

# Innovative Approaches to Value-Added Product Capture from Composting Waste Organics

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## Executive Summary

Plant and food production in temperate climates is limited to a small portion of the year due to reduced sunlight and freezing temperatures. In recent years, a surge of interest and investment into controlled environment agriculture (CEA), such as vertical farms, has new possible opportunities for year-round food production. Low power lighting technologies, e.g. LED lighting, are developing quickly as suitable alternatives to natural sunlight. Heating costs and optimizing production conditions during the fall and winter months in Canada still represent a significant challenge for closed environment production systems.

A project was undertaken to evaluate the feasibility of sustained capture of waste heat and CO<sub>2</sub> gas resulting from microbial aerobic respiration during the composting of waste organics. These by-products of composting processes were investigated as an innovative approach for utilization in closed environment greenhouse production. Value-added products from composting may reduce energy requirements for year-round agriculture and provide additional valorization pathways for CEA operations. The overall objectives of this project were to a) evaluate heat and carbon dioxide production during composting, b) design and implement a modular vertical greenhouse system capable of integration with an in-vessel composting unit (HotRot 1811; Fig. 1), and c) develop a life cycle analysis for integrated composting and controlled environment plant production utilizing waste heat and CO<sub>2</sub> from the processing of organic wastes.

Results from this project highlight the feasibility to use carbon dioxide gas generated during the composting of organic wastes to enhance plant growth in controlled environment plant production. In this project, elevated CO<sub>2</sub> gas environments created using compost material were able to increase Romaine lettuce yields relative to growing them under ambient conditions. This demonstrates the direct potential for utilization of organic waste biomass as a low-cost source of CO<sub>2</sub> gas. Other findings from the project also highlight some of the operational challenges and considerations that need to be overcome for this type of circular economy integration to be successful. A scalable prototype of an automated continuous monitoring respirometric system was also developed through this research. The respirometric system allows for rapid assessment of decomposability of organic waste mixtures in order to quickly optimize compost feedstock combinations for maximum gas generation. The project also resulted in the training of four graduate students (MSc) and a post-doctoral fellow.



**Fig. 1. HotRot 1811 composting system located at Dalhousie University, Nova Scotia, Canada.**

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## Table of Contents

Executive Summary .....	1
Acknowledgements .....	2
Table of Contents .....	3
Background.....	5
Literature Review .....	6
Objectives.....	9
Methodology .....	10
<i>Objective 1: Commissioning and operating the HotRot 1811 composting system</i>	<i>10</i>
<i>Objective 2: Quantifying CO<sub>2</sub> gas production and utilization for plant production under controlled environment conditions.....</i>	<i>11</i>
<i>Objective 3: Retrofit and establish a modular controlled environment plant production facility .....</i>	<i>11</i>
<i>Objective 4: Build automated respirometry system for rapid testing and assessment of decomposition rates in CEA biomass mixtures.....</i>	<i>12</i>
<i>Objective 5: Quantify heat (energy) utilization and CO<sub>2</sub> emissions during the composting of municipal source-separated organics (SSO) and other organic wastes [In progress]</i>	<i>12</i>
Project Results.....	13
<i>Objective 1: Commissioning and evaluating the operation of the HotRot 1811 composting system .....</i>	<i>13</i>
<i>Objective 2: Quantifying CO<sub>2</sub> gas production and utilization for plant production under controlled environment conditions.....</i>	<i>18</i>
<i>Objective 3: Retrofit and establish a modular controlled environment plant production facility .....</i>	<i>21</i>
<i>Objective 4: Build automated respirometry system for rapid testing and assessment of decomposition rates in CEA biomass mixtures.....</i>	<i>26</i>
<i>Objective 5: Quantify heat (energy) utilization and CO<sub>2</sub> emissions during the composting of municipal source-separated organics (SSO) and other organic wastes [In progress]</i>	<i>31</i>
Key Project Outcomes .....	33

Future Research Directions .....	34
References .....	35
Appendix A: HotRot 1811 Specifications Sheet.....	36
Appendix B: MSc Thesis (Anjie Luo), Dalhousie University	39

## Background

Landfilling organics are the primary source of two significant environmental issues—leachate production and methane greenhouse gas emissions (GHG). The uncontrolled anaerobic digestion of wet organic waste occurring in landfills, coupled with rain events, produces a potent mix of liquid organic by-products that require additional treatment and also generates hazardous and greenhouse gases, including H<sub>2</sub>S, NO<sub>x</sub>, and CH<sub>4</sub>. Diversion of organics from landfills removes many of the problems associated with GHG emissions and nuisance odour, as well as extending landfill life.

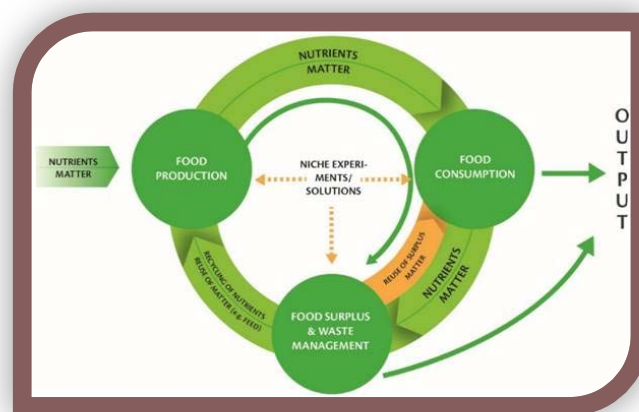


Fig. 2. Circular Economy Model for Recapture of Carbon from Composting Food Wastes (Jurgilevich et al., 2016)

Two conventional methods of dealing with municipal organic wastes in a responsible manner include: composting and anaerobic digestion for electrical energy generation. Composting is typically less capital intensive and generates a stable organic matter product, while anaerobic digestion includes the opportunity to produce and utilize biogas (essentially carbon dioxide (CO<sub>2</sub>) and methane) as an alternative fuel source. As composting is an exothermic process, there is also a significant amount of energy available that is typically lost to the surrounding environment. In general, the compost industry has not attempted to capture and utilize this energy emitted during composting, since conventional facility designs consist of buildings with large headspaces that makes the collection of energy and off-gases (such as CO<sub>2</sub>) unrealistic. In addition, the sub-optimal conditions that persist in typical compost plants create conditions that slowly release energy and product gases over a very long period of time (i.e. months) which further reduces the possibility of energy and gas collection.

Composting using in-vessel systems provides additional opportunities for control of airflow and recapture of energy and gas during the process. In-vessel composting systems enable homogeneous conditions to be established under continuous flow and also have the capacity for onboard monitoring of temperatures and airflow. Another significant advantage of in-vessel system is that they can be used to test difficult feedstocks, such as coffee cups, diapers, wet food wastes, and products labeled as biodegradable (including compostable bags) to determine the rate of decomposition. These types of systems can be modified to direct flow of exit air/gas into adjacent systems, such as a controlled environment production unit, for recapture of heat and utilization of CO<sub>2</sub>. The research focus of this project is to determine the feasibility of utilizing metabolic CO<sub>2</sub> from decomposing organic matter to enhance plant production under controlled environment conditions and to evaluate the potential to integrate an in-vessel composting system and CEA modular unit to recycle heat.

In November 2016, Hatch Ltd., through their subsidiary New Era Technologies in Goodwood, NS, donated their HotRot 1811 in-vessel composting system

(<https://www.globalcomposting.solutions/hotrot-1811-composting-unit>) to Dalhousie

University's Faculty of Agriculture (Hatch, 2016). The HotRot 1811 composting system includes a feedstock conveyor and hopper and a discharge conveyor that can process between 1.5-2.5 tonnes per day (Figure 2). The hopper can be filled and the conveyor system can be programmed to automatically introduce raw feedstocks into the composting unit at defined time intervals. This configuration allows continuous, unattended feeding and processing of material. The unit construction includes a steel/stainless steel u-shaped insulated hull and capped with fibreglass insulated lids. A central tine-bearing shaft runs the length the unit and is programmed to rotate in either direction to periodically advance material over pre-set time intervals. Air is periodically injected using low-pressure blowers to provide aeration. Temperature probes are incorporated along the length of the unit to provide real-time input on composting conditions at various stages of the process. Five sampling hatches are located along the length of the unit to test for properties such as moisture, pH, and oxygen levels. A negative pressure exhaust fan system draws air through the headspace and out of the system to an optional biofilter for control of odours. The HotRot system was transported from the New Era Technologies facility to the Agricultural Campus in Truro, NS and commissioned in July 2018.

A collaborating research team consisting of Dr. G. Price (Dalhousie University), Dr. P. Arnold (Acadia University), and Dr. T. Graham (University of Guelph) undertook to evaluate the opportunities to recapture metabolically generated CO<sub>2</sub> gas during composting and recycle it back into plant production under Controlled Environment Agriculture conditions.

## Literature Review

(The literature review is modified from the MSc thesis funded through this project by Mr. A. Luo, with the full thesis included in the Appendices)

Compost is applied as a soil amendment to improved fertility and structure by providing soil nutrients, building soil organic matter (OM), and promoting plant growth (Huang et al., 2016). Composting uses aerobic decomposition by a diverse array of microorganisms to convert raw OM into a stabilized humus-like material called compost (Irvine et al., 2010). Compost products also serve as a treatment process for organic wastes generated in agriculture, industry, and municipalities in order to reduce nuisance odors, pathogens, or weed seeds (Sweeten, 2008). During the decomposition process, gases such as carbon dioxide (CO<sub>2</sub>), ammonia (NH<sub>3</sub>), and methane (CH<sub>4</sub>) are generated by microorganisms. Current guidelines on compost stability suggest that composts are considered to be mature when the respiration rate from solid compost feedstocks (CF) is lower than 5 mg CO<sub>2</sub>-C kg<sup>-1</sup> OM hr<sup>-1</sup> (Moreira et al., 2008; CCME, 2005). Microbial activity as long as moisture and temperature conditions are adequate in organic materials. Eghball et al. (1997) reported carbon emissions as CO<sub>2</sub> ranging between 46 to 62% of total carbon reduction during cattle manure composting. Ahn et al. (2011) reported CO<sub>2</sub> emission rates ranging from 150 to 600 g kg<sup>-1</sup> of volatile dairy manure solids degraded (the VS was measured by Loss-on-Ignition; APHA, 1998). Poultry broiler manure has been reported to produce the highest quantity of CO<sub>2</sub> gas, ~25.5 kg CO<sub>2eq</sub> day<sup>-1</sup>, and hog manure the lowest, 8 kg CO<sub>2eq</sub> day<sup>-1</sup> (Brown et al., 2008). Eleazer et al. (1997) provided estimates of CO<sub>2</sub> emissions and biodegradation days for different municipal organic waste types (Table 1).

**Table 1. Gas generation (CO<sub>2</sub>) from organic wastes (dry basis) under simulated landfill conditions (Eleazer et al., 1997).**

Waste type	CO <sub>2</sub> (g·kg <sup>-1</sup> material)	Time (Days)
Grass	2.37	50
Leaves	0.5	100
Branches	1.03	100
Food	4.94	120
Coated paper	1.39	150
Old newsprint	1.22	300
Corrugated containers	2.5	400
Office paper	3.57	500

Mixed municipal organic wastes have decomposition rates ranging from 0.493 to 2.827 g CO<sub>2</sub>-C kg<sup>-1</sup> VS h<sup>-1</sup>, based on lowest to highest airflow rates, respectively (Evangelou et al., 2017). Rates of decomposition and evolution of CO<sub>2</sub> vary based on the operating conditions of the composting system, composition of the feedstock mixture, aeration rates, and moisture content of the compost. The C:N ratio of compost feedstocks plays an important role in understanding the potential emissions of CO<sub>2</sub>. Kranert (2010) reported that green waste compost with different organic matter contents (dry basis) had CO<sub>2</sub> emission rates of 1472, 941, and 597 kg CO<sub>2</sub> ton<sup>-1</sup> of green waste when mixed with 96%, 80%, and 60% woody material, respectively. Carbon-containing materials supply the energy required for microbial respiration and growth, while nitrogen-containing materials play a role as a protein source (Brinton & Seekings, 1988). A study by Dajana and Felicita (2017) reported that the cumulative CO<sub>2</sub> evolution per unit mass of volatile matter of composted tobacco waste (TW) and tobacco mixed with grape waste (TGW) was 94.01 g CO<sub>2</sub> kg<sup>-1</sup> (9.4%) and 208.18 g CO<sub>2</sub> kg<sup>-1</sup> (20.82%) volatile matter, respectively. Understanding and managing decomposition rates of carbon during composting play a role in how this process can be integrated within closed environment plant production systems. The type of composting technology, timing of processing, and operating conditions are important considerations for the quantity, quality, and timing of gas for CEA production systems.

Controlled environment agriculture (CEA) is used for the production of different commercial plant species. CEA can be defined as an enclosed environment used to create optimal growing conditions, i.e. nutrients, lighting, gas, for cultivating plants (Prasad et al., 2014). The benefit of CEA is greater flexibility and environmental control over plant production (Giroux et al., 2006) but at the expense of greater infrastructure and cost. CEA aims to modify the natural growing environment by improving root growth conditions, extending the growing season through differences in light exposure, and creating opportunities for production under circumstances that would typically not be suitable, i.e., during winter periods in temperate climatic zones (Jensen, 2001). Environmental modifications, including ambient temperature, relative humidity,



light quality, quantity, and photoperiod, nutrient supply, and carbon dioxide levels, are aimed to meet the optimum for plant growth and economic return. High capital costs for CEA infrastructure are one of the disadvantages, especially energy costs associated with managing temperature, humidity, and lighting (Benke & Tomkins, 2017). In temperate zones around North America, greenhouse operators face high thermal energy requirements to maintain the temperature and CO<sub>2</sub> levels of their greenhouses. In Canada, a large amount of supplemental heat is required during the cold winter season, amounting to about 10 to 35% of the total production costs (Ahamed et al., 2019). In traditional Canadian greenhouse production, to elevate the CO<sub>2</sub> concentrations in the growing areas, growers will purchase liquid or compressed gas CO<sub>2</sub> or burn sawdust wood pellets or natural gas/propane (Table 2). For example, in order to maintain a greenhouse at 1000 ppm of CO<sub>2</sub>, a greenhouse grower would need to supply CO<sub>2</sub> at the rate of 108 g m<sup>-2</sup> day<sup>-1</sup>, using 0.06 m<sup>3</sup> day<sup>-1</sup> m<sup>2</sup> of natural gas (Ahamed et al., 2019). Burning propane is a common way to increase CO<sub>2</sub> levels and temperature within a greenhouse, but this comes at an additional cost and at the expense of using a fossil fuel (Benke & Tomkins, 2017). For example, providing an additional 1000 ppm of CO<sub>2</sub> enrichment in a 1000 m<sup>2</sup> glass greenhouse will use 2.8 to 3.4 m<sup>3</sup> natural gas and 2.8 to 3.4 L propane *per hour* (Blom et al., 2002).

**Table 2. Energy consumption for generation of CO<sub>2</sub> gas in greenhouse operations through combustion of combustion of sawdust wood pellets, natural gas, and propane (Dion et al., 2013)**

	Wood pellets (kg)	Natural Gas (m <sup>3</sup> )	Propane (L)
<b>MJ per unit of fuel</b>	18.1	37.89	25.53
<b>g CO<sub>2</sub> per unit of fuel</b>	1729	1891	1510
<b>g CO<sub>2</sub>·MJ<sup>-1</sup></b>	96	50	59

Conventional supplementation of CO<sub>2</sub> gas into greenhouse environments has been shown to improve quality and yield (30%) of crops and change plant morphological characteristics, such as increasing leaf thickness (Raines, 2011; Becker & Kläring, 2016). In lettuce production, elevated CO<sub>2</sub> enhances the plant's health-promoting benefits by increasing phenolic compound content and antioxidant capacity (Pérez-López et al., 2018). In some commercial greenhouse operations, elevating CO<sub>2</sub> concentrations are used to increase crop yield and quantity. Many greenhouse growers elevate CO<sub>2</sub> levels to achieve higher yields of different ornamental and vegetable crops, such as basil (Al-Jaouni, 2018), tomato (Tripp et al., 1992), lettuce (Singh et al., 2020), and Chinese kale (La et al., 2009). Xu et al. (2016) suggested that soybean grown under an elevated atmospheric CO<sub>2</sub> (800 ppm) increased in biomass production by 54% to 136%. Food and flowering crops will see increases in photosynthetic rates and foliar carbohydrate of 36 and 43%, respectively, from increasing the ambient CO<sub>2</sub> concentrations from 395 to 550 ppm (Sreeharsha et al., 2015). Elevated CO<sub>2</sub> can positively alter plant morphological development, such as leaf area development, tiller production, and shoot to root ratios (Seneweera, 2011). For example, enriched CO<sub>2</sub> environments increase plants' resistance to environmental stress by modifying the profiles of secondary metabolites and increased virus resistance in tobacco plants

(Matros et al., 2006). The C:N ratio of plant tissues and C:N exchange between the growing medium and plants can also be influenced by the ambient concentration of CO<sub>2</sub> (Gifford et al., 2000). Masle (2000) reported increased plant production under elevated CO<sub>2</sub> in two wheat cultivars, Hartog and Birch grown under 900 ppm CO<sub>2</sub> grew and 350 ppm. Leaf area increases of 39% and 82% for Hartog and Birch, respectively, were measured at 900ppm CO<sub>2</sub>. Other plants, exposed to elevated CO<sub>2</sub>, have shown changes in carbohydrate partitioning between stems or roots with a limited capacity for leaf area enrichment (Stitt, 1999). A study by La et al. (2009) indicated that under conditions of CO<sub>2</sub> concentration increases from 330 ppm to 800 ppm, Chinese kale (*B. alboglabra*) had greater plant height (15.64%), stem thickness (11.79%), dry weights (11.91%), bolting stems (15.03%), roots (16.34%), and root/shoot ratios (3.9%).

The research to date from composting systems and CEA systems, especially under elevated CO<sub>2</sub>, suggests the potential exists to recapture CO<sub>2</sub> gas from composting and utilizing it for plant production. This project was developed to evaluate the potential of using CO<sub>2</sub> from composting processes and decomposition of organic matter for plant production. The goal was to develop an initial proof-of-concept of this approach using a commercial scale in-vessel composting system, HotRot 1811, and a modular vertical growing unit that was developed during this project.

## Objectives

The original objectives for this project were to:

1. Develop a heat and CO<sub>2</sub> capture and redistribution system integrated with an in-vessel composting system (HotRot 1811);
2. Quantify heat and CO<sub>2</sub> capture during the composting of municipal source-separated organics (SSO) and other organic wastes;
3. Design, develop, and evaluate a prototype modular greenhouse for production of horticultural crops using redistributed heat and CO<sub>2</sub> from the composting of municipal SSO and other organic wastes;
4. Develop a life-cycle analysis of municipal SSO composting with multi-level value streams including heat and CO<sub>2</sub> for horticultural greenhouse production.

The project had a number of setbacks that have resulted in modification or removal of some original objectives. Removal of the HotRot 1811 system from the New Era facility in Halifax, NS and subsequent commissioning at the Faculty of Agriculture's campus was delayed by 1 ½ years. The donated HotRot 1811 system was half-way through its identified lifespan and required replacement of integral parts that were worn out or weathered. As a result, the composting system has not operated for significant periods of time, impacting the ability to adequately integrate the system with the modular vertical farm unit that was built for this project. This has also delayed quantification of the energy utilization and heat recapture objective and the development of a life-cycle analysis. Despite the challenges, a significant number of outcomes and achievements from this project highlight the potential for recycling CO<sub>2</sub> gas into closed environment agriculture. As a result of delays in commissioning the HotRot 1811, and difficulties

in achieving a continuous flow operation of the system, alternate project objectives were established.

Revised objectives and plans for this project were:

1. Commissioning of HotRot 1811, establishing optimal process parameters, and operating the continuous flow composting system;
2. Quantify CO<sub>2</sub> gas production and evaluate the use of raw organic wastes and processed (partially composted) organic wastes as sources of CO<sub>2</sub> for plant production;
3. Retrofit and establish a modular controlled environment plant production facility for prototyping integration with an in-vessel composting system to recapture heat and CO<sub>2</sub> gas;
4. Build automated respirometry system for rapid testing and assessment of decomposition rates in CEA biomass mixtures for optimization of CO<sub>2</sub> delivery in plant production systems;
5. Quantify heat (energy) utilization and CO<sub>2</sub> emissions during the composting of municipal source-separated organics (SSO) and other organic wastes; [Research currently underway]
6. Develop a life-cycle analysis of municipal SSO composting with multi-level value streams including heat and CO<sub>2</sub> for horticultural greenhouse production. (HotRot); [Future research]



## Methodology

### Objective 1: Commissioning and operating the HotRot 1811 composting system

The HotRot 1811 was donated in 2016 by Hatch Ltd. and re-commissioned at the Agricultural Campus for Dalhousie University in July 2018. Over the period of July 2018 through to September 2021, the composting system has undergone a number of tests to evaluate:

- Effect of feedstock composition on process flow;
- Changes in physical and chemical composition of input feedstocks;
- Effect of modifying PLC parameters on flow-through rates;
- Monitoring of temperature profiles over the process flow.

### **Objective 2: Quantifying CO<sub>2</sub> gas production and utilization for plant production under controlled environment conditions**

A MSc student (A. Luo) was recruited in September 2017 to study the feasibility of using composted or partially composted feedstocks as sources of CO<sub>2</sub> under controlled environment agriculture production of lettuce. A number of initial experiments were conducted to evaluate plant responses under elevated CO<sub>2</sub> conditions, using chemical sources of carbon dioxide, and to examine CO<sub>2</sub> generation from compost sources. In Experiment 1, plant bioassays were established in 35cm x 35cm x 25cm boxes as a two factor study, elevated CO<sub>2</sub> (~1200ppm) vs. ambient (~400ppm) (Factor 1) and nutrient supply (Lystegro product at 3 rates; Factor 2). The treatments were established in a completely randomized design with four replications and in two different soils. Corn seeds were pre-germinated using moistened paper towels in petri dishes and subsequently transplanted to the soil+nutrient source media. Elevated CO<sub>2</sub> was provided by reacting 0.0178g NaHCO<sub>3</sub> and 5.53 ul H<sub>2</sub>SO<sub>4</sub> to increase the concentration in the container by 100ppm increments until the target level was reached. Plants were grown for 19 days and harvested. Shoots and roots were separated and weighed, then oven dried at 65°C and re-weighed. Corn shoot height, root length, plant tissue fresh & dry weight, and moisture content were measured, as well as soils were analyzed for available phosphorus (P) via an Olson P and Modified Morgan's (Ammonium Acetate and Acetic Acid solution at a pH of 4.8) extraction. Experiment 2 involved a series of tests evaluating different plant growing chamber sizes and conditions for supply of CO<sub>2</sub>, quantifying supply of CO<sub>2</sub> from raw and partially composted materials, and testing of CO<sub>2</sub> sensors for datalogging. The final series of studies are presented in full in **Appendix B** as the completed MSc thesis for A. Luo. These studies compared the use of pure CO<sub>2</sub> gas and CO<sub>2</sub> supplied from organic wastes partially composted and processed through the HotRot 1811. Plant production studies were conducted over different seasons in the year to evaluate the feasibility of off-season production. A production cycle of romaine lettuce grown hydroponically over a 35 day growing cycle was selected treatments were established based on source of CO<sub>2</sub>. Crops were grown hydroponically using established nutrient blends in a water solution and the elevated CO<sub>2</sub> concentrations, i.e. 800-1200 ppm, was set based on results in the scientific literature for lettuce.

### **Objective 3: Retrofit and establish a modular controlled environment plant production facility**

In April 2019, an insulated walk-in cooler freight container with a reefer unit, measuring 2.5m x 6.1m x 2.5m, was donated to the project from the Research Farm in the Faculty of Agriculture. The unit was transported and placed adjacent to the HotRot 1811 for retrofitting and eventual integration of the two units. An electrical supply and hook up was connected to the freight container for installation of a heat pump system and internal lighting for plant production. The reefer unit was removed and replaced with a Daikin model # RXS18LVJU outdoor heat pump condensing unit and Daikin model # FTXS18LVJU 18,000 BTU indoor evaporator coil to provide heating and cooling to the controlled environment plant production module. Internal temperature of the growing unit was set to 18°C and small fans were installed to draw fresh air through the system on a 20 minute sequence. Six 2.5m long red-blue-white LED lamp lighting systems (Intravision The Spectra Blade Model number 21GP66, provided 64.22  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$

lighting intensity) were provided by Cruus and the University of Guelph's Controlled Environment Systems Research Facility.

#### **Objective 4: Build automated respirometry system for rapid testing and assessment of decomposition rates in CEA biomass mixtures**

Respirometric approaches have been used for decades in scientific research and are a critical tool in determination of compost, or organic matter, stability. Measurement of evolved CO<sub>2</sub> is an important proxy for microbial activity, and therefore the accessibility of feedstock carbon for consumption or degradation. Conventional approaches are time and resource intensive, typically allowing only a few individual CO<sub>2</sub> measurements to be taken from decomposing material over a given time period. The aim of this objective, conducted in collaboration with Dr. T. Graham from the University of Guelph's Controlled Environment Systems Research Facility and through the Innovative Waste Management Laboratory in Dalhousie University, was to develop and test different automated respirometric manifolds capable of continuous or semi-continuous monitoring of CO<sub>2</sub> emissions during the decomposition process. The studies focused on the use of Nondispersive Infrared (NDIR) based CO<sub>2</sub> sensors under different experimental conditions and designs to capture near continuous to continuous respiration data from selected mixtures of municipal and agriculturally sourced organic wastes. These experiments were conducted in order to develop prototypes of automated respirometric systems for rapid assessment of decomposition potential from compost mixtures and to quantify cumulative CO<sub>2</sub> generation over time during composting. Two MSc students (C. Kiselchuk and A. Dsouza) were recruited to work on the development, testing, and validation of an automated respirometric system for use with waste biomass from CEA production systems.

#### **Objective 5: Quantify heat (energy) utilization and CO<sub>2</sub> emissions during the composting of municipal source-separated organics (SSO) and other organic wastes [In progress]**

The HotRot 1811 accommodates a feed rate of approximately 1.5 actual wet tonnes per day based on a 14 to 16 day retention time. The HotRot has thermocouples at five locations across the length of the unit and in the exhaust port. Temperature information is accessed through the control panel but is not logged directly by the system. Temperature and relative humidity sensors are installed in the exhaust port and post-particulate filter line to determine exit air temperatures and moisture. A Sensirion STC31 thermal conductivity sensor capable of measuring CO<sub>2</sub> at 0 to 25 vol% and 0 to 100 vol% will be installed in both locations as well. The sensor has a measurement repeatability of 0.2 vol%, with a stability of 0.025 vol% / °C. The measurement accuracy is either 0.5 vol% + 3% measured value for the low range or 1 vol% + 3% measured value for the high range. Inside the modular plant production unit, an EHWEM1-LV Eyedro energy meter with two sensors have been installed on the 110V main electrical line on the subpanel and are linked to an online energy monitoring website ([my.eyedro.com](http://my.eyedro.com)) to evaluate energy (electricity) usage over the Fall and Winter periods, with and without use of warm air from the HotRot. The Daikin heat pump system will be allowed to run for a period of time under ambient conditions during the Fall and Winter. The heat pump will subsequently be partially enclosed and connected to the air return system from the HotRot to establish a warm air envelope around it. The energy monitor will measure changes in energy usage as a result of

the warm return air from the HotRot to determine the amount of energy conserved or 'recaptured' from the recycled air.

## Project Results

### Objective 1: Commissioning and evaluating the operation of the HotRot 1811 composting system

The HotRot 1811 in-vessel composting system was donated by Hatch Ltd. in late 2016 but fully commissioned at Dalhousie University in July 2018.

The composting system consists of several active mechanical units including a feedstock hopper (4.77 m<sup>3</sup>) with four floor augers with an estimated capacity of 1.5 to 2 tons, an auger elevator to convey material into the main HotRot chamber, a 12.8 m long chamber with a 0.4 m diameter single rotating shaft and 40 flights across the span of the unit, and an auger elevator at the exit end. The unit includes two variable speed fans to draw air through and out of the system and air injectors at the bottom of the vessel. An exhaust port feeds into a particulate filter and condensation line, connecting to air ducts that are directed to a biofilter and air is recycled back into the front of the composting system. The composting system is controlled via a programmable logic controller panel (PLC) and can be set to move material in two directions, forward or reverse, for different amounts of time and also allows for a static period. Typical retention time for material in the system ranges from 14 to 16 days. Raw materials stored on the Agricultural Campus come from a range of different sources including: broiler chicken manure and wood shavings, used straw bedding from the sheep unit, liquid manure from the mink unit, used wood bedding from the dairy unit, and materials from the landscaping and grounds team (grass clippings, leaves, landscaping plant material). Future plans include diversion of cafeteria pre- and post-consumer organics and paper towels from the campus restrooms.

The composting system underwent an initial evaluation in early August 2018, over 16 days, to assess feed-in rates, programming of the main shaft directions, and determination of the differences in the chemical properties of the initial feedstocks and processed compost. The initial mixture of feedstocks included landscaping waste, straw bedding from the mink unit, and broiler chicken manure and bedding. The raw organic waste feedstock was added on days 1, 2, and 5 to the HotRot 1811 composting system over a 16 day period and temperatures were recorded daily from the control panel (Fig. 3). The temperature profile shows immediate thermophilic conditions, i.e. >55°C, when material is inserted into the composting vessel and as the feedstock is mixed and moved toward the exit over the 16 days. By the end of 16 days, thermophilic temperatures were reached across the whole system. The physical form of the material from raw feedstock to processed, partially composted product was also significantly changed (Fig. 4).



