EVALUATION OF ROMAINE LETTUCE (LACTUCA SATIVA L. CV. PARRIS ISLAND) PRODUCTION UNDER AN ELEVATED CARBON DIOXIDE (CO2) GAS ENVIRONMENT GENERATED FROM COMPOST MATERIALS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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_2) and ammonia (NH_3). The CO_2 emissions from the composting process can be used to supplement CO_2 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_2 and NH_3 emissions in a series of incubation experiments. The results indicated that the compost material had potential usage as a CO_2 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_2 gas enrichment in a hydroponic system. The mixed gas from compost material, after NH_3 filtering had similar effects on lettuce growth as a pure CO_2 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_2H_5OH	Ethanol
CD	Cow Dung
CEA	Controlled Environment Agriculture
CF	Compost Feedstock
CH_4	Methane
CO_2	Carbon Dioxide
DI	Deionized
DM	Dry Matter
EC	Electrical Conductivity
ETPS	Pharmaceutical Treatment Facility
GHG	Greenhouse Gases
H_3PO_4	Phosphoric Acid
HVAC	Heating, Ventilation, And Air Conditioning
IPCC	Intergovernmental Panel on Climate Change
IWM	Innovative Waste Management
K_2CO_3	Potassium Carbonate
KCl	Potassium Chloride
KOH	Potassium Hydroxide
MC	Moisture Content
N	Nitrogen
N_2O	Nitrous Oxide
NFT	Nutrient Film Technique
NH_3	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_2), ammonia (NH_3), and methane (CH_4) 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_2-C·kg^{-1} OM·hr^{-1} (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_2 (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_2 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_2 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_2 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_2 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_2 enrichment in a 1000 m^2 glass greenhouse will use 2.8 to 3.4 m^3 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_2 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_2 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_2 enrichment of the production space.

Elevated CO_2 can positively alter plant morphological development, such as leaf area development, tiller production, and shoot to root ratios (Seneweera, 2011). For example, enriched CO_2 environments increase plants' resistance to environmental stress by modifing 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_2 (Gifford et al., 2000).

Many greenhouse growers elevate CO_2 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_2 (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_2 concentration from 395 to 550 ppm (Sreeharsha et al., 2015).

1.2.1.1. Morphological changes at elevated CO_2

Atmospheric CO_2 enters intercellular air spaces through stomatal pores that regulate the partial pressure of CO_2 in those spaces. However, when ambient CO_2 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_2 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_2 conditions (Gutiérrez et al., 2009). For example, production of wheat (*Triticum aestivum* L.) under high nitrogen supplies but at different CO_2 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_2 in two wheat cultivars, Hartog and Birch. In Masle's research, wheat plants grown under 900 ppm CO_2 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_2, 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_2 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_2

The organic substance, ribulose 1,5-bisphosphate (RuBP), catalyzes two reactions in the cells: the fixation of CO_2 into photosynthetic metabolism; and the production of 2-phosphoglycolate in the photorespiratory pathway (Terashima et al., 2011). For short-term CO_2 enrichment, the ratio of these two reactions influences the partial pressure of CO_2 to O_2 (Dahal & Vanlerberghe, 2018). According to Rubisco kinetics, with increased ambient CO_2 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_2 doubled. Another study focusing on elevated root-zone CO_2 concentrations also showed a significant increase in photosynthetic CO_2 assimilation and stomatal conductance under proper light strength (He et al., 2007). Most of plants such as lettuce, potatoes, tobacco, and others use C3 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 C4 photosynthesis, which is different as C3 produces a four-carbon intermediate compound and splits into CO_2 and three-carbon compound during Calvin Cycle (Gowik & Westhoff, 2011). The benefits of C4 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_2

Increases in photosynthesis and Rubisco activity with elevated CO_2 levels enhances carbohydrate accumulation and influences CO_2 transport, which increases plant biomass (Ehlers et al., 2015). Under a doubling of CO_2 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_2 enriched environment (Giri et al., 2016; Wei et al., 2018). Lettuce, under a CO_2 concentration of 1000 ppm, gained over 70% greater head mass than those under a 200 ppm CO_2 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_2. For example, at different CO_2 concentration levels, the fruit yield of tomato was increased 22 to 41% at increasing CO_2 concentrations from 450 to 675 ppm. Soybean production studies have shown also similar results, with soybean shoots DM increasing 30% by elevating CO_2 from 450 to 550 ppm. The degree to which a plant responds to elevated CO_2 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_2 levels with increases in whole plant dry weight (Jongen & Jones, 1998).

Plant organs also respond differently to elevated CO_2, 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_2 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_2, was 179% higher than that in a standard atmosphere of 400 ppm CO_2. Another study by Behboudian and Tod (1995) examined the effects of CO_2 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_2 environments reached climacteric respiration (a stage of fruit ripening) earlier than in the higher CO_2 treatment resulting in lower ethylene concentration in plants. The research of Gillig et al. (2008) indicated that basil grown at 600 ppm CO_2 had significantly different dry biomass production from those exposed to a 1500 ppm CO_2 concentration. In their study, under an increased atmospheric CO_2 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_2, 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_2 enrichment on the growth of lettuce (Lactuca sativa) (Caporn, 1989)

	Ambient	**1200 ppm CO_2**
Shoot fresh mass (g)	9.22	13.94
Plant dry mass (g)	0.543	0.791
$\log_{10}$ (shoot/root) (dry)	0.756	0.885
Leaf number	14	16

However, their study also showed that CO_2 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_2 generation after complete combustion of sawdust wood pellets, natural gas, and propane (Dion et al., 2013)

	Wood pellets (kg)	**Natural Gas (m^3)**	**Propane (L)**
MJ per unit of fuel	18.1	37.89	25.53
g CO_2 per unit of fuel	1729	1891	1510
g $CO_2 \cdot MJ^{-1}$	96	50	59

