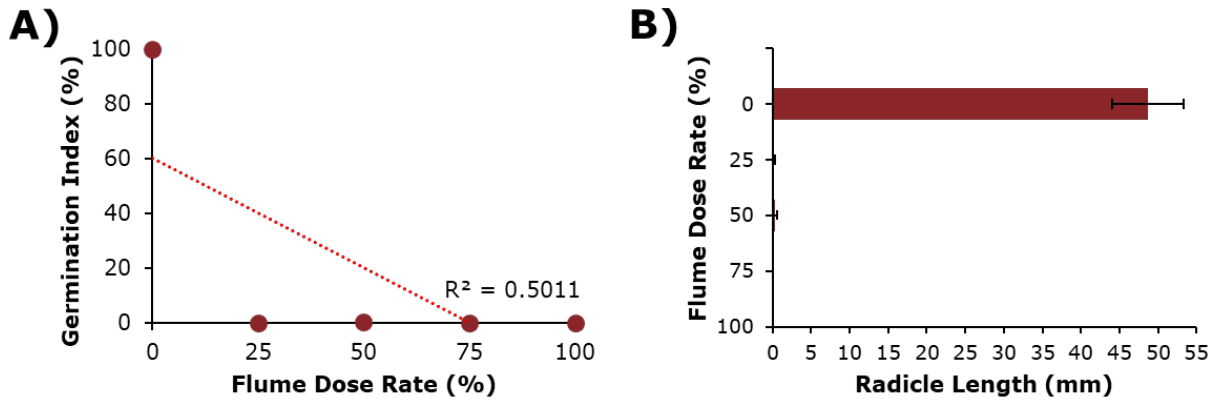


Figure 6. *Radish germination index (A) and mean radicle length (B) at varying flume dose rates (%). Error bars represent standard error.*

Cucumber

Table 6. Phytotoxicity indicators in cucumbers at various flume concentrations

Flume Dose Rate (% v/v)	Mean Germination Rate (%)	Mean Radicle Length (mm)	Mean Germination Index (%)
100	0.00	0.00	0.00
75	0.00	0.00	0.00
50	10.00	0.27	0.02
25	90.00	13.13	18.23
0	100.00	63.80	100.00

The mean GR, RL and GI were highest in the control, followed by a flume dose rate of 25%, with a mean GR of 90%, with steep declines at increasing rates (Table 8). The germination rate declined with increasing dose-rates. Both the mean RL and GI in cucumber decreased with higher doses of flume (Table 6, Figure 7). The GI was below 20% for all doses except the control, indicating a high phytotoxic effect on cucumber seedlings.

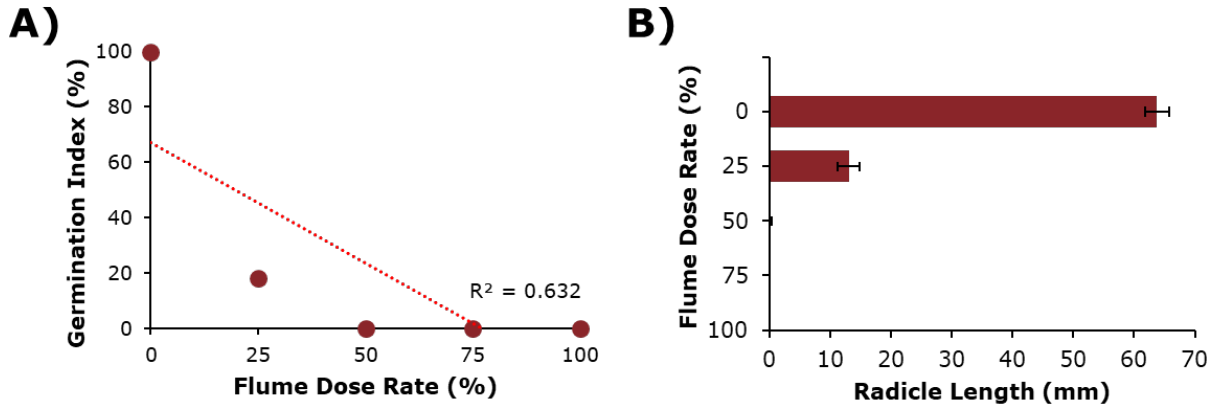


Figure 7. *Cucumber germination index (A) and mean radicle length (B) at varying flume dose rates (%). Error bars represent standard error.*

The results of this study demonstrate consistent phytotoxic effects of Highbury Canco flume on germination and early growth of lettuce, radish and cucumber seeds. The electrical conductivity of all flume dose rates was higher than the recommended ranges for each plant, impacting their germination rates relative to the control. Across the different seed types, control treatments with 0% (v/v) flume had the highest RL, GR and GI, with steep declines observed with increasing flume doses. When flume was used, lettuce showed no germination or radicle growth, while cucumbers and radishes saw sharp reductions in GI, falling below 1% and 20% respectively at 25% (v/v) flume, with rates decreasing until a flume dose rate of 75% (v/v) where the GI for both crops was 0%.

A clear relationship was observed across the data, with increasing flume concentrations resulting in progressively lower germination rates, shorter radicle lengths, and reduced GIs. Based on the observed rates, flume contains components that severely inhibit seed germination and early seedling growth. The results indicate that due to the high electrical conductivity of the flume sediment, germination of seeds will be an issue using this as a beneficial waste product. Further research should evaluate this material and its beneficial reuse for transplanted or established crops with higher tolerances to material with high electrical conductivity.

Activity 3: Microbial screening of flume via metagenomic analysis

Background

Both Highbury Canco and Conagra flume samples were sent for high throughput sequencing, targeting the three most agriculturally important groups of microbes: Bacteria, Fungi and Oomycetes. A targeted sequencing approach is preferred over untargeted sequencing when host DNA is expected to be abundant. In this case, tomato and other common flume component DNA would interfere with the analysis. The molecular method consists of DNA extraction, barcoding, and sequencing for the three groups, followed by analysis. Heat maps and other visualizations are presented here. Supplementary data is attached.

Materials and Methods

Composite samples were collected, homogenized, and sub-sampled into three sub-samples per flume source for DNA extraction. DNA was extracted with a commercially available kit (Qiagen, CA) and amplified with 16S, ITS1/2 and ITS6/7 primers for Bacteria, Fungi and Oomycetes, respectively. Libraries were constructed and sequenced on an Illumina MiSeq (Illumina, CA) using paired-end sequencing in a single run. Quality control was performed, and sequences were demultiplexed, filtered, denoised and clustered to produce Amplicon Sequence Variant (ASV) tables. A total of 813,109 sequences were retrieved and analyzed: 252,453 for bacteria, 289,696 for fungi and 270,960 for oomycetes. No sequences were flagged as poor quality after filtering. Rarefaction curves indicated that sequencing yields were high enough to capture the majority of taxa per sample for all of bacteria, fungi and oomycetes.

Rooted phylogenetic trees were produced from ASV sequence alignment. ASV and taxonomic tables were used to perform statistical analyses and metagenomics profiling in R (r-project.org;Figure 8). Further metagenomics analyses performed at Vineland also utilized R. PERMANOVA, p and R2 values for NMDS ordinations can be supplied as needed. Vineland ran the ASV matrix through the Python tools FUNGuilds (funguild.org) and FAPROTAX (loucalab.com) which parse fungal and bacterial sequencing results by ecological guilds.

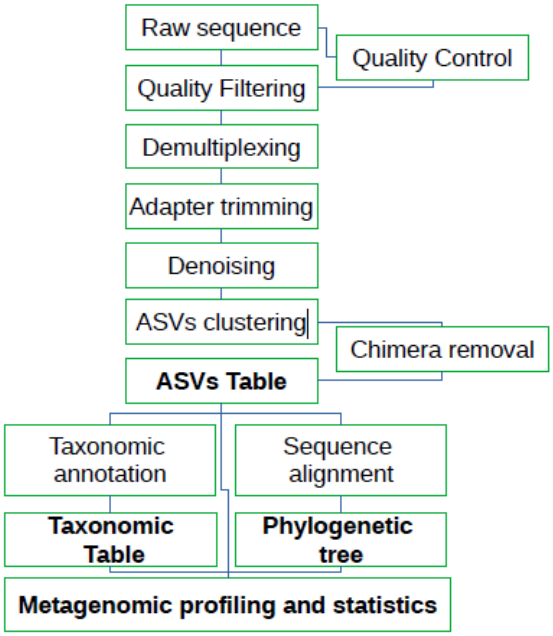


Figure 8. *Metagenomic pipeline overview (HGI)*

Results and Discussion

Summary

- Very distinct microbial community compositions were found between the flume samples from Highbury Canco and Conagra.
- Bacterial diversity was similar across flume samples; community composition differs somewhat at the Phylum level in each and is consistent across subsamples of the same flume.
- Oomycete diversity was lower in the Conagra Sample compared to the Highbury Canco sample, and Fungal diversity was lower in the Highbury Canco sample compared to the Conagra sample. Community composition differs in each sample and is consistent across subsamples of each flume. Plant pathogen communities differed between flume samples. Highbury Canco had relatively higher levels of plant pathogenic fungi: It was dominated by a sequence matching *Phytophthora capsica* whereas the Conagra sample was dominated by an amplicon sequence variant (ASV) matching *Candida tropicalis*.
- Microbial communities across preparations are dominated by environmental bacteria involved in nutrient transformations such as the Bacteroidetes and mainly yeasts or fungi such as *Geotrichum*. Conagra appeared to have more bacterial parasite organisms compared to Highbury Canco's high chemoheterotrophic bacteria. Highbury Canco flume appears to have relatively more fungal wood saprotrophs, undefined saprotrophs, and plant pathogens; whereas Conagra has relatively more epiphytes, endosymbionts, and endophytes. A high number of Oomycetes indicates the abundance of these aquatic and typically plant pathogenic organisms in the samples.

Diversity Indices

The diversity found across flume samples demonstrates a robust microbial community that should be capable of a broad range of ecological roles such as performing nutrient transformations and causing plant disease. Generally, microbial diversity in agricultural substrates is seen as a strength but, given the dominance of plant pathogens, this material should be treated with caution, especially when considering potential uses for non-agricultural source material (NASM). Bioprospecting for microbes of interest is also the goal of some analyses of microbial communities and the diversity of the flume microbiome could lead to interesting findings. This study looked at alpha diversity and beta diversity of the two flume samples. Alpha diversity refers to species diversity (abundance and richness) on a local scale, while Beta diversity is a measure of similarity or dissimilarity between two communities. In this case, Alpha diversity tells us about the diversity within each flume community, while the Beta diversity tells us how similar the two flume communities are.

Alpha Diversity

Alpha diversity is a measure of the internal diversity of a single sample. The Shannon Index estimates species richness and evenness but weights the analysis towards richness. The plots presented below are 'violin plots' which show smoothed histograms of the probability density function, which read similarly to boxplots. In violin plots, the bimodal distributions are highlighted, whereas these can be hidden in conventional boxplots. The red dot indicates the mean of the distribution. Group letters represent significant ($p<0.05$) differences per Tukey's post hoc HSD test on the linear model. Harvest Genomics (HGI) found that whereas the bacterial community diversity was similar between the two flume samples (Figure 9), significant differences were found between the fungal and oomycete diversities (Figures 10 and 11). Increased fungal diversity and reduced oomycete diversity could be found in the Highbury-Canco sample, and conversely, decreased fungal diversity and increased oomycete diversity was found in the Conagara flume sample.

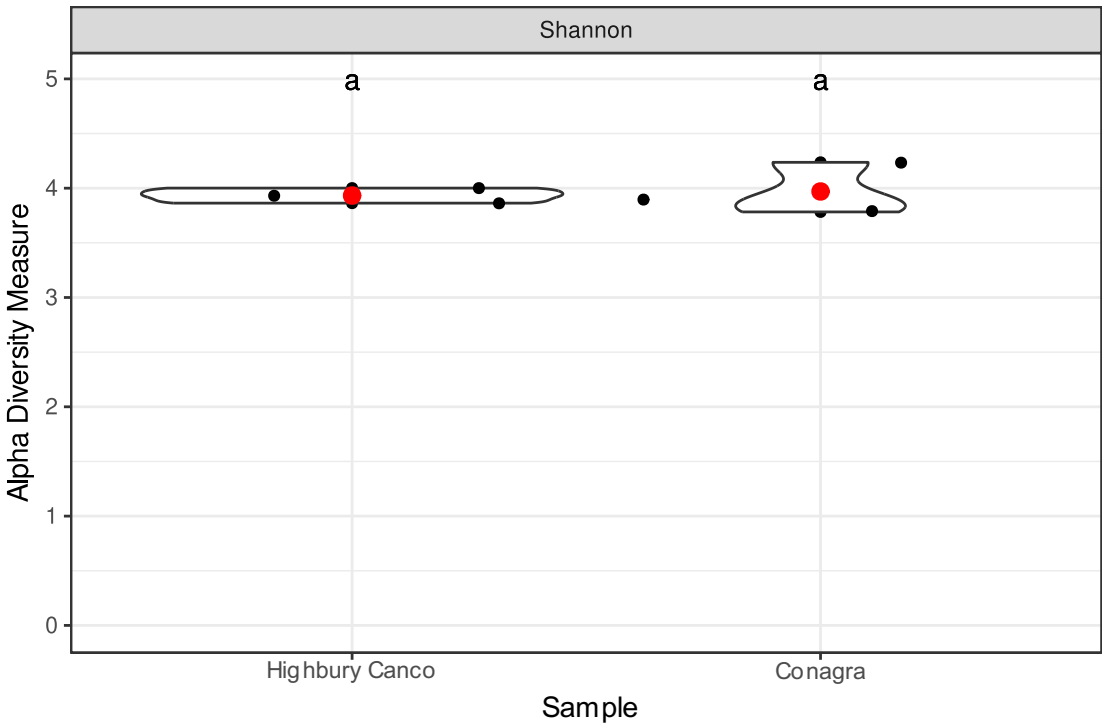


Figure 9. *Diversity (Shannon index) of bacterial community between Highbury Canco and Conagra flume samples. Red dot indicates mean of value distribution. Letters indicate significant differences (p<0.05) by Tukey's HSD Post-hoc test on linear model (HGI).*