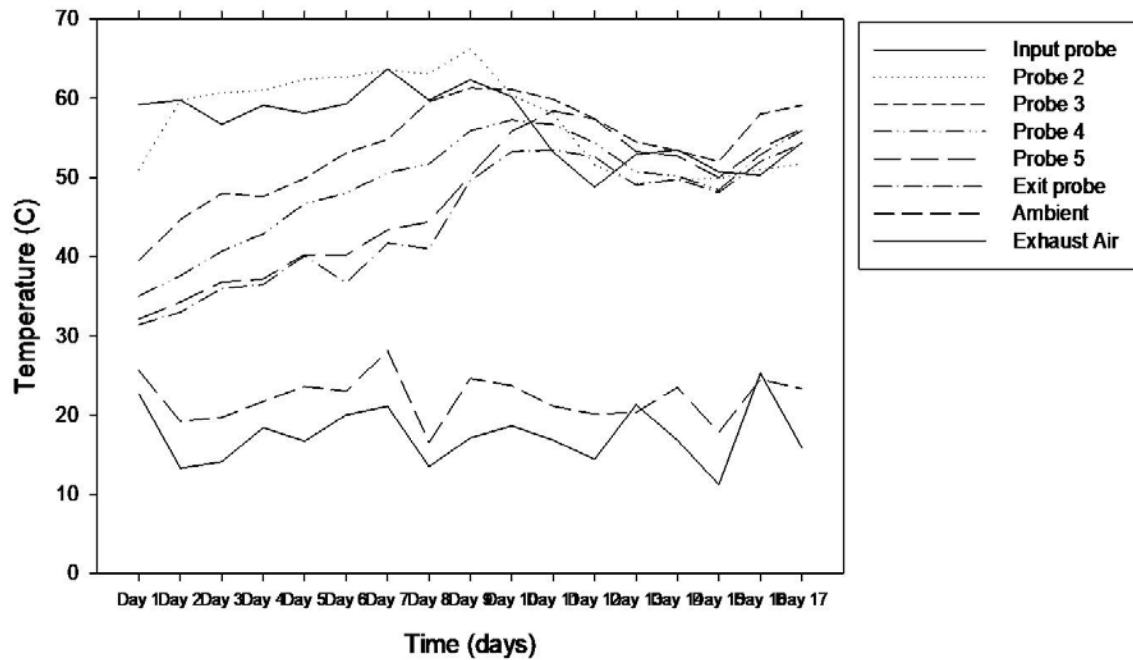


Fig. 3. HotRot 1811 temperature profile across the length of the unit (Input probe, Probe 2, 3, 4, 5, to Exit probe) during feed in of organic wastes over a 16 day period.



Fig. 4. Raw organic waste feedstock in HotRot 1811 hopper (left), processed material exiting the composting system (centre), and final material used for plant production experiments (right).

The raw biomass and partially composted product being generated from the HotRot 1811 were evaluated for chemical composition during the study. Table 3 highlights some of the differences between key chemical parameters between the raw feedstock and the partially composted biomass, to be used as a CO<sub>2</sub> source during the plant growth study. In particular, the HotRot 1811 significantly reduced the total carbon content over a 16 days processing period from 41%

to 27.7% and resulted in almost three times less ammonia emissions over a 24 hour incubation period.

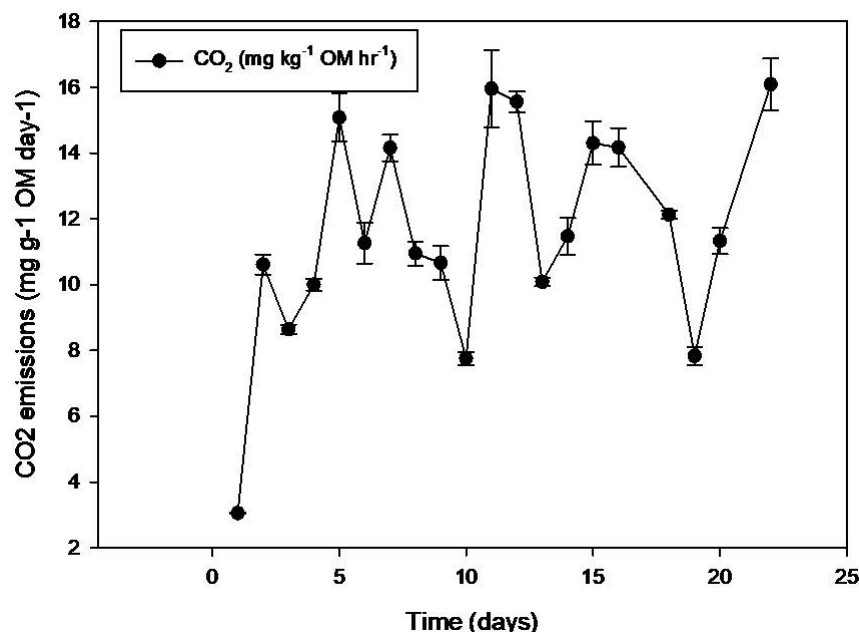
**Table 3.** Chemical properties of an untreated organic waste feedstock (raw) and a partially composted (processed) organic waste feedstock through the HotRot 1811 composting system.

Treatment	DM (g)	MC (%)	TC (%)	TN(%)	NH <sub>3</sub> -N (mg·kg <sup>-1</sup>
					DM·hr <sup>-1</sup> )
<b>Raw</b>	57.3±0.02	61.80±0.23	41.15±0.65	3.45±0.03	2.04 ±0.72 <sup>a</sup>
<b>Processed</b>	80.3±0.09	46.47±0.52	27.70±0.47	2.35±0.04	0.72±0.15 <sup>b</sup>

\*Values are means (N=6) ±SD. DM, dry matter content; MC, moisture content; TC, total percentage of carbon; TN, total percentage of nitrogen;

\*Values with the same letter in each column are not significantly different at  $p < 0.05$ .

Feedstock processed through the HotRot 1811 was subsequently incubated for 22 days to assess the rate of respiration, i.e. CO<sub>2</sub> evolution, and compare to the Canadian Council of Ministers of the Environment Compost Quality Guidelines (CCME, 2005) for maturity or stability. The mean respiration rate over 22 days was calculated to be 11.56 mg CO<sub>2</sub>-C g<sup>-1</sup> OM day<sup>-1</sup> and the CCME guidelines for compost maturity indicates a rate of <4 mg CO<sub>2</sub>-C g<sup>-1</sup> OM day<sup>-1</sup> (Fig. 5). The respiration rate of the material processed through the HotRot 1811 was not considered to have achieved maturity but was an order of magnitude lower than the raw feedstock (data not shown).

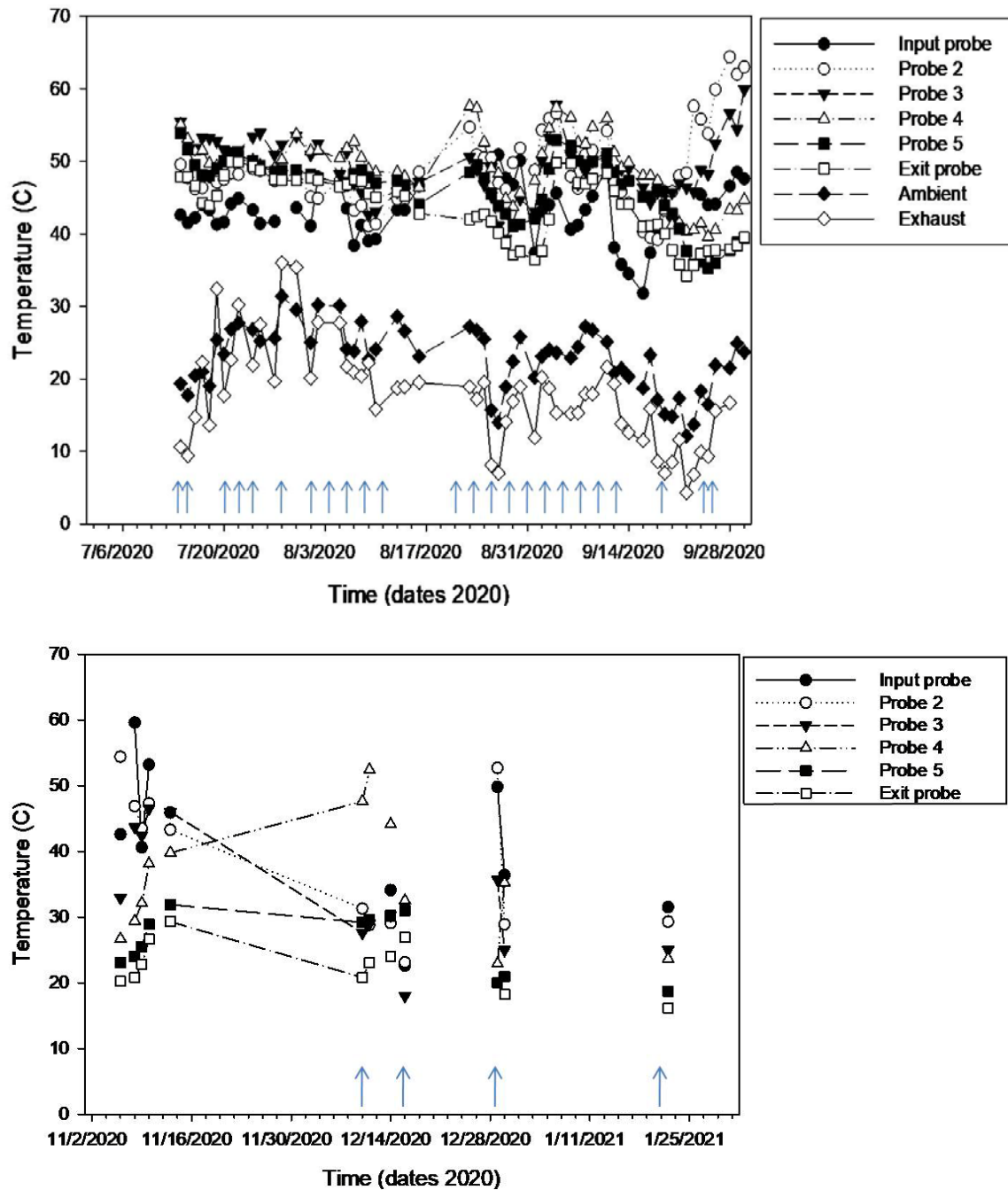


**Fig. 5.** Respiration rate of partially composted feedstocks from a HotRot 1811 over a 22 day incubation study.

The operation of the HotRot 1811 was evaluated intermittently over the Fall 2018 but the unit was not consistently able to be run over most of 2019 due to mechanical issues and repairs.



Over this time, the HotRot 1811 received replacement floor augers for the hopper and repairs to the auger elevator for the input material, a defective variable speed fan was replaced, and air flow ductwork was insulated and repaired. In the summer of 2020 and into 2021, the HotRot 1811 underwent a series of evaluations of feedstock mixtures and rates of input, changing of PLC parameters for the main shaft rotation and timing, and testing of continuous operations with regular feedstock additions. The temperature profiles were monitored and recorded from July 2020 through to August 2021, with the exception of a period February to June 2021 where the unit was not operating (Fig. 6).



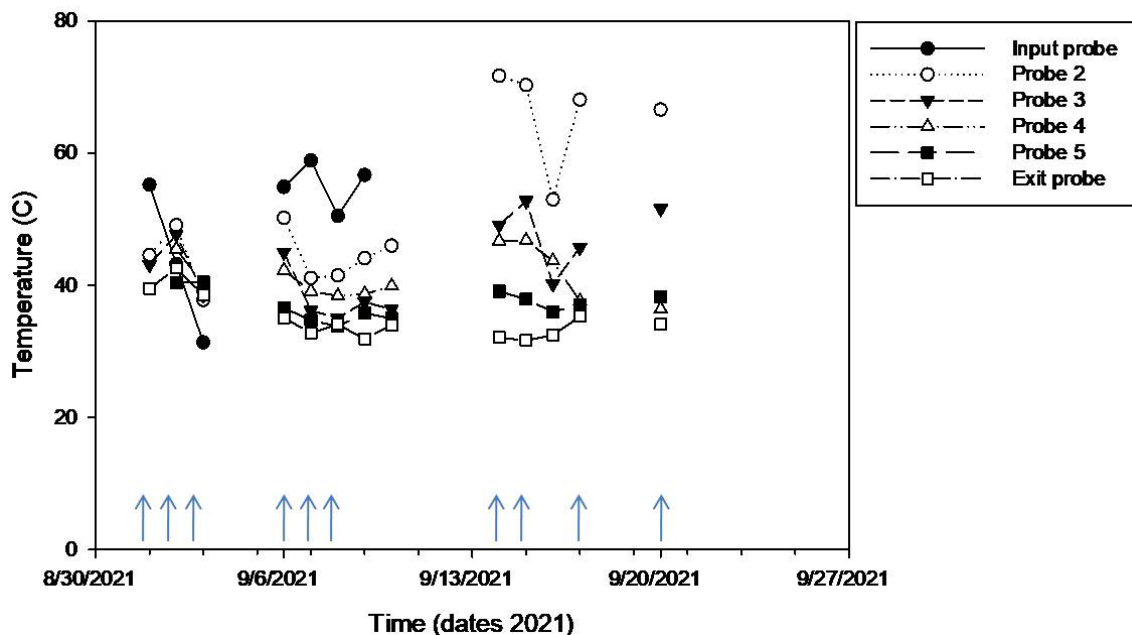


Fig. 6. Temperature profiles across the HotRot 1811 thermocouple probes from Input to Exit over a 12-month period in 2020 to 2021. Blue arrows indicate when organic waste feedstocks were added (typically 2 tons per load).

Microbially mediated decomposition of organic matter, particularly in composting systems, is an exothermic process that results in the release of heat. The thermocouples measure the released heat and this temperature reading is often used as a proxy to represent the biological activity of the system. There is a general correlation between temperature profiles in composting systems and CO<sub>2</sub> generated from microbial respiration (Fig. 7). Over a two month period in the summer of 2020, regular addition of organic waste feedstocks generated temperatures ranging from 40°C to 65°C across the composting unit. Based on the temperature profiles during this period from the HotRot 1811 the level of microbial activity was interpreted to be high when feedstock was added on a regular schedule.

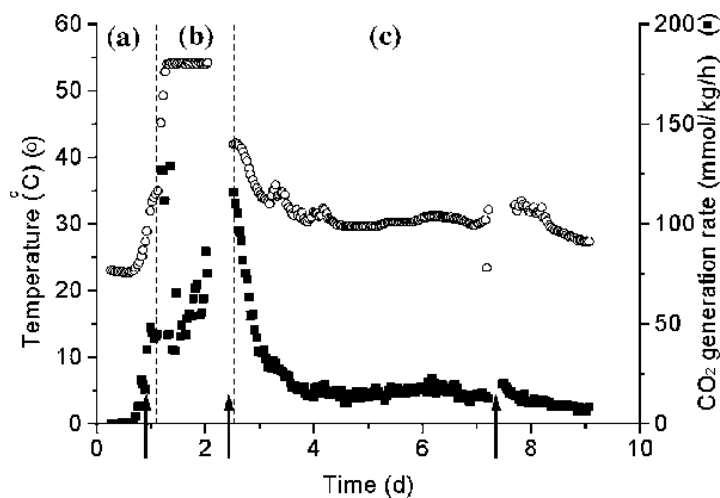


Fig. 7. Temperature profile and corresponding CO<sub>2</sub> generation rate in a composted contaminated soil bioreactor study (Haderlain et al., 2006).

Operation of the HotRot 1811 system is highly dependent on the moisture content of the incoming material and ensuring sufficient air flow through the system. High moisture, in combination with certain feedstocks such as straw, can lead to increased density of the mixture and generation of blocks that can hamper the turning of the main mixing shaft. The addition of bulking agents, such as bark and wood chips, significantly aided the reduction in compaction and helped flow of material out of the system. A significant blockage occurred at the end of September 2020 leading to the composting system being turned off and emptied manually until November. Over the late part of 2020 and through 2021 excess moisture in the feedstocks added and mechanical issues led to lower temperatures in the middle and rear of the unit, i.e. a reduction in thermophilic activity across the composting system (Fig. 7, bottom two graphs). A blockage in the composting unit was removed and material has been removed over the Fall 2021 in preparation for continuous operation over the Winter 2022 as a source of heat for the vertical farming module.

### **Objective 2: Quantifying CO<sub>2</sub> gas production and utilization for plant production under controlled environment conditions**

#### ***Plant Growth Bioassay (Elevated CO<sub>2</sub>)***

Production of CO<sub>2</sub> gas from decomposing organic matter under aerated or aerobic conditions is well established. An initial study was established to evaluate short-term plant growth (19 days) and phosphorus uptake in corn under ambient and elevated CO<sub>2</sub> conditions in two different agricultural soils (Fig. 8).



**Fig. 8.** Small container plant bioassays with corn seedlings in two soils under elevated CO<sub>2</sub> and ambient conditions.

The ambient and elevated CO<sub>2</sub> conditions were monitored using a TandD Tr-76ui (T & D Corp., Japan) CO<sub>2</sub> sensor and logger. Elevated CO<sub>2</sub> conditions were generated using a mixture of NaHCO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> to generate a controlled release of gas in the growing chamber. Results of gas concentration in the elevated CO<sub>2</sub> and ambient growing chambers are shown in Figure 9.

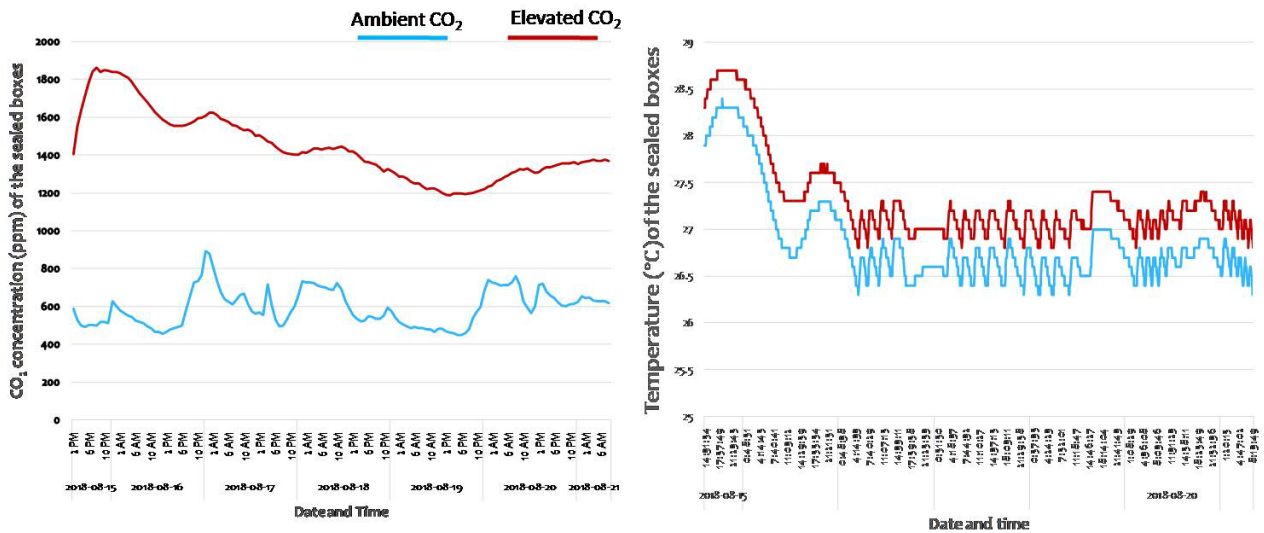


Fig. 9. CO<sub>2</sub> and temperature readings from Ambient and Elevated CO<sub>2</sub> growing chambers.

Initial CO<sub>2</sub> emissions were >1800ppm for the initial 24hrs and plateaued at 1300ppm for the remainder of the study. The temperature profiles in both growing chambers followed a similar pattern but were slightly elevated in the CO<sub>2</sub> chamber due to the plastic film cover. The plant responses after 19 days were not significant between the ambient and elevated CO<sub>2</sub> treatments (Fig. 10). Plant phosphorus uptake from the two soils in the 19 days bioassay experiment were also not significantly different between the elevated CO<sub>2</sub> and ambient treatments.

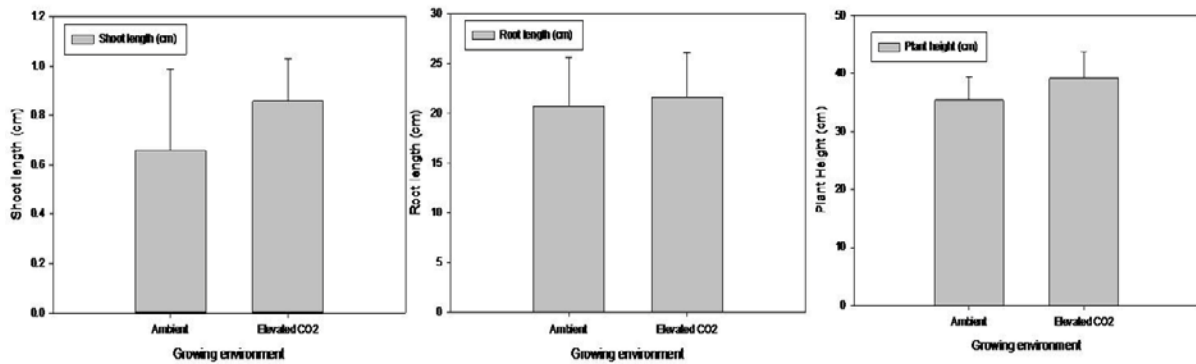


Fig. 10. Corn seedling responses to elevated CO<sub>2</sub> environment for shoot and root length (cm) and plant height (cm) after 19 days.

The selection of plant species and the duration may have impacted the initial results determined from this experiment. However, the experimental setup was capable maintaining an elevated CO<sub>2</sub> environment during early plant growth stages, despite no significant differences in growth or nutrient uptake.

**Food Waste In-Vessel Composting and CO<sub>2</sub> Generation**

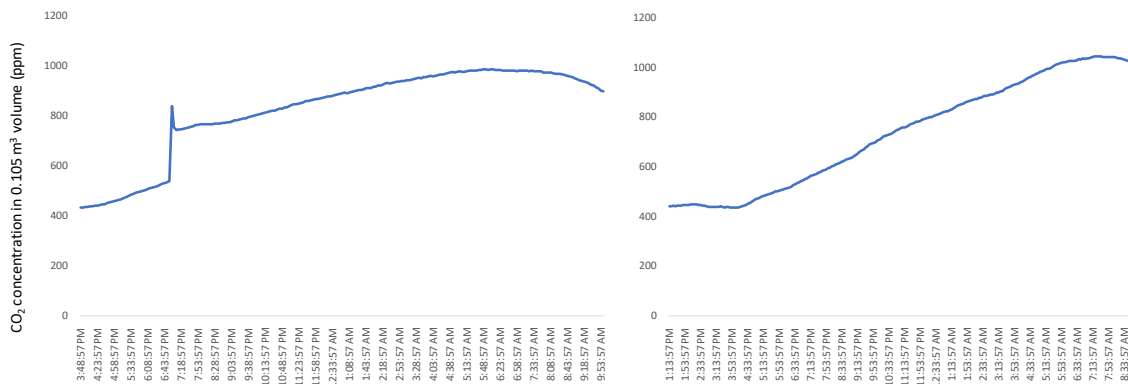
A preliminary in-vessel composting trial was conducted over a 30 day period to examine the physical decomposition of post-consumer organic wastes from the Agricultural Campus.

Separated food wastes (143 kg) from the AC cafeteria were mixed with sawdust (69.5 kg) and placed in a double-walled stainless steel in-vessel composting system with a 150 m<sup>3</sup> capacity (Fig. 11).



**Fig. 11.** In-vessel composting trial over 30 days with post-consumer cafeteria organic wastes (sequence from left to right, top to bottom).

The opening was covered with a lid connected to a variable speed fan to ensure airflow through the system and a shaft running vertically at the centre of the composter contained six thermocouples spaced at 5cm intervals and connected to a Campbell Scientific CRX100 data logger (Campbell Scientific, Edmonton, AB). The exit air was diverted through a container with dry sawdust to absorb moisture from the air and then into a plenum with a TandD Tr-76ui (T & D Corp., Japan) CO<sub>2</sub> sensor and logger. Gas sampling was collected over two time periods during the trial to determine whether food waste could generate sustained CO<sub>2</sub> gas. Sampling occurred over one 18 hour and one 19 hour period over two days (Fig. 12). The maximum yield in the plenum was 1000 ppm in a 68 L Rubbermaid container (0.105 m<sup>3</sup> volume) over both days.



**Fig. 12.** CO<sub>2</sub> gas generation over an 18 hour and 19 hour period from composting of food wastes in a small scale in-vessel composting system.

### Objective 3: Retrofit and establish a modular controlled environment plant production facility Controlled Environment Plant Growth Studies Under Elevated CO<sub>2</sub> Conditions

Subsequent studies were conducted using raw, partially composted, or composted biomass from the HotRot 1811 systems as a CO<sub>2</sub> source and compared relative to ambient conditions or a compressed gas CO<sub>2</sub> source. These studies were primarily conducted in a retrofitted walk-in cooler freight container to serve as a new vertical farming module. The insulated module had a heat pump system added in order to regulate internal temperature, a new electrical system was installed, and fans were added to ensure adequate air exchange. Sets of LED growing lamps were placed across the container for the plant growth studies under elevated CO<sub>2</sub> gas and ambient conditions. Romaine lettuce (*Lactuca sativa*) was chosen as the plant species for testing of the elevated CO<sub>2</sub> atmosphere conditions on growth and yield. The full results are presented in A. Luo's MSc Thesis (Appendix B) but a summary of results are presented in this section.

A hydroponic set up was established in a retrofitted walk-in cooler established for use as a vertical farming system. The hydroponic set up and the automated CO<sub>2</sub> sensor array to monitor the gas environments for the growing chambers are shown in Fig. 13 (A & B).