In temperate zones around North America, greenhouse operators face high thermal energy requirements to maintain the temperature and CO_2 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_2 concentrations in the growing areas, growers will use either pure CO_2 or burn sawdust wood pellets or natural gas/propane (**Table 2**). For example, in order to maintain a greenhouse at 1000 ppm of CO_2, a greenhouse grower would need to supply CO_2 at the rate of 108 $g \cdot m^{-2} \cdot day^{-1,}$ which requires 0.06 $m^3 \cdot day^{-1} \cdot m^2$ 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 $mS \cdot cm^{-1}$ (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, $g \cdot g^{-1} \cdot day^{-1}$) increases 12.5% when the greenhouse CO_2 concentration is raised from ambient conditions of 390 ppm to 1000 ppm (Duggan-Jones & Nichols, 2014). Additional CO_2 was supplied to the greenhouse by using liquid CO_2 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_2 ranges between 46 to 62% of total carbon reduction during cattle manure composting. Ahn et al. (2011) reported that emission rates of CO_2 ranged from 150 to 600 $g \cdot kg^{-1}$ of volatile dairy manure solids degraded (the VS was measured by loss on ignition according to Standard Methods, APHA, 1998). CO_2 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^{-1} animal unit day^{-1}, likely VS lost, total gas, and CO_2 equivalent (Brown et al., 2008)

Manure type	VS $animal^{-1} \cdot day^{-1}$ (kg)	Likely VS destruction (%)	Total gas ($m^3 \cdot d^{-1}$)	CO_2 equivalent ($kg \cdot day^{-1}$)
Beef	2.68	45	0.84	8.28
Dairy	3.91	48	1.23	12.14
Swine	2.18	50	0.81	8
Poultry layers	4.27	60	2.02	19.9
Poultry broilers	5.45	60	2.58	25.4

Poultry broilers manure produced the most CO_2 gas overall and the most CO_2 day^{-1}, which was proportional to the manure's total volatile solids content. Eleazer et al. (1997) provided estimates of CO_2 emission values and biodegradation days for different municipal organic waste types (**Table 4**).

Table 4. CO_2 gas generation for different types of organic wastes (dry basis) incubated to simulate decomposition in a municipal solid waste landfill (Eleazer et al., 1997)

Waste type	CO_2 (g·kg^{-1} 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

Several factors influence the emission of CO_2 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_2 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_2. Kranert (2010) reported that green waste compost with different organic matter content (dry basis) released different amounts of CO_2, with CO_2 emission rates of 1472, 941, and 597 kg CO_2 ton^{-1} of green waste with 96%, 80%, and 60% woody material, respectively. A study by Dajana and Felicita (2017) reported that the cumulative CO_2 evolution per unit mass of volatile matter of composted tobacco waste (TW) and tobacco mixed with grape waste (TGW) was 94.01

g CO_2 kg^{-1} (9.4%) and 208.18 g CO_2 kg^{-1} (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 10:1and 23:1, respectively. The higher initial C:N ratio material resulted in higher O_2 consumption, and a greater C:N ratio decrease. The mass balance determined for the biodegradation of TW and TGW was found to be:

TW: 1284 g DM (TW)+ 248 g $O_2 \rightarrow$ 117.5 g CO_2 + 330 g H_2O + 1075 g dry compost + 0.505 g NH_3

TGW: 1500 g DM (TGW)+ 471 g $O_2 \rightarrow$ 281 g CO_2 + 464 g H_2O + 1100 g dry compost + 0.122 g NH_3

In contrast, using other feedstocks at different mixing ratios resulted in varying CO_2 emission rates. For example, Santos et al. (2016) compared the CO_2 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_2 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_2 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_2 ($g \cdot m^{-2} \cdot day^{-1}$) from two mixtures of SFP and cotton during the thermophilic phase of composting (Santos et al., 2016)

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

Even non-traditional waste streams, such as pharmaceutical wastes, can be another source of CO_2 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_2 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_2 emissions after application of mature compost to soil

Agricultural activity plays an essential role in GHG's global fluxes, including CO_2, 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_2 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_2 fluxes. For instance, compared to the control group, a compost amendment (green waste, bagasse, and chicken manure) increased CO_2 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_2 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_2 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_3 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 NH_3 gas (Wang & Zeng, 2018).

Under aerobic conditions, 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 NH_3 emissions, but a lower rate slowly increases the 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 NH_3 (De Guardia et al., 2008; Jiang et al., 2013). The NH_3 gas emissions are a lost resource and cause nuisance odors (Fukumoto et al., 2011).

1.3. Summary

Plant responses to 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 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 CO_2 environment using gas released from compost material, from different stages of organic matter decomposition, compared to using a conventional chemical source for 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_2 gas.

It is hypothesized that after filtering NH_3 gas generated from compost material, the mixture gas has similar plant growth promotion ability as a pure CO_2 elevated environment.

2. Quantify the CO_2 and NH_3 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_2 enrichment in a controlled environment.

2. Short-term quantification of CO_2 and NH_3 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_2, NH_3, and other gases. Some of the gases emitted during composting may be detrimental to plant growth, such as ethylene, NH_3, and CH_4, 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_3 (Jiang et al., 2013; Fagbohungbe 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_2 and NH_3, 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_2 $kg^{-1}\cdot DM\cdot hr^{-1}$ compared to the immature and raw material, which produced 648 and 1433 mg CO_2 $kg^{-1}\cdot DM\cdot hr^{-1}$, respectively.

Composting has potential value as a CO_2 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_3), 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_3 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_3 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_3 and CO_2 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_2 and NH_3 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_2 and NH_3 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_2 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_3 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).

Compost feedstocks	**Raw**	**Processed**
MC (%)	42.7	19.70
Dry matter (%)	57.3	80.30
Total C (% Dry)	41.15	27.70
Total N (% Dry)	3.45	2.35
C:N	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_2 trap for carbon dioxide gas generated from the compost sample. Each mason jar was sealed with a lid to prevent ambient CO_2 gas from saturating the trap. There were five jars in total as replicates.