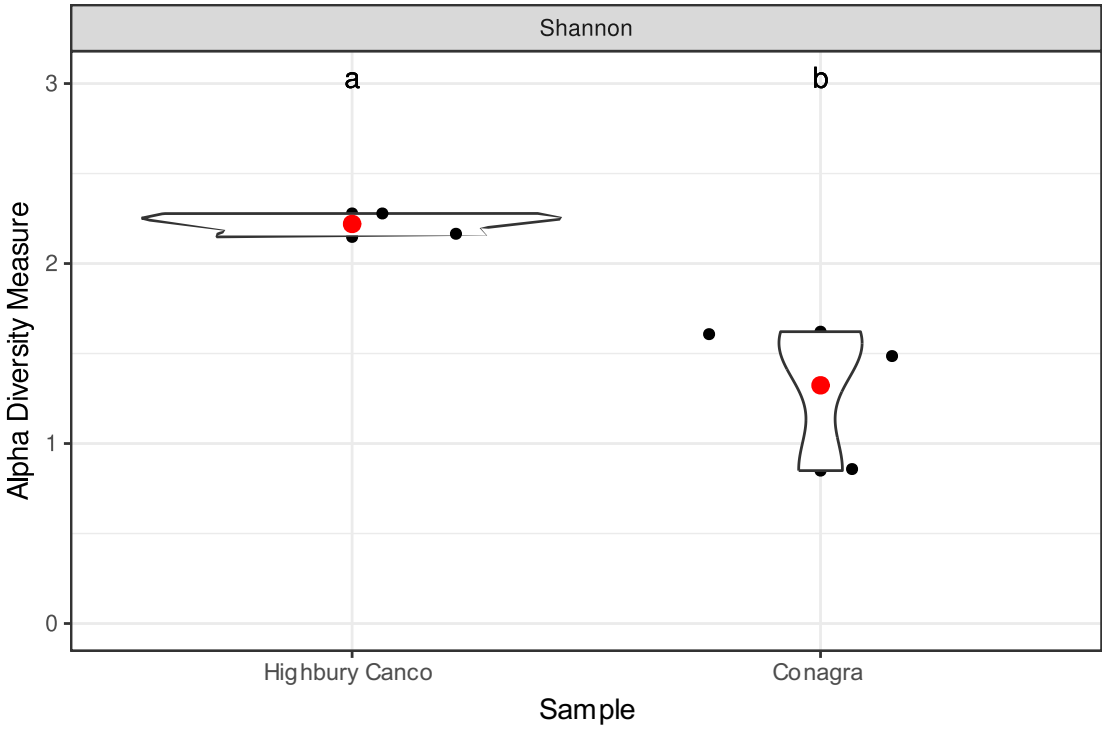


Figure 10*. Diversity (Shannon index) of fungal community between Highbury Canco and Conagra flume samples. Red dot indicates mean of value distribution. Letters indicate significant differences (p<0.05) by Tukey's HSD Post-hoc test on linear model (HGI).*

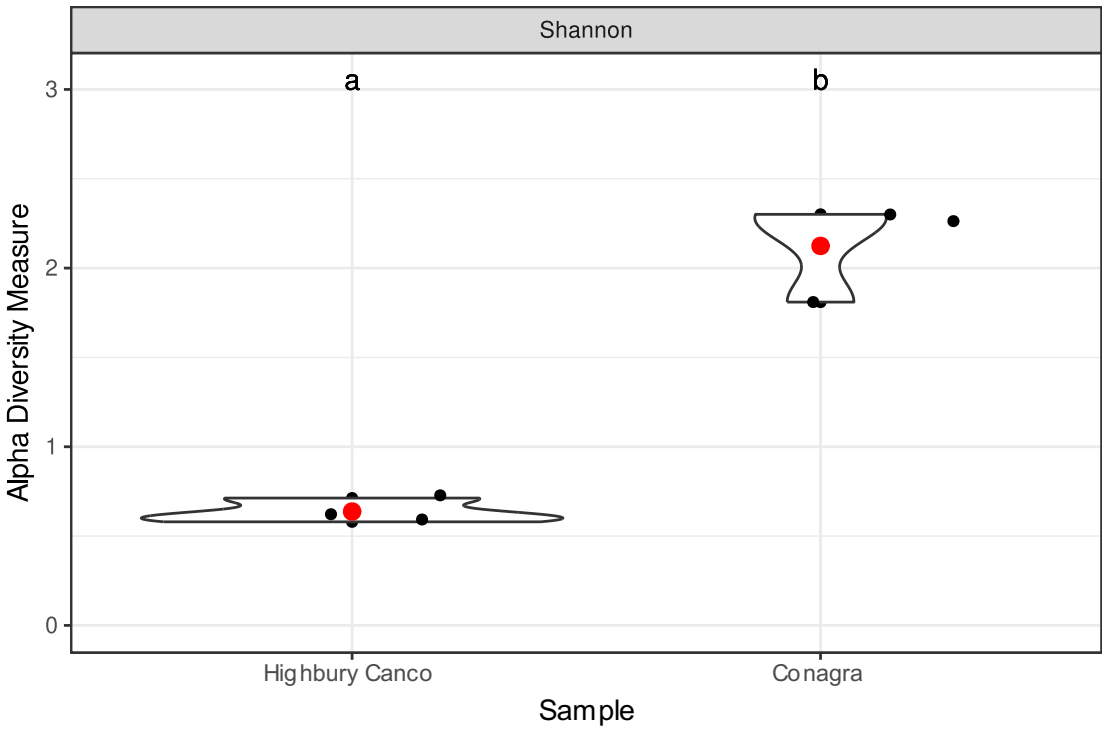


Figure 11. *Diversity (Shannon index) of oomycete community between Highbury Canco and Conagra flume samples. Red dot indicates mean of value distribution. Letters indicate significant differences ($p<0.05$) by Tukey's HSD Post-hoc test on linear model (HGI).*

Beta Diversity

Beta diversity compares the microbial composition of different samples against each other and quantifies how different they are. The plots below are multidimensional scaling (MDS) plots which are read similarly to the principal component analyses (PCA) plots shared in the metabolomics portion of this report; where clustering shows relatedness and distance indicates less relatedness. Both types of plots are dimensionality reduction techniques, however, they are optimized for different things. Whereas PCA projects the data in directions with the most variance, MDS plots the pairwise distances between points, which preserves the distances between the ranks of dissimilarity of data. This can better optimize the sample distribution to show more variation in species composition compared to PCA.

HGI found that the different flume sources strongly affected bacterial, fungal and oomycete communities. Compared to the Alpha Diversity above, the bacterial community compositions were very different, reflected in Figure 12. Fungal and Oomycete population compositions were also very different between flume sources (Figure 13 and 14) PERMANOVA results are included in the attached HGI report.

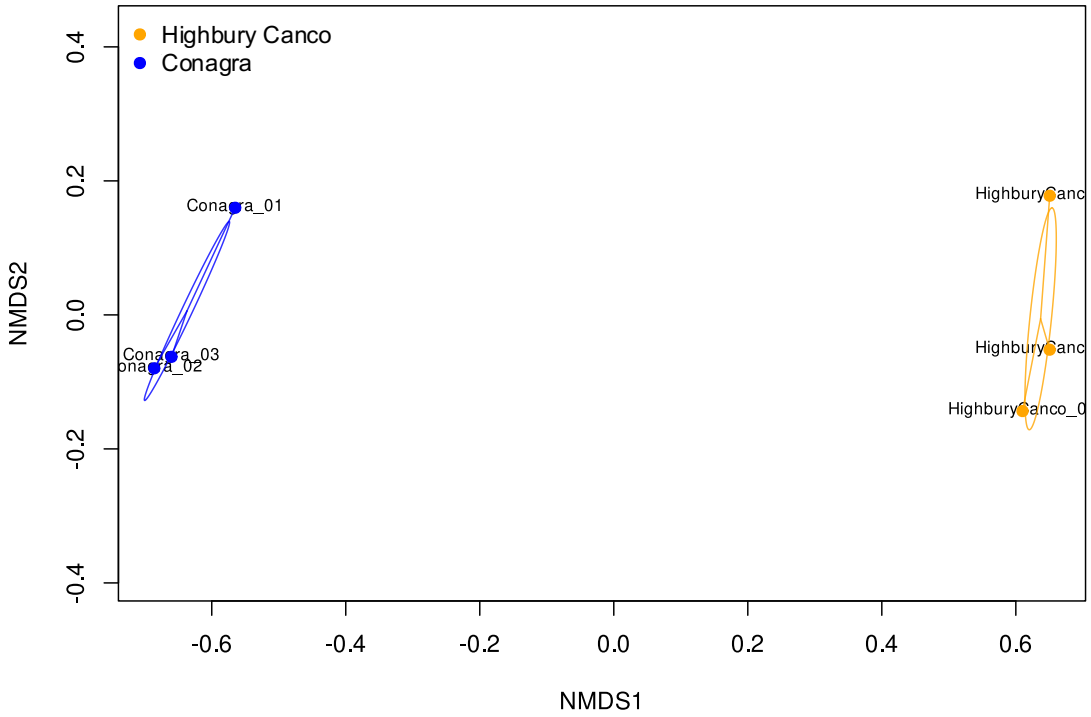


Figure 12. *Non-metric Multi-dimensional Scaling (NMDS) plot of bacterial communities from Highbury Canco and Conagra flume samples (HGI).*

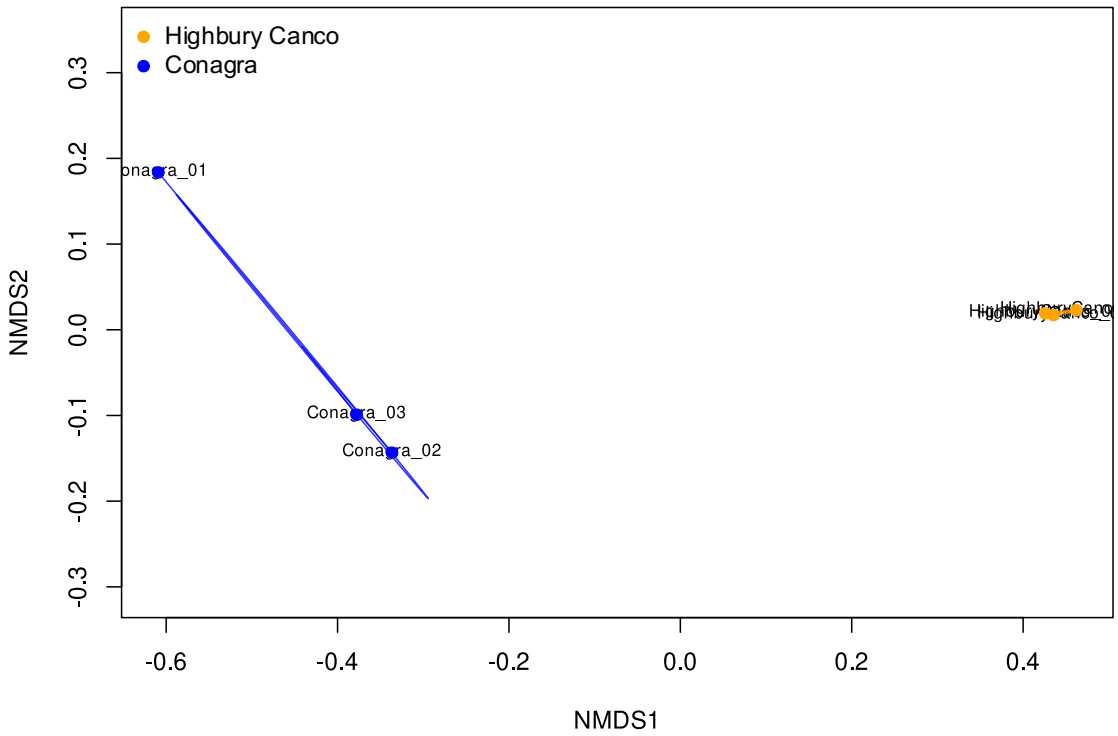


Figure 13. *Non-metric Multi-dimensional Scaling (NMDS) plot of fungal communities from Highbury Canco and Conagra flume samples (HGI).*

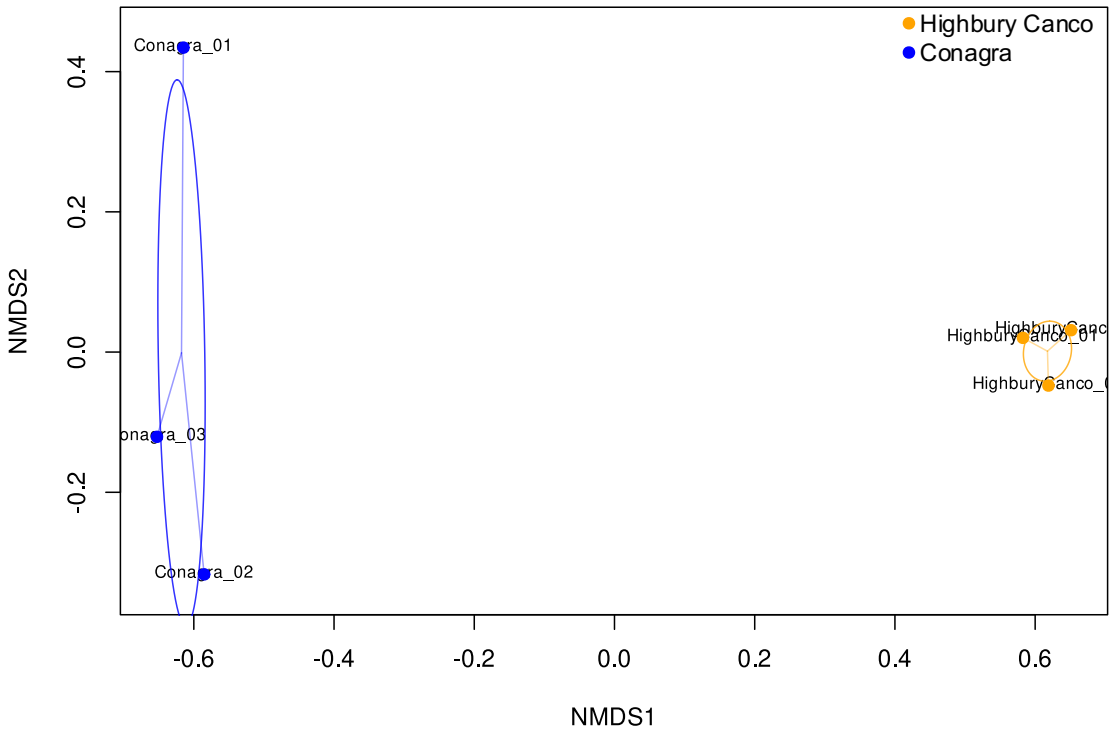


Figure 14. *Non-metric Multi-dimensional Scaling (NMDS) plot of oomycete communities from Highbury Canco and Conagra flume samples (HGI).*