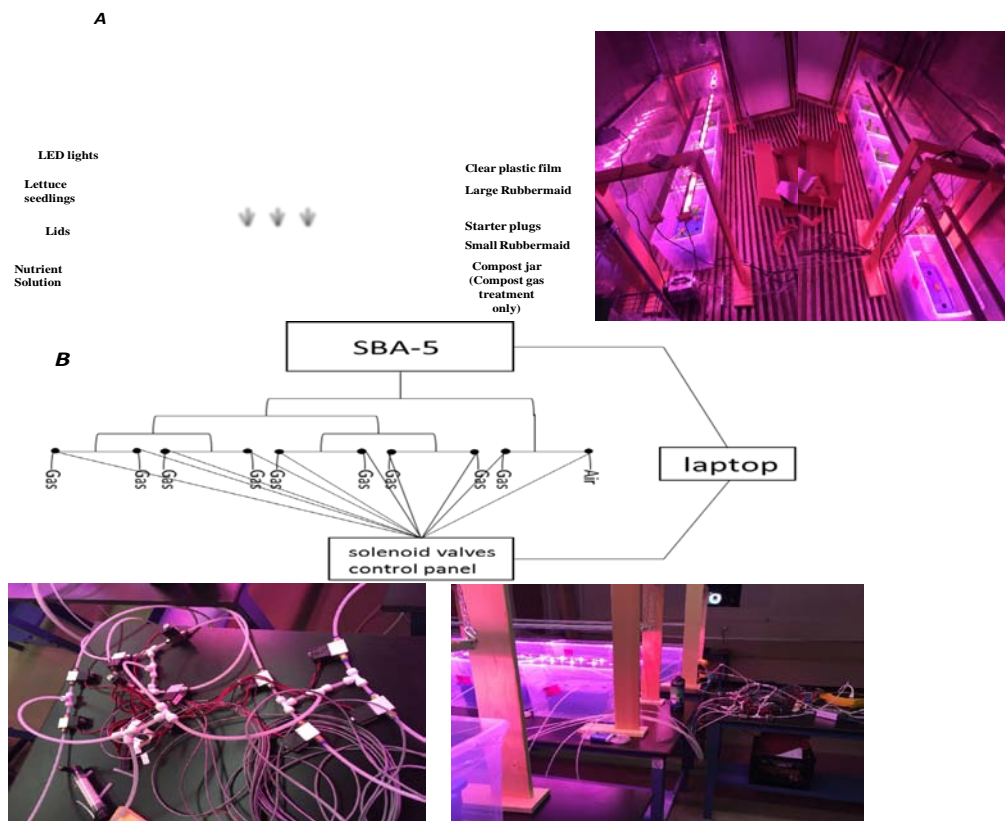
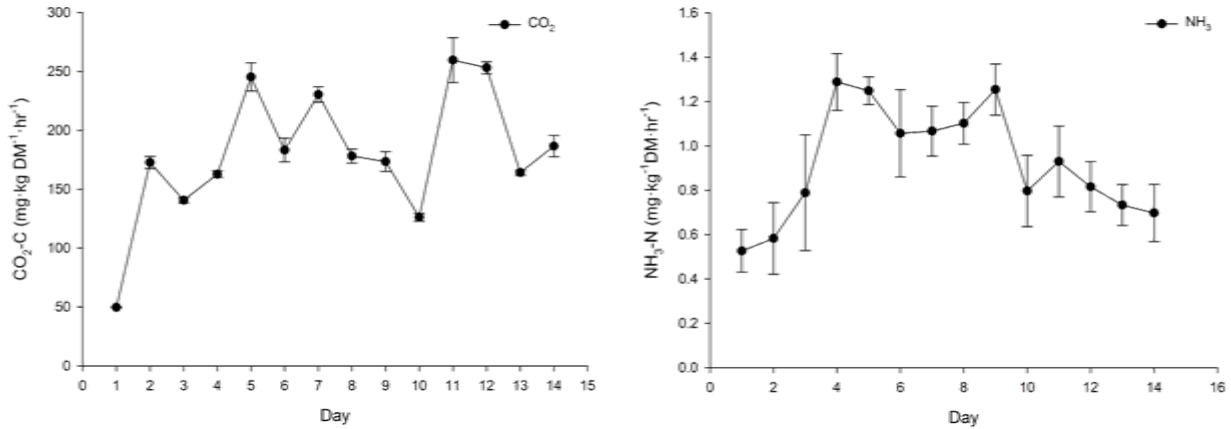


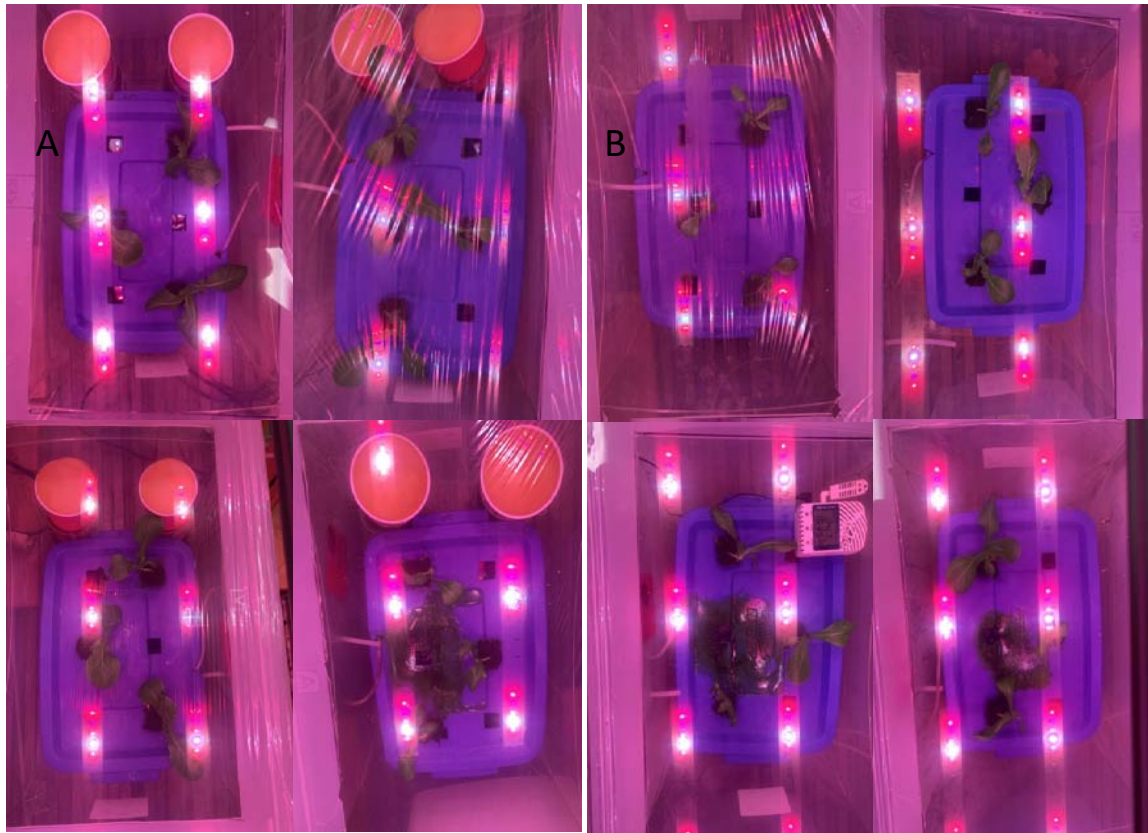
Fig. 13. A) Hydroponic set up for production of lettuce grown under i) ambient conditions, ii) using elevated CO<sub>2</sub> atmosphere partially composted biomass from the HotRot 1811, or iii) using elevated CO<sub>2</sub> atmosphere from a compressed gas source in a controlled environment agriculture module at the Faculty of Agriculture, Dalhousie University and B) PP Systems SBA-5 CO<sub>2</sub> sensor manifold developed to monitor the gas environment in each plant growth chamber over the study period.

The partially composted material was incubated over a 14 day period to assess CO<sub>2</sub> and NH<sub>3</sub> generation under 20°C. The partially composted material generated on average 180.48 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup> and between 0.72 to 0.92 mg NH<sub>3</sub>-N·kg<sup>-1</sup> DM·hr<sup>-1</sup> (Fig.14).



**Fig. 14.** CO<sub>2</sub> and NH<sub>3</sub> gas emissions from a partially composted organic waste feedstock over a 14 day incubation experiment at 20°C.

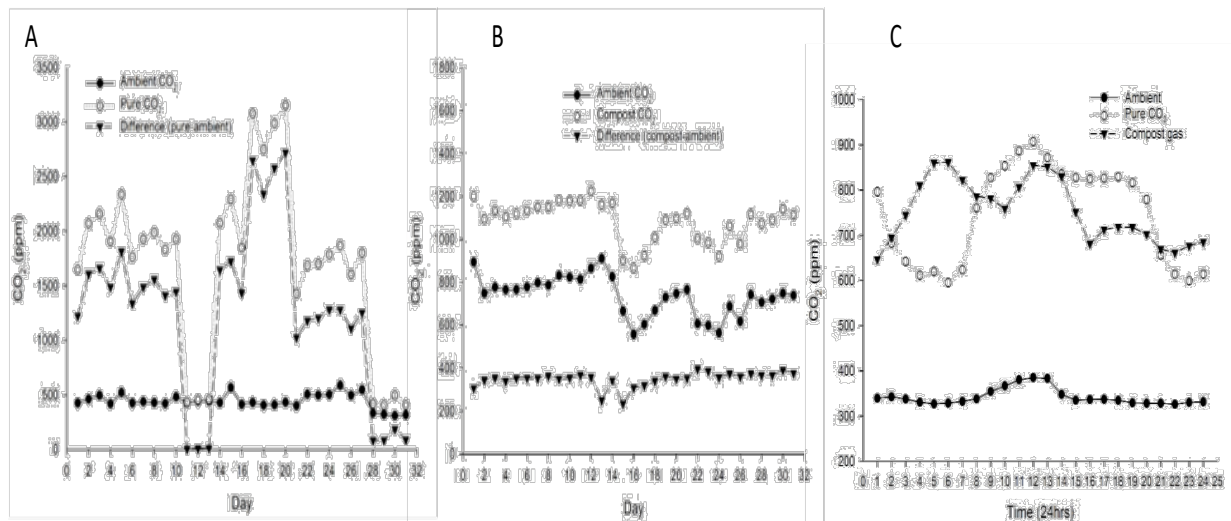
Three plant growth studies were undertaken to compare Romaine lettuce production, under hydroponic conditions, using compost material as a CO<sub>2</sub> gas source, a pure CO<sub>2</sub> gas source, and in ambient conditions. The growth studies were undertaken over a 31 day cycle and 24 hour daylight cycle with LED lighting and a deep-water hydroponic system in the vertical farm module at Dalhousie University. The growing temperature in the module was set at 21°C with fresh air circulation every 15 minutes. The growing containers were established in a completely randomized design. Growing containers using compost material as the CO<sub>2</sub> gas source had two Mason jars with a total of 300 g fresh weight partially composted material taken from the HotRot 1811 and a sponge trap to capture NH<sub>3</sub> (Fig. 15).



**Fig. 15. Romaine lettuce produced under an elevated CO<sub>2</sub> gas environment with NH<sub>3</sub> traps (A) and pure CO<sub>2</sub> gas (B) in a 31 day production cycle.**

Results from the three studies using Romaine lettuce receiving CO<sub>2</sub> gas from either a pure gas source, a compost source, or open to the ambient concentrations are shown in Fig. 16. The initial study (Fig. 16A) had wide variability in CO<sub>2</sub> gas concentrations from the pure gas source resulting from flow valve control issues and the average ambient concentration was 460 ppm. In the second study (Fig. 16B), the compost source was relatively stable, with slight variability at day 15 with an average concentration of 1085 ppm and ambient concentration at 736 ppm due to poor air exchange in the chamber. The CO<sub>2</sub> concentrations in the final study (Fig. 16C) between the two treatments (pure and compost) were more closely aligned, averaging 746 ppm and 754 ppm, respectively, with some day to day variability due to flow valve adjustments. Ambient concentrations in the third study averaged 342 ppm.





**Fig. 16.** Carbon dioxide concentrations in three lettuce production studies using a compost source, a pure gas source, and under ambient conditions under controlled environment conditions.

Lettuce production parameters were measured in each of the three studies including: fresh weight of the leaves and roots and moisture content. Table 5 highlights some of the key results from the lettuce production experiments. Under the current experimental conditions, in Study 1 the elevated CO<sub>2</sub> treatment from a pure gas source increased fresh weight lettuce leaf yield by 46% and root biomass by 76% relative to lettuce grown under ambient concentrations. In Study 2, the elevated CO<sub>2</sub> treatment from a compost gas source increased fresh weight lettuce leaf yield by 74% and root biomass by 157% relative to lettuce grown under ambient concentrations. In the final study, the elevated CO<sub>2</sub> treatment from a pure and compost gas sources increased fresh weight lettuce leaf yield by 135% and 182%, respectively, and root biomass by 78% and 151%, respectively, relative to lettuce grown under ambient concentrations. It is important to note that the lettuce yields obtained were significantly below a normal market weight (~680 g) due to the experimental setup (6 lettuce seedlings grown in close proximity per chamber).

In addition to lettuce leaf yield, the total carbon and nitrogen contents of the plant biomass were also analyzed for each of the three studies (Table 6). In all three studies, total carbon was significantly greater under elevated CO<sub>2</sub> gas than ambient conditions, ranging from 44% to 140% greater. Total nitrogen content was approximately two times greater under elevated CO<sub>2</sub> gas conditions than ambient in Studies 2 and 3, with pure gas and compost gas sources.

**Table 5. Plant harvest results from three Romaine lettuce studies under elevated CO<sub>2</sub> from a pure gas tank [P] and compost source [C] and under ambient conditions over a 31 day growing cycle (Source: A. Luo, MSc Thesis, 2020).**

Study	Treatment (ppm)	Leaves (Fresh, g)	Leaves (Dry, g)	Leaves (MC)	Roots (Fresh, g)	Roots (Dry, g)	Roots (MC)	Leave: root (Fresh)
Study 1	A (460)	70.68±24.98 <sup>b</sup>	3.04±0.95 <sup>b</sup>	0.96±0.01 (ns)	8.92±3.68 <sup>b</sup>	0.32±0.16 <sup>b</sup>	0.96±0.01 (ns)	9.28±5.82 (ns)
	C	NA	NA	NA	NA	NA	NA	NA
	P (1760)	103.51±28.02 <sup>a</sup>	4.28±0.99 <sup>a</sup>	0.96±0.01 (ns)	15.73±5.25 <sup>a</sup>	0.48±0.16 <sup>a</sup>	0.97±0.01 (ns)	7.33±3.49 (ns)
Study 2	A (736)	33.86±18.55 <sup>b</sup>	1.84±1.09 <sup>b</sup>	94.63±0.48 (ns)	3.47±1.77 <sup>b</sup>	0.23±0.21 <sup>b</sup>	93.77±2.60 (ns)	11.25±7.89 (ns)
	C (1085)	58.97±17.16 <sup>a</sup>	3.05±0.78 <sup>a</sup>	94.73±0.54 (ns)	8.93±4.09 <sup>a</sup>	0.48±0.24 <sup>a</sup>	94.85±0.49 (ns)	7.77±3.61 (ns)
	P	NA	NA	NA	NA	NA	NA	NA
Study 3	A (342)	37.85 ±9.28 <sup>c</sup>	2.01±0.57 <sup>c</sup>	94.52±1.58 <sup>b</sup>	4.32±2.72 <sup>c</sup>	0.20±0.11 <sup>b</sup>	95.30±0.82 <sup>b</sup>	10.79±2.34 (ns)
	C (754)	106.57±27.77 <sup>a</sup>	4.05±0.99 <sup>a</sup>	96.09±0.97 <sup>a</sup>	10.86±3.00 <sup>a</sup>	0.36±0.10 <sup>a</sup>	96.63±0.39 <sup>a</sup>	10.38±3.56 (ns)
	P (746)	88.76±10.34 <sup>b</sup>	3.50±0.69 <sup>a</sup>	96.07±0.51 <sup>a</sup>	7.70±2.64 <sup>b</sup>	0.29±0.10 <sup>a</sup>	96.22±0.68 <sup>a</sup>	12.66±4.07 (ns)

\*Values are means (N=4) ±SD. A, ambient condition as control; C, compost gas condition; P, pure CO<sub>2</sub> condition. NA, not available; ns, not significant;

\*Values with the same letter in each column are not significantly different at  $p < 0.05$ . ns: not significant.

**Table 6. Total carbon and nitrogen contents for lettuce leaves harvested from three studies comparing CO<sub>2</sub> gas sources (pure [P] and compost [C]) and ambient conditions.**

Study	Treatment (ppm)	C (%)	Log <sub>10</sub> (C%)	TC (g)	N (%)	Log <sub>10</sub> (N%)	TN (g)	C:N
Study 1	A (460)	37.36±1.31 (ns)	1.57±0.01 (ns)	1.14±0.37 <sup>b</sup>	6.36±0.37 (ns)	0.8±0.03 <sup>a</sup>	0.195±0.07 (ns)	5.89±0.36 <sup>b</sup>
	C	NA	NA	NA	NA	NA	NA	NA
	P (1760)	38.14±1.63 (ns)	1.58±0.02 (ns)	1.64±0.41 <sup>a</sup>	4.78±0.73 (ns)	0.67±0.07 <sup>b</sup>	0.20±0.04 (ns)	8.17±1.36 <sup>a</sup>
Study 2	A (736)	39.40±3.20 (ns)	1.59±0.04 (ns)	0.58±0.34 <sup>b</sup>	5.00±0.50 (ns)	0.70±0.04 <sup>a</sup>	0.07±0.04 <sup>b</sup>	7.94±0.85 <sup>b</sup>
	C (1085)	41.59±4.94 (ns)	1.62±0.05 (ns)	1.39±0.30 <sup>a</sup>	4.19±0.75 (ns)	0.62±0.07 <sup>b</sup>	0.14±0.04 <sup>a</sup>	10.18±1.96 <sup>a</sup>
	P	NA	NA	NA	NA	NA	NA	NA
Study 3	A (342)	36.81±1.46 (ns)	1.57±0.02 (ns)	0.74±0.23 <sup>b</sup>	5.50±0.35 (ns)	0.74±0.03 <sup>a</sup>	0.07±0.02 <sup>b</sup>	6.72±0.53 <sup>b</sup>
	C (754)	35.46±1.89 (ns)	1.55±0.02 (ns)	1.44±0.36 <sup>a</sup>	4.40±0.51 (ns)	0.64±0.05 <sup>b</sup>	0.18±0.04 <sup>a</sup>	8.18±1.13 <sup>a</sup>
	P (746)	36.49±1.57 (ns)	1.56±0.02 (ns)	1.28±0.26 <sup>a</sup>	4.12±0.56 (ns)	0.61±0.06 <sup>b</sup>	0.15±0.04 <sup>a</sup>	9.06±1.66 <sup>a</sup>

\*Values are means (N=4) ±SD. A, ambient condition as a control; C, compost gas condition; P, pure CO<sub>2</sub> treatment. TC, the total amount of carbon per plant; TN, the total amount of nitrogen per plant; NA, not available; ns, not significant;

\*Values with the same letter in each column are not significantly different at  $p < 0.05$ . ns: not significant.

#### Objective 4: Build automated respirometry system for rapid testing and assessment of decomposition rates in CEA biomass mixtures

Composting organic wastes is an aerobic process resulting in the microbial conversion of organic carbon into inorganic form and released as CO<sub>2</sub> gas. Assessing the decomposability of organic waste feedstocks is typically done on the basis of chemical measurements of the total carbon and nitrogen contents and attempting to establish a homogenous mixture that has a C:N ratio ranging between 25:1 and 35:1. This approach attempts to estimate the optimal ratio of available carbon and nitrogen for microbial metabolism in a feedstock mixture but does not address how much becomes available for decomposition. Respirometric techniques measure either the consumption of oxygen or release of CO<sub>2</sub> gas as an outcome of microbial consumption of carbon. Measuring *in situ* decomposition of organic waste mixtures using conventional analytical methodologies are time consuming, labour intensive, and may require costly analytical equipment such as gas chromatographs. Rapid assessment of potential degradability of feedstock mixtures is a critical gap in developing an integrated resource recovery system from organic wastes, i.e. recycling CO<sub>2</sub> gas, into plant production. This project has explored a number of low-cost, automated analytical approaches, in collaboration with the University of Guelph's Controlled Environment Systems Research Facility, for the quantification of CO<sub>2</sub> gas during decomposition of different source separated organics. Three MSc students from the University of Guelph were involved in development and testing of automated approaches for measuring CO<sub>2</sub> gas with biomass originating from controlled environment agriculture facilities.

The first prototype was developed to evaluate decomposing inedible biomass grown in Nutrient Film Technique (NFT) hydroponic system. The research was conducted with a MSc student (S. Ratcliffe) using a nine vessel incubation system linked to a SBA-4 CO<sub>2</sub> gas analyzer (PP Systems, MA, USA) for automated measurements of CO<sub>2</sub> gas from each vessel (Fig. 17). Measurements were controlled with open/close valves attached to each vessel and programmed in a random sequence to draw gas from the vessel over two hour intervals.



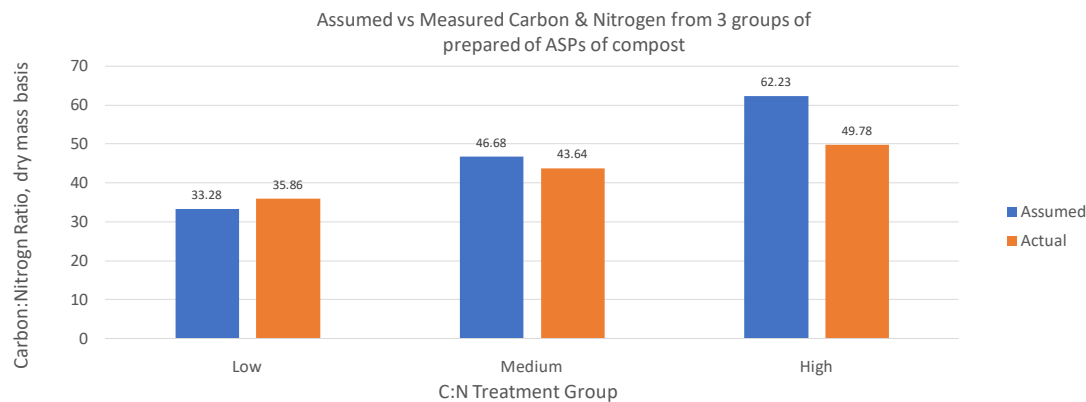
Fig. .17. Prototype automated respirometry system at the University of Guelph. (Source: S. Ratcliffe, 2019)

An incubation experiment was established using a combination of NFT roots from a lettuce production facility, wheat straw, and alpaca manure based on three different estimated C:N ratios (Table 7). The C:N ratios were estimated using literature values for the individual feedstocks, where available, and the biomass mixtures were subsequently analyzed for total carbon and nitrogen using a LECO CN analyzer.

**Table 7. Estimated and actual total carbon and nitrogen contents of three feedstocks: wheat straw, lettuce roots, and alpaca manure for use in a decomposition study (S. Ratcliffe, Guelph, 2019).**

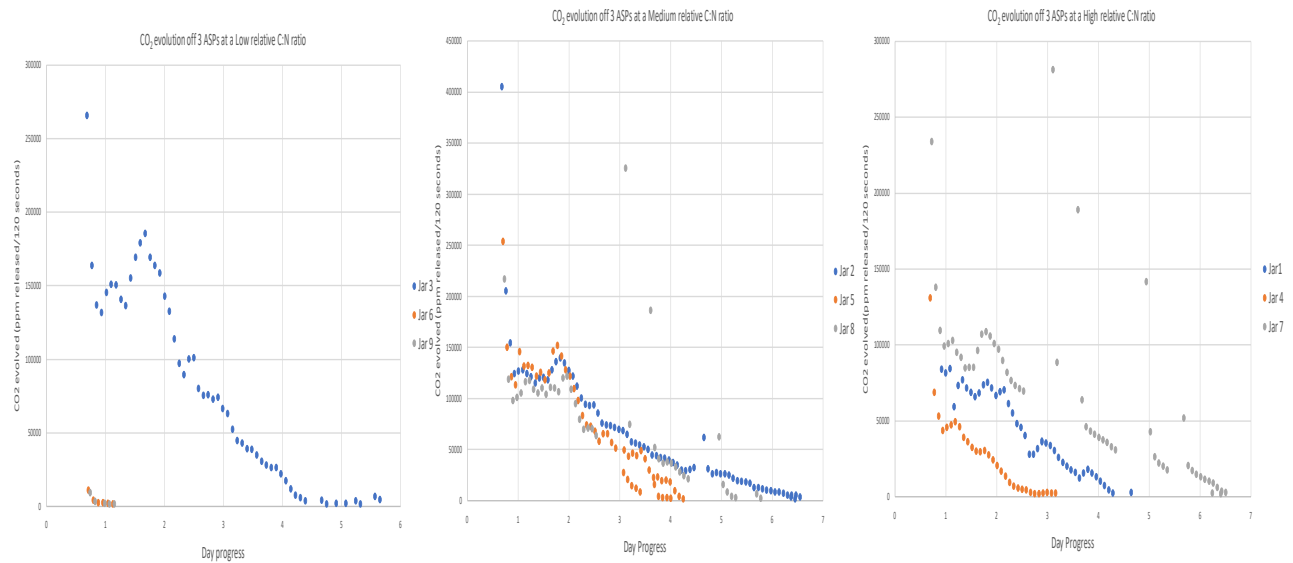
Feedstock	Estimated Values			Measured Values		
	Carbon (%)	Nitrogen (%)	C:N	Carbon (%)	Nitrogen (%)	C:N
Wheat straw	40	0.5	80	29.38	0.19	154.6
NFT Lettuce roots	45	4	11.25	17.24	1.26	13.7
Alpaca manure	20	1.3	15.38	33.24	6.57	5.06

Three treatment mixtures were established to represent Low, Medium, and High C:N ratios based on the estimated values but the measured C:N ratios resulted in the Medium and High treatments being very similar (Figs. 18 & 19).

**Fig. 18. Estimated vs. Actual total carbon and nitrogen ratios of three treatment groups based on mixtures of wheat straw, alpaca manure, and NFT lettuce roots. (Source: S. Ratcliffe, 2019)****Fig. 19. NFT lettuce roots on the plant (left), dried and chopped (middle), and ground (right). (Source: S. Ratcliffe, 2019)**

The goals of the experiment were to assess the precision of replicate data ( $n=3$ ) within each treatment group and to evaluate decomposability of the mixtures over a seven day incubation. Using non-dispersive infrared CO<sub>2</sub> gas analysis provides several advantages over conventional techniques including: automated continuous measurements and logging of data, shorter measurement time intervals, e.g. minutes, hours vs. days, weeks, and reduced labour requirements. The system was also set up with an electrical relay for the open/close valves and Arduino MEGA boards for data logging and control. The system operated on a sequence of two hour interval cycles for each vessel and a sampling window of two minutes. Results of the seven day incubation are shown in Figure 20. The data reflect variability between replicates, particularly in the Low and High C:N ratio treatments. The Low C:N ratio treatment had low CO<sub>2</sub> gas concentrations logged over the experimental period and it was unclear whether it was a result of the sensor, valves, or material reactivity. The Medium C:N ratio treatment replicates were much more aligned, suggesting that the automated respiration set up was capable of

capturing repeatable measurements from the same treatment. The High C:N ratio treatment data was variable in magnitude but two of the replicates followed similar patterns of CO<sub>2</sub> gas emissions.



**Fig. 20. Carbon dioxide concentrations over a seven day incubation study with mixtures of wheat straw, alpaca manure, and NFT lettuce roots. (Source: S. Ratcliffe, 2019)**

This experiment provided a clear proof-of-concept of the potential to use NDIR CO<sub>2</sub> gas sensors for rapid and continuous assessment of decomposing feedstock biomass from CEA production systems. The next phase of this work was to develop an automated respirometry system that was capable of collecting data from individual vessels on a continuous basis. The research was conducted two MSc students (C. Kiselchuk and A. Dsouza) using SCD-30 NDIR CO<sub>2</sub> gas sensors (Sensirion AG, Stäfa, Switzerland) in a manifold consisting of 17 incubation vessels (Kiselchuk, 2021; Dsouza et al., in review). The manifold consisted of air flow control valves and a pump moving air through individual vessels to collect the sample gas, condensate unit to remove moisture in the sample air, and into a vessel with the SCD-30 sensor (Fig. 21).



**Fig. 21. Automated respirometry system at the University of Guelph. (Source: A. Dsouza, 2021)**

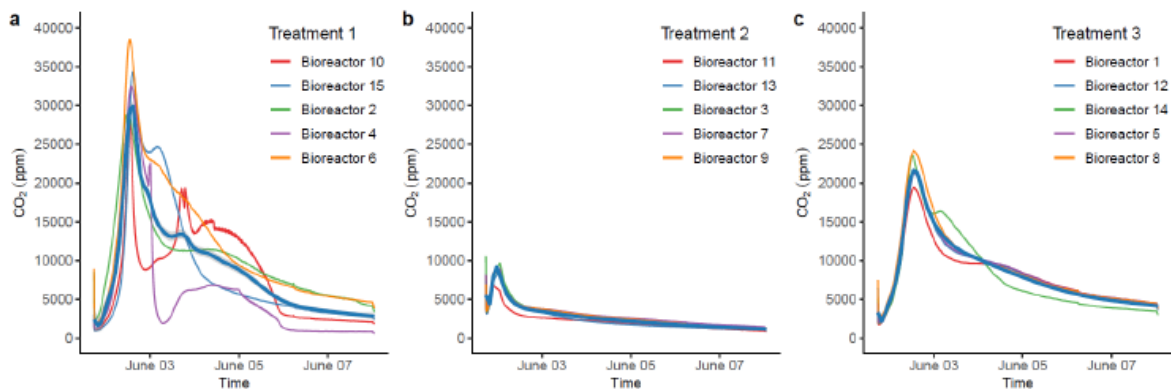
The study using the automated respirometry system evaluated hydroponically grown green bush bean (*Phaseolus vulgaris* L.) (GBBR) in combination with spent peat moss (PM) and/or leaf

litter (LL) over a seven day incubation period (Kiselchuk, 2021). The feedstocks were established in combinations to achieve a C:N ratio of 25:1 (Table 8.)

**Table 8. Physical and chemical parameters of three feedstocks (green bush bean-GBBR, spent peat moss-PM, and leaf litter-LL) and three treatment combinations to achieve a 25:1 C:N ratio (Kiselchuk, 2021).**

	Constituent	C:N Ratio	Constituent Wet Mass (g)	Bulk Density (kg/m <sup>3</sup> )	pH	Total Carbon (g)	Total Nitrogen (g)	Initial Moisture Content (%)	Final Moisture Content (%)
Feedstock	GBBR	9:1	-	-	6.6	-	-	64	-
	PM	101:1	-	-	6	-	-	88.9	-
	LL	48:1	-	-	5.9	-	-	1	-
Treatment 1	GBBR	25:1	110	460.65	5.9	78.9	3.1	28.7	60
	LL		140						
Treatment 2	GBBR	25:1	110	341.9	6.1	55.35	2.2	80.1	58.2
	PM		200						
Treatment 3	GBBR	25:1	110	360.2	5.9	73.95	2.9	57.5	57.5
	PM		175						
	LL		110						

The study was established in a completely randomized design with five replicates and humidified air pulled through each vessel to reduce moisture loss during the incubation period. The final moisture content of each treatment was approximately 58%, with the expectation based on previous testing that some moisture would be lost. Results of CO<sub>2</sub> gas evolution from the feedstock mixture treatments over seven days are shown in Figure 22.