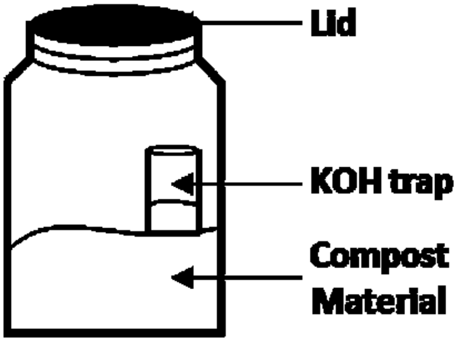

Fig. 1. CO_2 trap apparatus

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_2CO_3) to determine the CO_2 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_2 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_2 sensor (TR-76Ui, GENEQ Inc., Montreal, QC) was used in each chamber for continuous measurement of CO_2 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_2 concentration changes in the growth chamber with some air exchanges. Every 100-ppm increase in CO_2 in the chamber represents 0.64 mg CO_2-C emitted from the CF (based on internal dimensions of the chamber of 35 cm × 35 cm × 25 cm = 30,625 cm^3; every 100 ppm CO_2 increase in the chamber required 3.06 mL CO_2 gas, under standard temperature and pressure, 24.4 L CO_2 is 1 mol and 44g CO_2, so 3.06 mL equal 0.602 mg CO_2).

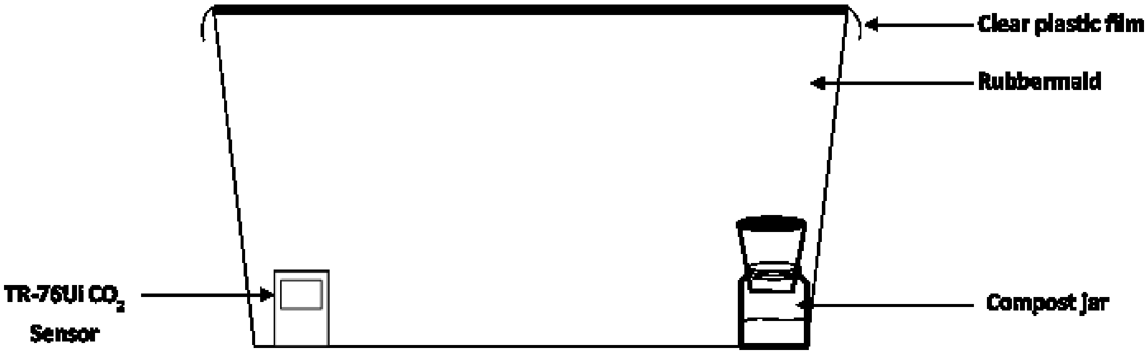

Fig. 2. Setup for experiment two to measure continuous CO_2 emissions from compost feedstock in sealed chamber over a 24-hour period.

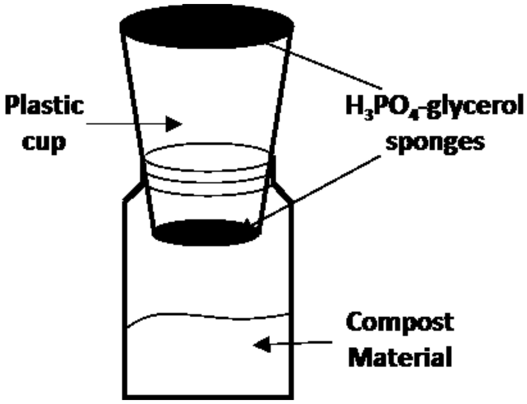

Fig. 3. NH_3 trap apparatus

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_3 and a large sponge (10.16 cm) in the other end to prevent NH_3 from the surrounding air from reaching the small sponges. The sponges were submerged in a 25 mL H_3PO_4-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 (Nômmik, 1973). After 24 hours of trapping the NH_3, 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_4^+-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_2 in the closed environment from the partially processed compost material were calculated as follows (Wollum & Gomez, 1970):

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

Where F denotes the gas CO_2 emission rate ($CO_2 \cdot kg^{-1}$ DM $\cdot min^{-1}$), EC_{raw} is the electric conductivity value of pure 0.5 N KOH (Sm^{-1}), EC_s is the electrical conductivity value of KOH trap for CO_2 sample (Sm^{-1}), EC_{sat} is the electric conductivity value of 0.25N K_2CO_3 (Sm^{-1}), $m_{compost}$ is the experimental material weight (kg), θ is the solids content (1-MC, %), k_t is the total timing of the gas sampling period (mins), P is the maximum capacity of KOH trap solution can absorb CO_2 (1 mL 0.5N KOH absorb 11 mg CO_2)

The values of NH_3 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_3 emission rate (mg $NH_3 \cdot kg^{-1}$ DM$\cdot min^{-1}$), $[NH_4^+]$ is the concentration of ammonium ion in 0.025 L 2 N KCl extraction solution ($mg \cdot L^{-1}$), Q_{NH3} is the ratio of ammonia gas to ammonium ion conversion (mg $NH_3 \cdot mg^{-1}$ NH_4^+), $m_{compost}$ is the experimental material weight (kg), θ is the solids content (1-MC, %), k_t 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_2 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_2-C·kg^{-1} DM·hr^{-1}) and day 11 (259.66 mg CO_2-C·kg^{-1} DM·hr^{-1}). The average gas emission rate was 180.48 mg CO_2-C·kg^{-1} DM·hr^{-1}, with a total of 4.2 g (150g CF wet basis, 47.46% MC) CO_2-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**).