Community Compositions

Bacterial, fungal and oomycete communities have distinct taxonomic compositions across the two flume samples at the phylum (Bacteria) and Genus (Fungi and Oomycetes) levels (Figure 15, 21, 26). Bacterial taxa were the most similar between the flume sources at the phylum level despite different abundances within each phyla and tended to be dominated by Bacteriodota. Fungal communities were dominated either by a combination of *Geotrichum* and a *Dipodascaceae* (Highbury Canco) or by *Candida* (Conagara). Oomycate communities were dominated by *Phytophthora* (Highbury Canco), or a combination of Pythialeans – *Phythium* and *Globisporangium*, as well as *Pseudoperonospora* (Downy Mildew) and *Phytophthora.*

Bacterial Communities

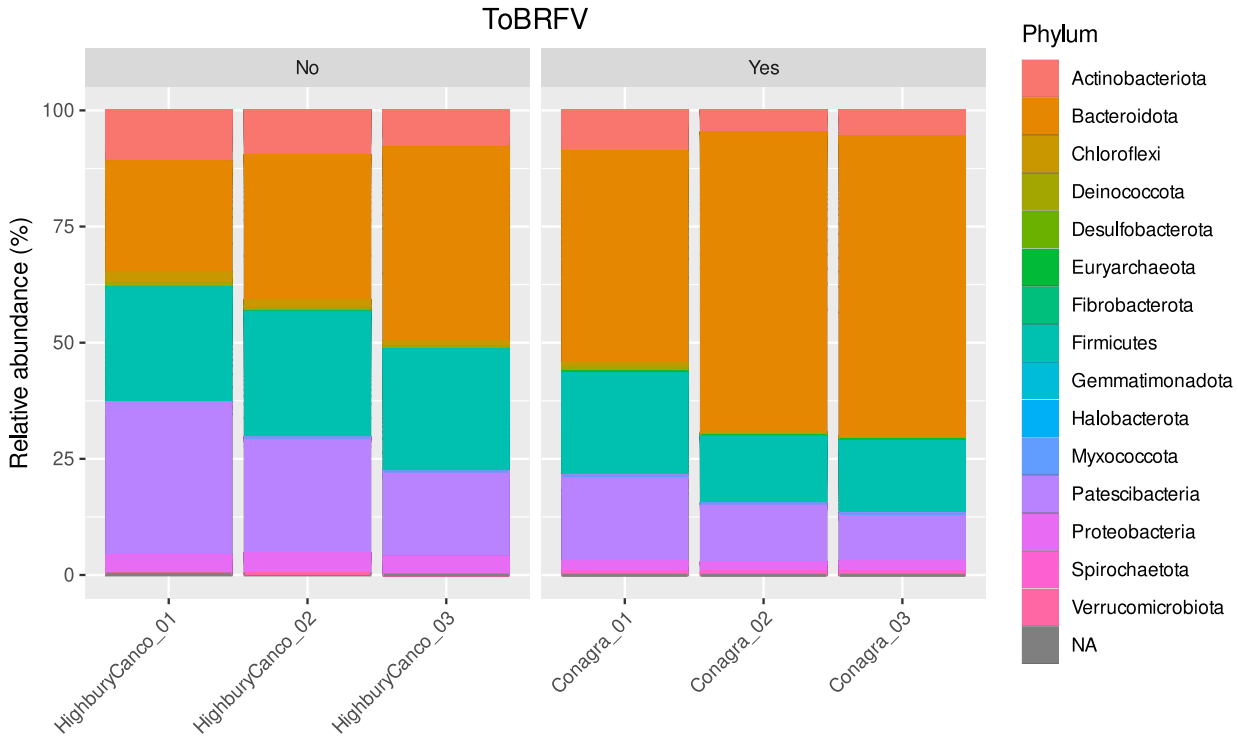


Figure 15. *Bacterial community abundance between Highbury Canco and Conagra flume samples, at the Phylum level (HGI). The size of the different coloured segments represent the relative abundance. Bar-chart are coloured based on Phylum. ToBRFV indicates whether the sample tested positive for ToBRFV at arrival at Vineland.*

Figure 16. *Most prevalent Bacterial species present in Highbury Canco and Conagra flume samples determined by 16S targeted sequencing analysis. Vector version attached in supplementary material (VRIC).Lines on axis indicates phylogenetic tree of the species – how each species is related. Names on the right side indicate the names of the species detected. The darker the colour the more abundant the species is in the sample.*

Bacterial communities in the Conagara flume samples were dominated by Prevotellaceans – members of the dominant Bacterioidales seen in the Phylum rankings above (Figure 16). These are widely distributed gram-negative bacterial anaerobes, suggesting that this sample was oxygen-depleted. *Prevotella* is commonly found at high prevalence in the gut of cattle, swine and sheep where it is involved in carbohydrate and protein breakdown (Tett et al., 2021; Wang et al., 2019), as well as from humans, especially those consuming a plant-rich diet (De Filippis et al., 2019). These can be opportunistic pathogens of several systems within humans. This genus is implicated in periodontal disease in humans (Tanaka et al., 2008) and interestingly its prevalence in the human gut is inversely related to Parkinsons Disease (Hill-Burns et al., 2017).

Bacterial populations in the Highbury Canco flume also contained high numbers of Prevotellaceae, but were dominated instead by Saccharimonadales, a group including sugar fermenting anoxic bacteria also found in wastewater processing sludge operated for simultaneous nitrification-denitrification/phosphorus removal where it functions in glycolysis and phosphorus removal (Zhange et al., 2024), suggesting that the flume material originally may have had high sugar, phosphorus and nitrate components. This order has also been found by sequencing from peat, rainforest soil, animals, and subsurface mineral samples.

In both samples, other abundant and notable bacteria include the Lachnospiraceae family, which ferment plant materials in animal guts including the human gut, as well as the *Ruminococcus* genus (found at higher abundance in the Highbury Canco sample) a *Clostridium* bacterium which breaks down plant cell walls in the human colon. The Coriobacteria found in both samples point to the oxygen-limited conditions in the samples, as does the presence of *Clostridium sensu stricto*, another typically anaerobic gut bacteria, and several of the other genera of bacteria and actinobacteria found in both samples. The HTML tables located in the 3.Abundance/Bacteria folder are a great place to explore the wealth of bacterial ASVs found in these samples.

The FAPROTAX analysis quantifies the functional groups of bacteria in the sample, and it should be noted that only 30% of the bacterial taxa identified in the flume samples were functionally annotated by FAPROTAX, so this should be taken as a measure of relative abundances of groups rather than an exhaustive list of all members and their functions. There are abundant chemoheterotrophs in flume, and although Conagra seems to have relatively more animal parasites or symbionts and Highbury Canco has relatively more chemoheterotrophs (Figure 17), we are only comparing between two samples, so it is impossible to draw any strong conclusions.

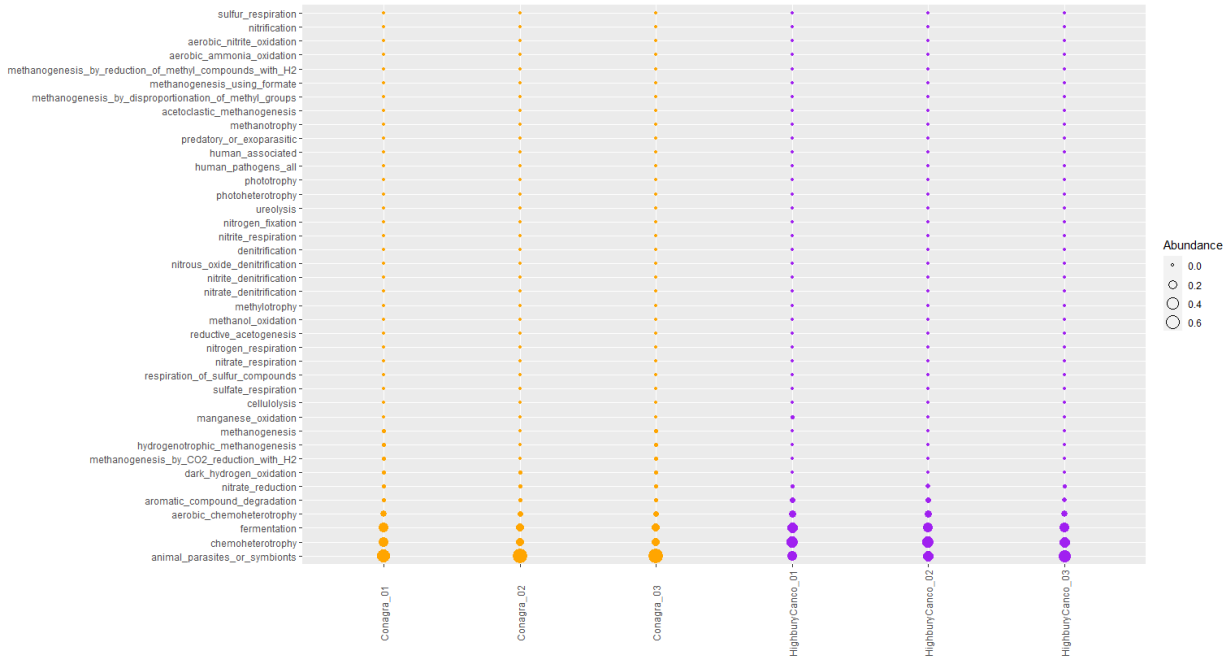


Figure 17*. FAPROTAX bubble plot of bacterial functional groups in two different flume samples, reporting the top 40 annotated categories (VRIC) Names on the left axis indicate functional groups of bacteria. Size of plots correspond to abundance of bacteria of those functional groups in each sample.*

Other functional groups seem relatively matched between the samples. An additional insight from FAPROTAX was gleaned by constructing Molecular Ecological Networks from the data. When we input the bacteria from both environments, the molecular ecological network shows two distinct communities or clusters of bacteria. This makes sense, as there are two

completely separate environments, so we should expect two separate bacterial communities. Molecular ecological networks were constructed for the bacteria in each environment separately. When the environments are separated there is only one cluster in the network which validates the subsampling protocol.

We also made ecological networks for all the microorganisms (fungi/oomycetes/bacteria) in both environments (Figure 18) and for each environment separately. Cytoscape files and individual environment maps are included in the supplementary material if further exploration of these networks is of interest.

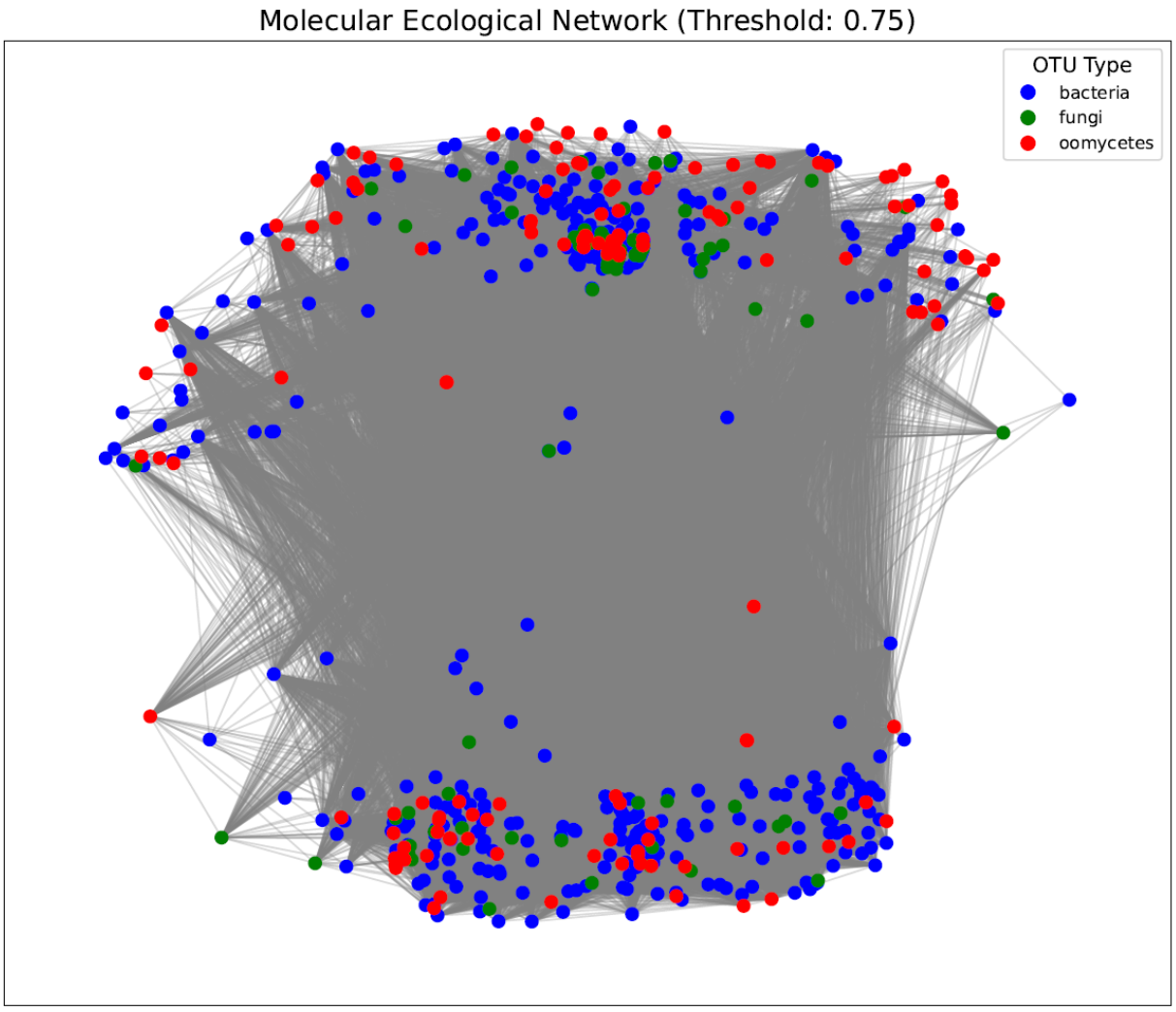


Figure 18. *Molecular Ecological Network for two flume samples showing clustering in two directions for the two different samples (VRIC).*

Differential abundance analyses are used to examine the most different variants or taxa between treatments to detect species that are particular to a specific sample or caused by a different treatment, such as different vegetable processing sources. Two heat maps are

produced for each sample, one for amplicon sequence variants (ASVs) and one for taxa. Multiple ASVs can map to one given taxon, so it is important to look at both formats. Clustering of communities was not completely related to each preparation, and both clustering was found to vary between ASV and taxa analyses due to ASVs and taxa mapping. As with other figures, easy-to-read vector versions of these plots are found in the submitted folder.