**Fig. 22. Evolved CO<sub>2</sub> gas from three treatments: a) GBBR-LL (Treatment 1), b) GBBR-PM (Treatment 2), and c) GBBR-LL-PM (Treatment 3) over a seven day incubation study (n=5) The thick dark blue line represents the mean of five replicates. [Source: Kiselchuk, 2021].**

The data revealed that replicate samples in all three treatments overlapped closely, with the exception of Treatment 1 where greater variability between replicates was observed. The variability was greater in Treatment 1 due to the reactivity, or high decomposability, associated with the root biomass and leaf litter. In contrast, Treatment 3 CO<sub>2</sub> gas evolution, which was a combination including PM and LL, was more stable between the replicates primarily due to the spent PM. This suggests that individual sensors are capable of capturing variability across

replicate samples resulting from the natural heterogeneity of these types of feedstocks. The cumulative CO<sub>2</sub> gas evolution was greatest in Treatments 1 and 3, associated with the higher decomposability of the leaf litter relative to the spent peat moss (Fig. 23). Proportionally, Treatments 1 and 3 evolved approximately 13% of the total initial carbon in the mixture of feedstocks, while Treatment 2 evolved approximately 5% of the initial carbon.

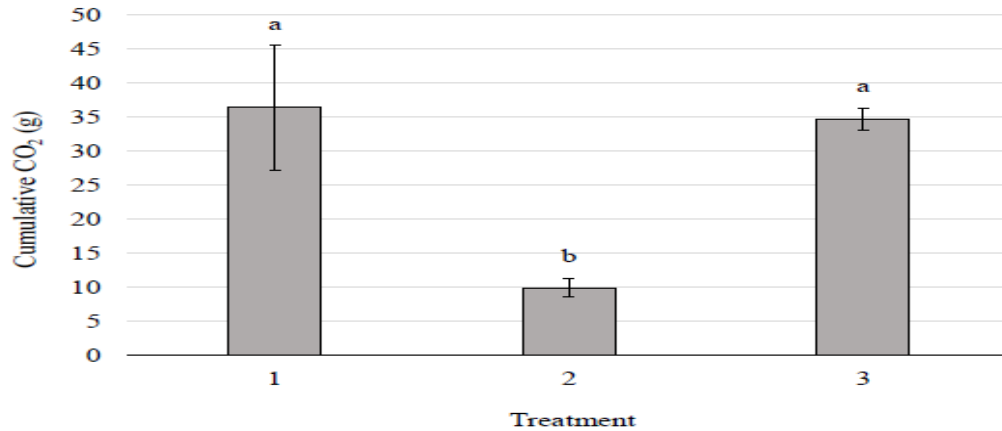


Fig. 23. Cumulative CO<sub>2</sub> gas from three treatments: a) GBBR-LL (Treatment 1), b) GBBR-PM (Treatment 2), and c) GBBR-LL-PM (Treatment 3) over a seven day incubation study (n=5). [Source: Kiselchuk, 2021].

The temperature profiles for each treatment vessel are shown in Figure 24. Treatment 1 resulted in highly variable temperature profiles across the five replicates but were more stable in the two other treatments. Based on the small quantity of material used in this study (~40 g of biomass), it would not be anticipated to achieve sustained thermophilic temperatures. In Treatment 1, only one replicate was able to achieve a thermophilic temperature range, with a mean over all replicates of 35°C.

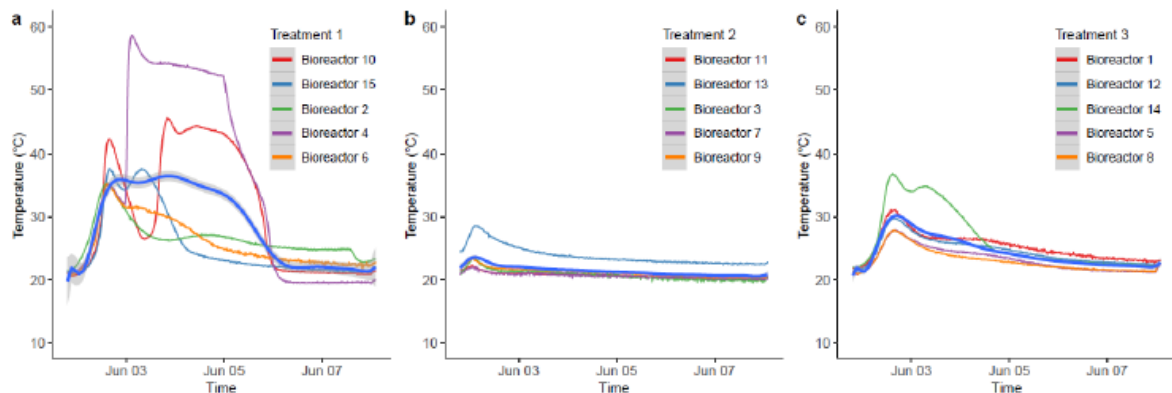


Figure 24. Temperature profiles from three treatments: a) GBBR-LL (Treatment 1), b) GBBR-PM (Treatment 2), and c) GBBR-LL-PM (Treatment 3) over a seven day incubation study (n=5). The thick dark blue line represents the mean of five replicates. [Source: Kiselchuk, 2021].

Additional experiments were conducted using leaf litter and spent coffee grounds in the automated respirometry system. Results from these experiments validated the capability of the

respirometry system to provide repeatable data from a wide range of feedstock mixtures and generate high resolution datasets on CO<sub>2</sub> gas evolution (Kiselchuk, 2021).

Additional research on the decomposition of CEA biomass is being conducted by a third MSc student (A. Dsouza). The focus of this research is to evaluate decomposability of various biomass waste feedstocks being generated from local CEA production facilities, e.g. GoodLeaf Farms, as opportunities to generate CO<sub>2</sub> gas for recycling back into plants.

### Objective 5: Quantify heat (energy) utilization and CO<sub>2</sub> emissions during the composting of municipal source-separated organics (SSO) and other organic wastes [In progress]

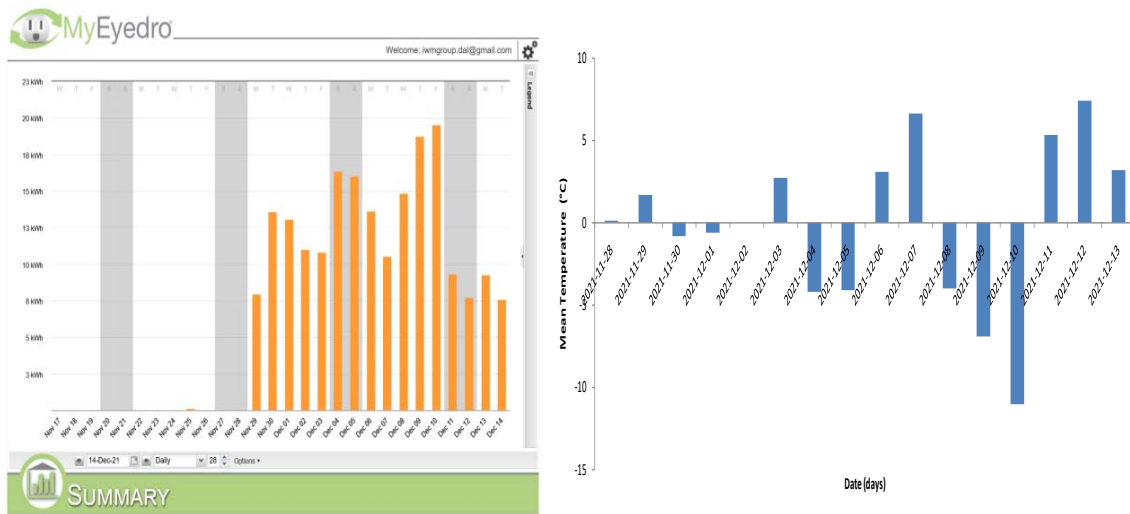
The original objectives of this project were to quantify CO<sub>2</sub> gas emissions from composting to recycle back into plant production, examine ways to recycle heat lost from composting processes back into plant production, and evaluate energy consumption/conservation approaches for sustainable year-round CEA production. Work on achieving these objectives are currently underway due to delays in commissioning of the HotRot 1811 system and mechanical issues affecting on-going operation of the composting system. The vertical farming production module currently has an EHWEM1-LV Eyedro energy meter with two sensors installed on the 110V main electrical line on the subpanel and are linked to an online energy monitoring website (my.eyedro.com) (Fig. 25). The energy monitoring system tracks daily electrical usage and has features such as conversion of energy use into carbon dioxide equivalents or cost per kWh.



Fig. 25. MyEyedro Green Solutions cloud-based energy usage monitoring of the Faculty of Agriculture's vertical farming module for Controlled Environment Agriculture.

Figure 26 illustrates the current energy consumption to heat and operate the vertical farming module from November 30<sup>th</sup>, 2021 up to December 14<sup>th</sup>, 2021 using the Daikin heat pump system. It is clear that on the coldest days in December (8<sup>th</sup> to 10<sup>th</sup>) the highest energy consumption was recorded to maintain the module temperature at 18°C.





**Fig. 26. MyEyedro electrical energy usage for the period of November 29<sup>th</sup> to December 14<sup>th</sup>, 2021 and the corresponding mean temperature in Truro, Nova Scotia, Canada.**

During the Winter 2022, an encasement will be constructed around the heat pump unit to capture warm air directed from the HotRot 1811 composting system. The energy monitoring system will be run continuously over the next 12 months to evaluate the effect of utilizing heat from the composting process to reduce energy consumption of the heat pump during cold periods.

To directly quantify CO<sub>2</sub> gas emissions during the processing of organic wastes processed through the HotRot 1811, a Sensirion STC31 thermal conductivity sensor capable of measuring CO<sub>2</sub> at 0 to 25 vol% and 0 to 100 vol% has been installed at the exhaust port of the unit. The sensor is connected to a datalogger for continuous recording of gas concentrations and during the Fall and Winter operations. The data will be correlated to the type and quantity of material being added into the system over the monitoring period. These data will be aggregated and used to develop an initial Life Cycle Analysis framework for processing organics and recycling carbon into plant production.

## Key Project Outcomes

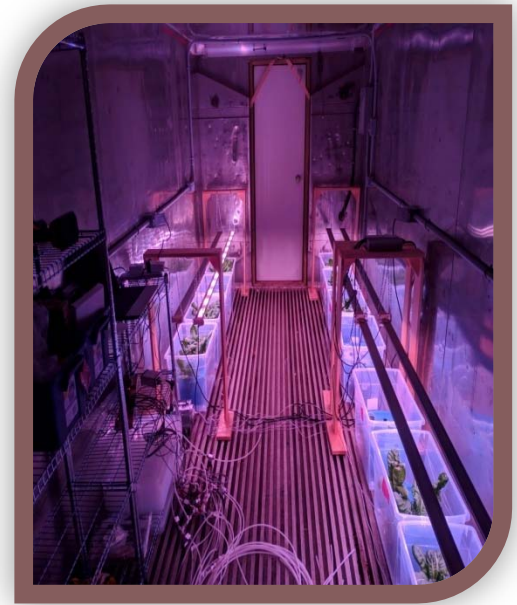
A number of outcomes resulting from this project include:

1. Training of four Masters of Science students: Anjie Luo (MSc, Dalhousie University), Connor Kiselchuk (MSc, University of Guelph), and Ajwal Dsouza (MSc, University of Guelph), and Sean Ratcliffe (MSc, ABD).
2. Completion of three MSc theses including:
  - a. Anjie Luo: 2020. *Evaluation of Romaine lettuce (*Lactuca sativa* L. cv. Parris Island) production under an elevated CO<sub>2</sub> gas environment generated from compost materials*. MSc thesis, Dalhousie University
  - b. Connor Kiselchuk: 2021. *Carbon Dioxide Production from Organic Waste Recycled in Controlled Environment Agriculture Systems*. MSc thesis, University of Guelph
  - c. Ajwal Dsouza: 2022. *Quantifying carbon dioxide production from processing of controlled environment agriculture residues*. MSc thesis, University of Guelph
3. Training of a PhD student and Post-Doctoral Fellow (Allan Thomson, Dalhousie University)
4. Establishment of collaborations with the University of Guelph's PhytoGro Research Chair in Controlled Environment Systems, Dr. Thomas Graham (<https://ses.uoguelph.ca/people/thomas-graham>), and the Controlled Environment Systems Research Facility
5. Three peer-reviewed publications (published or submitted)
  - a. Thomson, A., Price, G.W., Arnold, P., Dixon, M., Graham, T. Review of the potential for recycling CO<sub>2</sub> from organic waste composting into plant production under Controlled Environment Agriculture. *J. Cleaner Prod.* (accepted)
  - b. Dsouza, A.; Price, G.W.; Dixon, M.; Graham, T. A Conceptual Framework for Incorporation of Composting in Closed-Loop Urban Controlled Environment Agriculture. *Sustainability* **2021**, *13*, 2471. <https://doi.org/10.3390/su13052471>
  - c. Dsouza, A.; Price, G.W.; Kiselchuk, C., Lawson, J., Dixon, M.; Graham, T. Development of an automated, multi-vessel respirometric system to evaluate decomposition of composting feedstocks. *Biosys. Engin.* (in review)
6. Design and development of a controlled environment plant production system that can be integrated with an in-vessel composting system, located in the Faculty of Agriculture, Dalhousie University.

## Future Research Directions

The intention of this project was to establish an empirical platform to support the conceptual framework of a circular economy of organic wastes to food production. Composting is a well established method of managing food wastes, source separated and agricultural organic wastes. The science of composting is still incomplete but the certainty is that two valuable microbial metabolic outputs, CO<sub>2</sub> gas and heat, are untapped resources from the process. This project clearly demonstrated the potential for a partially composted material to act as a source of CO<sub>2</sub> gas in a plant production environment. Additional work from this project has resulted in prototypes of continuous CO<sub>2</sub> gas monitoring respirometry systems for rapid assessment of compost mixture decomposition rates. This tool allows for quantification of CO<sub>2</sub> gas production over time and optimization of feedstock combinations. The advantage of the respirometric system developed through this project is the lower cost (\$100s vs. \$1000s), reduction in labour requirements for sampling, much higher intervals of gas sampling, and eliminating the need for expensive analytical equipment. Future research will need to focus on integrating bioreactor systems with Controlled Environment Agriculture facilities to recover and recycle these two valuable by-products. Significant research has already been conducted on elevated CO<sub>2</sub> gas under LED lighting and hydroponic nutrient solutions. Integration of organic waste recycling and CEA will require evaluating gas filtering to remove unwanted components, such as VOCs and NH<sub>3</sub>, and managing gas flow rates to production units. The delivery mechanism of CO<sub>2</sub> gas to plant production rooms and ensuring availability of gas from decomposing system will be the next big challenge in research for this area.

The continuing expansion of urban areas, leading to greater concentration of organic wastes, lends itself to CEA production systems. Circularization of the economy is about reducing energy waste and optimizing opportunities to cycle resources back into production. Future research in this area will lead to the development of integrated waste management and plant production facilities that operate year-round to supply fresh food locally.



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## Appendix A: HotRot 1811 Specifications Sheet



# HotRot®

COMPOSTING SYSTEMS



## HotRot 1811



GROWTH FROM WASTE

# HotRot 1811

## TYPICAL APPLICATIONS

Suitable for on-site applications (commercial food, mining camps, zoos, poultry mortalities) or smaller municipal transfer stations and sewage treatment plants.

## FEED SYSTEM

A feed hopper enables 24/7 unattended operation. Multiple units can be installed in parallel with individual or larger common feed hoppers. Alternatively, a bin lifter may be used for homogenous wastes where staff are undertaking other activities in the vicinity (e.g. a waste management facility at a remote camp). Ancillary equipment such as feed hoppers, bin-lifters, augers, conveyors, shredders and dewatering units are options that can be supplied as part of a turnkey installation.

## ODOUR

A bio filter package incorporating a condensate tank and plenum floor is supplied with each unit. Installed plant is eligible for HotRot's Odour-Free Guarantee (conditions apply). Biofilter media is required in addition.

## LEACHATE

Nil. HotRots produce a small amount of condensate which can be used for irrigation, wetting maturation piles or discharged to sewer.

## CONTROL INTERFACE

High resolution colour touch screen with mimic display, graphical data trends, parameter settings, alarms, multiple language option and online connectivity option.

## CONSTRUCTION MATERIALS

Standard: Painted mild steel frame and outer hull with stainless steel liner. Mild steel shaft and tines.

Optional: Stainless steel shaft, tines and end-plate liners where low pH conditions are anticipated.

## DESIGN LIFE \*

10 years. \*Excluding maintenance and wear items.

Design and specifications may change without notice at any time due to ongoing research and development.

Specifications	
Physical Dimensions	Length overall – 12.78m
	Width overall – 2.2m
	Height overall – 2.24m – Plus ancillaries
Typical Footprint	120m <sup>2</sup> includes feed hopper, biofilter, feed and discharge augers
Weight (Empty)	11,500kg
Power Supply	Typically 400V, 32A, 3-phase (+neutral+ earth) 50Hz. Other voltages available
Power Consumption	26kwh/tonne
Noise	75dB typical when running, 1.5m from unit
Nominal Processing Capacity	1.7t/day (600 tpa) with bin lifter
	2.5t/day (900 tpa) with feed hopper
	Both prior to dewatering unit. The product may require some passive maturation prior to use or final testing.

## Appendix B: MSc Thesis (Anjie Luo), Dalhousie University



EVALUATION OF ROMAINE LETTUCE (LACTUCA SATIVA L. CV.  
PARRIS ISLAND) PRODUCTION UNDER AN ELEVATED CARBON  
DIOXIDE (CO<sub>2</sub>) GAS ENVIRONMENT GENERATED FROM COMPOST  
MATERIALS

by

Anjie Luo

Submitted in partial fulfillment of the requirements  
for the degree of Master of Science

at

Dalhousie University  
Halifax, Nova Scotia  
December 2020

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## TABLE OF CONTENTS

LIST OF TABLES .....	iv
LIST OF FIGURES .....	v
ABSTRACT .....	vi
LIST OF ABBREVIATIONS USED .....	vii
ACKNOWLEDGEMENTS .....	viii
1. Introduction.....	1
1.1. Background Introduction.....	1
1.2. Literature review .....	2
1.2.1. Effects of elevated CO <sub>2</sub> on greenhouse production .....	2
1.2.1.1. Morphological changes at elevated CO <sub>2</sub> .....	3
1.2.1.2. Photosynthesis at elevated CO <sub>2</sub> .....	3
1.2.1.3. Plant biomass and production at elevated CO <sub>2</sub> .....	4
1.2.1.4. Modern controlled environment agriculture.....	6
1.2.1.5. Romaine lettuce .....	9
1.2.2. Gas generated from the composting process .....	9
1.2.2.1. How the C:N ratio of compost material affects CO <sub>2</sub> generation .....	11
1.2.2.2. CO <sub>2</sub> emissions after application of mature compost to soil .....	14
1.2.2.3. NH <sub>3</sub> emissions after application of mature compost to soil .....	14
1.3. Summary .....	15
1.4. Objectives.....	16
2. Short-term quantification of CO <sub>2</sub> and NH <sub>3</sub> gas generation from composted material .....	17
2.1. Introduction .....	17
2.2. Materials and methods .....	18
2.2.1. Composting materials .....	18
2.2.2. Carbon dioxide and ammonia emissions .....	19
2.3. Calculations and analytical methodology .....	22
2.4. Results .....	23
2.4.1. Gas generated from CF material under controlled environment conditions.....	23
2.4.2. CO <sub>2</sub> changes in the semi-sealed growth chamber.....	26

2.4.3. Differences in NH <sub>3</sub> gas emissions between raw and processed compost feedstock	27
2.5. Discussion	28
2.5.1. CO <sub>2</sub> generated from CF material	28
2.5.2. NH <sub>3</sub> generated from CF material	29
2.6. Conclusion	31
3. Evaluating romaine lettuce ( <i>Lactuca sativa</i> L. cv. Parris Island) production under carbon dioxide enrichment by using composting and conventional gas sources	32
3.1. Introduction	32
3.2. Materials and methods	33
3.2.1. Hydroponic plant production system design	33
3.2.2. Composting material	35
3.2.3. Plant material	36
3.2.4. Automated CO <sub>2</sub> monitoring system	36
3.3. Analytical methodology	38
3.4. Results	39
3.4.1. Biomass production and moisture content	39
3.4.2. Growth environment	42
3.4.3. Total carbon and nitrogen uptake in lettuce	45
3.4.4. Nutrient solution conditions at the end of the study	47
3.5. Discussion	48
3.5.1. Effects of elevated CO <sub>2</sub> concentrations on lettuce production	48
3.5.2. Effects of elevated CO <sub>2</sub> concentrations on lettuce	50
3.5.3. Effects of elevated CO <sub>2</sub> concentrations on hydroponic nutrient solution	51
3.5.4. Effects of compost generated gas on lettuce growth	52
3.6. Conclusions	53
4. Future research	55
5. Overall conclusion	57
References	59

## LIST OF TABLES

<b>Table 1.</b> Effect of CO <sub>2</sub> enrichment on the growth of lettuce ( <i>Lactuca sativa</i> ) (Caporn, 1989) .....	6
<b>Table 2.</b> Energy and CO <sub>2</sub> generation after complete combustion of sawdust wood pellets, natural gas, and propane (Dion et al., 2013) .....	7
<b>Table 3.</b> Volatile solids (VS) produced 455 kg <sup>-1</sup> animal unit day <sup>-1</sup> , likely VS lost, total gas, and CO <sub>2</sub> equivalent (Brown et al., 2008) .....	10
<b>Table 4.</b> CO <sub>2</sub> gas generation for different types of organic wastes (dry basis) incubated to simulate decomposition in a municipal solid waste landfill (Eleazer et al., 1997) .....	11
<b>Table 5.</b> Emission rates of CO <sub>2</sub> (g·m <sup>-2</sup> ·day <sup>-1</sup> ) from two mixtures of SFP and cotton during the thermophilic phase of composting (Santos et al., 2016) .....	13
<b>Table 6.</b> Chemical properties of raw mixed feedstocks and the processed material after 14 days in the HotRot 1811 (N=5) .....	19
<b>Table 7.</b> Parameters including dry matter, moisture content, total percentage and amount of carbon, total percentage and amount of nitrogen of CF material at the start and end of the 14-day CO <sub>2</sub> incubation study (N=5) .....	24
<b>Table 8.</b> Parameters including dry matter, moisture content, total percentage and amount of carbon, total percentage and amount of nitrogen from CF material at the start and end of the 14-day NH <sub>3</sub> incubation study (N=5) .....	25
<b>Table 9.</b> NH <sub>3</sub> -N (mg·kg <sup>-1</sup> DM·hr <sup>-1</sup> ) emissions from raw compost mixture feedstock vs. processed compost feedstock over 24 hours (N=6) .....	28
<b>Table 10.</b> Growth parameters of lettuces under different CO <sub>2</sub> concentrations and various gas sources (from compost emissions or pure CO <sub>2</sub> cylinder) .....	41
<b>Table 11.</b> Total carbon and nitrogen content of lettuce leaf (dry basis) under different CO <sub>2</sub> concentrations and various gas sources (from compost emissions or pure CO <sub>2</sub> cylinder) .....	46
<b>Table 12.</b> The concentration of nitrogen, pH, and EC of the nutrient solution under different CO <sub>2</sub> gas conditions in growth chambers at the end of 31 days .....	47

## LIST OF FIGURES

<b>Fig. 1.</b> CO <sub>2</sub> trap apparatus.....	19
<b>Fig. 2.</b> Setup for experiment two to measure continuous CO <sub>2</sub> emissions from compost feedstock in sealed chamber over a 24-hour period.....	20
<b>Fig. 3.</b> NH <sub>3</sub> trap apparatus .....	21
<b>Fig. 4.</b> CO <sub>2</sub> and NH <sub>3</sub> gas emissions from the partially composted material over a 14-day incubation period (N=5).....	24
<b>Fig. 5.</b> CO <sub>2</sub> level changes in a semi-sealed growth chamber for 24 hours at different amounts of wet CF.....	26
<b>Fig. 6.</b> Hourly fluctuations in CO <sub>2</sub> concentrations from a semi-sealed growth chamber containing a hydroponic nutrient solution with an air stone over a 24-hour period.....	27
<b>Fig. 7.</b> The hydroponic system set up for lettuce growth .....	34
<b>Fig. 8.</b> Compost jar in compost gas treatment of hydroponic system.....	35
<b>Fig. 9.</b> Automatically CO <sub>2</sub> gas sampling and analyzing setup .....	37
<b>Fig. 10.</b> Examples of CO <sub>2</sub> data collected for 240 s from elevated CO <sub>2</sub> chamber and an ambient condition .....	38
<b>Fig. 11.</b> Daily fluctuations in CO <sub>2</sub> concentration over 31 days in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure CO <sub>2</sub> gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 1. ....	42
<b>Fig. 12.</b> Daily fluctuations in CO <sub>2</sub> concentration over 31 days in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure CO <sub>2</sub> gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 2 .....	43
<b>Fig. 13.</b> Hourly fluctuations in CO <sub>2</sub> concentration over 24 hours in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure CO <sub>2</sub> gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 3 .....	44

## ABSTRACT

Composting is a process in which organic matter is biologically degraded leading to stabilized organic matter and waste gases such as carbon dioxide (CO<sub>2</sub>) and ammonia (NH<sub>3</sub>). The CO<sub>2</sub> emissions from the composting process can be used to supplement CO<sub>2</sub> in controlled environment agriculture (CEA) production. Partially composted animal bedding and manure feedstocks processed and collected from a HotRot 1811 composting system was evaluated for CO<sub>2</sub> and NH<sub>3</sub> emissions in a series of incubation experiments. The results indicated that the compost material had potential usage as a CO<sub>2</sub> source for use in a controlled environment agricultural growing system. Romaine lettuce (*Lactuca sativa* L. cv. Parris Island) was grown under compost gas and pure CO<sub>2</sub> gas enrichment in a hydroponic system. The mixed gas from compost material, after NH<sub>3</sub> filtering had similar effects on lettuce growth as a pure CO<sub>2</sub> enriched treatment, leading to increased lettuce biomass production (82% to 180%), relative to plants under ambient conditions and increased total amount of nitrogen (100% to 157%) and carbon contents (95% to 140%) in the leaves.

## LIST OF ABBREVIATIONS USED

C	Carbon
C <sub>2</sub> H <sub>5</sub> OH	Ethanol
CD	Cow Dung
CEA	Controlled Environment Agriculture
CF	Compost Feedstock
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon Dioxide
DI	Deionized
DM	Dry Matter
EC	Electrical Conductivity
ETPS	Pharmaceutical Treatment Facility
GHG	Greenhouse Gases
H <sub>3</sub> PO <sub>4</sub>	Phosphoric Acid
HVAC	Heating, Ventilation, And Air Conditioning
IPCC	Intergovernmental Panel on Climate Change
IWM	Innovative Waste Management
K <sub>2</sub> CO <sub>3</sub>	Potassium Carbonate
KCl	Potassium Chloride
KOH	Potassium Hydroxide
MC	Moisture Content
N	Nitrogen
N <sub>2</sub> O	Nitrous Oxide
NFT	Nutrient Film Technique
NH <sub>3</sub>	Ammonia
OL	Oak Leaf
OM	Organic Matter
PB	Paris Batavia
RuBP	Ribulose 1,5-bisphosphate
SFP	Solid Fraction of Pig Slurry
SM	Spent Mycelia
TGW	Tobacco & Grape Waste
TN	Total Nitrogen
TW	Tobacco Waste
VS	Volatile Solids

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## **1. Introduction**

### **1.1. Background Introduction**

As a soil amendment and source of fertility, compost can be used to maintain soil nutrient content, build soil organic matter (OM), and promote plant growth (Huang et al., 2016). Composting is an aerobic decomposition process in which microorganisms convert raw OM into a stabilized humus-like material called compost (Irvine et al., 2010). Compost products can benefit the soil as organic fertilizers while also serving as a treatment process for organic waste that can reduce nuisance odors, pathogens, or weed seeds (Sweeten, 2008). During the composting process, gases such as carbon dioxide (CO<sub>2</sub>), ammonia (NH<sub>3</sub>), and methane (CH<sub>4</sub>) are generated. Some studies suggest that composts are considered to be relatively stable or mature when the solid compost feedstock (CF) respiration rate is lower than 5 mg CO<sub>2</sub>-C·kg<sup>-1</sup> OM·hr<sup>-1</sup> (Moreira et al., 2008; CCME, 2005). However, microbial activity occurs continually within the final or partially processed compost material even though it is considered stable and mature. After applying the compost into the soil, soil microbes may continue to use this added substrate as an energy source resulting in higher microbial activity in the soil OM pool and releasing more CO<sub>2</sub> (Perelo & Munch, 2005). There has been limited investigation on the role of composting and organic matter decomposition on gas emissions for use in other processes.

Supplemental CO<sub>2</sub> gas added to greenhouse environments can improve the quality and yield (30%) of greenhouse crops and change plants' morphological characteristics, such as increasing leaf thickness (Raines, 2011; Becker & Kläring, 2016). In lettuce production, elevated CO<sub>2</sub> enhances the plant's health-promoting benefits by increasing phenolic compound content and antioxidant capacity (Pérez-López et al., 2018). In some commercial production operations, CO<sub>2</sub> concentrations in greenhouses are elevated using various approaches to increase crop yield and

quantity. For instance, burning propane is a common way to increase CO<sub>2</sub> levels and temperature within a greenhouse, but this comes at an additional cost (Benke & Tomkins, 2017). For example, the traditional way to supply additional 1000 ppm CO<sub>2</sub> enrichment in a 1000 m<sup>2</sup> glass greenhouse will use 2.8 to 3.4 m<sup>3</sup> natural gas and 2.8 to 3.4 L propane per hour (Blom et al., 2002). This study will focus on the use of compost generated CO<sub>2</sub> under a controlled growing environment to promote photosynthetic growth and production of Romaine lettuce (*Lactuca sativa*).