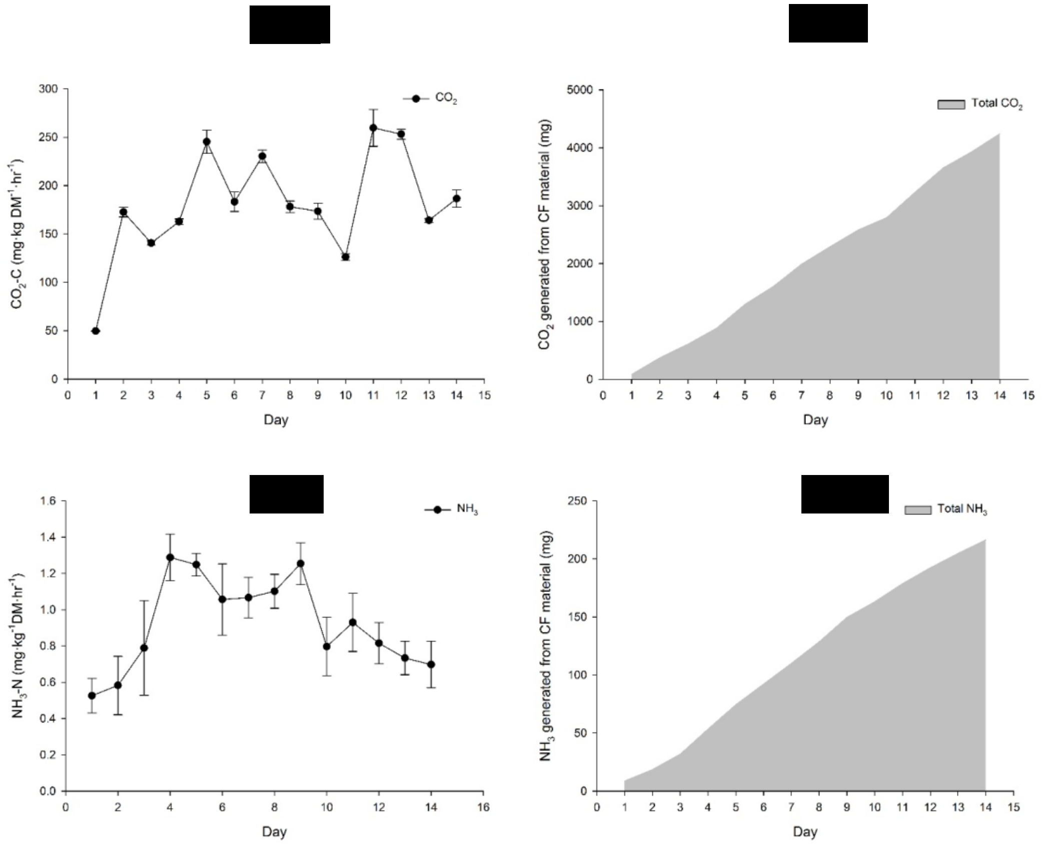

Fig. 4. CO_2 and NH_3 gas emissions from the partially composted material over a 14-day incubation period (N=5).

(a) the average of CO_2 generation rate per sampling point; (b) the cumulative amount of CO_2 generated from CF; (c) the average of NH_3 generation rate per sampling point; (d) the cumulative amount of NH_3 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_2 incubation study (N=5)

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

The NH_3 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_3-N·kg^{-1} DM·hr^{-1}) and day 9 (1.25 mg NH_3-N·kg^{-1} DM·hr^{-1}). The average gas emission rate was 0.92 mg NH_3-N·kg^{-1} DM·hr^{-1} with a total of 216 mg (150g CF wet basis, 47.46% MC) NH_3-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_3 incubation study (N=5)

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

Both CO_2 and NH_3 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_2 changes in the semi-sealed growth chamber

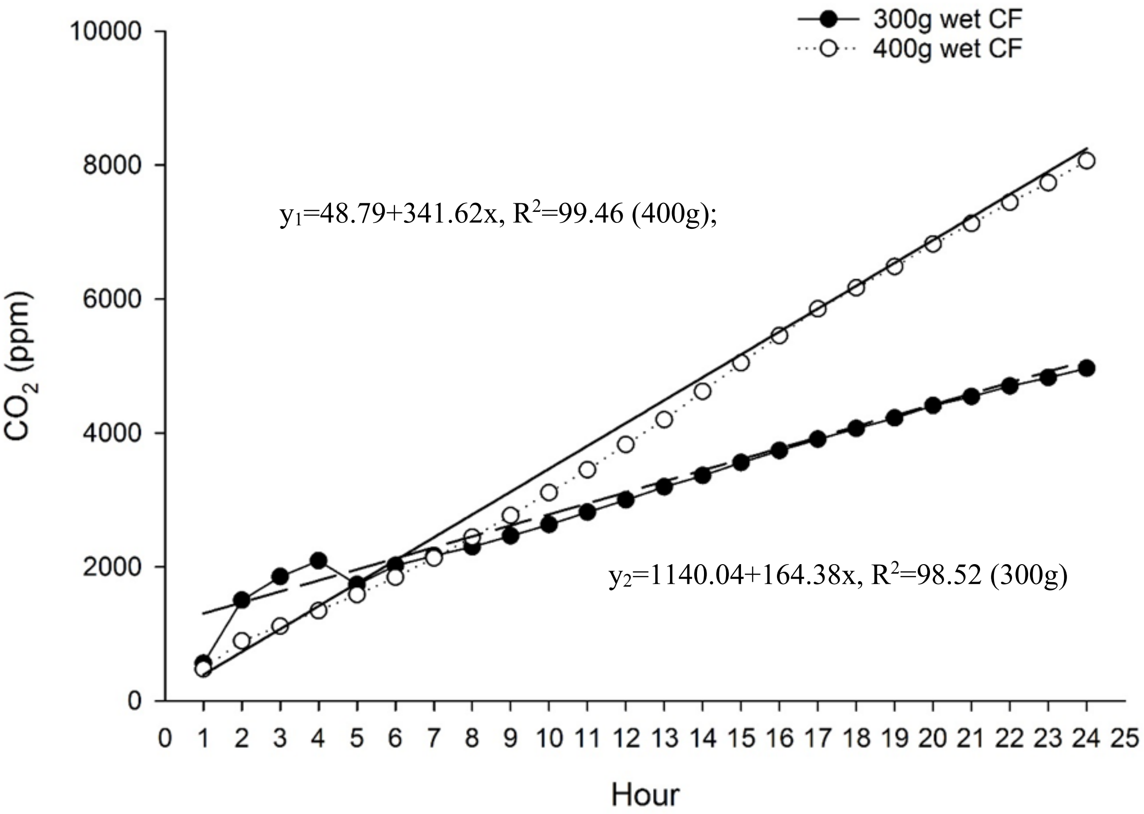

Fig. 5. CO_2 level changes in a semi-sealed growth chamber for 24 hours at different amounts of wet CF.