The difference between the two flume samples is also demonstrated by the differential abundance analyses elucidated in the attached HGI report. For example, a heat map of the most differentially abundant taxa in the bacterial sequence analysis shows a very stark contrast between the samples with little overlap (Figure 19,20) The ASV differential plots and associated bubble plots tell the same story of two very different microbiomes (see HGI report).

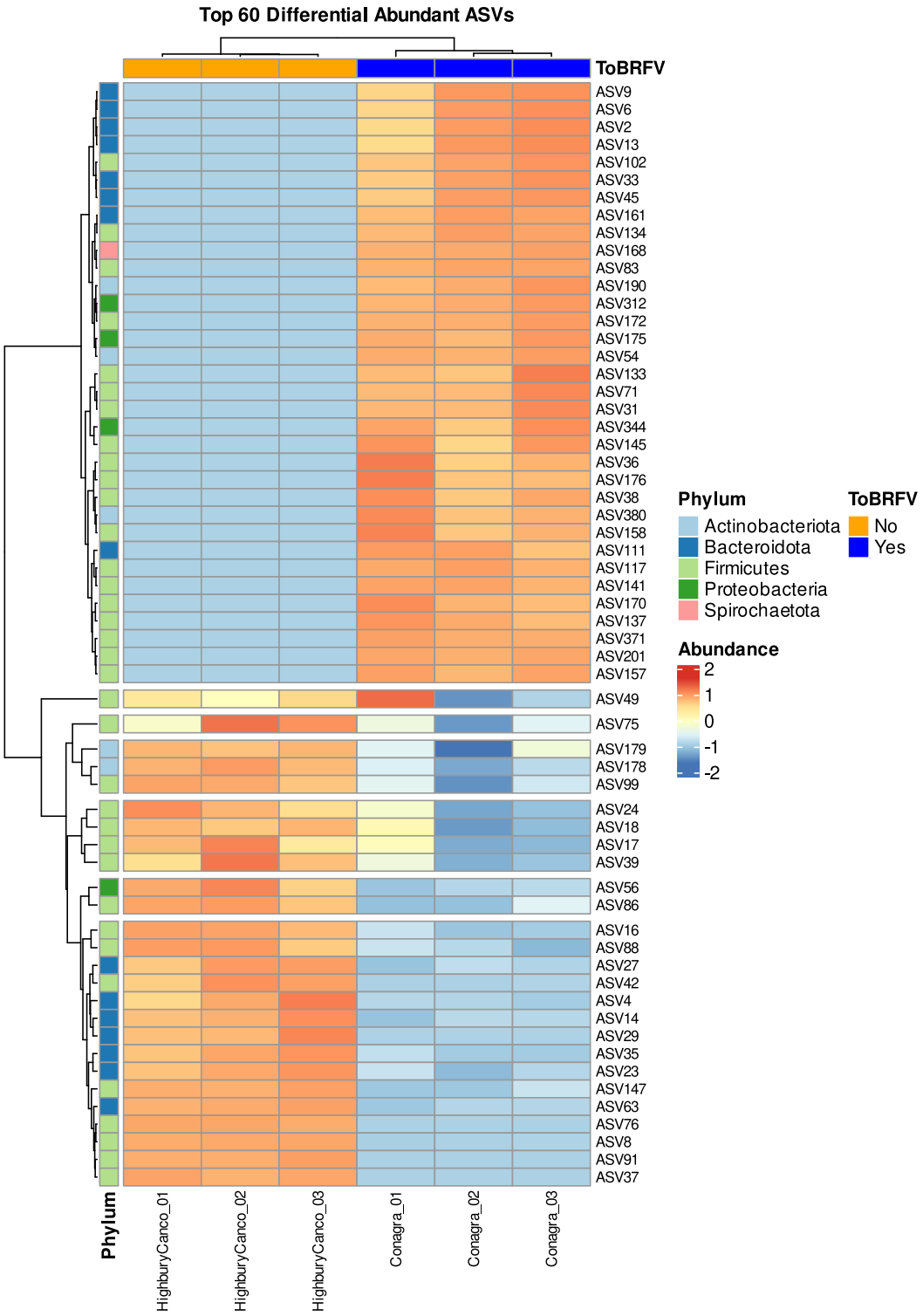


Figure 19. *Most differently abundant bacterial ASVs between treatments Names on the right of the Y axis are ASV's name. Heatmap has been clustered by rows and columns. To build this heatmap, abundances were Hellinger normalized and colors were generated from scaled transformed counts (HGI).*

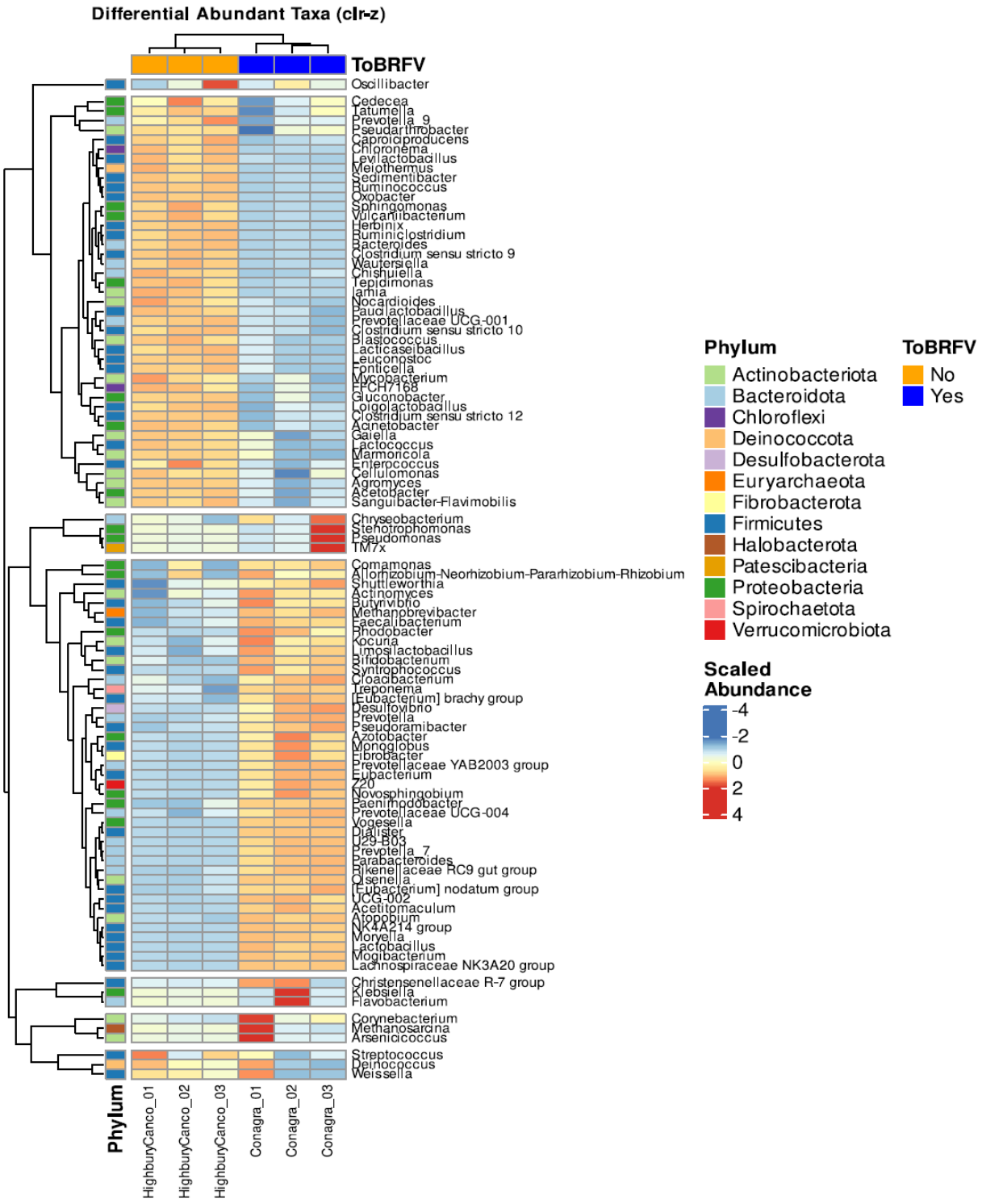


Figure 20. *Most differently abundant bacterial Taxa between two flume samples. Names on the right of the Y axis are taxonomic annotations at the Species rank. Counts have been crl-z transformed. This heatmap was built with ASV clustered at the Species rank. Samples have been clustered based on their counts. Please see attached HGI report for vector version (HGI)*

Fungal Communities

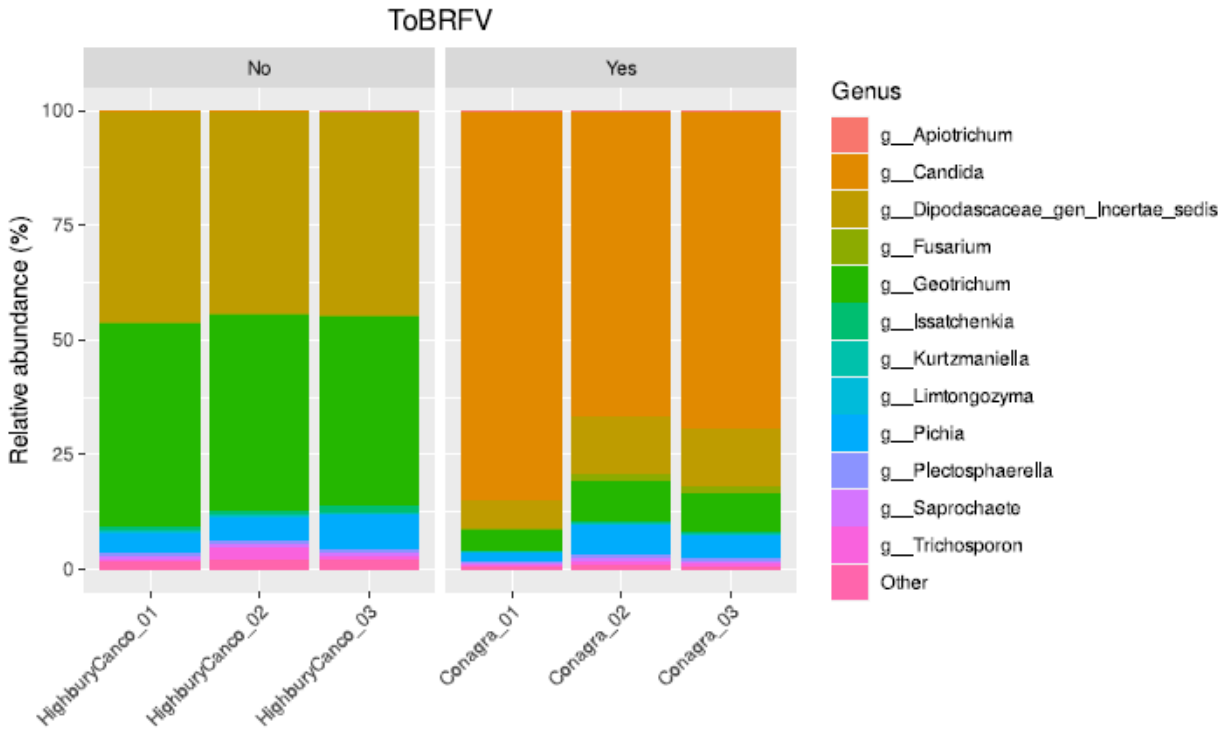


Figure 21. *Fungal community abundance between Highbury Canco and Conagra flume samples, at the Genus level (HGI). The size of the different coloured segments represents the relative abundance. Bar-charts are coloured based on Genus. Taxa not in the top 10 have been clustered in the "Other" category. ToBRFV indicates whether the sample tested positive for ToBRFV at arrival at Vineland.*

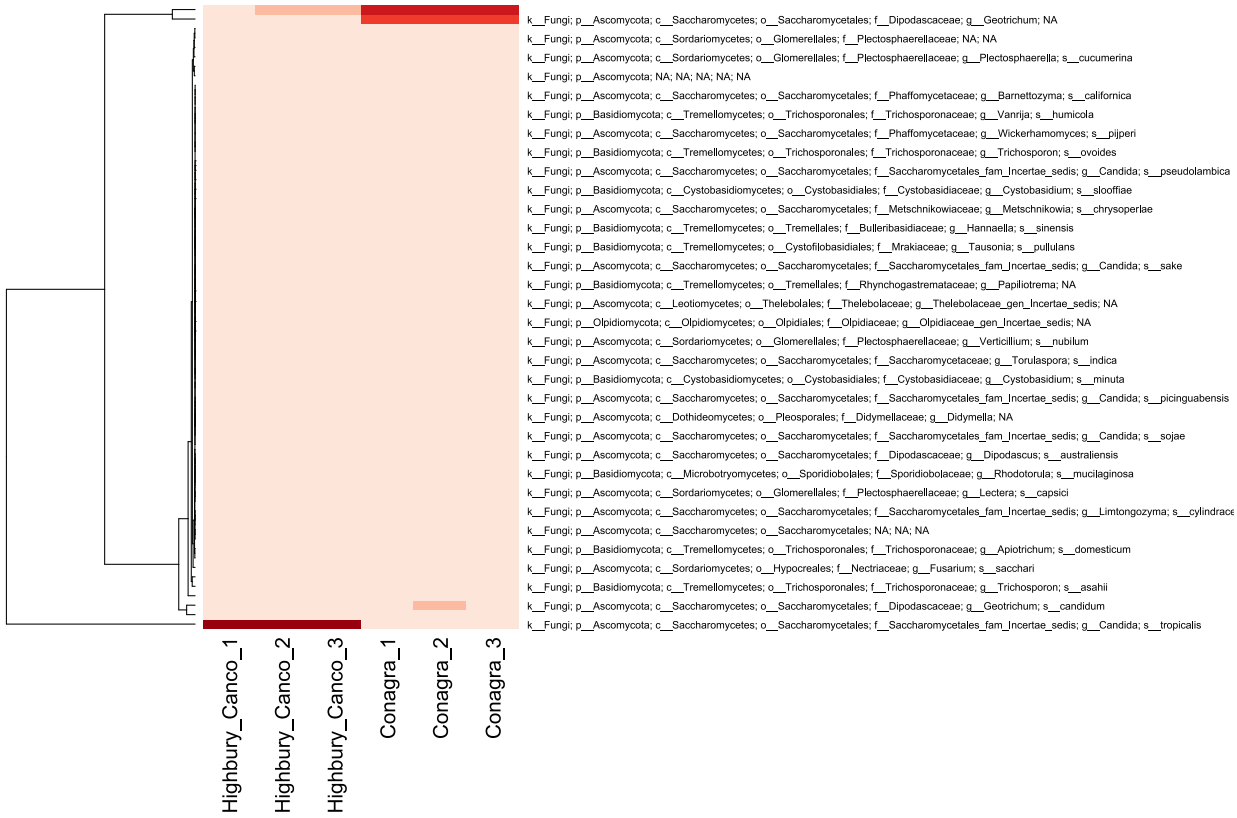


Figure 22. *Most prevalent fungal species present in Highbury Canco and Conagra flume samples determined by ITS1/2 targeted sequencing analysis. Vector version attached in supplementary material (VRIC). Lines on axis indicates phylogenetic tree of the species – how each species is related. Names on the right side indicate the names of the species detected. The darker the colour the more abundant the species is in the sample*

Fungal communities in the Conagra flume samples were dominated by *Candida* followed by *Dipodascaceae* (Figure 21, 22)*. Candida,* more specifically *Candida tropicalis* is an opportunistic pathogen often found in the soil, known to affect individuals with compromised immune systems, and causes urinary tract infections and soft tissue infections (Queiroz et al., 2023). Subspecies of *C. tropicalis* have been shown to have plant growth promoting activities on rice and have been used in a commercial biofertilizer product called BioGro (Amprayn et al., 2011). This organism is of interest due to its close similarity to other industrially valuable yeasts and is being explored as a bioproduction platform – using the organism to synthesize compounds of interest rather than industrial chemical synthesis platforms. (Queiroz et al., 2023). *Dipodascaceae* is a genus of fungi which is less prevalent than other genera of the family Dipodascaceae*. Dipodascaceae* is often found in woody areas such as forests, and has been noted as being able to infect immuno-compromised individuals (Lakshmi et al., 2023). In addition to this, species are being explored as bioproduction platforms (Cooper, 2011).