## **1.2. Literature review**

### **1.2.1. Effects of elevated CO<sub>2</sub> on greenhouse production**

Carbon dioxide is one of the basic building blocks used in photosynthesis to create the raw material for nearly all biomass on Earth. In modern controlled environment agriculture (CEA) production, there are benefits gained through CO<sub>2</sub> enrichment of the production space.

Elevated CO<sub>2</sub> can positively alter plant morphological development, such as leaf area development, tiller production, and shoot to root ratios (Seneweera, 2011). For example, enriched CO<sub>2</sub> environments increase plants' resistance to environmental stress by modifying the profiles of secondary metabolites and increased virus resistance in tobacco plants (Matros et al., 2006). The C:N ratio of plant tissues and C:N exchange between the growing medium and plants can also be influenced by the ambient concentration of CO<sub>2</sub> (Gifford et al., 2000).

Many greenhouse growers elevate CO<sub>2</sub> levels to achieve higher yields of different ornamental and vegetable crops, such as basil (Al-Jaouni, 2018), tomato (Tripp et al., 1992), lettuce (Singh et al., 2020), and Chinese kale (La et al., 2009). Xu et al. (2016) suggested that soybean grown under an elevated atmospheric CO<sub>2</sub> (800 ppm) increased in biomass production by 54% to 136%. Scientists have also concluded that food and flowering crops will improve around 36 and 43% in photosynthetic rates and foliar carbohydrate respectively by increasing the ambient

CO<sub>2</sub> concentration from 395 to 550 ppm (Sreeharsha et al., 2015).

#### **1.2.1.1. Morphological changes at elevated CO<sub>2</sub>**

Atmospheric CO<sub>2</sub> enters intercellular air spaces through stomatal pores that regulate the partial pressure of CO<sub>2</sub> in those spaces. However, when ambient CO<sub>2</sub> partial pressure increases, stomata tend to close, decreasing the stomatal conductance and transpiration, causing increased water use efficiency (Prior et al., 2010). When soybean grown under the higher temperature, 40 to 80% of citrate, malate, malonate, fumarate and succinate in leaflets decreased, but CO<sub>2</sub> enrichment reduced the impact of elevated growth temperature on organic acid (Sicher, 2015). Morphological adjustments, especially the leaf area development, contribute to additional opportunities to capture light and nutrients at high CO<sub>2</sub> conditions (Gutiérrez et al., 2009). For example, production of wheat (*Triticum aestivum* L.) under high nitrogen supplies but at different CO<sub>2</sub> concentrations from 360 to 700 ppm had increased leaf elongation rates of expanding blade (32%), higher total leaf area (18%), and greater shoot mass (36%) (Seneweera & Conroy, 2005). Masle (2000) reported different responses to elevated CO<sub>2</sub> in two wheat cultivars, Hartog and Birch. In Masle's research, wheat plants grown under 900 ppm CO<sub>2</sub> grew significantly more than those under 350 ppm, with leaf area increases of 39% and 82% for Hartog and Birch, respectively. Other plants, exposed to elevated CO<sub>2</sub>, showed changes in carbohydrate partitioning between stems or roots with a limited capacity for leaf area enrichment (Stitt, 1999). A study by La et al. (2009) indicated that under conditions of CO<sub>2</sub> concentration increases from 330 ppm to 800 ppm, Chinese kale (*B. alboglabra*) had greater plant height (15.64%), stem thickness (11.79%), dry weights (11.91%), bolting stems (15.03%), roots (16.34%), and root/shoot ratios (3.9%).

#### **1.2.1.2. Photosynthesis at elevated CO<sub>2</sub>**

The organic substance, ribulose 1,5-bisphosphate (RuBP), catalyzes two reactions in the

cells: the fixation of CO<sub>2</sub> into photosynthetic metabolism; and the production of 2-phosphoglycolate in the photorespiratory pathway (Terashima et al., 2011). For short-term CO<sub>2</sub> enrichment, the ratio of these two reactions influences the partial pressure of CO<sub>2</sub> to O<sub>2</sub> (Dahal & Vanlerberghe, 2018). According to Rubisco kinetics, with increased ambient CO<sub>2</sub> partial pressure from 36 Pa to 72 Pa at 25°C, the carboxylation rate is enhanced, which means photosynthesis is increased (Kitaya et al., 1998). The result showed that dry matter increased where lettuce exposure to CO<sub>2</sub> doubled. Another study focusing on elevated root-zone CO<sub>2</sub> concentrations also showed a significant increase in photosynthetic CO<sub>2</sub> assimilation and stomatal conductance under proper light strength (He et al., 2007). Most of plants such as lettuce, potatoes, tobacco, and others use C<sub>3</sub> photosynthesis, which involves producing a three-carbon compound (3-g-phosphoglyceric acid) during Calvin Cycle, and goes on to become glucose. Some plants, such as corn and sorghum, use C<sub>4</sub> photosynthesis, which is different as C<sub>3</sub> produces a four-carbon intermediate compound and splits into CO<sub>2</sub> and three-carbon compound during Calvin Cycle (Gowik & Westhoff, 2011). The benefits of C<sub>4</sub> photosynthesis is that plants have more tolerant under light and water limitation or high temperature to produce more carbon (Watcharamongkol et al., 2018; Young et al., 2020).

### **1.2.1.3. Plant biomass and production at elevated CO<sub>2</sub>**

Increases in photosynthesis and Rubisco activity with elevated CO<sub>2</sub> levels enhances carbohydrate accumulation and influences CO<sub>2</sub> transport, which increases plant biomass (Ehlers et al., 2015). Under a doubling of CO<sub>2</sub> concentrations, which causes higher relative moisture content (MC) and a more stable temperature environment, the biomass of foliage and plant dry matter (DM) increases by about 31-51%, primarily in leafy vegetables (Jia et al., 2010). In leafy vegetables, carbohydrates are formed in leaves and ultimately used to support plant growth (Li et al., 2017).

Higher yield and quality of products, such as lettuce, spinach and tomatoes, can be typical outcomes from a CO<sub>2</sub> enriched environment (Giri et al., 2016; Wei et al., 2018). Lettuce, under a CO<sub>2</sub> concentration of 1000 ppm, gained over 70% greater head mass than those under a 200 ppm CO<sub>2</sub> environment (Becker & Kläring, 2016). Research by Reinert et al. (1997) indicated that the tomato's total vegetative dry mass was enhanced by exposure to elevated CO<sub>2</sub>. For example, at different CO<sub>2</sub> concentration levels, the fruit yield of tomato was increased 22 to 41% at increasing CO<sub>2</sub> concentrations from 450 to 675 ppm. Soybean production studies have shown also similar results, with soybean shoots DM increasing 30% by elevating CO<sub>2</sub> from 450 to 550 ppm. The degree to which a plant responds to elevated CO<sub>2</sub> is species or variety-specific depending on overall environmental conditions of exposure. For example, *A. capillaris* (56%), *H. lanatus* (60%), and *L. perenne* (34%) showed significant responses to elevated CO<sub>2</sub> levels with increases in whole plant dry weight (Jongen & Jones, 1998).

Plant organs also respond differently to elevated CO<sub>2</sub>, based on physiological requirements at specific growth stages. For example, Seneweera (2011) showed that the dry mass of sheaths, blades, and rice plants' roots increased by 47%, 1%, and 162%, respectively, as CO<sub>2</sub> increased from 370 ppm to 700 ppm. Pérez-López et al. (2015) indicated that the antioxidant capacity of lettuce, which were grown in 700 ppm CO<sub>2</sub>, was 179% higher than that in a standard atmosphere of 400 ppm CO<sub>2</sub>. Another study by Behboudian and Tod (1995) examined the effects of CO<sub>2</sub> enrichment (340 and 1000 ppm) on fruit quality (ripening and concentration of mineral elements, soluble sugars, and total soluble solids) for the New Zealand' cultivar of tomato, '*Virosa*.' grown in lower CO<sub>2</sub> environments reached climacteric respiration (a stage of fruit ripening) earlier than in the higher CO<sub>2</sub> treatment resulting in lower ethylene concentration in plants. The research of Gillig et al. (2008) indicated that basil grown at 600 ppm CO<sub>2</sub> had significantly different dry

biomass production from those exposed to a 1500 ppm CO<sub>2</sub> concentration. In their study, under an increased atmospheric CO<sub>2</sub> level, the plants proliferated and absorbed more carbon than nitrogen, but without an adequate nutrient supply, plants did not fulfill their nitrogen demand, reducing their ability to produce proteins, increasing the C:N ratio. Results of a study by Caporn (1989) showed that under elevated CO<sub>2</sub>, lettuce yield increased 37% and 51%, after 30 and 36 days, respectively, more than those under ambient conditions (**Table 1**)

**Table 1.** Effect of CO<sub>2</sub> enrichment on the growth of lettuce (*Lactuca sativa*) (Caporn, 1989)

	<b>Ambient</b>	<b>1200 ppm CO<sub>2</sub></b>
<b>Shoot fresh mass (g)</b>	9.22	13.94
<b>Plant dry mass (g)</b>	0.543	0.791
<b>Log<sub>10</sub> (shoot/root) (dry)</b>	0.756	0.885
<b>Leaf number</b>	14	16

However, their study also showed that CO<sub>2</sub> enrichment only slightly affected the fourth leaf expansion rate after day 14 when the third leaf had emerged.

#### **1.2.1.4. Modern controlled environment agriculture**

Controlled environment agriculture (CEA) is used for the production of a wide range of plant species, and the setup can be as simple as a small greenhouse. Generally, CEA can be defined as an enclosed environment to achieve optimal growth conditions for cultivating plants (Prasad et al., 2014). The CEA's benefit is flexibility and control, allowing agricultural production to occur anywhere, including extreme locations such as outer space (Giroux et al., 2006). CEA may also modify the natural growing environment by improving root growth conditions, extending the growing season through differences in light exposure, and creating opportunities for production

under circumstances that would typically not be suitable, i.e., during winter periods in temperate climatic zones (Jensen, 2001). All environmental modifications, including ambient temperature, relative humidity, light quality, quantity, and photoperiod, nutrient supply, and carbon dioxide levels, are aimed to meet the optimum for plant growth and economic return. However, CEA requires added attention to every detail of production, including the infrastructure and environmental conditions. High capital costs for CEA infrastructure are one of the disadvantages, especially energy costs associated with managing temperature, humidity, and lighting (Benke & Tomkins, 2017).

**Table 2.** Energy and CO<sub>2</sub> generation after complete combustion of sawdust wood pellets, natural gas, and propane (Dion et al., 2013)

	<b>Wood pellets (kg)</b>	<b>Natural Gas (m<sup>3</sup>)</b>	<b>Propane (L)</b>
<b>MJ per unit of fuel</b>	18.1	37.89	25.53
<b>g CO<sub>2</sub> per unit of fuel</b>	1729	1891	1510
<b>g CO<sub>2</sub>·MJ<sup>-1</sup></b>	96	50	59

In temperate zones around North America, greenhouse operators face high thermal energy requirements to maintain the temperature and CO<sub>2</sub> levels of their greenhouses. Especially in Canada, a large amount of supplemental heat is required during the cold winter season, amounting to about 10 to 35% of the total production costs (Ahamed et al., 2019). In traditional Canadian greenhouse production, to elevate the CO<sub>2</sub> concentrations in the growing areas, growers will use either pure CO<sub>2</sub> or burn sawdust wood pellets or natural gas/propane (**Table 2**). For example, in order to maintain a greenhouse at 1000 ppm of CO<sub>2</sub>, a greenhouse grower would need to supply CO<sub>2</sub> at the rate of 108 g·m<sup>-2</sup>·day<sup>-1</sup>, which requires 0.06 m<sup>3</sup>·day<sup>-1</sup>·m<sup>2</sup> of natural gas (Ahamed et al., 2019).

Compared to combusting propane or natural gas, current biomass heating systems have some disadvantages, such as low efficiency (higher emissions) and potential plant growth effects (Ahamed et al., 2019).

Hydroponic techniques are among the most widely used plant growth methods in modern CEA systems, mainly used in vegetable production. Hydroponic systems are a method of growing plants without soil or other solid media, using a nutrient solution that meets the plant's physiological requirements (Trejo-Téllez & Gómez-Merino, 2012). Several hydroponic systems are used in modern CEA production, including deep-water culture, nutrient film technique (NFT), drip irrigation, and aeroponic systems. One study comparing the quantity of lettuce grown in a hydroponic system or under soil cultivation showed a 115% increase in biomass production under the hydroponic system (Manzocco et al., 2011). Plants grown in hydroponic systems focus more on vegetative parts instead of expanding their root system because there is no big challenge to their growth requirements (Goto et al., 1996). Compared to traditional soil culture, less soil-borne diseases appear in hydroponic production systems but also require pathogen and diseased monitoring during the hydroponic production (Lee & Lee, 2015).

Moreover, growing crops continuously under soil conditions and without rotation causes soil nutrient deficiencies and soil pathogen accumulation (Lee & Lee, 2015). There are more significant water requirements with soil cultivation due to evaporation, and leaching, compared to an enclosed hydroponic system. Even though the hydroponic system has many advantages, there are a few issues, including high initial costs, daily monitoring requirements, and greater risk of instrument malfunction (Treftz & Omaye, 2016). For example, pH and nutrient content require daily monitoring to ensure appropriate ion concentrations are maintained for optimal uptake. Plants in hydroponic systems are dependent on the maintenance of nutrient solution supply and condition,



which can be adjusted through automated systems using sensors and injectors (Barbosa et al., 2015).

#### **1.2.1.5. Romaine lettuce**

Romaine lettuce (*Lactuca sativa* L.) is one of Canadians' most popular vegetables and is usually sold as whole heads. The best growth condition for romaine lettuce in a hydroponic system is at 19 to 24°C, 50 to 70% relative humidity, a pH at 5.6-6, and electrical conductivity of 1.15 to 1.25 mS·cm<sup>-1</sup> (Mathieu et al., 2006). Under traditional hydroponic production, lettuce seeds will start in a germination area such as Rockwool cubes and are shaded from the light source on the first day after germination. Then, as the leaves emerge to become seedlings, they are transported to the hydroponic system and connected to the nutrient reservoir. For different types of lettuce the harvest time is different, and most lettuce can be harvested between 30 to 70 days and depends on what it will be used for, such as heading and semi-heading lettuce (45 days), butterhead lettuce (60 to 70 days), loose-leaf lettuce (45 to 60 days) (Steve, 2020). The romaine lettuce production cycle requires 30 to 35 days, depending on the cultivars used for the short-term research period (Mathieu et al., 2006).

In greenhouses, lettuce relative growth rate (compared to a control group, g·g<sup>-1</sup>·day<sup>-1</sup>) increases 12.5% when the greenhouse CO<sub>2</sub> concentration is raised from ambient conditions of 390 ppm to 1000 ppm (Duggan-Jones & Nichols, 2014). Additional CO<sub>2</sub> was supplied to the greenhouse by using liquid CO<sub>2</sub> or burning propane.

#### **1.2.2. Gas generated from the composting process**

The process of composting involves the generation of greenhouse gases (GHG), including carbon dioxide, nitrous oxide, and methane, which contribute approximately 4% to the total global anthropogenic GHG emissions (Pipatti & Savolainen, 1996; Papageorgiou et al., 2009). During

the composting process, aerobic microorganisms are the primary decomposers releasing heat and GHG into the environment through their metabolic activities.

According to Eghball et al. (1997), carbon loss as CO<sub>2</sub> ranges between 46 to 62% of total carbon reduction during cattle manure composting. Ahn et al. (2011) reported that emission rates of CO<sub>2</sub> ranged from 150 to 600 g·kg<sup>-1</sup> of volatile dairy manure solids degraded (the VS was measured by loss on ignition according to Standard Methods, APHA, 1998). CO<sub>2</sub> emission values for different types of animal manure, based on data from Brown et al. (2008), are shown in **Table 3**.

**Table 3.** Volatile solids (VS) produced 455 kg<sup>-1</sup> animal unit day<sup>-1</sup>, likely VS lost, total gas, and CO<sub>2</sub> equivalent (Brown et al., 2008)

<b>Manure type</b>	<b>VS animal<sup>-1</sup> · day<sup>-1</sup> <sup>1</sup> (kg)</b>	<b>Likely VS destruction (%)</b>	<b>Total gas (m<sup>3</sup>·d<sup>-1</sup>)</b>	<b>CO<sub>2</sub> equivalent (kg·day<sup>-1</sup>)</b>
<b>Beef</b>	2.68	45	0.84	8.28
<b>Dairy</b>	3.91	48	1.23	12.14
<b>Swine</b>	2.18	50	0.81	8
<b>Poultry layers</b>	4.27	60	2.02	19.9
<b>Poultry broilers</b>	5.45	60	2.58	25.4

Poultry broilers manure produced the most CO<sub>2</sub> gas overall and the most CO<sub>2</sub> day<sup>-1</sup>, which was proportional to the manure's total volatile solids content. Eleazer et al. (1997) provided estimates of CO<sub>2</sub> emission values and biodegradation days for different municipal organic waste types (**Table 4**).

**Table 4.** CO<sub>2</sub> gas generation for different types of organic wastes (dry basis) incubated to simulate decomposition in a municipal solid waste landfill (Eleazer et al., 1997)

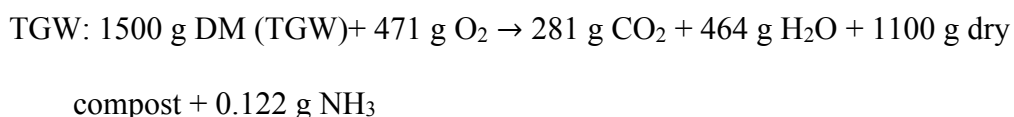
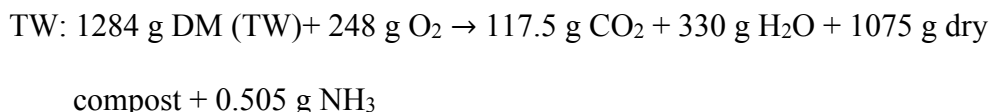
<b>Waste type</b>	<b>CO<sub>2</sub> (g·kg<sup>-1</sup> material)</b>	<b>Time (Days)</b>
<b>Grass</b>	2.37	50
<b>Leaves</b>	0.5	100
<b>Branches</b>	1.03	100
<b>Food</b>	4.94	120
<b>Coated paper</b>	1.39	150
<b>Old newsprint</b>	1.22	300
<b>Corrugated containers</b>	2.5	400
<b>Office paper</b>	3.57	500

Several factors influence the emission of CO<sub>2</sub> from composts, such as the Carbon-Nitrogen (C:N) ratio, temperature, MC, and composition of the feedstock materials.

#### **1.2.2.1. How the C:N ratio of compost material affects CO<sub>2</sub> generation**

Carbon-containing materials supply the energy required for microbial respiration and growth, while nitrogen-containing materials play a role as a protein source (Brinton & Seekings, 1988). The C:N ratio of compost feedstocks plays an important role in understanding the potential emissions of CO<sub>2</sub>. Kranert (2010) reported that green waste compost with different organic matter content (dry basis) released different amounts of CO<sub>2</sub>, with CO<sub>2</sub> emission rates of 1472, 941, and 597 kg CO<sub>2</sub> ton<sup>-1</sup> of green waste with 96%, 80%, and 60% woody material, respectively. A study by Dajana and Felicita (2017) reported that the cumulative CO<sub>2</sub> evolution per unit mass of volatile matter of composted tobacco waste (TW) and tobacco mixed with grape waste (TGW) was 94.01

g CO<sub>2</sub> kg<sup>-1</sup> (9.4%) and 208.18 g CO<sub>2</sub> kg<sup>-1</sup> (20.82%) volatile matter, respectively. The initial C:N ratio of TW and TGW was 21: 1 and 35: 1, respectively. At the end of composting, the conversion of TW and TGW was 47% and 55%, with a C:N ratio decreased to 10:1 and 23:1, respectively. The higher initial C:N ratio material resulted in higher O<sub>2</sub> consumption, and a greater C:N ratio decrease. The mass balance determined for the biodegradation of TW and TGW was found to be:



In contrast, using other feedstocks at different mixing ratios resulted in varying CO<sub>2</sub> emission rates. For example, Santos et al. (2016) compared the CO<sub>2</sub> emission rates of a mixture of the solid fraction of pig slurry (SFP) and gin waste at different initial feedstock mixing ratios. The mixture at a volume ratio of 4:3 (SFP: cotton gin lower C:N ratio) had significantly higher CO<sub>2</sub> emissions than at a ratio of 3:4 (SFP: cotton gin) (**Table 5**). However, The C:N ratios of initial cotton gin waste and solid fraction of pig slurry were 51.3:1 and 10.9:1, respectively. The higher SFP content mixture had a lower C:N ratio but more CO<sub>2</sub> released, which contradicts Kranert's (2010) research. In this case, the cotton gin waste was used as a bulking agent, which balanced the SFP's excessive moisture supporting the higher temperatures and ventilation demands while reducing gaseous emissions during the composting process.

**Table 5.** Emission rates of CO<sub>2</sub> (g·m<sup>-2</sup>·day<sup>-1</sup>) from two mixtures of SFP and cotton during the thermophilic phase of composting (Santos et al., 2016)

<b>Composting time (days)</b>	<b>SFP: Cotton (4:3)</b>	<b>SFP: Cotton (3:4)</b>
<b>0</b>	104	0
<b>6</b>	131	88
<b>15</b>	253	167
<b>32</b>	459	141
<b>41</b>	245	147
<b>56</b>	22	27
<b>Total</b>	1214	570

Even non-traditional waste streams, such as pharmaceutical wastes, can be another source of CO<sub>2</sub> during the decomposition process and under suitable environmental conditions (Majumdar et al., 2006). Their results indicated that total carbon emissions from spent mycelia (SM) and sludge from a pharmaceutical treatment facility (ETPS) mixed with cow dung (CD), at a C:N ratio of 67:1 were significantly different from one another. The SM and ETPS were collected from Alembic Pharmaceutical Ltd., India, with C:N ratios of 5:1 and 8:1. The SM and ETPS contained a higher organic carbon content, which created a more favorable living condition for microorganisms' growth. Further addition of CD with a high C:N ratio of 50:1 made the mixtures suitable for biological activity (Karak et al., 2014). Compared to the mixtures of CD with SM (1:1) and CD with ETPS (1:1), the loss relative to the initial carbon added of the former mixture had increased to 0.077%. Different mixture rates of the same material also caused other CO<sub>2</sub> emissions, with mixtures of CD with ETPS (1:1, 1:3, and 3:1), total carbon emissions increased 5.06 (1: 3)

and 2.6 times (3: 1) compared to the 1: 1 ratio.

#### **1.2.2.2. CO<sub>2</sub> emissions after application of mature compost to soil**

Agricultural activity plays an essential role in GHG's global fluxes, including CO<sub>2</sub>, which contributes 10 to 12% of total global GHG anthropogenic emissions. Organic amendments such as plant residues, animal manures, and compost provide a significant source of these gases (IPCC, 2007). Mature composts provide some OM for microbes in the soil for further decomposition resulting in CO<sub>2</sub> emissions into the atmosphere but additional soil C storage. According to Bass et al. (2016), adding organic amendments to soil elevated the soil's CO<sub>2</sub> fluxes. For instance, compared to the control group, a compost amendment (green waste, bagasse, and chicken manure) increased CO<sub>2</sub> emissions by 88% during the initial period (first four weeks) and rapidly decreased until there was no significant difference between treatments by the mid-point (next eight weeks). However, composted material applied to the soil still has a biodegradation potential that contributes to gas emissions (Moreira et al., 2008). Compost as a soil amendment also shows more potential of generating CO<sub>2</sub> gas into the atmosphere than biochar. Given the potential for residual activity in mature composts, as measured in soil amendment studies, the use of the material for the generation of CO<sub>2</sub> for indoor production systems needs investigation. To date, few studies have focused on the generation of gas from partially processed composts for use under controlled environment agricultural production.

#### **1.2.2.3. NH<sub>3</sub> emissions after application of mature compost to soil**

Ammonia gas emission from the composting process can be a primary odor problem (Hong et al. 2005). Ammonia is the by-product of anaerobic and aerobic decomposition of organic materials during the composting process (Yasuda et al., 2009). The N was mineralized to ammonia from the degradation of OM during composting process, which can be oxidized to nitrate. In the

static compost pile or anaerobic condition, the limitation of oxygen reduces the oxidized of ammonia and generates more  $\text{NH}_3$  gas (Wang & Zeng, 2018).

Under aerobic conditions,  $\text{NH}_3$  gas emissions increase sharply after the beginning of the composting process, two days to reach the peak, and then slow down for 15 days (Jiang et al., 2013). A sharp increase of ammonia and high temperatures can be detected during the early thermophilic phase (Osada et al., 2001). A higher aeration rate increases  $\text{NH}_3$  emissions, but a lower rate slowly increases the  $\text{NH}_3$  emission rate and delays the peak emission to 7-10 days (Szanto et al., 2007; Jiang et al., 2011). Other researchers have also shown strong correlations between high temperature and oxygen uptake rate with the emission peaks of  $\text{NH}_3$  (De Guardia et al., 2008; Jiang et al., 2013). The  $\text{NH}_3$  gas emissions are a lost resource and cause nuisance odors (Fukumoto et al., 2011).

### **1.3. Summary**

Plant responses to  $\text{CO}_2$  enrichment include greater nutrient uptake, morphological changes, increased photosynthetic ability, and higher biomass production. Compared to the high cost of traditional enrichment methods, elevating  $\text{CO}_2$  concentration by capturing emissions during the composting process or partially processed compost material may be an economical approach for greenhouse production during colder seasons and to circularize waste resources. However, the effects of composting gas on plant production have not been thoroughly studied. This study will focus on examining the responses of plants grown in an elevated  $\text{CO}_2$  environment using gas released from compost material, from different stages of organic matter decomposition, compared to using a conventional chemical source for  $\text{CO}_2$  enrichment.

#### **1.4. Objectives**

The objectives of this study were to:

1. Evaluate responses of romaine lettuce (*Lactuca sativa* L. cv. Parris Island) to a carbon dioxide enrichment level using mixed gas from a partially composted material versus pure CO<sub>2</sub> gas.

It is hypothesized that after filtering NH<sub>3</sub> gas generated from compost material, the mixture gas has similar plant growth promotion ability as a pure CO<sub>2</sub> elevated environment.

2. Quantify the CO<sub>2</sub> and NH<sub>3</sub> gas emissions from a partially composted material over time to determine potential impacts on plant production;

It is hypothesized that the partially composted material still has a large amount of gas generation ability to elevate and maintain CO<sub>2</sub> enrichment in a controlled environment.



## **2. Short-term quantification of CO<sub>2</sub> and NH<sub>3</sub> gas generation from composted material**

### **2.1. Introduction**

Composting is a process where the organic matter (OM) is biologically degraded, which generates waste gases, including CO<sub>2</sub>, NH<sub>3</sub>, and other gases. Some of the gases emitted during composting may be detrimental to plant growth, such as ethylene, NH<sub>3</sub>, and CH<sub>4</sub>, and may need to be managed before use in a CEA. Previous studies have shown that 10% to 46% of the initial total nitrogen (TN) in the raw material (depending on the feedstock component) is lost in the form of NH<sub>3</sub> (Jiang et al., 2013; Fagbohunbe et al., 2017), which accounts for 79 to 94% of TN loss during the whole composting period (Jiang et al., 2011). In some composting facilities, since ammonia is lighter than air (density is 58.8% of the air), the odors spread to the surrounding area. Waste gas, including CO<sub>2</sub> and NH<sub>3</sub>, are generated during the composting process and when the mature or partially processed compost products are curing in a pile. If it is not managed correctly, an immature compost pile may result in anaerobic conditions leading to different N gases being generated. According to Hue and Liu (1995), different kinds of mature commercial compost yielded from 9 to 99 mg CO<sub>2</sub> kg<sup>-1</sup>·DM·hr<sup>-1</sup> compared to the immature and raw material, which produced 648 and 1433 mg CO<sub>2</sub> kg<sup>-1</sup>·DM·hr<sup>-1</sup>, respectively.

Composting has potential value as a CO<sub>2</sub> source, and in the short-term can generate high quantities of gas during decomposition. However, other gas emissions from the composting process, such as ammonia (NH<sub>3</sub>), may damage plants. Ammonia gas is generated from the composting process from the aerobic decomposition organic matter as the by-products. The N lost from raw material in the form of NH<sub>3</sub> gas ranges from 9 to 24% of the total nitrogen in swine manure and poultry manure (El Kader et al., 2007; Jiang et al., 2013). Higher NH<sub>3</sub> accumulation in a controlled environmental agricultural system can lead to plants being negatively impacted,

displaying toxicity symptoms, due to the alkaline nature caused by ammonia dissolving in the nutrient solution (Britto & Kronzucker, 2002).

This study's objective was to evaluate the rates of NH<sub>3</sub> and CO<sub>2</sub> gas emissions from partially processed animal manure and used bedding materials.