A comparison of CO_2 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_2 concentration inside the growth chamber. Using 300 g wet CF material, CO_2 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_2 concentration increase in the chamber required 0.602 mg CO_2 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_2 and 0.27 mg CO_2-C generated from the wet CF material per hour compared to 2.06 mg CO_2 and 0.56 mg CO_2-C per hour in the 400 g treatment with 341.62 ppm.

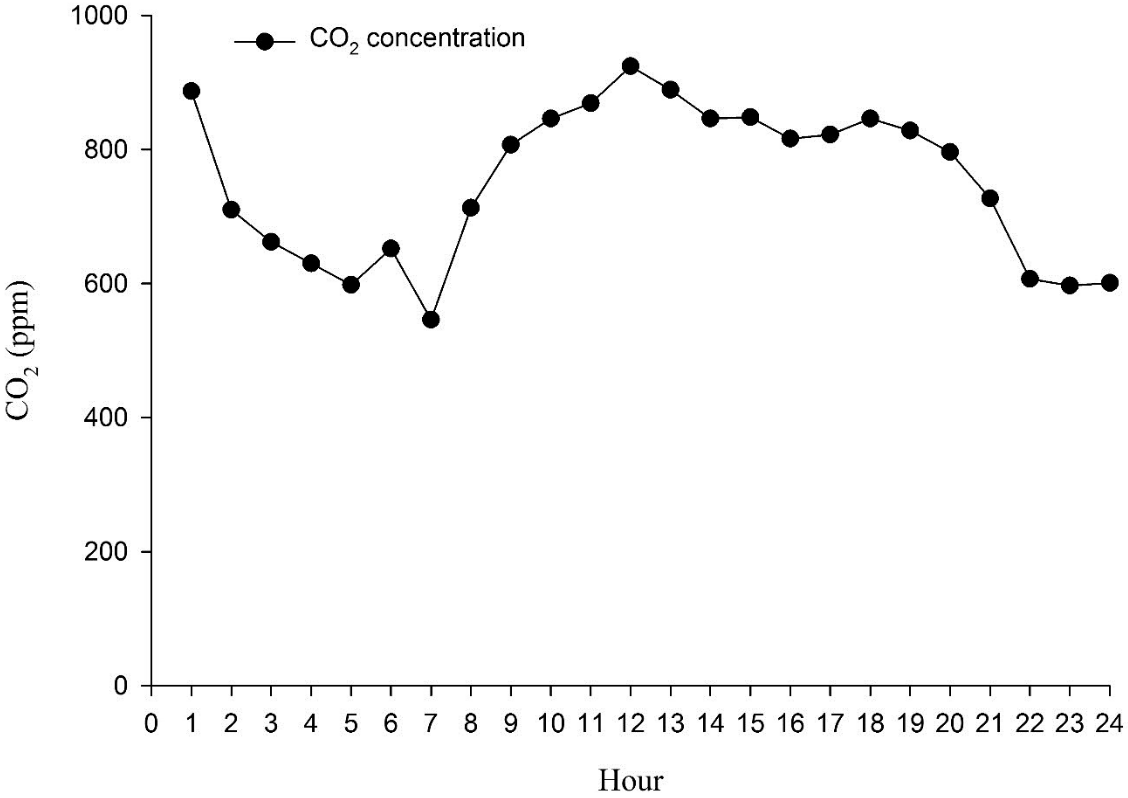

Fig. 6. Hourly fluctuations in CO$_2$ concentrations from a semi-sealed growth chamber containing a hydroponic nutrient solution with an air stone over a 24-hour period

Fluctuations in CO$_2$ 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$_2$ 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$_3$ 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$_3$ gas was generated more rapidly from raw CF than in processed CF (**Table 9**). Under the same environmental conditions, raw CF material generated NH$_3$ gas nearly two times faster than in the processed CF group.

Table 9. NH_3-N (mg·kg^{-1} DM·hr^{-1}) emissions from raw compost mixture feedstock vs. processed compost feedstock over 24 hours (N=6)

Treatment	DM (g)	MC (%)	TC (%)	TN(%)	NH_3-N (mg·kg^{-1} DM·hr^{-1})
Raw	57.3±0.02	61.80±0.23	41.15±0.65	3.45±0.03	2.04 ±0.72^{a}
Processed	80.3±0.09	46.47±0.52	27.70±0.47	2.35±0.04	0.72±0.15^{b}

**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_2 generated from CF material

In the 14-day incubation experiment, CO_2 emission rates rapidly increased to a peak on day 5 by 245.37 mg CO_2-C·kg^{-1} DM·hr^{-1} 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_2 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_2 evolved over the 14 days study (60 g CO_2-C kg^{-1} DM) were lower than other compost components (yard trimmings 112 g and dairy manure 85 g CO_2-C kg^{-1} 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_2 emission rate and total generation amounts were less than other studies.

The average CO_2 emission rates from the CF material in study 1 on the first day were 83.42 mg CO_2-C (150 g wet basis, 49.65 mg CO_2-C$\cdot$ kg^{-1} DM$\cdot$hr^{-1}) in a controlled incubation environment. Compared to the results of study 2, a semi-sealed chamber used for evaluating the CO_2 generated from different amounts (300 g wet CF: 1.68 mg CO_2-C$\cdot$ kg^{-1} DM$\cdot$hr^{-1}; 400 g wet CF: 2.61 mg CO_2-C$\cdot$ kg^{-1} DM$\cdot$hr^{-1}) of CF material (with the same CF material and same MC as in study 1) showed a different concentration of CO_2 increasing rates. The amount of CO_2 generated at the first 24 hours in study 1 was higher than those recorded by the CO_2 sensor in the semi-sealed chamber in study 2. At least 94-97 % of CO_2 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_2 sensor.