Fungal communities in Highbury Canco were dominated by a combination of *Geotrichum* and *Dipodascaceae* as well. G*eotrichum* species are found in many substrates, including soil, plants, milk, and water, or associated with insects. Infection by some species of *Geotrichum*

lead to sour rot, where infected fruits and vegetables appear water-soaked and soft, followed by mycelium growth on the surface and sour odour (Paes et al., 2021).

Saprotrophs: Decomposers that feed on nonliving organic matter (detritus) through absorptive nutrition, secreting enzymes to break down complex molecules e.g. cellulose.

Epiphytes: Fungi that grow on other plants for physical support without parasitizing them, deriving nutrients from air, rain, or debris

Endosymbionts: organisms that form a symbiotic relationship with another cell or organism. Some endosymbionts can be found either inside cells (intracellular), while others attach to the surface of cells (extracellular)

Endophytes: Are microorganisms (fungi or bacteria) that live within plant tissues (between tissue cells) without causing harm.

When comparing the two Flume samples using FUNGuild, Highbury Canco appears to have relatively more wood saprotrophs, undefined saprotrophs, and plant pathogens, while Conagra has relatively more epiphytes, endosymbionts, and endophytes (Figure 23). Saprotrophs contribute to soil fertility by breaking down organic materials and releasing mineral nutrients into the surrounding soil (Niego, 2023). Epiphytes support the development of habitats within habitats supporting further microbial life (Verma, 2023). Endosymbionts can form beneficial relationships with host organisms promoting nutrient acquisition and retention. Endophytes are similar to endosymbionts and are able to form a relationship with plant's roots extending into rhizosphere, promoting growth (Gupta 2022).

A total of 91/113 fungal ASV were differently abundant between the samples. Across both samples, the FUNGuild analysis found that 29/113 ASVs from the ITS1/2 readers were primarily undefined Saprotrophs, followed by 27/113 being Animal parasites and 22/113 being animal pathogens. Animal parasites include *Pichia*, which is found in wood and digestive systems of the beetle *Odontotaenius disjunctus. Pichia can* decay animal material and may likely serve as an opportunistic human pathogen (Irinyi et al. 2015, Põlme et al. 2020). Candida was identified as the endosymbiont and has been previously discussed. The identified epiphyte is *Symmetrospora oryzicola*, which is known as a red yeast and occurs on leaf surfaces (Haelewaters et al., 2020).

Focusing just on the plant pathogen fungi in the two flume samples, Highbury Canco has significantly more plant pathogenic fungi than Conagra, according to ANOVA & TukeyHSD analyses. This is of course relative to each other; we cannot determine whether these levels are high compared to other environmental samples, but potentially pathogenic taxa are found in these samples. They include the plant pathogenic fungi include *Geotrichum*, which was previously discussed earlier, sour rot of fruits and vegetables such as tomatoes, and citrus that occurs commonly across the globe including in Canada, the United Kingdom, Republic of South Africa, Brazil and Peru (Thornton et al. 2010).Also present in the samples is *Plectosphaerella cucumerina* (annotated as an Endophyte), a filamentous Ascomycete fungus which leads to fruit, root and collar rot and collapse in cucurbits, and other horticultural crops (Carlucci et al., 2012). Also identified was *Fusarium sacchari* a plant

pathogen and wood saphrophyte, which grows optimally in tropical climates, leading to ‘Pokkah Boeng” disease and wilting in Sugar Cane, as well as fruit rot in bananas (Yao et al., 2020).

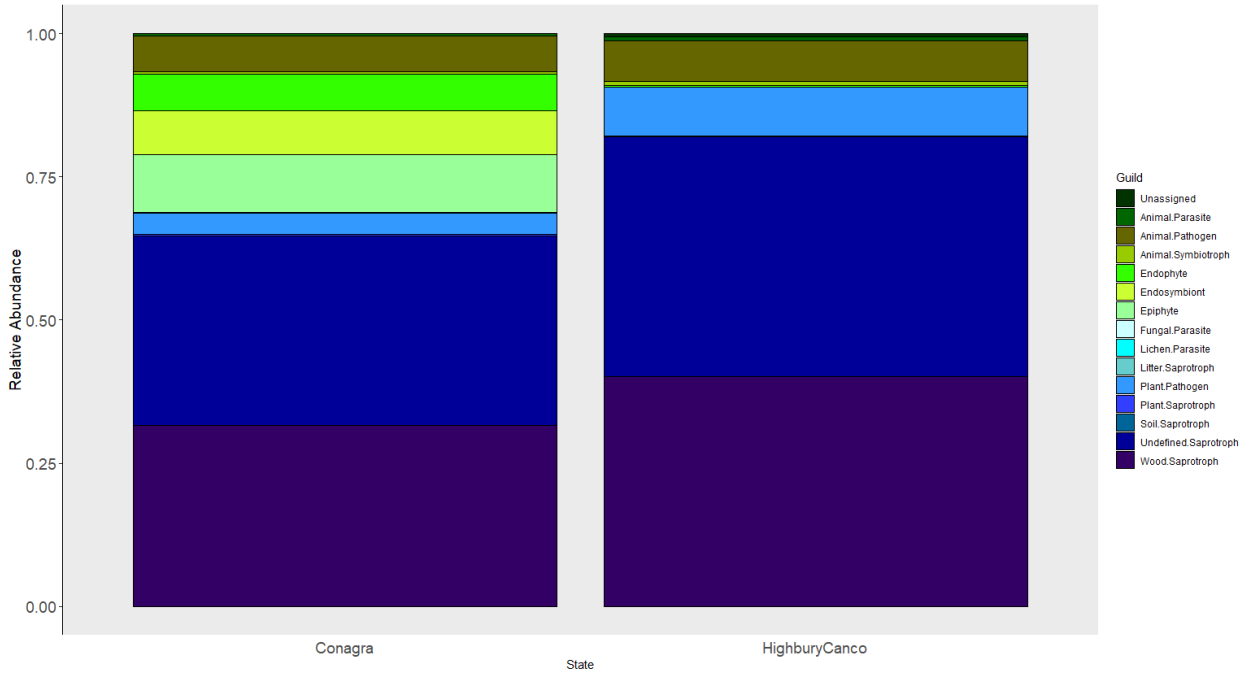


Figure 23. *Annotated fungal guilds for Highbury Canco and Conagra flume samples, generated using FUNGUild (VRIC).*

The difference between the two flume samples is also demonstrated by the differential abundance analyses elucidated in the attached HGI report. For example, a heat map of the most differentially abundant taxa in the fungal sequence analysis shows a very stark contrast between the samples with little overlap (Figure 24,25). The ASV differential plots and associated bubble plots tell the same story of two very different microbiomes (see HGI report).

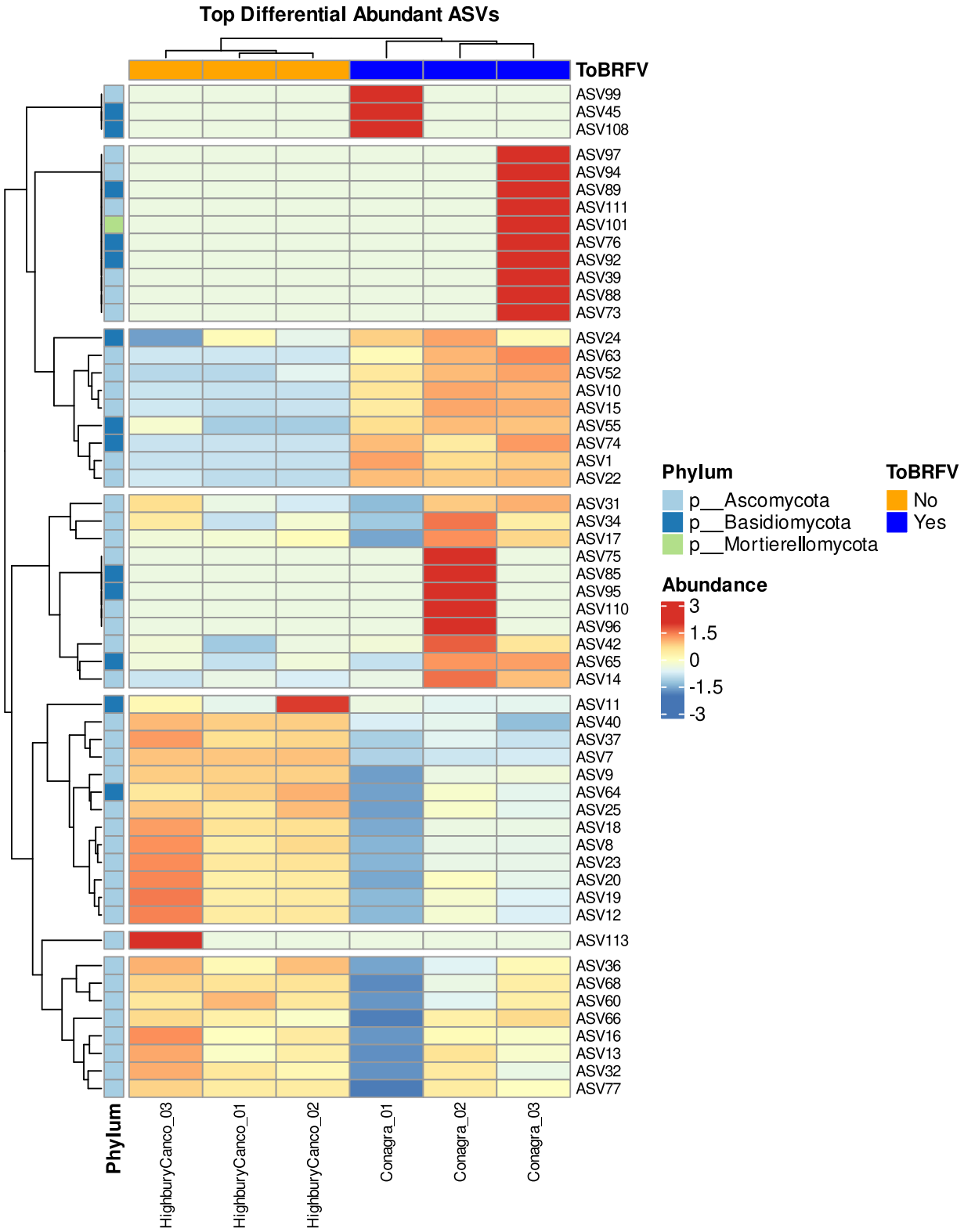


Figure 24. *Most differently abundant fungal ASVs between treatments. Names on the right of the Y axis are ASV's name. Heatmap has been clustered by rows and columns. To build this heatmap, abundances were Hellinger normalized and colors were generated from scaled transformed counts. (HGI).*

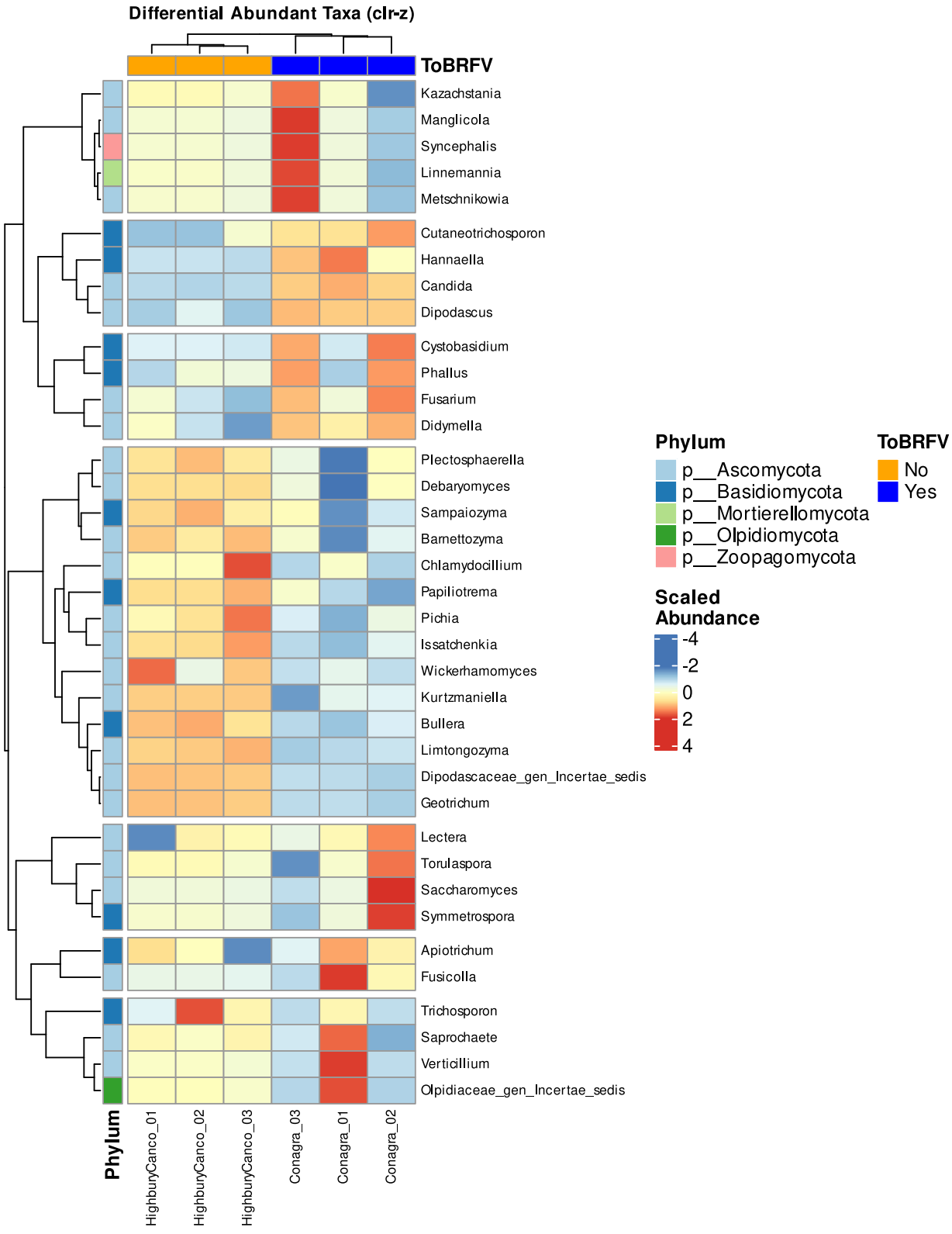


Figure 25. *Most differently abundant fungal taxa between treatment. Names on the right of the Y axis are taxonomic annotations at the Genus rank. Heatmap has been clustered by rows. Abundances are scaled transformed count. Counts have been crl-z transformed. (HGI).*

Oomycete Communities.