## **2.2. Materials and methods**

Three experiments were conducted in this study to evaluate CO<sub>2</sub> and NH<sub>3</sub> emissions from a partially composted mix of animal manure and used bedding feedstocks. Experiment 1 was a controlled environment incubation experiment established to determine CO<sub>2</sub> and NH<sub>3</sub> gas emissions over 14 days from 150 g of processed compost feedstock (CF) material (46.47% MC, five replicates for each). Experiment 2 was established to determine the rate of CO<sub>2</sub> emissions using processed CF over 24 hours under the room condition and in a hydroponic system. Experiment 3 was a 24-hour incubation established to compare NH<sub>3</sub> emissions using materials taken from the raw CF material and processed CF material based on the same amount of wet mass (150 g, 46.47% MC). All the experiments were conducted at an incubation temperature of 21°C.

### **2.2.1. Composting materials**

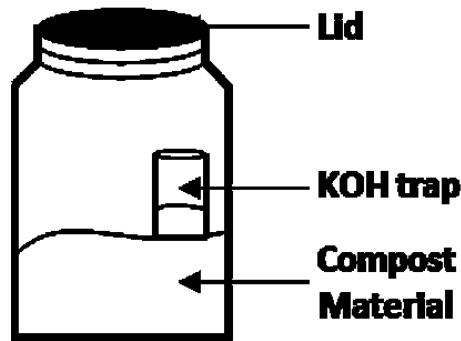
The raw and processed compost feedstocks were collected from a HotRot 1811 (Global Composting Solutions Ltd. New Zealand) in-vessel composting system housed in the Faculty of Agriculture, Dalhousie University. The HotRot 1811 is a horizontal, continuous agitation, flow-through, in-vessel composting system. The raw feedstock consisted of a mixture of grass clippings, cattle feed, poultry manure, and wood shavings. The chemical characteristics of the feedstocks used in this study are shown in **Table 6**.

**Table 6.** Chemical properties of raw mixed feedstocks and the processed material after 14 days in the HotRot 1811 (N=5).

<b>Compost feedstocks</b>	<b>Raw</b>	<b>Processed</b>
<b>MC (%)</b>	42.7	19.70
<b>Dry matter (%)</b>	57.3	80.30
<b>Total C (% Dry)</b>	41.15	27.70
<b>Total N (% Dry)</b>	3.45	2.35
<b>C:N</b>	11.93	11.79

### 2.2.2. Carbon dioxide and ammonia emissions

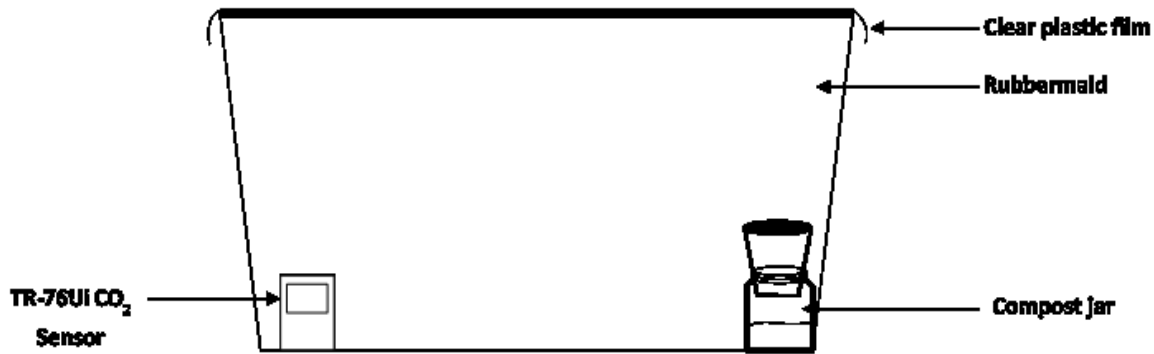
In Experiment 1, as illustrated in **Fig. 1**, each 1-liter mason jar contained a vial with 25 mL of 0.25N potassium hydroxide (KOH) as a CO<sub>2</sub> trap for carbon dioxide gas generated from the compost sample. Each mason jar was sealed with a lid to prevent ambient CO<sub>2</sub> gas from saturating the trap. There were five jars in total as replicates.



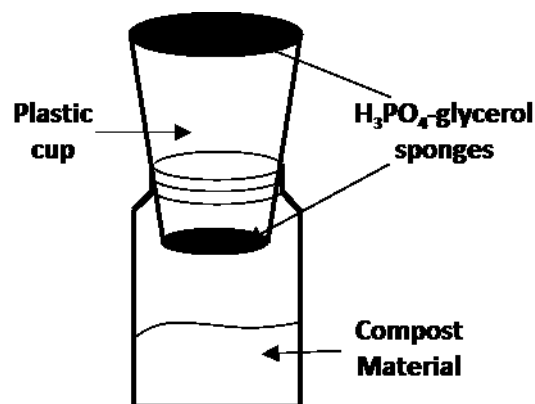
**Fig. 1.** CO<sub>2</sub> trap apparatus

The KOH traps from each experimental unit were measured for electrical conductivity using an EC meter (Economy pH/EC Meter, Spectrum Technologies, Inc.) and compared to a reference solution of 0.125N potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) to determine the CO<sub>2</sub> emission rates from the compost (Smirnova et al., 2014). The trapped KOH was collected for two hours every

day over a 14-day experiment. In Experiment 2, a CO<sub>2</sub> emission experiment was set up in a semi-sealed chamber to measure the first 24 hours of gas generation from the partially composted feedstocks (**Fig. 2**). For the room condition, there were two groups of compost material used in this experiment (same CF material as study 1, 300 g, and 400 g wet basis, 46.47% MC), and a portable CO<sub>2</sub> sensor (TR-76Ui, GENEQ Inc., Montreal, QC) was used in each chamber for continuous measurement of CO<sub>2</sub> concentrations over the 24 hour period. For the experiment conducted in a hydroponic system, 300 g wet CF (46.47% MC) was used to measure the CO<sub>2</sub> concentration changes in the growth chamber with some air exchanges. Every 100-ppm increase in CO<sub>2</sub> in the chamber represents 0.64 mg CO<sub>2</sub>-C emitted from the CF (based on internal dimensions of the chamber of 35 cm × 35 cm × 25 cm = 30,625 cm<sup>3</sup>; every 100 ppm CO<sub>2</sub> increase in the chamber required 3.06 mL CO<sub>2</sub> gas, under standard temperature and pressure, 24.4 L CO<sub>2</sub> is 1 mol and 44g CO<sub>2</sub>, so 3.06 mL equal 0.602 mg CO<sub>2</sub>).



**Fig. 2.** Setup for experiment two to measure continuous CO<sub>2</sub> emissions from compost feedstock in sealed chamber over a 24-hour period.



**Fig. 3.** NH<sub>3</sub> trap apparatus

For ammonia gas measurements, as illustrated in **Fig. 3**, each mason jar (1 liter) was covered using a two open-ended cup that had a small sponge (7.62 cm) for trapping compost NH<sub>3</sub> and a large sponge (10.16 cm) in the other end to prevent NH<sub>3</sub> from the surrounding air from reaching the small sponges. The sponges were submerged in a 25 mL H<sub>3</sub>PO<sub>4</sub>-glycerol solution (every 500 mL of the solution had 20 mL glycerol, 25 mL of concentrated phosphoric acid, 455 mL deionized water (DI)) for 24 hours before the next sampling time (Nõmmik, 1973). After 24 hours of trapping the NH<sub>3</sub>, the small sponges were extracted with 25 mL of 2 N potassium chloride KCl in sealed plastic Ziplock bags and squeezed for 2 min. The extraction of KCl solution samples was analyzed in a SEAL Autoanalyzer III (AA3) (SEAL Analytical Ltd. 7 Regis Pl, North Lynn Industrial Estate, King's Lynn PE30 2JN, United Kingdom) for NH<sub>4</sub><sup>+</sup>-N analysis. The samples were collected every 24 hours, over 14 days in total.

Experiment 3 was set up to compare the ammonia gas emissions from the raw feedstock mixture and the partially composted feedstocks. The two feedstock sample groups were tested using the same setup, as shown in **Fig. 2**, but with 150 g of material (wet weight basis), 61.8% MC for raw material and 47.46% MC for processed material. There were six replicates for each group, and the total gas trapping time was 24 hours. The ammonia gas sample was extracted by a 2M KCl solution and analyzed colorimetrically using a Bran and Luebbe AutoAnalyzer 3 (Seal Analytical,

Wisconsin, USA). Compost samples from each experiment were also collected to analyze gravimetric moisture content, total carbon, and nitrogen content at the end of the study. A LECO-CNS 2000 (LECO Corporation, St. Joseph, Ontario) was used to measure the total carbon and nitrogen content of the initial and final samples from each experiment.

### 2.3. Calculations and analytical methodology

The values of CO<sub>2</sub> in the closed environment from the partially processed compost material were calculated as follows (Wollum & Gomez, 1970):

$$F = \frac{(EC_{raw} - EC_s)}{m_{compost} \cdot \theta \cdot k_t} \cdot P$$

Where F denotes the gas CO<sub>2</sub> emission rate (CO<sub>2</sub> · kg<sup>-1</sup> DM · min<sup>-1</sup>), EC<sub>raw</sub> is the electric conductivity value of pure 0.5 N KOH (Sm<sup>-1</sup>), EC<sub>s</sub> is the electrical conductivity value of KOH trap for CO<sub>2</sub> sample (Sm<sup>-1</sup>), EC<sub>sat</sub> is the electric conductivity value of 0.25N K<sub>2</sub>CO<sub>3</sub> (Sm<sup>-1</sup>), m<sub>compost</sub> is the experimental material weight (kg), θ is the solids content (1-MC, %), k<sub>t</sub> is the total timing of the gas sampling period (mins), P is the maximum capacity of KOH trap solution can absorb CO<sub>2</sub> (1 mL 0.5N KOH absorb 11 mg CO<sub>2</sub>)

The values of NH<sub>3</sub> in the closed environment from the CF were calculated as follows:

$$F = \frac{0.025 \cdot [NH_4^+] \cdot Q_{NH_3}}{m_{compost} \cdot \theta \cdot k_t}$$

Where F denotes the gas NH<sub>3</sub> emission rate (mg NH<sub>3</sub> · kg<sup>-1</sup> DM · min<sup>-1</sup>), [NH<sub>4</sub><sup>+</sup>] is the concentration of ammonium ion in 0.025 L 2 N KCl extraction solution (mg · L<sup>-1</sup>), Q<sub>NH<sub>3</sub></sub> is the ratio of ammonia gas to ammonium ion conversion (mg NH<sub>3</sub> · mg<sup>-1</sup> NH<sub>4</sub><sup>+</sup>), m<sub>compost</sub> is the experimental material weight (kg), θ is the solids content (1-MC, %), k<sub>t</sub> is the total gas sampling period (mins).

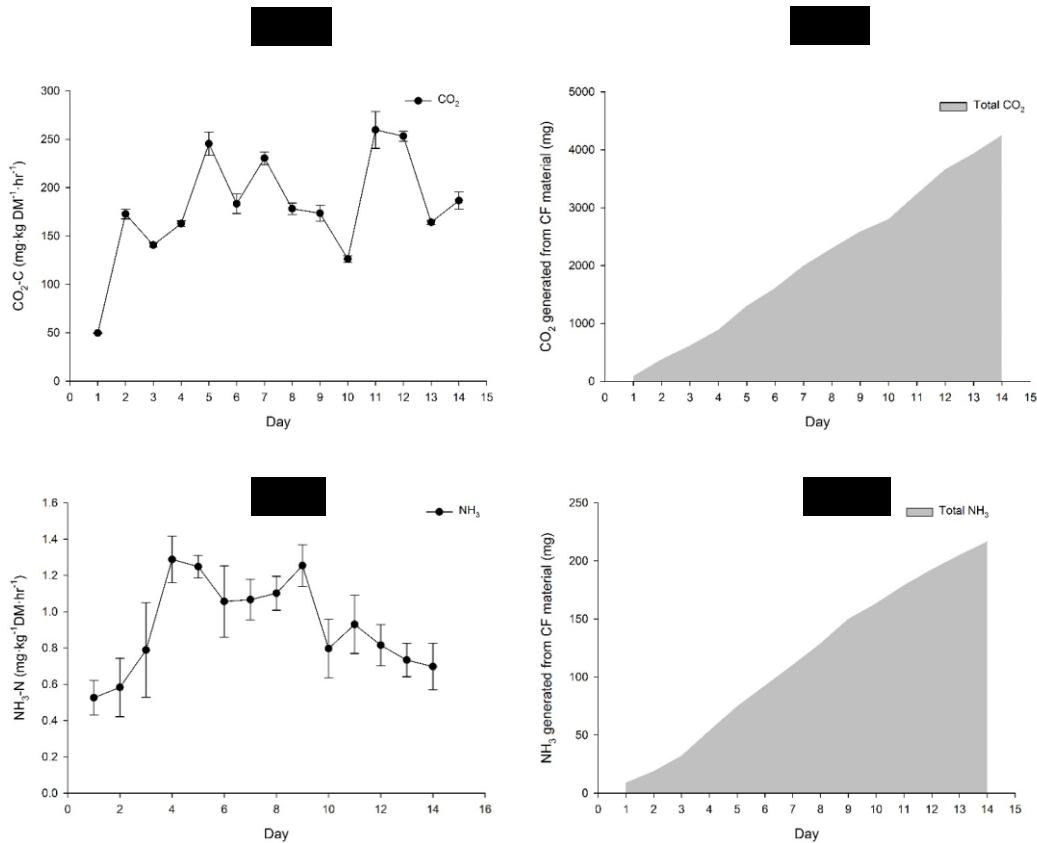
Statistical analysis was conducted using a one-way ANOVA to compare the gas volumes generated from CF (between raw material and processed material in experiment 3) using SAS

(Statistical Analysis System version 9.4, SAS Institute, Raleigh, North Carolina). Significance was based on an alpha value of 0.05. Multiple means comparison, where necessary, were conducted using Tukey's multiple means comparison test at an alpha value of 0.05.

## **2.4. Results**

### **2.4.1. Gas generated from CF material under controlled environment conditions**

The CO<sub>2</sub> gas generation rate increased rapidly after the experiments were started (**Fig. 4.a**). The maximum gas emission rates were observed on day 5 (245.37 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup>) and day 11 (259.66 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup>). The average gas emission rate was 180.48 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup>, with a total of 4.2 g (150g CF wet basis, 47.46% MC) CO<sub>2</sub>-C emitted over the 14-day period (**Fig. 4.b**). Total dry matter declined by 6.36 g, while carbon decreased by 2.25%, and the total mass of carbon lost was 3.42 g C (**Table 7**).



**Fig. 4.** CO<sub>2</sub> and NH<sub>3</sub> gas emissions from the partially composted material over a 14-day incubation period (N=5).

(a) the average of CO<sub>2</sub> generation rate per sampling point; (b) the cumulative amount of CO<sub>2</sub> generated from CF; (c) the average of NH<sub>3</sub> generation rate per sampling point; (d) the cumulative amount of NH<sub>3</sub> emission from CF.

**Table 7.** Parameters including dry matter, moisture content, total percentage and amount of carbon, total percentage and amount of nitrogen of CF material at the start and end of the 14-day CO<sub>2</sub> incubation study (N=5)

Treatment	DM (g)	MC (%)	TC (%)	TN (%)	C:N	Total C	Total N
						(g)	(g)
<b>Initial</b>	80.30	46.47	27.7	2.35	11.79	22.24	1.89
<b>Final</b>	73.94±	45.38±	25.45±	2.05±	12.41±	18.82±	1.51±
	0.74	0.55	0.57	0.08	0.56	0.44	0.06



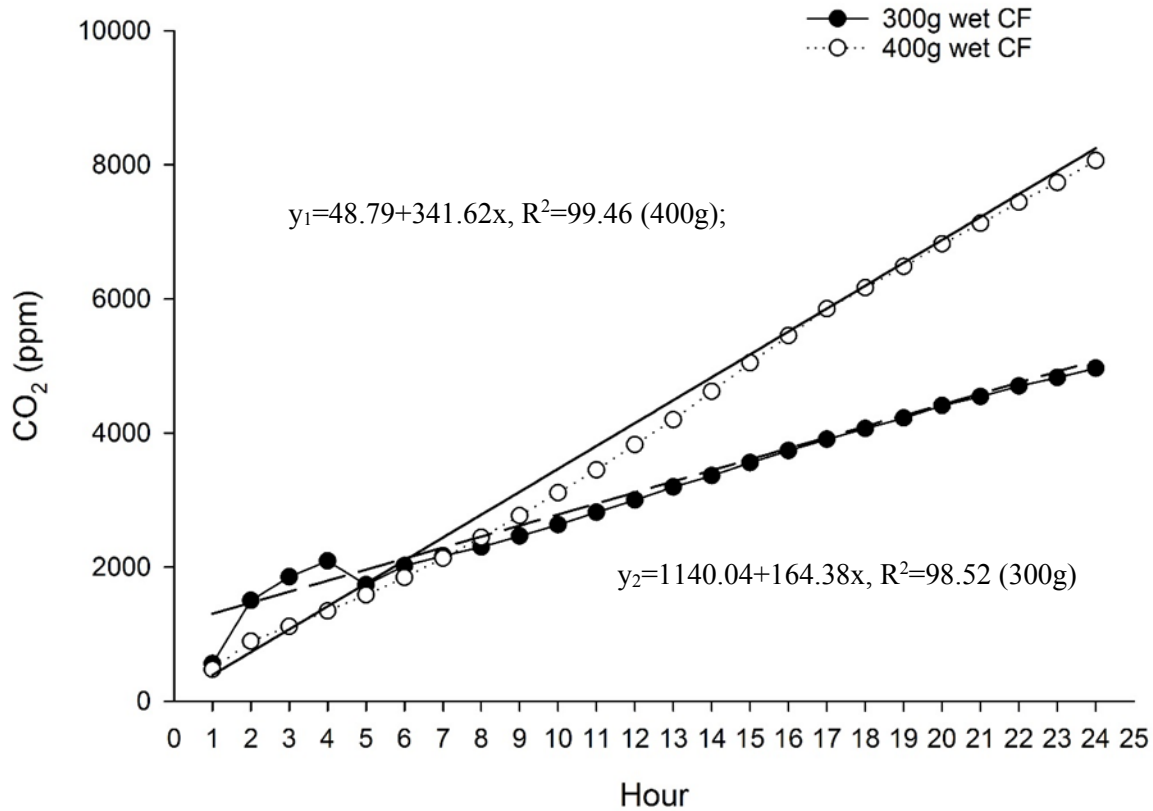
The NH<sub>3</sub> gas generation rate increased rapidly after the start of the experiment (**Fig. 4.c**). The maximum gas emission rates were observed on day 4 (1.29 mg NH<sub>3</sub>-N·kg<sup>-1</sup> DM·hr<sup>-1</sup>) and day 9 (1.25 mg NH<sub>3</sub>-N·kg<sup>-1</sup> DM·hr<sup>-1</sup>). The average gas emission rate was 0.92 mg NH<sub>3</sub>-N·kg<sup>-1</sup> DM·hr<sup>-1</sup> with a total of 216 mg (150g CF wet basis, 47.46% MC) NH<sub>3</sub>-N during the 14-day period (**Fig. 4.d**). The total dry matter declined by 7.56 g, with nitrogen decreasing by 0.26%, and the total mass of nitrogen lost was 370 mg N (**Table 8**).

**Table 8.** Parameters including dry matter, moisture content, total percentage and amount of carbon, total percentage and amount of nitrogen from CF material at the start and end of the 14-day NH<sub>3</sub> incubation study (N=5)

<b>Treatment</b>	<b>DM (g)</b>	<b>MC (%)</b>	<b>TC (%)</b>	<b>TN (%)</b>	<b>C:N</b>	<b>Total C (g)</b>	<b>Total N (g)</b>
<b>Initial</b>	80.30	46.47	27.7	2.35	11.79	22.24	1.89
	72.74±	45.14±	25.28±	2.09±	12.10±	18.39±	1.52±
<b>Final</b>	0.87	0.43	0.77	0.08	0.51	0.42	0.05

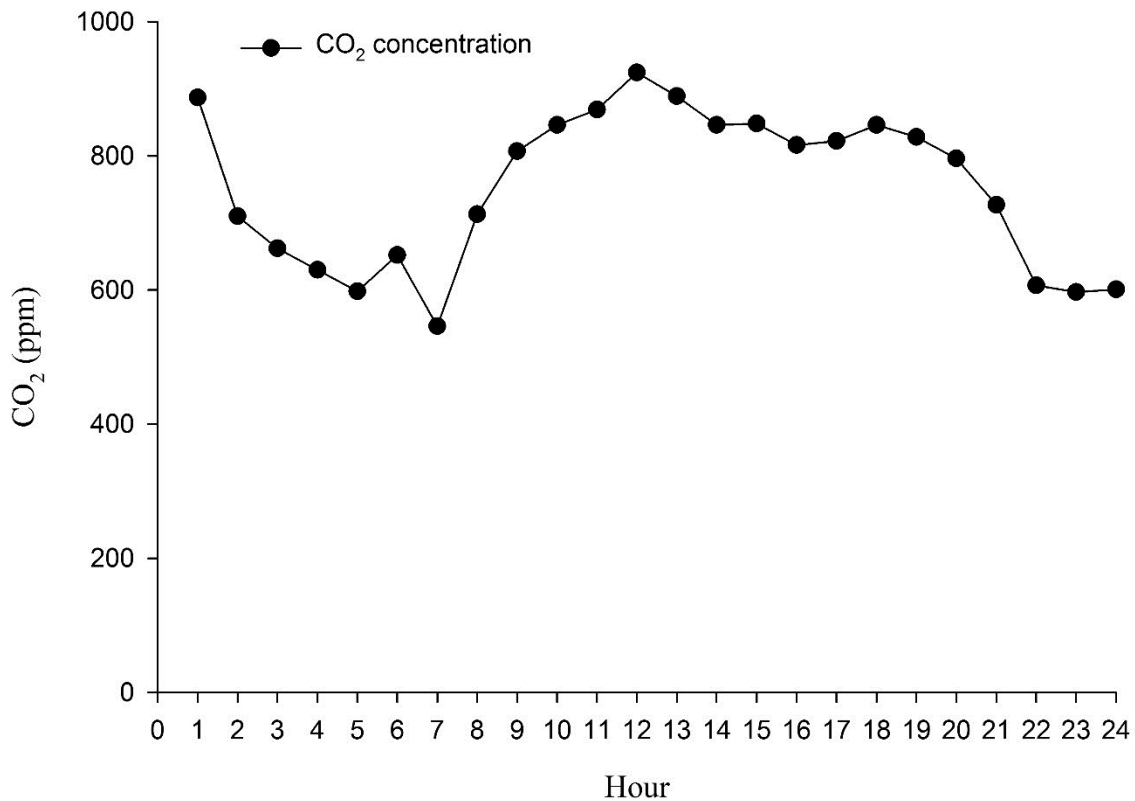
Both CO<sub>2</sub> and NH<sub>3</sub> displayed a similar pattern of change over the experiments, a rapid increase to a peak within the first several days and fluctuating for 5 to 6 days.

### 2.4.2. CO<sub>2</sub> changes in the semi-sealed growth chamber



**Fig. 5.** CO<sub>2</sub> level changes in a semi-sealed growth chamber for 24 hours at different amounts of wet CF.

A comparison of CO<sub>2</sub> emissions using two different CF quantities (wet basis 46.47% MC) in a semi-sealed growth chamber is shown in **Fig. 5**. Data from the beginning of the 300 g group was fluctuated due to the breath increased the CO<sub>2</sub> concentration inside the growth chamber. Using 300 g wet CF material, CO<sub>2</sub> levels in the chamber increased 164.38 ppm per hour compared to the 400 g CF treatment, which generated 341.62 ppm per hour under the same experimental conditions. It was estimated that every 100 ppm CO<sub>2</sub> concentration increase in the chamber required 0.602 mg CO<sub>2</sub> emissions from the CF material. Gas emissions from the 300 g treatment averaged an increase of 164.38 ppm per hour, which equaled 0.99 mg CO<sub>2</sub> and 0.27 mg CO<sub>2</sub>-C generated from the wet CF material per hour compared to 2.06 mg CO<sub>2</sub> and 0.56 mg CO<sub>2</sub>-C per hour in the 400 g treatment with 341.62 ppm.



**Fig. 6.** Hourly fluctuations in CO<sub>2</sub> concentrations from a semi-sealed growth chamber containing a hydroponic nutrient solution with an air stone over a 24-hour period

Fluctuations in CO<sub>2</sub> concentrations were also measured over a 24-hour period using the same semi-sealed growth chamber that the lettuce would be grown in and included the hydroponic nutrient solution container with an air stone (**Fig.6**). The CO<sub>2</sub> levels fluctuated between 500 to 900 ppm, around double the ambient conditions using 300 g wet compost material (46.47% MC).

#### **2.4.3. Differences in NH<sub>3</sub> gas emissions between raw and processed compost feedstock**

The raw material had higher moisture content, total carbon, and total nitrogen than processed material (116%, 48%, and 47% higher). NH<sub>3</sub> gas was generated more rapidly from raw CF than in processed CF (**Table 9**). Under the same environmental conditions, raw CF material generated NH<sub>3</sub> gas nearly two times faster than in the processed CF group.

**Table 9.** NH<sub>3</sub>-N (mg·kg<sup>-1</sup> DM·hr<sup>-1</sup>) emissions from raw compost mixture feedstock vs. processed compost feedstock over 24 hours (N=6)

Treatment	DM (g)	MC (%)	TC (%)	TN(%)	NH <sub>3</sub> -N (mg·kg <sup>-1</sup> DM·hr <sup>-1</sup> )
<b>Raw</b>	57.3±0.02	61.80±0.23	41.15±0.65	3.45±0.03	2.04 ±0.72 <sup>a</sup>
<b>Processed</b>	80.3±0.09	46.47±0.52	27.70±0.47	2.35±0.04	0.72±0.15 <sup>b</sup>

*\*Values are means (N=6) ±SD. DM, dry matter content; MC, moisture content; TC, total percentage of carbon; TN, total percentage of nitrogen;*

*\*Values with the same letter in each column are not significantly different at p < 0.05.*

## 2.5. Discussion

### 2.5.1. CO<sub>2</sub> generated from CF material

In the 14-day incubation experiment, CO<sub>2</sub> emission rates rapidly increased to a peak on day 5 by 245.37 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup> and started to decrease by day 11. This study's results were similar to decomposition results from a study by Zeng et al. (2017) with biosolids, in which the CO<sub>2</sub> gas generation rate reached a peak on day 6. Compared to other compost materials, such as dairy manure, chicken litter, and yard trimmings composts, the peak rates were slower in the CF material used in our study and biosolids (Hao et al., 2004). Several factors resulted in anaerobic decomposition conditions leading to reduced gas production rates. In the case of biosolid sewage sludge it was the low porosity, while and for CF in our study was due to less air permeability of the static pile in the jar (Hernández et al., 2006). Moreover, the total amounts of CO<sub>2</sub> evolved over the 14 days study (60 g CO<sub>2</sub>-C kg<sup>-1</sup> DM) were lower than other compost components (yard trimmings 112 g and dairy manure 85 g CO<sub>2</sub>-C kg<sup>-1</sup> DM, Zeng, et al., 2017) not only due to the static anaerobic condition, but the material used in our study was collected from the processed pile of the HotRot 1811 composting system. As a result of the static

composting condition and processed material used in the incubation experiment, the CO<sub>2</sub> emission rate and total generation amounts were less than other studies.

The average CO<sub>2</sub> emission rates from the CF material in study 1 on the first day were 83.42 mg CO<sub>2</sub>-C (150 g wet basis, 49.65 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup>) in a controlled incubation environment. Compared to the results of study 2, a semi-sealed chamber used for evaluating the CO<sub>2</sub> generated from different amounts (300 g wet CF: 1.68 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup>; 400 g wet CF: 2.61 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup>) of CF material (with the same CF material and same MC as in study 1) showed a different concentration of CO<sub>2</sub> increasing rates. The amount of CO<sub>2</sub> generated at the first 24 hours in study 1 was higher than those recorded by the CO<sub>2</sub> sensor in the semi-sealed chamber in study 2. At least 94-97 % of CO<sub>2</sub> generated from CF material was lost from the semi-sealed plastic film covered on the chamber's top or could not be recorded by the CO<sub>2</sub> sensor.

The average CO<sub>2</sub> generation rates in study 1 were 180.48 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup> and significantly higher than the recommendation of CCME (2005) that the compost maturity requirement 4 mg CO<sub>2</sub>-C·kg<sup>-1</sup> OM·day<sup>-1</sup>. As a result, the material collected from the processed pile of the HotRot 1811 composting system had immature compost characterizations.