The average CO_2 generation rates in study 1 were 180.48 mg CO_2-C$\cdot$kg^{-1} DM$\cdot$hr^{-1} and significantly higher than the recommendation of CCME (2005) that the compost maturity requirement 4 mg CO_2-C$\cdot$kg^{-1} OM$\cdot$day^{-1}. As a result, the material collected from the processed pile of the HotRot 1811 composting system had immature compost characterizations.

2.5.2. NH_3 generated from CF material

The NH_3 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_3-N kg^{-1} DM$\cdot$day^{-1}). After that, the emissions of NH_3 decreased to a low level for the remainder of the study. Other researchers have also observed a similar emission pattern with NH_3 generation from pig manure compost (Jiang et al., 2011). In their study, NH_3 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_3-N kg^{-1} DM·day^{-1}). 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_3 gas generating ability (Szanto et al., 2007). The total amount of NH_3 gas generated from study 1 was 3.09 mg NH_3-N kg^{-1} DM for 14 days and had similar results compared to other studies of NH_3 gas emission from the composting process (Shen et al., 2011). In their study, three aeration rates: 0.01, 0.1, and 0.2 $m^3 \cdot min^{-1} \cdot m^{-3}$ resulted in a significant difference in NH_3 emissions with a total 0.01, 2.34, and 4.38 NH_3-N kg^{-1} DM (NH_3 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_3 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_4 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_3-N emissions in the 14-day incubation experiment accounted for 11% of initial total nitrogen. Compared to the other decomposition processes, the NH_3 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_3 (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_2 and NH_3 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_2O) gas emission, which can be solved by increasing the aeration rates and replacing the new material in a controlled environment. Lower amounts of NH_3 emissions and the potential long-term and stable CO_2 generating of the processed CF materials, demonstrate the capacity to use organic material that is undergoing composting processes as a CO_2 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_2 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_2 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_2 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 off 400 ppm (Mckeehen et al., 1996). During composting, organic waste material is aerobically degraded by microorganisms, generating CO_2 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_2 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_2 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_2 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_2 enrichment conditions. All three studies were established in a completely randomized design with three treatments (pure CO_2, CO_2 from compost gas, and ambient CO_2 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_2 gas cylinder (Air Liquide, ≤99.9% compressed CO_2). 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 ($35 \times 35 \times 25 = 30625$ cm^3), 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 $mg \cdot L^{-1}$ N with 5.6% nitrate and 0.6% ammoniacal nutrients, Nutri+ NUTRIENT GROW A&B solution) (Azis et al., 2020).

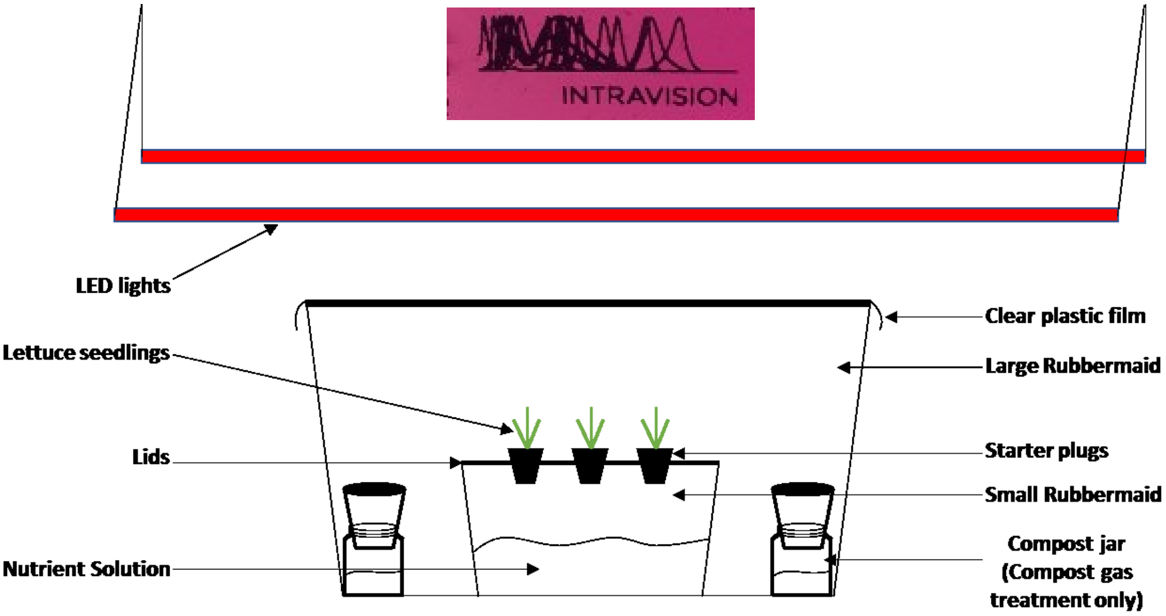

Fig. 7. The hydroponic system set up for lettuce growth

Two red-blue-white LED lamp lighting systems (Cruus® Model number 21GP66 , provided 64.22 $umol \cdot s^{-1} \cdot m^{-2}$ 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 $umol \cdot s^{-1} \cdot m^{-2}$ had significantly biomass increased (38.35% higher in lettuce aboveground as fresh basis) than 16 hours 600 $umol \cdot s^{-1} \cdot m^{-2}$ 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 mS·cm^{-1} (Singh & Bruce, 2016). The initial pH and EC of the nutrient solution was 5.88 and 1.28 mS 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 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 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.**).