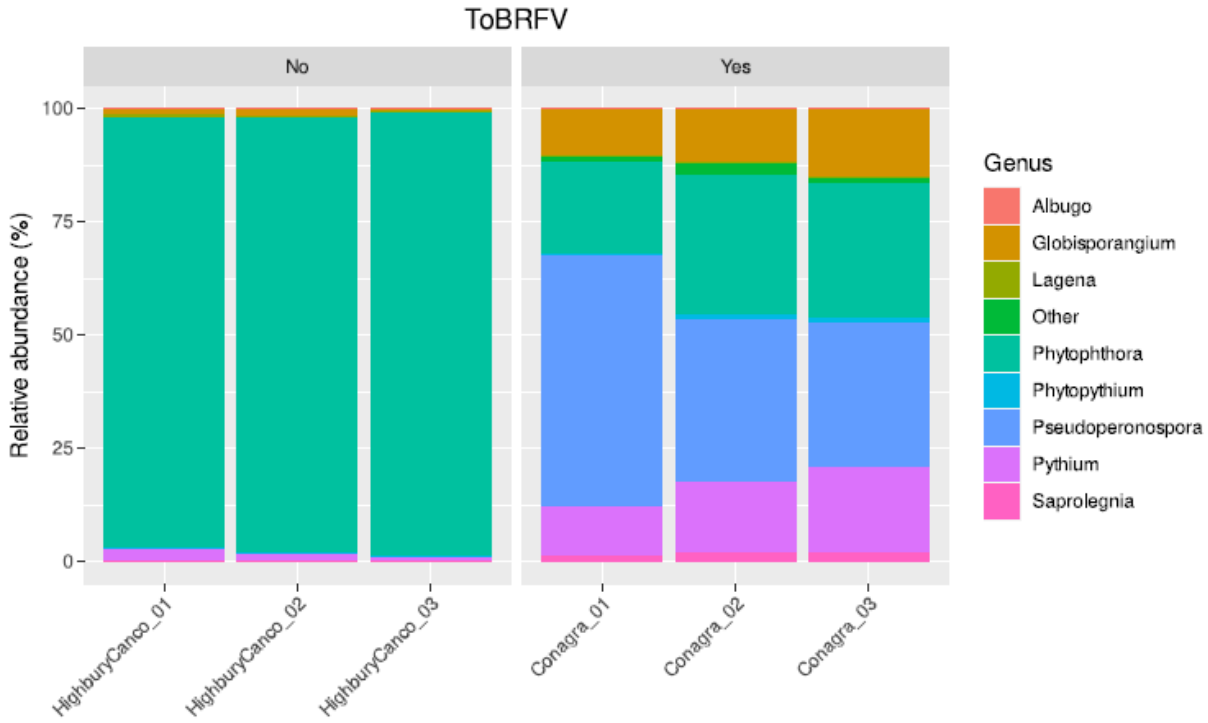


Figure 26. *Histogram of the top 10 most abundant Oomycete taxa in at the Genus rank from two samples of Tomato Flume (HGI). The size of the different coloured segments represents the relative abundance. Bar-charts are coloured based on Genus. Taxa not in the top 10 have been clustered in the "Other" category. ToBRFV indicates whether the sample tested positive for ToBRFV at arrival at Vineland.*

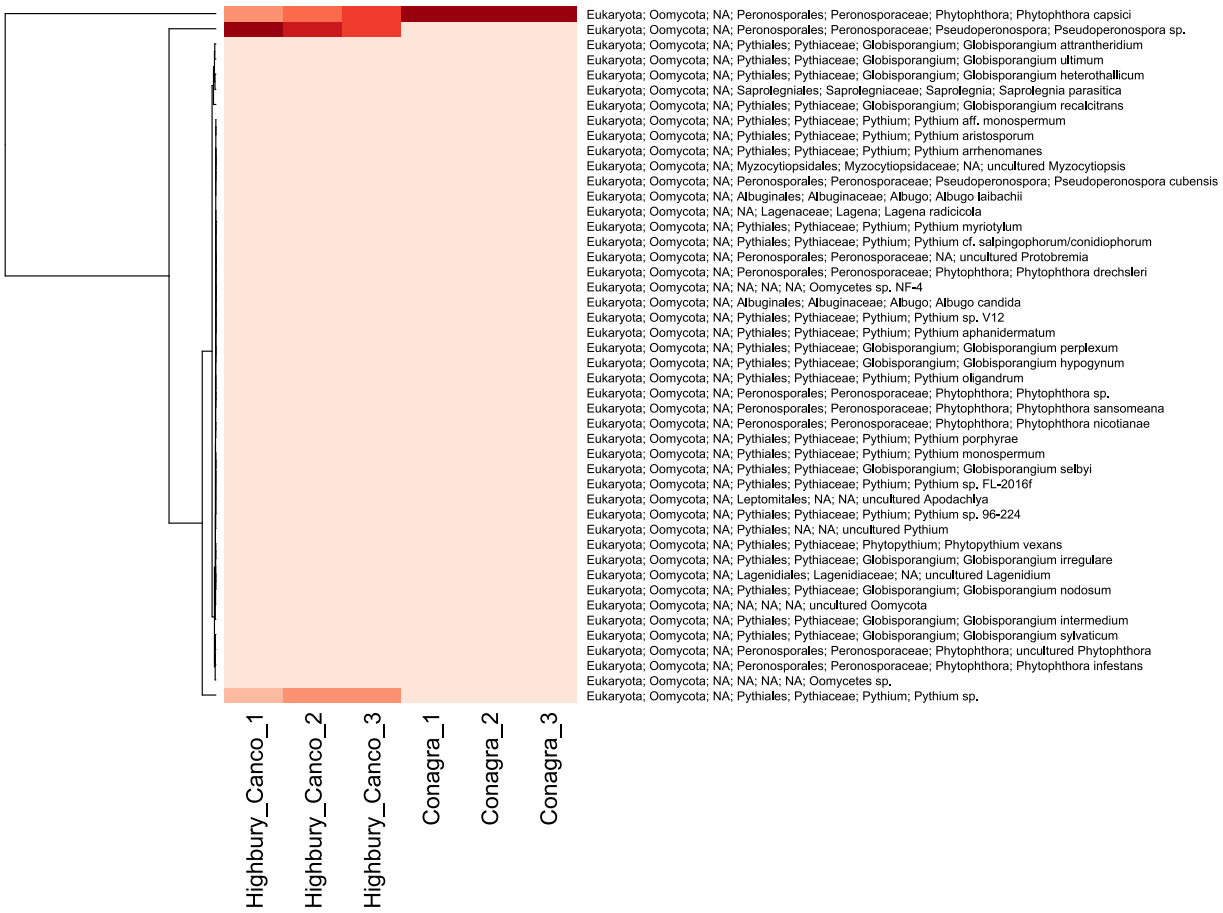


Figure 27. *Most prevalent Oomycete species present in Highbury Canco and Conagra flume samples determined by ITS1/2 targeted sequencing analysis. Vector version attached in supplementary material (VRIC). Lines on axis indicates phylogenetic tree of the species – how each species is related. Names on the right side indicate the names of the species detected. The darker the colour the more abundant the species is in the sample*

As seen in the Beta diversity report, the two communities are distinct between Highbury Canco and Conagra. When looked at more in-depth, it is seen that Highbury Canco Oomycete communities were dominated by *Pytophthora*, while Conagra contained a mixture of Pythium, Pseudoperonospora, *Phytophthora* and *Globisporangium.* A total of 31/143 Oomycetes ASVs were differently abundant between the Highbury Canco and Conagra samples.

Both samples had high abundances of *Phytophthora capsici,* a well-known plant pathogen*.* This organism has a wide host range, including peppers, eggplants, cucurbits, snap beans, lima beans, some weed species and tomatoes. *P. capsici* thrives in warm and humid or wet conditions*. P. capsici* leads to fruit rot in tomatoes, where symptoms include crown rot, buckeye rot, soft rot and white mycelial growth. *P. capsici* can form Oospores, which have thick walls, which are resistant to degradation and can remain dormant in the soil for up to 10 years. We are unable to determine if *P. capsica's* presence is due to recent infection, or dormant oospores.

Also detected was Pseudoperonospora, however not at a species level. Species in this group are known to cause downy mildews on hemp, hops and cucurbits. Once established in a

region, this pathogen can spread rapidly via airborne inoculum, leading to severe defoliation, decreased crop quality, marketability and yield. Often growers see water-soaked lesions on leaves, with the lesions growing in number and size as the disease progresses. This disease circulates throughout much of the year in the Southern US but does not overwinter in Ontario; rather it travels gradually from the Gulf of Mexico during the summer and would not be expected to be found in plant or environmental samples before late summer.

Globisporangium and *Pythium* both lead to root rot and damping off in a variety of species. *Pythium* can affect spinach, cucumbers, peppers and tomatoes, while *Globisporangium* affects apples, carrots, garlic, lettuce, and spinach. With root rot and damping off, plants often have discolored roots, with attenuation and decay. In turn this harms yield, plants that grow experience stunting, wilting and thinning. In the case of spinach, damping off is regularly seen between April to June. Both organisms favour wet soil conditions, and infection occurs when drainage is poor and in heavy soil types. Management strategies for root rot and damping off include using sterile flats, preventing overwatering of seedlings and using registered products (Ministry of Agriculture and Rural Affairs, 2024.)

The difference between the two flume samples is also demonstrated by the differential abundance analyses elucidated in the attached HGI report. A heat map of the most differentially abundant taxa in the Oomycete sequence analysis shows a very stark contrast between the samples with little overlap (Figure 28,29).

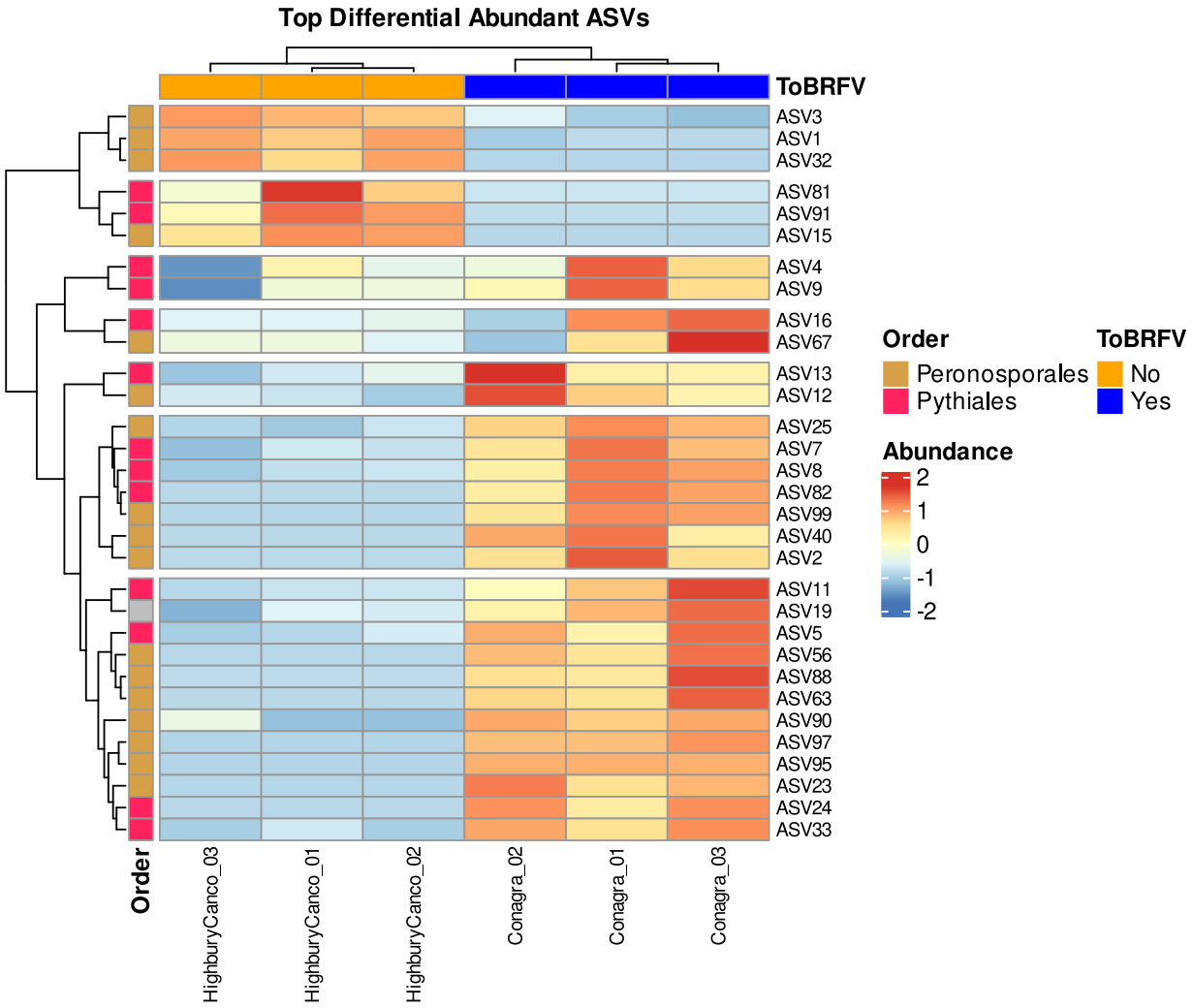


Figure 28. *Most differently abundant Oomycetes ASVs between treatments. Names on the right of the Y axis are ASV's name. Heatmap has been clustered by rows and columns. To build this heatmap, abundances were Hellinger normalized and colors were generated from scaled transformed counts. (HGI)*