### **2.5.2. NH<sub>3</sub> generated from CF material**

The NH<sub>3</sub> emissions from a static compost material in a mason jar from study 1 increased slowly over the first two days and needed 4 to 9 days to reach the peak emission (maximum 30.96 mg NH<sub>3</sub>-N kg<sup>-1</sup> DM·day<sup>-1</sup>). After that, the emissions of NH<sub>3</sub> decreased to a low level for the remainder of the study. Other researchers have also observed a similar emission pattern with NH<sub>3</sub> generation from pig manure compost (Jiang et al., 2011). In their study, NH<sub>3</sub> emissions at a low aeration rate slowly increased over the initial 7 days and reached the peak emission rate after

10 days, then decreased to a low level (maximum 500 mg NH<sub>3</sub>-N kg<sup>-1</sup> DM·day<sup>-1</sup>). Our study's emission rates were significantly lower than those in the fresh compost process, resulting in processed material used in study 1, which had less NH<sub>3</sub> gas generating ability (Szanto et al., 2007). The total amount of NH<sub>3</sub> gas generated from study 1 was 3.09 mg NH<sub>3</sub>-N kg<sup>-1</sup> DM for 14 days and had similar results compared to other studies of NH<sub>3</sub> gas emission from the composting process (Shen et al., 2011). In their study, three aeration rates: 0.01, 0.1, and 0.2 m<sup>3</sup>·min<sup>-1</sup>·m<sup>-3</sup> resulted in a significant difference in NH<sub>3</sub> emissions with a total 0.01, 2.34, and 4.38 NH<sub>3</sub>-N kg<sup>-1</sup> DM (NH<sub>3</sub> emission rates did not significantly increase after 14 days), respectively. The higher C:N with lower nitrogenous emissions under similar conditions can be explained by the lower ammonia content at the same dry matter levels (El Kader et al., 2007). In El Kader et al.'s study, NH<sub>3</sub> emissions from chicken manure were 167% greater than those in cattle manure resulting in C:N ratio differences of material (chicken manure: 8.4; cattle manure: 23.6).

Nearly three times the amount of NH<sub>3</sub> gas emissions were generated in the raw CF material collected from HotRot 1811 composting system than the processed CF at the first 24 hours, caused by higher carbon and nitrogen content (raw CF: 41.14% C and 3.45% N; processed CF: 27.70% C and 2.35% N) and more stable material in processed CF material (Szanto et al., 2007). The total N lost by NH<sub>3</sub>-N emissions in the 14-day incubation experiment accounted for 11% of initial total nitrogen. Compared to the other decomposition processes, the NH<sub>3</sub> loss rate can be affected by the C:N ratio, MC, porosity, and rate of turning, which fluctuated from 9-13% for cattle manure and 10 to 24% for turkey manure (El Kader et al., 2007). More nitrogen lost from initial composting material is also indicated in the mixture of pig manure with corn stalk by 20 to 39% in the form of NH<sub>3</sub> (Jiang et al., 2013).

## 2.6. Conclusion

The experimental feedstock collected from the discharge of the HotRot 1811 composting system had immature compost characteristics such as high CO<sub>2</sub> and NH<sub>3</sub> gas emission rates compared to the mature compost standards. A high gas generation appeared one day after the study started, and the respiration and chemical reaction rates showed significantly higher after day 2 and 3 than the first day. The material used in this study was collected from a processed CF pile, which showed more stable decomposition activity and gas emission than raw material. The characterization of CF material used in our study is more likely to pass the thermophilic period of four composting process stages. Other gas may be generated from compost material, especially in a static pile, such as nitrous oxide (N<sub>2</sub>O) gas emission, which can be solved by increasing the aeration rates and replacing the new material in a controlled environment. Lower amounts of NH<sub>3</sub> emissions and the potential long-term and stable CO<sub>2</sub> generating of the processed CF materials, demonstrate the capacity to use organic material that is undergoing composting processes as a CO<sub>2</sub> gas source in a controlled growth chamber.

### **3. Evaluating romaine lettuce (*Lactuca sativa* L. cv. Parris Island) production under carbon dioxide enrichment by using composting and conventional gas sources**

#### **3.1. Introduction**

Lettuce (*Lactuca sativa* L.) is one of the most common salad vegetables, known to be rich in phytochemicals such as vitamins, carotenoids, and other antioxidants (Nicolle et al., 2004). The lettuce production in the U.S. was 8,087 million pounds with a \$1.9 billion farm-gate value in 2015 (USDA, 2016). The farm-gate value of lettuce production in Canada in 2018 was \$82.9 million, representing 3.45% of total fruit and vegetable revenues (Statistics Canada, 2018). As a result of the growing demand for food and increasing awareness of the importance of vegetables in people's diets, farmers have demonstrated increasing interest in enhancing vegetables' quality and productivity. In order to achieve more significant plant growth promotion, it is not only essential to increase the nutrient supply, but also to optimize the growing environment. An elevated CO<sub>2</sub> environment substantially increases the photosynthetic rate in plants such as lettuce, improving plant growth and productivity and altering plant morphological development (Lake et al., 2017). Elevated CO<sub>2</sub> also alleviates the loss of production caused by environmental stress, resulting in improved tolerance mechanisms (Pérez-López et al., 2012). Burning propane has been used to increase the CO<sub>2</sub> levels in a greenhouse, but is costly and consumes a non-renewable resource, which is the second-largest cost for greenhouse production after the labor cost and takes 70 to 80% of the total energy cost (Sanford, 2005). In controlled environments, lettuce shows significantly higher carbohydrate accumulation under 1000 ppm and 5000 ppm than ambient conditions of 400 ppm (McKeehen et al., 1996). During composting, organic waste material is aerobically degraded by microorganisms, generating CO<sub>2</sub> that releasing it into the surrounding environment. Compost amendments to soil provide valuable benefits for nutrient recycling, enhancing soil OM, and



improving soil structural properties. On the other hand, few studies have examined gas emissions from the composting process to elevate CO<sub>2</sub> levels for plant production in the greenhouse. In a preliminary study conducted (data not shown), when lettuce was grown under a controlled environment with CO<sub>2</sub> gas supplied directly from static compost piles, plants displayed stunted growth and low development, with symptoms of ammonium toxicity (brown leaf, stunt or die).

This study's objective was to evaluate the response of Romaine lettuce (cv. Parris Island) under carbon dioxide enrichment using a mixture of composted manure and used bedding compared to a pure CO<sub>2</sub> gas source in a hydroponic growing system.

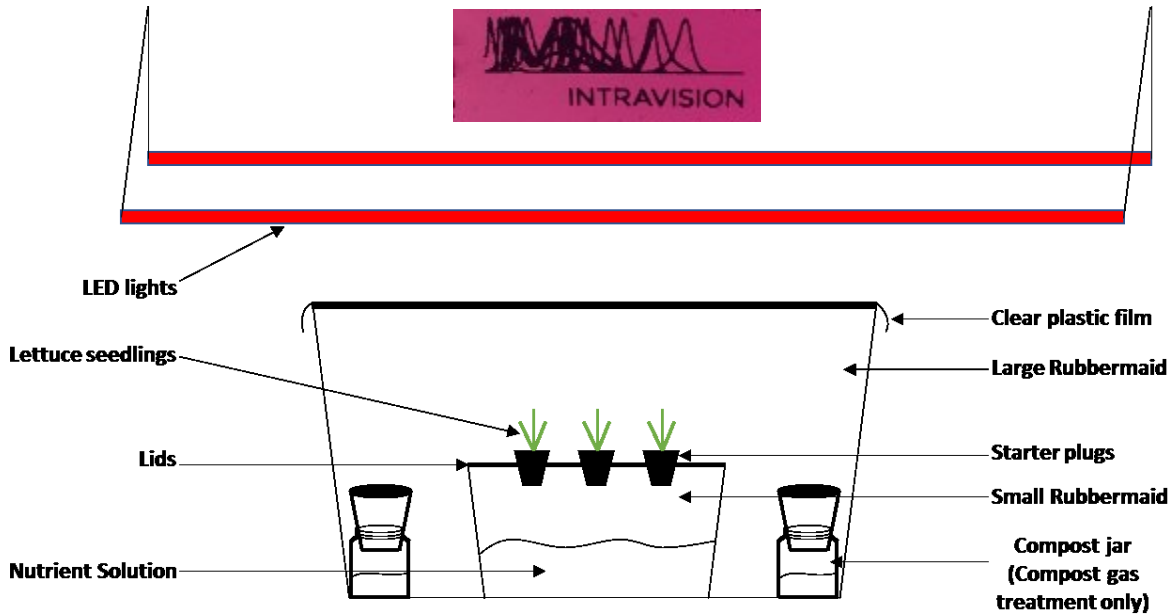
### **3.2. Materials and methods**

Three-time replicated studies were conducted to compare the lettuce growth under different CO<sub>2</sub> enrichment conditions. All three studies were established in a completely randomized design with three treatments (pure CO<sub>2</sub>, CO<sub>2</sub> from compost gas, and ambient CO<sub>2</sub> as the control group) with four replications. Each treatment had four chambers, which contained three plants, a total of 3 (treatment)×4 (replicates) ×3 (plants)=36 lettuce plants in each study period. Pure gas used in this study was supplied from a pure CO<sub>2</sub> gas cylinder (Air Liquide, ≤99.9% compressed CO<sub>2</sub>). All studies were conducted for 31 days, and lettuce biomass was dried under 65 °C for five days before analyzed.

#### **3.2.1. Hydroponic plant production system design**

A deep-water culture hydroponic system was established for these studies (**Fig. 7**) to grow Romaine lettuce (*Lactuca sativa* L. cv. Parris Island) in the Faculty of Agriculture, Dalhousie University. The hydroponic plant production system consisted of a large clear polypropylene container (35×35×25=30625 cm<sup>3</sup>), which served as a growing environment. A smaller Rubbermaid™ polyethylene container (12 liters) was placed inside the larger one, which contained

the nutrient reservoir and plants. The small container held 11 L of nutrient solution. The nutrient solution was a mixture of 4 mL concentrated nutrient solution into 11 L DI water (5-0-2 and 1-5-8 N-P-K with 120 mg·L<sup>-1</sup> N with 5.6% nitrate and 0.6% ammoniacal nutrients, Nutri+ NUTRIENT GROW A&B solution) (Azis et al., 2020).



**Fig. 7.** The hydroponic system set up for lettuce growth

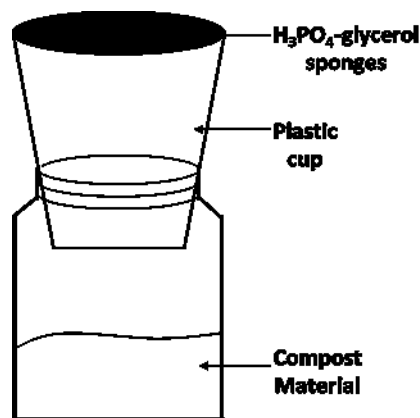
Two red-blue-white LED lamp lighting systems (Cruus® Model number 21GP66 , provided 64.22  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  lighting intensity) with six 8 ft long lamps were hung at 45 cm over the top of reservoirs, perpendicular to the containers, providing constant lighting over 24 hours. The lettuce under 24 hours with 400  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  had significantly biomass increased (38.35% higher in lettuce aboveground as fresh basis) than 16 hours 600  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  when the total light input was kept the same (Shen et al., 2014). The more extended photoperiod promoted lettuce biomass accumulation, and by using continuous light radiation on lettuce, the higher yield was achieved with fewer lamps required (Koontz & Prince, 1986; Kitaya et al., 1998; Kang et al., 2013).

An electrical conductivity and pH meter (Economy pH/EC Meter, Spectrum Technologies,

Inc.) were used to monitor the nutrient solution in the reservoir in order to maintain a pH between 5.5 and 6.5 (Domingues et al., 2012). The fresh nutrient solution was added when the EC or pH was out of the optimal range, with the optimum range of EC for lettuce being 1.2 to 1.8  $\text{mS}\cdot\text{cm}^{-1}$  (Singh & Bruce, 2016). The initial pH and EC of the nutrient solution was 5.88 and 1.28  $\text{mS}\cdot\text{cm}^{-1}$ , respectively. The room temperature was maintained at 19 to 21 °C during the whole study period.

### 3.2.2. Composting material

The compost feedstock (CF) was collected from the discharge end of a HotRot 1811 composting system (Global Composting Solutions Ltd. New Zealand) housed in the Faculty of Agriculture, Dalhousie University. The HotRot 1811 is a horizontal, continuous agitation, flow-through, in-vessel composting system. Based on previous experiments, it was determined that 300g of compost feedstock would generate sufficient  $\text{CO}_2$  gas to elevate the volume of headspace in the large plastic growing container. Two jars of compost feedstock were used in each growth chamber as the  $\text{CO}_2$  gas source for the composting treatment in this study. Each jar contained 150 g wet-processed compost feedstock obtained from the HotRot discharge line (100 g CF with 50 g DI water) in each jar (Fig. 8).



**Fig. 8.** Compost jar in compost gas treatment of hydroponic system

The jar was covered with a sponge that had been submerged in an  $\text{H}_3\text{PO}_4$ -glycerol solution (20 mL glycerol, 25 mL of concentrated phosphoric acid, 455 mL DI water) for 24 hours to absorb

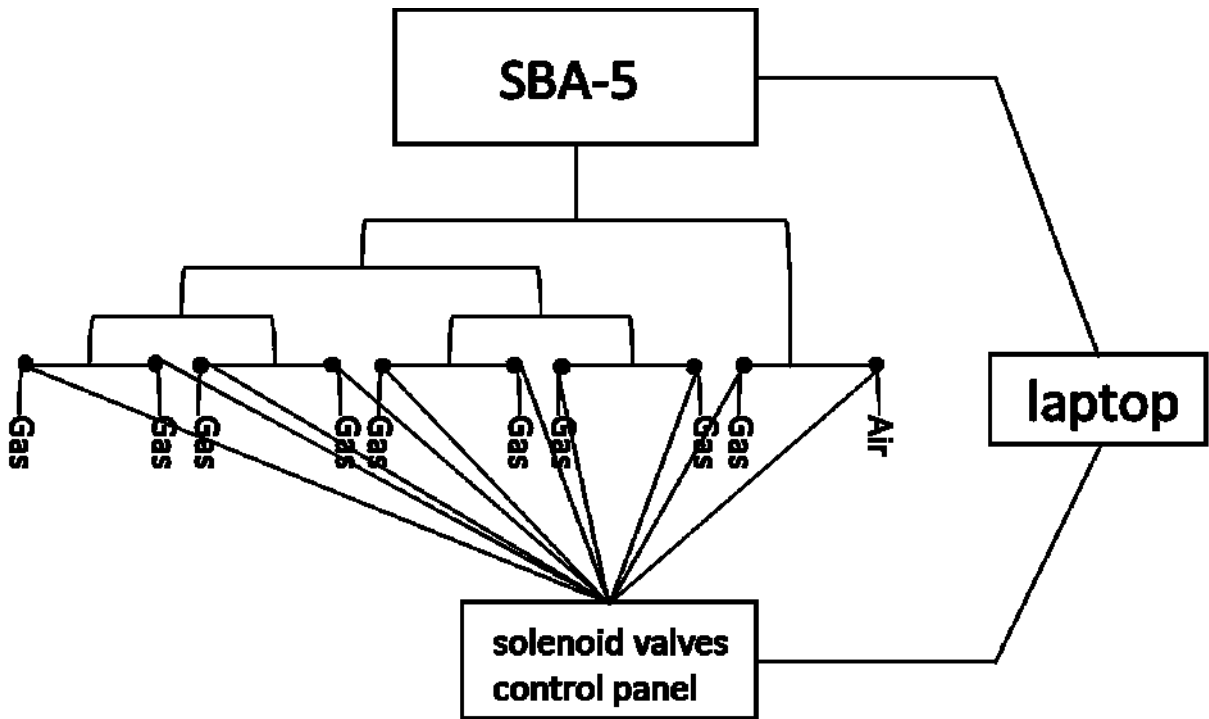
any ammonia gas generated from CF (Nõmmik, 1973). Compost material in each chamber was changed every 15 days, and each jar contained 150 g of compost material (wet basis with 46.47% MC) processed through the HotRot 1811 composting system. Each compost jar generated 4.2 g CO<sub>2</sub>-C over the 14 days (based on the previous study, 180.48 mg CO<sub>2</sub>-C·kg<sup>-1</sup> DM·hr<sup>-1</sup> emission rate).

### **3.2.3. Plant material**

Romaine lettuce seeds (*Lactuca sativa* L. cv Romaine) were pre-germinated in mineral wool cubes (3×3 cm with a density of 0.015 g·m<sup>-3</sup>) at room temperature and in the dark. The cut cubes were pre-soaked in DI water before depositing each seed inside the cube, then placed in a tray for seed germination at room temperature. Once the seeds germinated in the cubes, they were placed in the hydroponic system, and each cube was put into the holes located on the lids of the nutrient reservoir tanks. A variety of plant measurements were taken from this hydroponic system at the end of the study. Plant measurements included dry mass, root to shoot ratio, leaf area, and plant nutrient content. In each experimental reservoir, the nutrient content, light density, and CO<sub>2</sub> concentration were measured and recorded during the study period. After harvesting, plant root and shoot tissue were dried in an oven at 65 °C for three days and ground to test for total carbon and nitrogen analysis in the LECO CNS-2000 (LECO Corp. St. Joseph, MI), in the Innovative Waste Management Laboratory, Dalhousie University.

### **3.2.4. Automated CO<sub>2</sub> monitoring system**

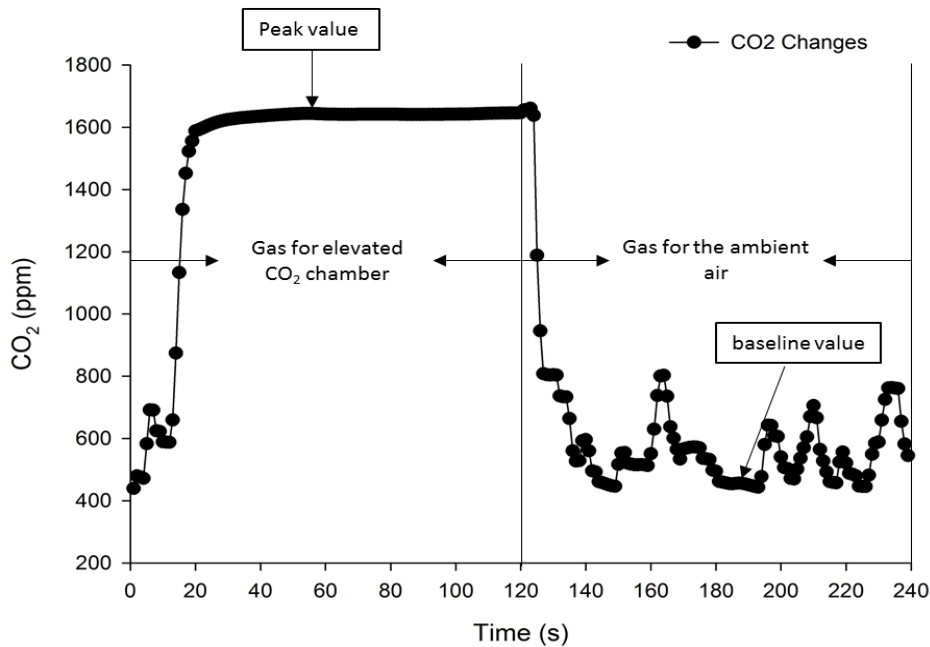
An automated CO<sub>2</sub> gas sensor system was established for continuous monitoring of gas evolved over the study period **Fig 9**.



**Fig. 9.** Automatically CO<sub>2</sub> gas sampling and analyzing setup

Every growth chamber was connected to a solenoid-controlled open/close valve (AOMAG, 2 Way normally closed electric solenoid air valve) through a plastic tube (3.5 mm inner diameter) and controlled through an electrical relay attached to a small Raspberry Pi (Raspberry Pi Foundation, 37 Hills Road, Cambridge, CB2 1NT.). An SBA-5 CO<sub>2</sub> analyzer (PP Systems Inc. 110 Haverhill Rd, 301, Amesbury, MA 01913, United States) with an attached pump was used to monitor gas within each chamber. The gas tube length from the growth chamber to the solenoid valves (9') and the CO<sub>2</sub> analyzer (20") were all equal. The solenoid valves opened sequentially for 120 s to draw the gas sample to the SBA-5 sensor and closed before collecting the next sample. There were 120 s between each sample from different chambers to clean the gas line with fresh ambient air drawn into the system. The SBA-5 analyzer was run continuously throughout the study and recorded CO<sub>2</sub> data every second. A PC based laptop was used to record the data from the SBA-5 analyzer and to control the electrical relay sequence for the solenoid valve. In order to avoid the

contamination error from gas remaining in the gas line, the peak value (from elevated CO<sub>2</sub> chamber) and the baseline value (from ambient air) within 120s were used as the marked data (Fig. 10.).



**Fig. 10.** Examples of CO<sub>2</sub> data collected for 240 s from elevated CO<sub>2</sub> chamber and an ambient condition

### 3.3. Analytical methodology

A one-way ANOVA analyzed the relationship of CO<sub>2</sub> source, wet and dry plant biomass yield, plant total C and N uptake (logarithmic transformation were applied to the values of percentage of C and N), and nutrient content including NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> of the nutrient solution collected at the end of the study in SAS (Statistical Analysis System version 9.4, SAS Institute, Raleigh, North Carolina). Significance was based on an alpha value of 0.05. Multiple means comparison, where necessary, were conducted using Tukey's multiple means comparison test at an alpha value of 0.05.

### 3.4. Results

#### 3.4.1. Biomass production and moisture content

**Table 10** shows the biomass production and moisture content of harvested lettuce at the end of the study. In study 1, plants were grown under the control group (average of 460 ppm CO<sub>2</sub>) and lettuce leaf biomass production was 71 g fresh weight per plant after 31 days. Under pure CO<sub>2</sub> gas enrichment conditions, biomass production was 46% higher (104 g fresh weight per plant). In plants grown under elevated CO<sub>2</sub> conditions using compost material as the gas source, most lettuce seedlings died within the first three days after germination. The plants' death was determined to be due to a high concentration of NH<sub>3</sub> gas emitted from the composting material. The ammonia gas emissions were confirmed from the incubation study results, with 3.21 mg NH<sub>3</sub>-N generated from each compost treatment.

In study 2, plants were in a freight container being retrofitted into a vertical farming system, with some limited ventilation fans installed, as a result of variable air exchange in the freight container the ambient control treatment was exposed to an average of 736 ppm CO<sub>2</sub> over the study period. Lettuce leaf biomass production after 31 days in the ambient control treatment was 34 g fresh weight per plant, while lettuce grown under elevated CO<sub>2</sub> from the compost material treatment was 74% greater (59 g fresh weight per plant). Lettuce seedlings under high CO<sub>2</sub> conditions from a pure gas source grew poorly, and many did not survive. It was determined that concentrations of CO<sub>2</sub> in the growth chambers were >10,000 ppm due to poor air circulation in the new environment and a malfunctioning flow regulator into the growth chambers. Higher CO<sub>2</sub> and moisture conditions in the pure gas chambers also led to algae's rapid growth and stem rot on the seedlings.

In study 3, plants were grown again in the retrofitted freight container but with newly

installed fans to improve the fresh air exchange and the ambient control treatments were exposed to an average of 342 ppm CO<sub>2</sub> over the study period. Lettuce leaf biomass production after 31 days in the ambient control treatment was 38 g fresh weight per plant.

In all three studies, the leaf to root ratio did not show any significant differences when CO<sub>2</sub> concentrations in the growing environments changed. The weights of leaves and roots, including wet and dry biomass, were significantly higher under enriched CO<sub>2</sub> conditions than those under the control group. The source of CO<sub>2</sub>, i.e., compost or pure gas from a tank, to elevate the gas environments was not significantly different with respect to lettuce biomass production.



**Table 10.** Growth parameters of lettuces under different CO<sub>2</sub> concentrations and various gas sources (from compost emissions or pure CO<sub>2</sub> cylinder)

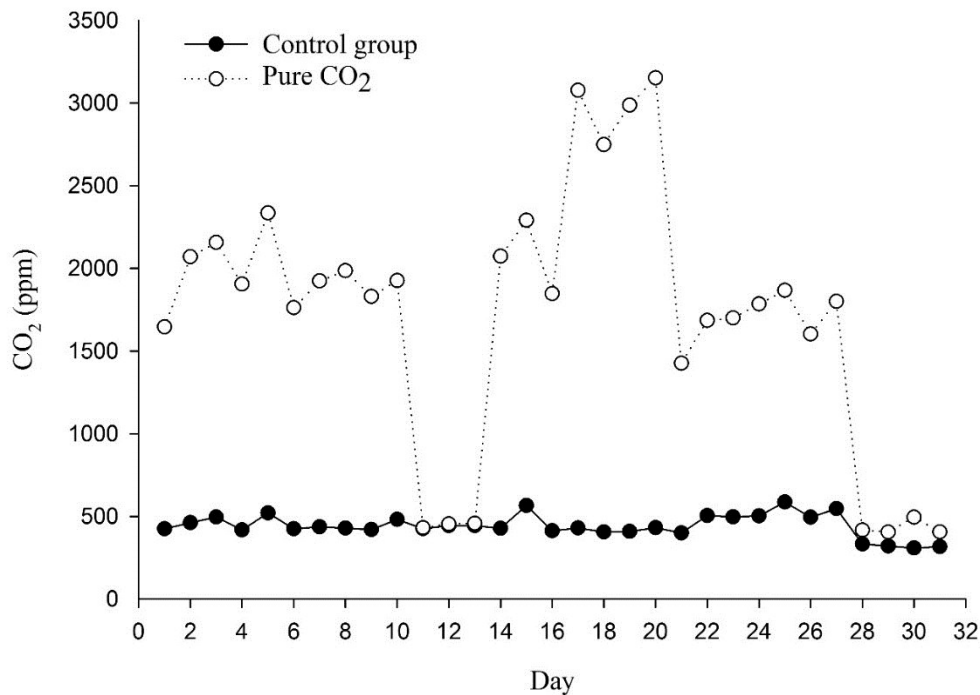
Study	Treatment (ppm)	Leaves (Fresh, g)	Leaves (Dry, g)	Leaves (MC)	Roots (Fresh, g)	Roots (Dry, g)	Roots (MC)	Leaf: root (Fresh)
Study 1	A (460)	70.68±24.98 <sup>b</sup>	3.04±0.95 <sup>b</sup>	0.96±0.01 (ns)	8.92±3.68 <sup>b</sup>	0.32±0.16 <sup>b</sup>	0.96±0.01 (ns)	9.28±5.82 (ns)
	C	NA	NA	NA	NA	NA	NA	NA
Study 2	A (736)	103.51±28.02 <sup>a</sup>	4.28±0.99 <sup>a</sup>	0.96±0.01 (ns)	15.73±5.25 <sup>a</sup>	0.48±0.16 <sup>a</sup>	0.97±0.01 (ns)	7.33±3.49 (ns)
	C (1085)	33.86±18.55 <sup>b</sup>	1.84±1.09 <sup>b</sup>	94.63±0.48 (ns)	3.47±1.77 <sup>b</sup>	0.23±0.21 <sup>b</sup>	93.77±2.60 (ns)	11.25±7.89 (ns)
Study 3	A (342)	58.97±17.16 <sup>a</sup>	3.05±0.78 <sup>a</sup>	94.73±0.54 (ns)	8.93±4.09 <sup>a</sup>	0.48±0.24 <sup>a</sup>	94.85±0.49 (ns)	7.77±3.61 (ns)
	P (746)	NA	NA	NA	NA	NA	NA	NA
Study 4	A (342)	37.85 ±9.28 <sup>c</sup>	2.01±0.57 <sup>c</sup>	94.52±1.58 <sup>b</sup>	4.32±2.72 <sup>c</sup>	0.20±0.11 <sup>b</sup>	95.30±0.82 <sup>b</sup>	10.79±2.34 (ns)
	C (754)	106.57±27.77 <sup>a</sup>	4.05±0.99 <sup>a</sup>	96.09±0.97 <sup>a</sup>	10.86±3.00 <sup>a</sup>	0.36±0.10 <sup>a</sup>	96.63±0.39 <sup>a</sup>	10.38±3.56 (ns)
Study 5	A (342)	88.76±10.34 <sup>b</sup>	3.50±0.69 <sup>a</sup>	96.07±0.51 <sup>a</sup>	7.70±2.64 <sup>b</sup>	0.29±0.10 <sup>a</sup>	96.22±0.68 <sup>a</sup>	12.66±4.07 (ns)
	P (746)	NA	NA	NA	NA	NA	NA	NA

\*Values are means (N=4) ±SD. A, ambient condition as control; C, compost gas condition; P, pure CO<sub>2</sub> condition. NA, not available; ns, not significant;

\*Values with the same letter in each column are not significantly different at  $p < 0.05$ . ns: not significant.