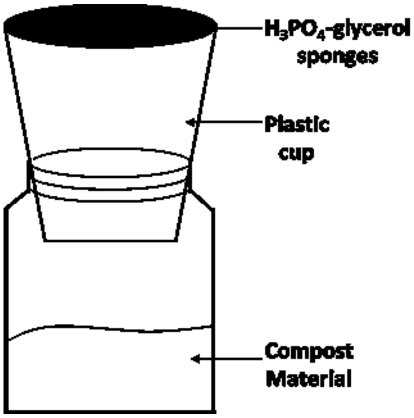

Fig. 8. Compost jar in compost gas treatment of hydroponic system

The jar was covered with a sponge that had been submerged in an H_3PO_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 (Nômmik, 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_2-C over the 14 days (based on the previous study, 180.48 mg CO_2-C·kg^{-1} DM·hr^{-1} 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^{-3}) 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_2 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_2 monitoring system

An automated CO_2 gas sensor system was established for continuous monitoring of gas evolved over the study period **Fig 9**.

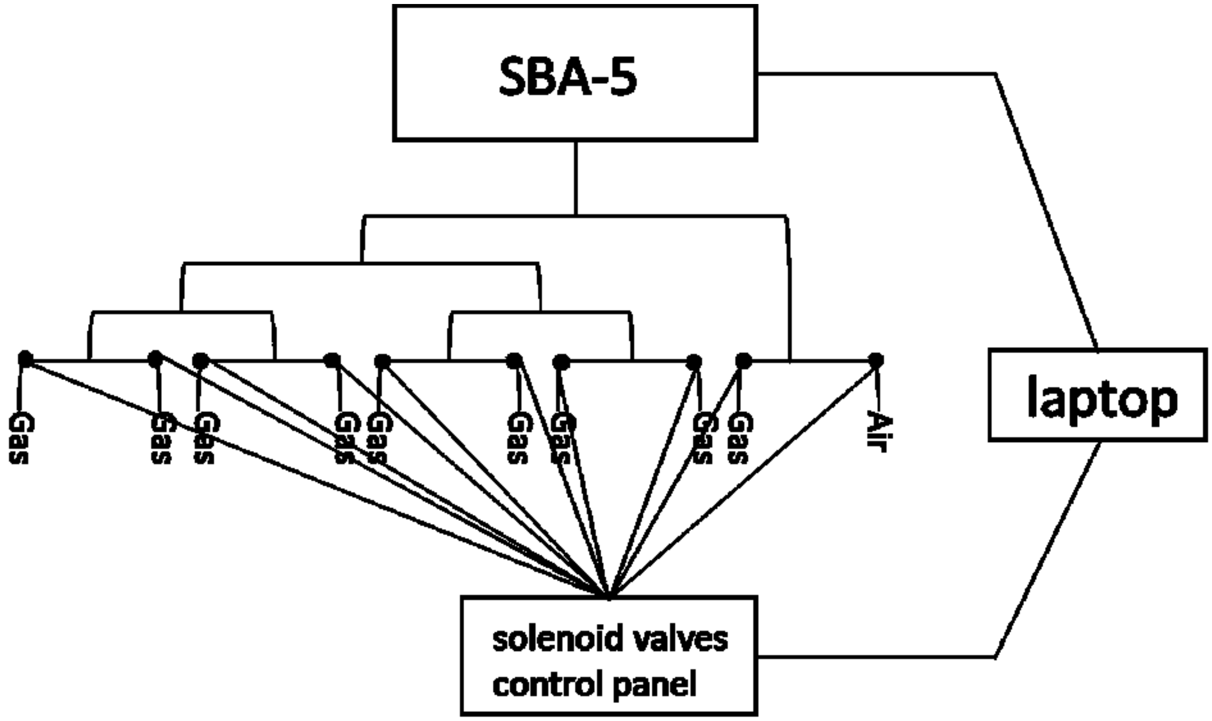

Fig. 9. Automatically CO_2 gas sampling and analyzing setup

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_2 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_2 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_2 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_2 chamber) and the baseline value (from ambient air) within 120s were used as the marked data (**Fig. 10.**).

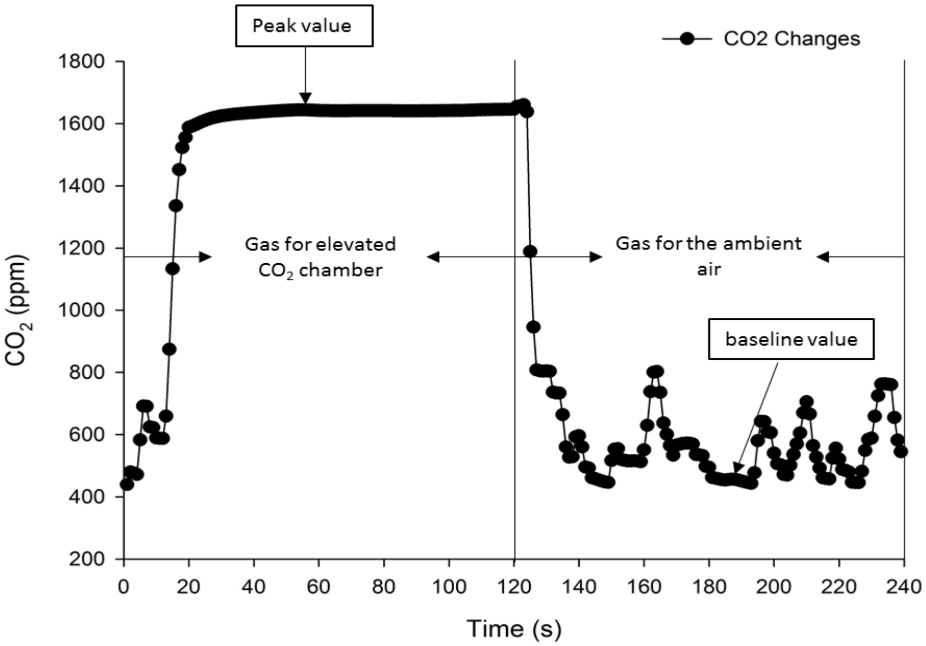

Fig. 10. Examples of CO_2 data collected for 240 s from elevated CO_2 chamber and an ambient condition