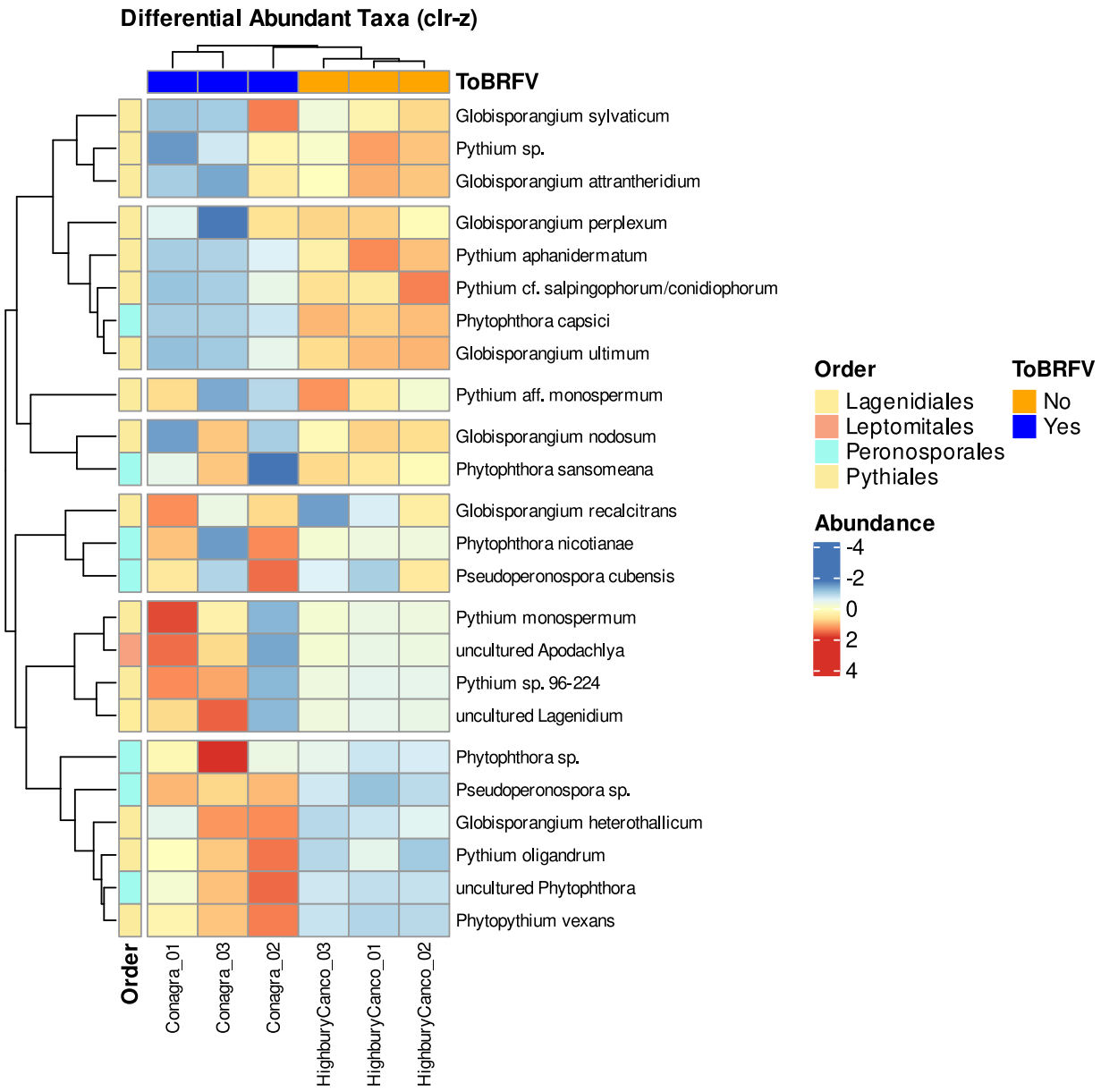


Figure 29. *Most differently abundant Oomycetes taxa between treatment. Names on the right of the Y axis are taxonomic annotations at the Species rank. Heatmap has been clustered by rows and columns. Counts have been crl-z transformed. Abundances colors are scaled transformed count. (HGI)*

Conclusions

Distinct microbial community compositions were found between the Highbury Canco and Conagra samples. Bacterial diversity was similar across the flume samples, with differences at the phylum level. Fungal diversity was lower in the Highbury Canco sample compared to the Conagra sample, while Oomycete diversity was lower in the Conagra Sample compared to the Highbury Canco sample. Plant pathogen communities differed between flume samples and Highbury Canco had relatively higher levels of plant pathogenic fungi: It was dominated by a sequence matching *Phytophthora capsica* whereas the Conagra sample was dominated by an amplicon sequence variant (ASV) matching *Candida tropicalis.* Microbial communities

across preparations are dominated by environmental bacteria involved in nutrient transformations such as the Bacteroidetes. Highbury Canco flume appears to have relatively more fungal wood saprotrophs, undefined saprotrophs, and plant pathogens whereas Conagra has relatively more epiphytes, endosymbionts, and endophytes. Both samples had high reads of Oomycetes. Both the Fungi and Oomycete can cause disease in humans, animals and importantly plants.

Evidence-Based Research and Recommendations

1	Caution should be exercised when using both Highbury Canco and Conagra flume as non-agricultural source materials. Many animal and plant pathogenic species sequences were retrieved. The current analysis does not determine the incidence of live pathogens in the samples tested only whether their DNA was present at the time of study, and therefore while caution is warranted, the level of risk due to pathogens to health, crop quality and yield is unknown. It is not recommended to apply susceptible crops, or in areas near susceptible crops.
2	Flume samples are able to support the growth of anaerobic bacteria such as Prevotellaceans, as well as Saccharimonadales. Flume microbial composition should be monitored over time, and in different storage conditions, to understand how the microbiome changes and potential impacts when considering applications.
3	It would be interesting to determine what factors in the Highbury Canco and Conagra samples (e.g. plant material, pH, crops, soil composition) contributed to the differences in microbial diversity.
4	The collected flume was only tested for ToBRFV, however it would be valuable to understand what viruses and viroids are present in the flume samples, as that can impact potential applications. There is no standard practice to evaluate a wide range of known and novel viral genomes in a cost-effective manner.
5	Genome mining enables the identification of genes encoding beneficial enzymes and secondary metabolites that facilitate resource acquisition and modulate plant hormone level. Further mining the genome of flume samples could identify enzymes and secondary metabolites that aid crop growth (Paterson, 2017).

Activity 4: Chemical analyses of flume samples collected in 2024

Background

- Volatile profiling identified toluene and methylphenol (cresol), a microbial metabolite of toluene as a primary component of wet tomato flume sediment collected in 2023.
- Tomato flume sediment samples collected in 2023 had high levels of steroidal alkaloids, natural metabolites from tomato plants.

Methodology

- Multiple technical replicates (aliquots) of the bulk wet flume sample provided by Highbury Canco was used for characterization samples/replicates
- Mass spectrometry-based chemical analyses were used to characterize similarities and differences between tomato flume sediment samples provided by Highbury Canco in 2024, and 2023 samples from Conagra and Highbury Canco.
- Chemical components that may underlie malodorous attributes or influence potential uses for repurposed flume were identified and characterized

The following analytical methods were used to characterize the organic chemical composition of flume sediment provided by Highbury Canco in 2024:

GC-EI-MS (Gas chromatography electron ionization mass spectrometry)

- Gas chromatography–mass spectrometry (GC-MS). Odor-causing volatile chemicals emitted from flume samples were trapped and concentrated, and primary chemical components were identified by GC-MS
- This provides a comprehensive analysis of organic aroma chemicals that might contribute to odor
- This "volatile" chemical fingerprint contributes to understanding the source of odorant chemicals in flume, and ways to mitigate their production

UPLC-ESI-QTOF-MS (Ultra-performance liquid chromatography couple to electrospray ionization quadrupole-time-of-flight mass spectrometry)

- Water-soluble extracts of flume samples were analyzed using untargeted UPLC-MS analysis, providing a high-resolution profile of the organic chemicals present
- This approach provides a comprehensive profile of thousands of flume-associated chemicals
- This fingerprint of non-volatile chemical helps identify potential sources of odorant chemicals identified by GC-MS, as well as other plant-, or microbe-derived chemicals that could affect potential uses in the re-purposing of flume.

Detailed methods and software versions are provided in last year's final project report ("Flume Repurposing") using volumes/amounts noted for the Conagra wet flumes samples.

Results and Discussion

Volatile profiling identifies methylphenol as a primary component of wet tomato flume sediment in 2024 sample (Figure 30)

- The volatile phenolic methylphenol was the primary component of volatile organic chemicals collected from wet tomato flume sediment samples provided by Highbury-Canco.
- Methylphenol (cresol), is a microbial metabolite of toluene, which was the most abundant volatile chemical in wet flume samples in 2023 and contributes to the odor of the material.
- Toluene is still detectable, but most has been converted to methylphenol presumably by microbial metabolism.

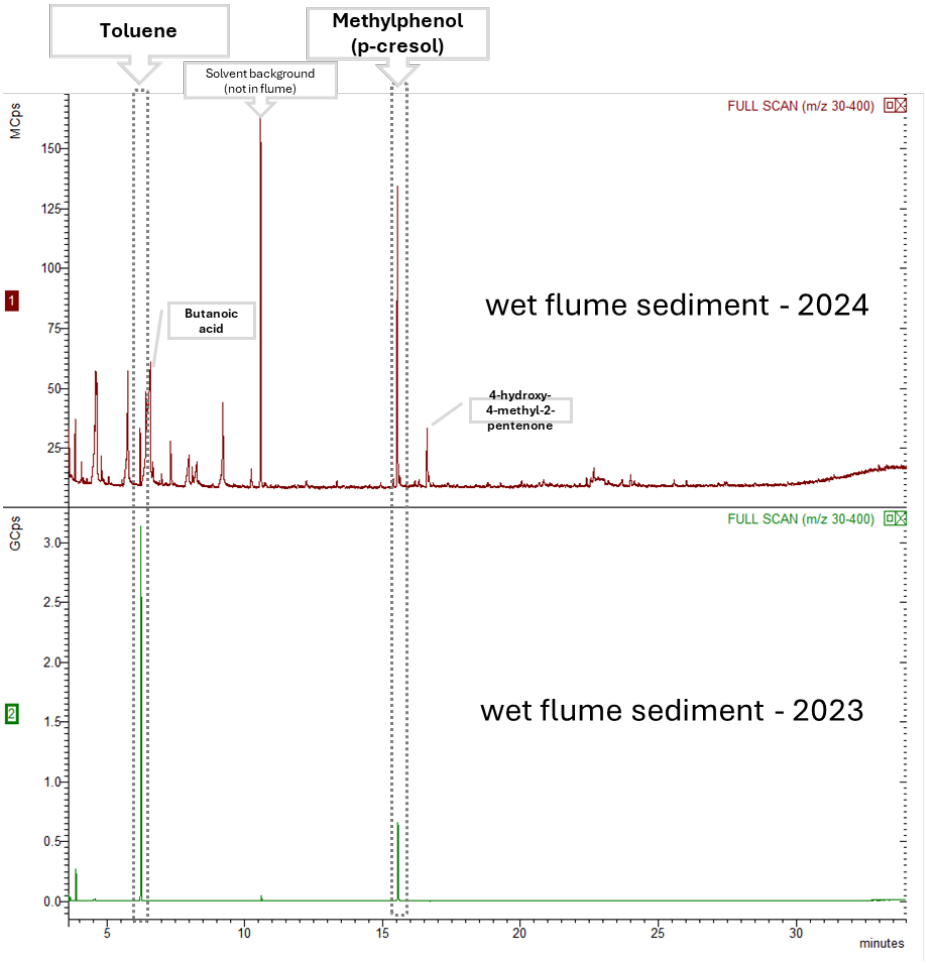


Figure 30. *Gas chromatography analysis of volatile chemical profiles for tomato flume sediment provided by Highbury Canco in 2024 compared to wet flume sediment from Conagra collected and analyzed in 2023.*

- The 2024 flume sample has more organic acid contents (i.e. butanoic acid) likely derived from decomposing tomato material.

LC-MS metabolomics confirms substantial steroidal alkaloid content in tomato flume sediment

- The five most abundant chemicals detected in tomato flume sediment samples from Highbury Canco in 2024 belong to a class of tomato-derived defense biochemicals called steroidal alkaloids, known for their antibacterial and antifungal properties (Table 9). These were also the most abundant chemical class in the 2023 samples.
- Tomatidine and related steroidal alkaloids from tomato are also phytotoxins (Hoagland, 2009; Itkin et al, 2011), so the abundant steroidal alkaloid content of flume sediment provides further explanation for the potent phytotoxic effects of flume water in the germination trials (see Activity 2).

Table 9. Top 5 most abundant chemicals detected in flume sediment collected in 2024

Compound	Putative compound ID	Predicted Molecular Formula	Mass Error (ppm)
15.60_446.3259m/z	steroidal alkaloid derivative	$C_{27}H_{43}NO_4$	-1.3
20.23_489.3450n	steroidal alkaloid derivative	$C_{29}H_{47}NO_5$	-0.92
21.52_487.3295n	steroidal alkaloid derivative	$C_{29}H_{45}NO_5$	-0.59
22.26_415.3443n	**tomatidine** (a steroidal alkaloid)	$C_{27}H_{45}NO_2$	-1.87
22.41_489.3455n	steroidal alkaloid derivative	$C_{29}H_{47}NO_5$	0.06

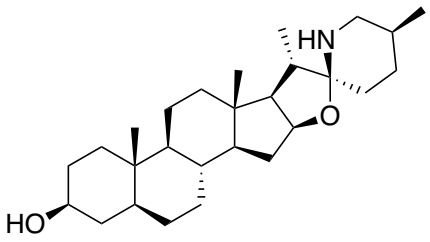


Figure 31. *Structure of tomatidine, a phytotoxin and major component of tomato flume sediment.*

Conclusion

Although having phytotoxic effects on the germination and early growth of lettuce, radish and cucumber seeds, Highbury Canco flume could be used on farm for increasing available nitrogen in the soil as demonstrated by the increase in ammonia/ammonium in the incubation trial. Application of flume would ideally target specific crops after transplanting, as these later crop cycles typically have higher tolerances to amendments with high electrical conductivity. Given the acidic conditions of the Highbury Canco flume, its application to support acidic soil conditions could be used to support the growth of acid-loving horticultural crops including magnolias, hydrangeas and potatoes. In addition to this, given the high electrical conductivity, crops such as potatoes, citrus fruits and alfalfa can tolerate higher electrical conductivity with little crop loss. Further, as the soil respiration is quite high and may release nutrients such as nitrogen to quickly for plant uptake; therefore, a lower application rate of flume than what has been tested here may be a beneficial reuse.