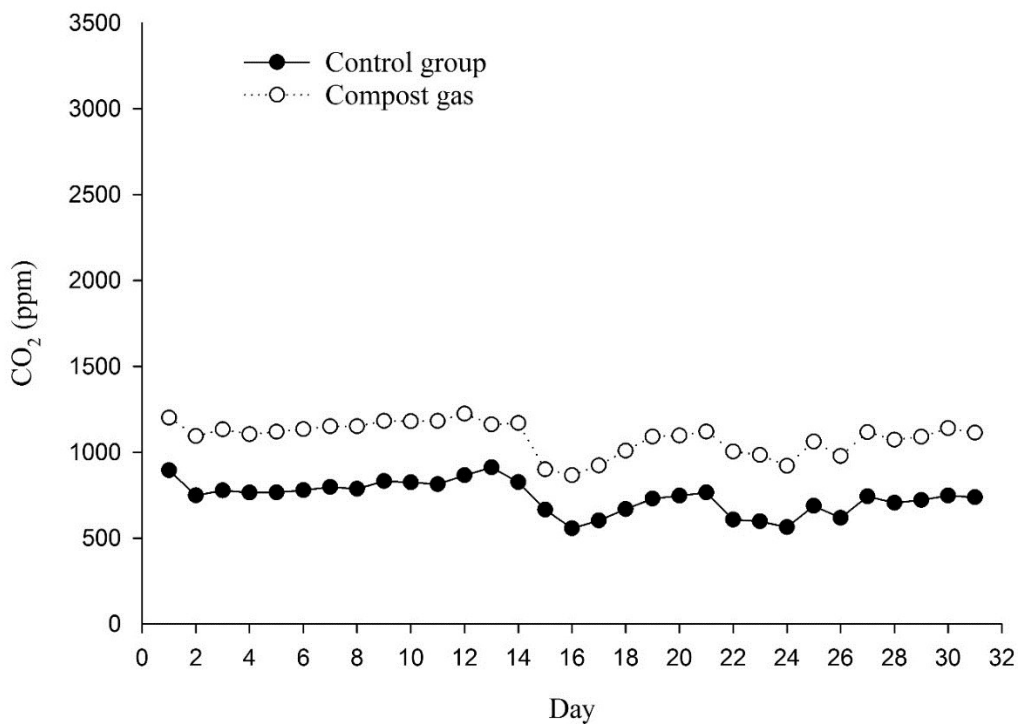
### 3.4.2. Growth environment

Over the three studies, the average CO<sub>2</sub> concentrations in control groups were 460, 736, and 346 ppm, mainly affected by differing issues associated with the growing spaces. In study 1, a classroom was retrofitted to serve as the growing environment and was serviced with a modern HVAC (heating, ventilation, and air conditioning) system. This was one of the early attempts to set up the full growing system with LED lighting and a CO<sub>2</sub> gas supply. The replacement of pure CO<sub>2</sub> tank caused the CO<sub>2</sub> concentration dropped to ambient level from day 11 to 13. As a result, lettuce grown under unfiltered compost gas died in the early stages after germination from NH<sub>3</sub> accumulation. Pure gas treatments had approximately three times higher CO<sub>2</sub> concentrations (1760 ppm) than the growing room's control group (Fig. 11).



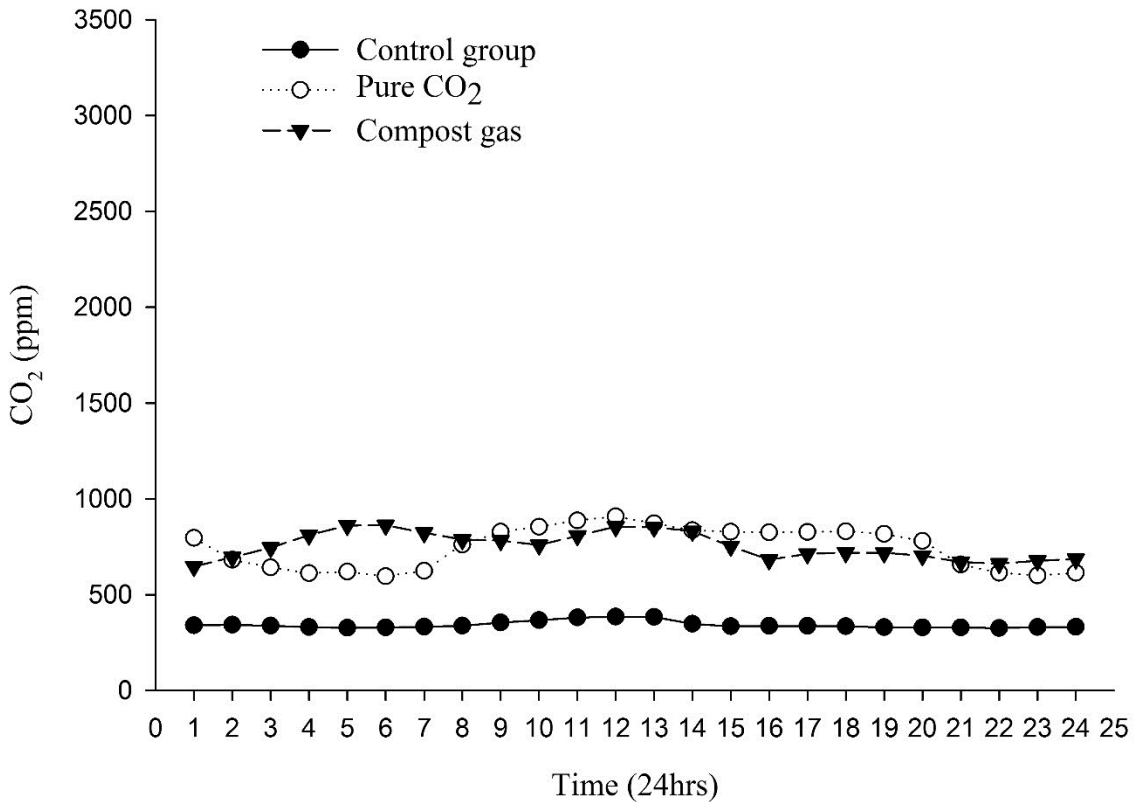
**Fig. 11.** Daily fluctuations in CO<sub>2</sub> concentration over 31 days in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure CO<sub>2</sub> gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 1.

In study 2, the experimental set up was shifted to a freight container being retrofitted into a vertical farming system. The growing chambers were installed into the new controlled environment, which was temperature-controlled but lacked adequate ventilation. Moreover, issues with the gas regulator and flow control valves from the pure gas tanks led to high CO<sub>2</sub> concentrations in the growing chambers for this treatment. Poor ventilation and accumulation of CO<sub>2</sub> in the chambers promoted algae growth and fungal rot. The replacement of compost jars caused CO<sub>2</sub> concentration dropped at day 15 and then increased. The average CO<sub>2</sub> concentration in the growing chamber was 1085 ppm from the compost treatment, 47% higher than the control group (Fig. 12).



**Fig. 12.** Daily fluctuations in CO<sub>2</sub> concentration over 31 days in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure CO<sub>2</sub> gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 2

In study 3, the retrofitted freight container was modified to include additional ventilation, and a filter was used in compost treatments to remove  $\text{NH}_3$  during the decomposition of compost material. Both compost gas and pure gas treatments had better control than study 1; fewer fluctuations in gas delivery over the study period, which was maintained approximately two times greater than the control group (754 ppm in compost gas treatments, 746 ppm in pure gas treatments)(Fig. 13). Resulting in the pump of SBA-5  $\text{CO}_2$  sensor broken, the  $\text{CO}_2$  value could not be recorded continuously for 31 days.



**Fig. 13.** Hourly fluctuations in  $\text{CO}_2$  concentration over 24 hours in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure  $\text{CO}_2$  gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 3

### **3.4.3. Total carbon and nitrogen uptake in lettuce**

There were no significant differences in percentage C (%) of harvested lettuce across treatments in any studies (**Table 11**). In contrast, the TC of lettuce under elevated CO<sub>2</sub> environments was higher than the control group. The elevated CO<sub>2</sub> concentrations significantly increased the TC accumulation in both elevated CO<sub>2</sub> environment, compost gas (140% in study 2; 95% in study 3) and pure gas (44% in study 2; 73% in study 3) exposed lettuce compared to those in the control group.

**Table 11.** Total carbon and nitrogen content of lettuce leaf (dry basis) under different CO<sub>2</sub> concentrations and various gas sources (from compost emissions or pure CO<sub>2</sub> cylinder)

Study	Treatment (ppm)	C (%)	Log <sub>10</sub> (C%)	TC (g)	N (%)	Log <sub>10</sub> (N%)	TN (g)	C:N
Study 1	A (460)	37.36±1.31 (ns)	1.57±0.01 (ns)	1.14±0.37 <sup>b</sup>	6.36±0.37 (ns)	0.8±0.03 <sup>a</sup>	0.195±0.07 (ns)	5.89±0.36 <sup>b</sup>
	C	NA	NA	NA	NA	NA	NA	NA
	P (1760)	38.14±1.63 (ns)	1.58±0.02 (ns)	1.64±0.41 <sup>a</sup>	4.78±0.73 (ns)	0.67±0.07 <sup>b</sup>	0.20±0.04 (ns)	8.17±1.36 <sup>a</sup>
Study 2	A (736)	39.40±3.20 (ns)	1.59±0.04 (ns)	0.58±0.34 <sup>b</sup>	5.00±0.50 (ns)	0.70±0.04 <sup>a</sup>	0.07±0.04 <sup>b</sup>	7.94±0.85 <sup>b</sup>
	C (1085)	41.59±4.94 (ns)	1.62±0.05 (ns)	1.39±0.30 <sup>a</sup>	4.19±0.75 (ns)	0.62±0.07 <sup>b</sup>	0.14±0.04 <sup>a</sup>	10.18±1.96 <sup>a</sup>
	P	NA	NA	NA	NA	NA	NA	NA
Study 3	A (342)	36.81±1.46 (ns)	1.57±0.02 (ns)	0.74±0.23 <sup>b</sup>	5.50±0.35 (ns)	0.74±0.03 <sup>a</sup>	0.07±0.02 <sup>b</sup>	6.72±0.53 <sup>b</sup>
	C (754)	35.46±1.89 (ns)	1.55±0.02 (ns)	1.44±0.36 <sup>a</sup>	4.40±0.51 (ns)	0.64±0.05 <sup>b</sup>	0.18±0.04 <sup>a</sup>	8.18±1.13 <sup>a</sup>
	P (746)	36.49±1.57 (ns)	1.56±0.02 (ns)	1.28±0.26 <sup>a</sup>	4.12±0.56 (ns)	0.61±0.06 <sup>b</sup>	0.15±0.04 <sup>a</sup>	9.06±1.66 <sup>a</sup>

\*Values are means (N=4) ±SD. A, ambient condition as a control; C, compost gas condition; P, pure CO<sub>2</sub> treatment. TC, the total amount of carbon per plant; TN, the total amount of nitrogen per plant; NA, not available; ns, not significant;

\*Values with the same letter in each column are not significantly different at  $p < 0.05$ . ns: not significant.

The percent total N of lettuce grown under elevated CO<sub>2</sub> environments was significantly lower than those grown in the control group. The percent total N of lettuce exposed to pure gas exposed lettuce was 25% lower in both studies 1 and 3, while the percent total N of lettuce exposed to compost gas was 16.2% lower in study 1 and 20% lower in study 3. The TN of lettuce in the group exposed to compost gas was significantly higher (100% in study 2 and 114% in study 3)

than the control group but not in pure gas exposed lettuce. In all three studies, the lettuce C:N ratio under high CO<sub>2</sub> conditions was lower than those in the control group.

### 3.4.4. Nutrient solution conditions at the end of the study

Nitrogen concentration, pH, and EC of the nutrient solution, collected at the end of each study, showed significant differences between the CO<sub>2</sub> enriched treatments and the control group (Table 12).

**Table 12.** The concentration of nitrogen, pH, and EC of the nutrient solution under different CO<sub>2</sub> gas conditions in growth chambers at the end of 31 days.

Study	Treatment (ppm)	pH	EC (mS·cm <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mg·L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg·L <sup>-1</sup> )
Study 1	A (460)	4.36±0.10 <sup>b</sup>	1.34±0.18 <sup>(ns)</sup>	3.43±1.52 <sup>(ns)</sup>	284.4±56.6 <sup>(ns)</sup>
	C	NA	NA	NA	NA
	P (1760)	5.58±0.22 <sup>a</sup>	0.97±0.26	2.24±1.48 <sup>(ns)</sup>	288.6±55.1
Study 2	A (736)	4.11±0.14 <sup>b</sup>	1.06±0.35 <sup>(ns)</sup>	1.84±1.57 <sup>(ns)</sup>	217.7±46.1 <sup>(ns)</sup>
	C (1085)	5.38±0.43 <sup>a</sup>	0.97±0.05 <sup>(ns)</sup>	2.11±1.67 <sup>(ns)</sup>	252.6±48.5 <sup>(ns)</sup>
	P	NA	NA	NA	NA
Study 3	A (342)	4.11±0.17 <sup>b</sup>	1.26±0.07 <sup>a</sup>	1.13±1.90 <sup>(ns)</sup>	168.5±100.2 <sup>(ns)</sup>
	C (754)	6.01±0.28 <sup>a</sup>	0.98±0.07 <sup>b</sup>	0.16±0.05 <sup>(ns)</sup>	133.6±56.7 <sup>(ns)</sup>
	P (746)	5.87±0.53 <sup>a</sup>	0.99±0.14 <sup>b</sup>	0.23±0.20 <sup>(ns)</sup>	121.8±23.1 <sup>(ns)</sup>

\*Values are means (N=4) ±SD. A, ambient condition as a control; C, compost gas condition; P, pure CO<sub>2</sub> condition. NA, not available; ns, not significant;

\*Values with the same letter in each column are not significantly different at  $p < 0.05$ . ns: not significant.

The pH of the nutrient solution collected from pure gas treatments was 28% and 43% higher than those in the control group in studies 1 and 3. The initial pH and EC values of the nutrient solution at the beginning were 5.72 and 1.67 mS·cm<sup>-1</sup>. The pH values from the control group decreased in all three treatments than the initial nutrient solution. However, in elevated CO<sub>2</sub> treatments, including compost and pure gas resources, pH did not significantly change during the

whole study period. The pH of the nutrient solution collected from compost gas treatments were 31% and 46% higher than those in the control group in study 2 and 3. In all three studies, the EC of each high CO<sub>2</sub> treatment was lower than those in the control group. The nitrogen content (both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) of the nutrient solution collected from higher CO<sub>2</sub> treatments was lower than the control group. The nitrogen concentrations remaining in the solution were negatively correlated to lettuce biomass yield.

### **3.5. Discussion**

#### **3.5.1. Effects of elevated CO<sub>2</sub> concentrations on lettuce production**

In all three studies, both leaf and root biomass production were higher in the elevated CO<sub>2</sub> treatments, including compost and pure gas (**Table 10**). This study had similar trends to an enriched CO<sub>2</sub> environment study conducted by Pérez-López et al. (2013). Their study examined lettuce (cv. Paris Batavia (PB); cv. Oak Leaf (OL)) under elevated CO<sub>2</sub> at different light intensities and salt stress grown in plastic pots (containing a mixture of perlite and vermiculite). Under a high CO<sub>2</sub> environment (700 ppm), the production of both lettuce cultivars, PB, and OL increased by 55% and 77% of total fresh biomass compared to those grown under the control group (400 ppm). Under similar gas conditions to study 3, treatments under elevated CO<sub>2</sub> conditions in this study resulted in higher biomass production (182% in compost gas at 754 ppm and 134% in pure gas at 746 ppm) relative to plants grown in the control group (342 ppm). Based on results obtained in study 3, the fresh weight per plant in the control group was not much different from Pérez-López et al.'s (2013) study, while lettuce production in a high carbon dioxide environment was significantly greater than in their study.

In study 1, the unfiltered gas from compost material had a toxic effect on lettuce seedlings resulting in death or reduced growth. Zandvakili et al. (2019) reported that compost as a slow-



release organic fertilizer could mineralize N for plant use but at early stages of growth might be lost through  $\text{NH}_3$  volatilization and cause ammonia toxicity. Previous studies have shown that  $\text{H}_3\text{PO}_4$  effectively removed ammonia gas released from the litter in broiler houses (Reece et al., 1979). In studies 2 and 3, a phosphoric acid ( $\text{H}_3\text{PO}_4$ ) trap was used as a filter to trap ammonia gas from the compost material and had a significant effect on removing ammonia before reaching the plants. It contributed to making the compost mixture gas less toxic but provided the benefits of elevated  $\text{CO}_2$  in the chambers for increased lettuce production. As observed in study 1 relative to studies 2 and 3, the filter traps containing  $\text{H}_3\text{PO}_4$  mixed with glycerol used to remove  $\text{NH}_3$  gas from the compost generator jars had tangible effects on lettuce production.

Controlling the gas flow and delivery to the growing chambers was found to be exceedingly important in our pure gas treatments over all three studies. Lettuce grown using a pure  $\text{CO}_2$  gas source in study 2 had very high concentrations, leading to algal growth and plant stunting. Other studies have shown that green algae, such as *Chlorella* spp., have positive effects by generating plant-promoting substances (Ordog, 1999; Schwarz & Gross, 2004). *Chlorella* spp. is very sensitive to the  $\text{CO}_2$  changes and very common to be used for carbon dioxide removing results of the high photosynthetic efficiency to convert  $\text{CO}_2$  to  $\text{O}_2$  (Singh & Singh, 2014). However, too much algae accumulation within a limited space, especially in a controlled environment and hydroponic system, may cause nutrient competition, oxygen depletion, and pH swings that might inhibit plant growth (Radin et al., 2009; Caixeta et al., 2010). In study 3, ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) was used as a disinfectant and sterilizing agent, especially in elevated  $\text{CO}_2$  treatments to prevent algae growth. Adjusting gas flow regulation supply and ensuring more air circulation in the retrofitted freight container vertical farming system provided a better opportunity to regulate  $\text{CO}_2$  delivery to the growth chambers and maintain the control group  $\text{CO}_2$  concentrations closer to outdoor

conditions.

### **3.5.2. Effects of elevated CO<sub>2</sub> concentrations on lettuce**

Elevated CO<sub>2</sub> promotes CO<sub>2</sub> fixation and increases the synthesis of 3-phosphate in leaves, which is then transformed into carbohydrates (Dong et al., 2018). In Baslam et al.'s study (2012), a lettuce cultivar, cv. Batavia Rubia Munguia, did not show significant changes in the percentage of carbon in the plant under CO<sub>2</sub> enrichment conditions (710 ppm) relative to the control group (395 ppm). In study 3, lettuce's total dry biomass increased 10 to 22% relative to the control group, resulting in significant changes in total carbon accumulation per plant even though the percent total carbon was not significantly different.

Elevated CO<sub>2</sub> concentrations appeared to decrease total N uptake in the lettuce shoots and leaves, resulting in a narrowing of the C:N ratio. The decrease in nitrogen uptake under high CO<sub>2</sub> conditions can be explained in various ways: the abundance of carbon supply results in the accumulation of carbohydrates, which directly increases the leaf biomass, thus dilutes and decreases the concentrations of other components, including nitrogen or proteins (Stitt, 1999). Under high CO<sub>2</sub> conditions, nitrogen use efficiency increases by using more N components to invest in both resource acquisition and defense due to the reallocation of proteins (Cavagnaro et al., 2011). Many researchers have also reported that lettuce biomass production increases under high CO<sub>2</sub> conditions but with lower protein content. Under elevated CO<sub>2</sub> compared to the control group, Giri et al. (2016) found no significant effects on the carbon accumulation in lettuce shoots but a reduction of 30% for nitrogen and sulfur and a 20% decrease in copper and zinc. Elevated CO<sub>2</sub> effects on nutrient concentrations can differ between lettuce cultivars: cv. Blonde of Paris Batavia showed more significant nitrate accumulation but cv. Oak Leaf, which had similar nitrate accumulation in the control group, decreased under high CO<sub>2</sub> conditions (Pérez-López et al.,

2015). The accumulation of carbon also differs between cultivars under elevated CO<sub>2</sub> conditions. For instance, cv. Maravilla de Verano has shown significantly increased carbon (13%) at 700 ppm, while Batavia Rubia Munguia showed no changes in the same environment (Baslam et al., 2012). The red-blue-white LED lamps used in this study only can provide 64.22  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ , which was much lower than the lettuce recommendation light intensity of 250  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  (Brechner, 1996). Plants grown in low light conditions generate less ATP and NADPH for carbon fixation and become the limitation for lettuce grown under an elevated CO<sub>2</sub> environment (Dong et al., 2018).

### **3.5.3. Effects of elevated CO<sub>2</sub> concentrations on hydroponic nutrient solution**

A hydroponic system's nutrient solution is critically important to ensure adequate growth and supply of nutrients, including micro-and macro-nutrients, over the plant growth cycle. Nutrients have various physiological functions within the plants, where a deficiency or toxicity can result in lower plant growth (Domingues et al., 2012). The nutrient solution's electrical conductivity provides an indication of the conditions for the root absorption of plant-available nutrients. A decline in the nutrient solution EC decreased proportionally with the total amount of nutrients available for plant absorption. In hydroponic systems, the EC of the nutrient solution when growing lettuce, red spinach, and Pak Choy have been shown to decrease over time (Siregar et al., 2017). In all three studies, the EC of the nutrient solution collected after harvest showed a difference between elevated CO<sub>2</sub> and the control group. The EC was 22% lower in elevated CO<sub>2</sub> treatments, including pure gas and compost gas treatment, than those in the control group.

The nutrient solution pH can be impacted by the cation and anion uptake of lettuce roots: increasing NH<sub>4</sub><sup>+</sup> uptake by the roots results in electrochemical compensation enhancing the release of protons, which results in a lower pH. In contrast, higher NO<sub>3</sub><sup>-</sup> uptake results in more proton influx or anion extrusion that increases pH (Imas et al., 1997; Savvas et al., 2006). In many studies,

researchers have shown that  $\text{NO}_3^-$  as the primary source of nitrogen solution and lettuce roots typically absorb more anions than cations, and the solution pH increases (Hershey, 1992; Savvas et al., 2006). The initial nutrient solution in all three studies contained 5.6% nitrate and 0.4% ammoniacal N ( $\text{NO}_3^-$  14:1  $\text{NH}_4^+$ ), which indicated that the  $\text{NO}_3^-$  became the primary N resource for plant growth. Compared to the similar results of Savvas et al. 's research, at the end of the studies, with the  $\text{NO}_3^-$  absorbed by the roots, the nutrient solution's pH increased while the plant biomass accumulated.

The correlation of nitrogen uses from the nutrient solution relative to lettuce's yield can also be used to measure N use efficiency. In the elevated  $\text{CO}_2$  treatments, there was a greater correlation between N consumption in the nutrient solution and final lettuce biomass yields (Fig.5.4). For lettuce grown in the control group, the average consumption of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  per milligram in the nutrient solution increased lettuce biomass by 4.21 and 0.15 grams, respectively. In contrast, under elevated  $\text{CO}_2$  environments, the average consumption of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  per milligram in the nutrient solution increased plant biomass by 28.73 and 0.3 gram, respectively in pure gas and 21.64 and 0.19 gram, respectively, in compost gas treatments.

#### **3.5.4. Effects of compost generated gas on lettuce growth**

Microbes play an essential role in the decomposition process, converting organic matter into plant-available inorganic nutrient forms over time (Bi et al., 2020). The accumulation of  $\text{NH}_3$  gas in a controlled environment results in toxic damage to plants (Zandvakili et al., 2019). For N volatilization, like  $\text{NH}_3$ , can be significant during the composting process and the decomposition of organic matter. Based on the incubation experimental results, 99.58 mg  $\text{NH}_3$  gas was generated from the compost treatments (based on a  $0.92 \text{ mg NH}_3\text{-N}\cdot\text{kg}^{-1} \text{ DM}\cdot\text{hr}^{-1}$  emission rate). Many researchers have shown that  $\text{NH}_3$  can spread across membranes into plant cells, resulting in cell

respiration inhibition of metabolic reactions (Coskun et al., 2013; Esteban et al., 2016; Silva et al., 2020). The toxic symptoms of lettuce impacted by high  $\text{NH}_3$  exposure are lower seed germination rate, rotting of the roots, stunted growth, and wilting in the plants (Santamaria, 2006; Hoque et al., 2007).

The usage of  $\text{H}_3\text{PO}_4$  in this study was effective in removing ammonia gas generated from the composting process. The lettuce grown under the compost treatments showed increased biomass yield in studies 2 and 3 with the  $\text{H}_3\text{PO}_4$ -gas filter, but growth was stunted in study 1 without the filter. The lettuce harvested from the compost gas elevated  $\text{CO}_2$  treatments showed no significant differences than those in pure gas treated lettuce, including similar biomass production, C:N ratio, and pH & EC of nutrient solution collected at the end of studies.

Some research has indicated that other gas by-products generated from compost material rather than  $\text{NH}_3$ , such as methane ( $\text{CH}_4$ ), may increase leaf surface ozone and result in harmful chlorosis or yellowing (Yamulki, 2006; Salgotra & Zargar, 2020). Compared with the results of pure gas elevated treatments, few differences were observed between filtered compost gas and pure  $\text{CO}_2$  conditions to promote lettuce growth. In this study, other gases generated from compost material that may have negatively affected plant development were not evident.

### **3.6. Conclusions**

Under elevated  $\text{CO}_2$  conditions, lettuce biomass production was higher than that in the control group. Elevated  $\text{CO}_2$  enhanced the total amount of carbon accumulation for both root and shoot but reduced the nitrogen percent in the plant. Lettuce (cv. Parris Island) used in this study had similar responses to the high  $\text{CO}_2$  environment as other cultivars, such as cv. Batavia Rubia Munguia, including no leaf percent carbon changes, decreased total N in the leaf and increased total biomass accumulation. Using unfiltered gas from decomposing compost material elevated

CO<sub>2</sub> conditions and caused damage to plant growth due to high concentrations of NH<sub>3</sub> gas generation. H<sub>3</sub>PO<sub>4</sub> filters made significant contributions to removing NH<sub>3</sub> gas while not affecting CO<sub>2</sub> gas emissions from the decomposing compost materials to promote lettuce growth. Lettuce under elevated CO<sub>2</sub> conditions, enhanced by H<sub>3</sub>PO<sub>4</sub>-filtered compost gas, was not significantly different from those under pure CO<sub>2</sub> treated conditions. As a result of this study, it is evident that CO<sub>2</sub> gas generated from compost material has the potential usage in greenhouse or controlled environment agriculture to promote crop growth by increasing yield and reducing cost through an elevated carbon dioxide atmosphere. A requirement to filter out other gases, such as ammonia, was also determined from these experiments. The amounts and types of other gases will need further investigation and will be related to the types of feedstocks being composted. In this study, the use of poultry manure as part of the processed animal bedding compost will have been a significant contributor to the ammonia emissions measured.

#### **4. Future research**

The compost material used in both the incubation and the hydroponic studies was collected from animal bedding and poultry litter processed through a HotRot 1811 composting system over 16 days. The effects of using gas directly from the HotRot 1811 composting system in a controlled hydroponic growing system has not been studied. Using material generated from this composting system, a mixed gas was generated with components, such as ammonia, that required removal prior to introduction into a plant growth environment. Further investigation of the types and quantities of gases generated from composting different feedstocks will be required to prevent potential toxic damage to plant development. Different compost material types need to be studied in future research to determine gas emission rates, total volumes generated over time, and other gas components. Compost gas effects on different plant species under controlled environment conditions also needs to be studied in the future, including the micro- and macro-nutrient requirements under elevated CO<sub>2</sub> conditions.

The compost source CO<sub>2</sub> generation has the potential usage on the urban vertical controlled environment agriculture system. After filtering the toxic gas such as ammonia from the compost material, the mixture gas may be used to reduce or replace the usage of traditional CO<sub>2</sub> enrichment methods (burning carbon-based fuels such as nature gas, propane, or directly from the CO<sub>2</sub> tanks). There will also be more advantages by using composting generated gas in CEA near urban areas. Composting is one of the most common organic waste management methods to control and treat the source-separated organics generated from urban areas. The recycling and re-use of CO<sub>2</sub> and heat emitted from the composting process in controlled environment agriculture reduces the negative environmental impacts such as methane emissions from landfills. Another important area of study will be on the additional light supply

requirements for lettuce growth under elevated CO<sub>2</sub> conditions to achieve the optimum production yields and quality.



## 5. Overall conclusion

Elevated CO<sub>2</sub> promoted romaine lettuce (cv. Parris Island) growth, similar to other lettuce cultivars under similar conditions. Direct use of compost material as the CO<sub>2</sub> gas source caused ammonia damage to the lettuce seedlings in our study and led to death of the plants. Additional testing confirmed these results and demonstrates the need to understand the other by-products from the composting process. Phosphoric acid was used to significantly reduce the NH<sub>3</sub> gas generated from CF material and allow CO<sub>2</sub> gas to emit and accumulate in the controlled environment. Positive effects on lettuce biomass production resulted from using the compost gas as a CO<sub>2</sub> supply into the semi-sealed hydroponic system. Lettuce biomass doubled, relative to the control group of lettuce grown under ambient conditions, in all the CO<sub>2</sub> enrichment environment treatments in our study.

More nutrient absorption into lettuce under the high CO<sub>2</sub> conditions was inferred from changes in solution pH. The incubation study results suggest that nitrogen may have not only been lost as NH<sub>3</sub> but also potentially as N<sub>2</sub>O.

On a commercial scale, gas generation of processed compost material from larger composting systems, such as the HotRot 1811 composting system, could enhance the CO<sub>2</sub> levels in larger controlled environment agriculture facilities. Additional research to quantify daily gas generation and approaches to integrate the two types of systems is still required. According to the incubation results, one metric ton of dry compost material would be able to generate enough CO<sub>2</sub> to elevate the concentrations in a 600 m<sup>2</sup> or 2400 m<sup>3</sup> greenhouse up to 1000 ppm CO<sub>2</sub>. Similar CO<sub>2</sub> enrichment in a greenhouse requires 40.32 to 48.96 m<sup>3</sup> natural gas and 40.32 to 48.96 L propane every day.

In conclusion, the filtered gas generated from the processed CF has potential uses on

elevating the CEA CO<sub>2</sub> levels to promote plant growth. Composting integrated into CEA systems provide a potential opportunity to process organic residuals generated from the production process and return value back to the operations. In the future, scaling delivery of CO<sub>2</sub>, and possibly heat, into CEA systems will demonstrate the true potential for circularization of urban economies.

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