The challenge with flume use under NASM is that available nutrients are calculated based on set equations with set multipliers that estimate nutrient release for plant uptake. Flume sediment inherently contains low amounts of total nitrogen, phosphorus and potassium, which is a challenge for beneficial reuse as NASMs require a set threshold to be allowed for application on a farm (13,000 ppm). Flume sediment may release nutrients as higher rates compared to this multiplier, which may underestimate the potential value of the nutrients supplied in the growing season, especially right after application.

Beyond the nutrients within flume sediment, flume provides microorganisms that are able to breakdown soil organic matter and build soil health. Although having a rich microbiome, the presence of human, animal and plant pathogens should remain a key consideration when deciding how to use flume for field application. Additionally, given the high transmissibility of ToBRFV, and its widespread nature in Ontario, all flume samples should undergo ToBRFV testing before further use, to determine the risk to rugose-susceptible crops.

Bibliography

Amprayn, K., Rose, M. T., Kecskés, M., Pereg, L., Nguyen, H. T., & Kennedy, I. R. (2012). Plant growth promoting characteristics of soil yeast (Candida tropicalis HY) and its effectiveness for promoting rice growth. *Applied Soil Ecology : A Section of Agriculture, Ecosystems & Environment*, *61*, 295–299. https://doi.org/10.1016/j.apsoil.2011.11.009

Carlucci, A., Raimondo, M. L., Santos, J., & Phillips, A. J. L. (2012). Plectosphaerella species associated with root and collar rots of horticultural crops in southern Italy. *Persoonia*, *28*(1), 34–48. https://doi.org/10.3767/003158512X638251

Cooper, C. R. (2011). Chapter 2 - Yeasts Pathogenic to Humans. In *The Yeasts* (Fifth Edition, pp. 9–19). Elsevier B.V. https://doi.org/10.1016/B978-0-444-52149-1.00002-1

De Filippis F, Pasolli E, Tett A, Tarallo S, Naccarati A, De Angelis M, Neviani E, Cocolin L, Gobbetti M, Segata N, Ercolini D. (2019). Distinct genetic and functional traits of human intestinal *prevotella copri* strains are associated with different habitual diets. *Cell Host Microbe*. 25:444–453. 10.1016/j.chom.2019.01.004.

Gale, E.S., Sullivan, D.M., Cogger, C.G., Bary, A.I., Hemphill, D.D., and Myhre, E.A. 2006. Estimating Plant-Available Nitrogen Release from Manures, Composts, and Specialty Products. Journal of Environmental Quality, 35, 2321-2332.

Gupta, A., Raina, M., & Kumar, D. (2022). Endophytic microorganisms: utilization as a tool in present and future challenges in agriculture. In *Biocontrol Mechanisms of Endophytic Microorganisms* (pp. 285–301). Elsevier. https://doi.org/10.1016/B978-0-323-88478-5.00013-4

Haelewaters, D., Toome-Heller, M., Albu, S., & Aime, M. C. (2020). Red yeasts from leaf surfaces and other habitats: three new species and a new combination of Symmetrospora (Pucciniomycotina, Cystobasidiomycetes). *Fungal Systematics and Evolution*, *5*(1), 187–196. https://doi.org/10.3114/fuse.2020.05.12

Hill-Burns, E. M.; Debelius, J. W.; Morton, J. T.; Wissemann, W. T.; Lewis, M. R.; Wallen, Z. D.; Peddada, S. D.; Factor, S. A.; Molho, E.; Zabetian, C. P.; Knight, R.; Payami, H. (2017). Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Movement Disorders*. 32(5): 739–749. 10.1002/mds.26942.

Hoagland, R. E. (2009). Toxicity of tomatine and tomatidine on weeds, crops and phytopathogens fungi. *Allelopathy Journal*, *23*(2), 425-436.

Irinyi, L., Serena, C., Garcia-Hermoso, D., Arabatzis, M., Desnos-Ollivier, M., Vu, D., Cardinali, G., Arthur, I., Normand, A.-C., Giraldo, A., da Cunha, K. C., Sandoval-

Denis, M., Hendrickx, M., Nishikaku, A. S., de Azevedo Melo, A. S., Merseguel, K. B., Khan, A., Parente Rocha, J. A., Sampaio, P., … Meyer, W. (2015). International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database—the quality controlled standard tool for routine identification of human and animal pathogenic fungi. *Medical Mycology*, *53*(4), 313–337. https://doi.org/10.1093/mmy/myv008

Itkin, Maxim, et al. (2011). "GLYCOALKALOID METABOLISM1 is required for steroidal alkaloid glycosylation and prevention of phytotoxicity in tomato." *The Plant Cell* 23.12 (2011): 4507-4525.

Lakshmi, D. V., Prasad, B. V. S., & Prasad, D. V. R. (2023). Dipodascus capitatus: A rare and emerging yeast like fungal infection in immuno-compromised subjects. *IP International Journal of Medical Microbiology and Tropical Diseases*, *9*(1), 17–25. https://doi.org/10.18231/j.ijmmtd.2023.004

Luo, Y., Liang, J., Zeng, G., Chen, M., Mo, D., Li, G., & Zhang, D. (2018). Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. *Waste Management*, *71*, 109–114. https://doi.org/10.1016/j.wasman.2017.09.023

Ministry of Agriculture and Rural Affairs. (2024). *Guide to Vegetable Production in Ontario*.

Moebius-Clune, B.N., D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu, R.R. Schindelbeck, A.J. Ristow, H.M. van Es, J.E. Thies, H.A. Shayler, M.B. McBride, K.S.M Kurtz, D.W. Wolfe, and G.S. Abawi, 2016. Comprehensive Assessment of Soil Health – The Cornell Framework, Edition 3.2, Cornell University, Geneva, NY.

Nash, D., Ellmen, I., Knapp, J. J., Menon, R., Overton, A. K., Cheng, J., Lynch, M. D. J., Nissimov, J. I., & Charles, T. C. (2024). A Novel Tiled Amplicon Sequencing Assay Targeting the Tomato Brown Rugose Fruit Virus (ToBRFV) Genome Reveals Widespread Distribution in Municipal Wastewater Treatment Systems in the Province of Ontario, Canada. *Viruses*, *16*(3), 460. https://doi.org/10.3390/v16030460

Niego, A. G. T., Rapior, S., Thongklang, N., Raspé, O., Hyde, K. D., & Mortimer, P. (2023). Reviewing the contributions of macrofungi to forest ecosystem processes and services. *Fungal Biology Reviews*, *44*, 100294. https://doi.org/10.1016/j.fbr.2022.11.002

Paterson, J., Jahanshah, G., Li, Y., Wang, Q., Mehnaz, S., & Gross, H. (2017). The contribution of genome mining strategies to the understanding of active principles of PGPR strains. *FEMS Microbiology Ecology*, *93*(3), fiw249. https://doi.org/10.1093/femsec/fiw249

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., Nguyen, N., Kjøller, R., Bates, S. T., Baldrian, P., Frøslev, T. G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järv, H., Madrid, H., Nordén, J., … dos Santos Chikowski, R. (2020). FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, *105*(1), 1–16. https://doi.org/10.1007/s13225-020-00466-2

Queiroz, S. S., Jofre, F. M., Bianchini, I. A., Boaes, T. D. S., Bordini, F. W., Chandel, A. K., & Felipe, M. D. G. A. (2023). Current advances in Candida tropicalis: Yeast overview and biotechnological applications. *Biotechnology and applied biochemistry*, *70*(6), 2069–2087. https://doi.org/10.1002/bab.2510

Tanaka S, Yoshida M, Murakami Y, et al. (2008). The relationship of *Prevotella intermedia*, *Prevotella nigrescens* and *Prevotella melaninogenica* in the supragingival plaque of children, caries and oral malodor. *J Clin Pediatr Dent.* 32(3): 195–200. 10.17796/jcpd.32.3.vp6571778156l8l1.

Tett A, Pasolli E, Masetti G, Ercolini D, Segata N. (2021). *Prevotella* diversity, niches and interactions with the human host. Nature Reviews. Microbiology. 19 (9): 585–599. 10.1038/s41579-021-00559-y.

Thornton, C. R., Slaughter, D. C., & Davis, R. M. (2010). Detection of the sour-rot pathogen Geotrichum candidum in tomato fruit and juice by using a highly specific monoclonal antibody-based ELISA. *International Journal of Food Microbiology*, *143*(3), 166–172. https://doi.org/10.1016/j.ijfoodmicro.2010.08.012

Tracey, A. (2019, May 31). *Is Phytophthora capsici Causing your Tomato Fruit Rot?* ONVegetables.

Verma, A., Bind, S., & Bajeli, J. (2023). Inflection of the root microbiome by plants: Plant growth promotion and disease management. In *Unravelling Plant-Microbe Synergy* (pp. 151–173). Elsevier. https://doi.org/10.1016/B978-0-323-99896-3.00004-7

Wang X, Tsai T, Deng F, Wei X, Chai J, Knapp J, Apple J, Maxwell CV, Lee JA, Li Y, Zhao J (2019). Longitudinal investigation of the swine gut microbiome from birth to market reveals stage and growth performance associated bacteria. *Microbiome*. 7(1): 109. 10.1186/s40168-019-0721-7.

Wuest, S.B., and Gollany, H.T. 2012. Soil organic carbon and nitrogen after application of nine organic amendments. Soil and Water Management and Conservation, Soil Science Society of American Journal, 77(1), 237-245.

Zhang, J., Lei, S., Zhang, X., Xie, S., Zheng, Y., Yang, W., Chen, A. & Zhao, J. (2024). Enhanced nitrogen and phosphorus removal by Saccharimonadales sp. in a sequencing batch reactor. *Biochemical Engineering Journal*, 211, 109456. 10.1016/j.bej.2024.109456

Appendix A

Table 1. *Observed values for micro- and macro-nutrients as well as cation exchange capacity for control and Flume treatments (Low = 12.5 tonnes/ha, Moderate = 25 tonnes/ha, and High = 50 tonnes/ha) during the incubation trial day 1.*

Treatment	Cation Exchange Capacity meq/100g	Phosphorus (Bray-1) ppm	Calcium ppm	Magnesium ppm	Sulphur ppm	Sodium ppm	Zinc ppm	Manganese ppm	Iron ppm	Copper ppm	Boron ppm	Aluminum ppm
Control	17.73	22.33	3330.00	100.67	24.00	32.00	3.10	51.00	58.33	1.23	0.57	317.67
Low	17.83	21.33	3346.67	101.67	23.33	30.00	2.97	53.67	59.33	1.20	0.50	286.33
Moderate	17.67	21.67	3303.33	103.67	22.67	29.67	2.93	52.00	61.00	1.20	0.50	274.33
High	17.23	22.33	3206.67	106.33	19.67	32.00	2.97	50.67	62.00	1.20	0.50	274.67

Table 2. *Observed values for micro- and macro-nutrients as well as cation exchange capacity for control and Flume treatments (Low = 12.5 tonnes/ha, Moderate = 25 tonnes/ha, and High = 50 tonnes/ha) during the incubation trial day 7.*

Treatment	Cation Exchange Capacity meq/100g	Phosphorus (Bray-1) ppm	Calcium ppm	Magnesium ppm	Sulphur ppm	Sodium ppm	Zinc ppm	Manganese ppm	Iron ppm	Copper ppm	Boron ppm	Aluminum ppm
Control	17.47	19.33	3273.33	99.67	23.33	31.00	3.33	52.33	57.33	1.17	0.53	299.67
Low	17.53	19.67	3286.67	100.67	21.67	29.33	2.87	53.00	58.00	1.20	0.50	290.67
Moderate	17.67	20.67	3296.67	104.33	21.33	30.00	2.90	55.00	60.00	1.20	0.50	308.67
High	17.23	21.33	3216.67	103.67	18.67	26.33	3.00	53.00	60.00	1.17	0.50	277.33

Table 3. *Observed values for micro- and macro-nutrients as well as cation exchange capacity for control and Flume treatments (Low = 12.5 tonnes/ha, Moderate = 25 tonnes/ha, and High = 50 tonnes/ha) during the incubation trial day 14.*

Treatment	Cation Exchange Capacity meq/100g	Phosphorus (Bray-1) ppm	Calcium ppm	Magnesium ppm	Sulphur ppm	Sodium ppm	Zinc ppm	Manganese ppm	Iron ppm	Copper ppm	Boron ppm	Aluminum ppm
Control	17.93	19.67	3363.33	102.67	24.67	32.67	3.03	50.67	56.67	1.27	0.50	281.33
Low	17.37	23.67	3246.67	102.33	23.67	30.67	3.13	54.00	58.67	1.17	0.50	304.33
Moderate	18.83	22.33	3520.00	111.33	23.67	31.00	3.20	61.67	65.33	1.37	0.53	355.33
High	18.77	24.33	3500.00	112.67	23.00	30.67	3.27	64.67	69.00	1.37	0.50	357.00

Table 4. *Observed values for micro- and macro-nutrients as well as cation exchange capacity for control and Flume treatments (Low = 12.5 tonnes/ha, Moderate = 25 tonnes/ha, and High = 50 tonnes/ha)during the incubation trial day 21.*

Treatment	Cation Exchange Capacity meq/100g	Phosphorus (Bray-1) ppm	Calcium ppm	Magnesium ppm	Sulphur ppm	Sodium ppm	Zinc ppm	Manganese ppm	Iron ppm	Copper ppm	Boron ppm	Aluminum ppm
Control	19.60	21.67	3620.00	105.67	25.00	33.33	3.17	61.33	64.67	1.27	0.50	368.00
Low	19.07	24.33	3566.67	111.33	25.67	33.33	3.23	65.67	67.33	1.40	0.50	347.00
Moderate	19.10	24.33	3566.67	111.33	24.67	32.00	3.37	66.33	69.00	1.33	0.50	363.33
High	18.40	27.33	3426.67	113.00	19.00	28.00	3.60	70.67	70.67	1.37	0.50	364.67

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