3.3. Analytical methodology

A one-way ANOVA analyzed the relationship of CO_2 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_4^+/NO_3^- 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_2) and lettuce leaf biomass production was 71 g fresh weight per plant after 31 days. Under pure CO_2 gas enrichment conditions, biomass production was 46% higher (104 g fresh weight per plant). In plants grown under elevated CO_2 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_3 gas emitted from the composting material. The ammonia gas emissions were confirmed from the incubation study results, with 3.21 mg NH_3-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_2 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_2 from the compost material treatment was 74% greater (59 g fresh weight per plant). Lettuce seedlings under high CO_2 conditions from a pure gas source grew poorly, and many did not survive. It was determined that concentrations of CO_2 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_2 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_2 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_2 concentrations in the growing environments changed. The weights of leaves and roots, including wet and dry biomass, were significantly higher under enriched CO_2 conditions than those under the control group. The source of CO_2, 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_2 concentrations and various gas sources (from compost emissions or pure CO_2 cylinder)

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 ^{b}	3.04±0.95 ^{b}	0.96±0.01 $^{(ns)}$	8.92±3.68 ^{b}	0.32±0.16 ^{b}	0.96±0.01 (ns)	9.28±5.82 (ns)
	C	NA	NA	NA	NA	NA	NA	NA
	P (1760)	103.51±28.02 ^{a}	4.28±0.99 ^{a}	0.96±0.01 (ns)	15.73±5.25 ^{a}	0.48±0.16 ^{a}	0.97±0.01 (ns)	7.33±3.49 (ns)
Study 2	A (736)	33.86±18.55 ^{b}	1.84±1.09 ^{b}	94.63±0.48 (ns)	3.47±1.77 ^{b}	0.23±0.21 ^{b}	93.77±2.60 (ns)	11.25±7.89 (ns)
	C (1085)	58.97±17.16 ^{a}	3.05±0.78 ^{a}	94.73±0.54 (ns)	8.93±4.09 ^{a}	0.48±0.24 ^{a}	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 ^{c}	2.01±0.57 ^{c}	94.52±1.58 ^{b}	4.32±2.72 ^{c}	0.20±0.11^{b}	95.30±0.82 ^{b}	10.79±2.34 (ns)
	C (754)	106.57±27.77 ^{a}	4.05±0.99 ^{a}	96.09±0.97 ^{a}	10.86±3.00 ^{a}	0.36±0.10 ^{a}	96.63±0.39 ^{a}	10.38±3.56 (ns)
	P (746)	88.76±10.34 ^{b}	3.50±0.69 ^{a}	96.07±0.51 ^{a}	7.70±2.64 ^{b}	0.29±0.10 ^{a}	96.22±0.68 ^{a}	12.66±4.07 (ns)

**Values are means (N=4) ±SD. A, ambient condition as control; C, compost gas condition; P, pure CO_2 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_2 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_2 gas supply. The replacement of pure CO_2 tank caused the CO_2 concentration droped 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_3 accumulation. Pure gas treatments had approximately three times higher CO_2 concentrations (1760 ppm) than the growing room's control group (**Fig. 11**).

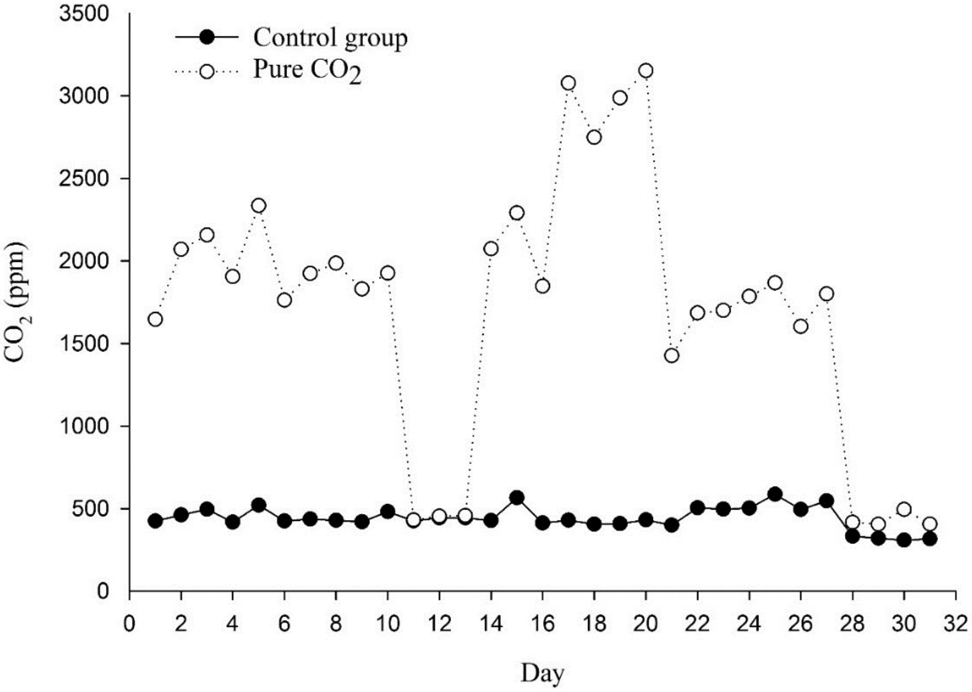

Fig. 11. Daily fluctuations in CO_2 concentration over 31 days in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure CO_2 gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 1.

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_2 concentrations in the growing chambers for this treatment. Poor ventilation and accumulation of CO_2 in the chambers promoted algae growth and fungal rot. The replacement of compost jars caused CO_2 concentration dropped at day 15 and then increased. The average CO_2 concentration in the growing chamber was 1085 ppm from the compost treatment, 47% higher than the control group (**Fig. 12**).

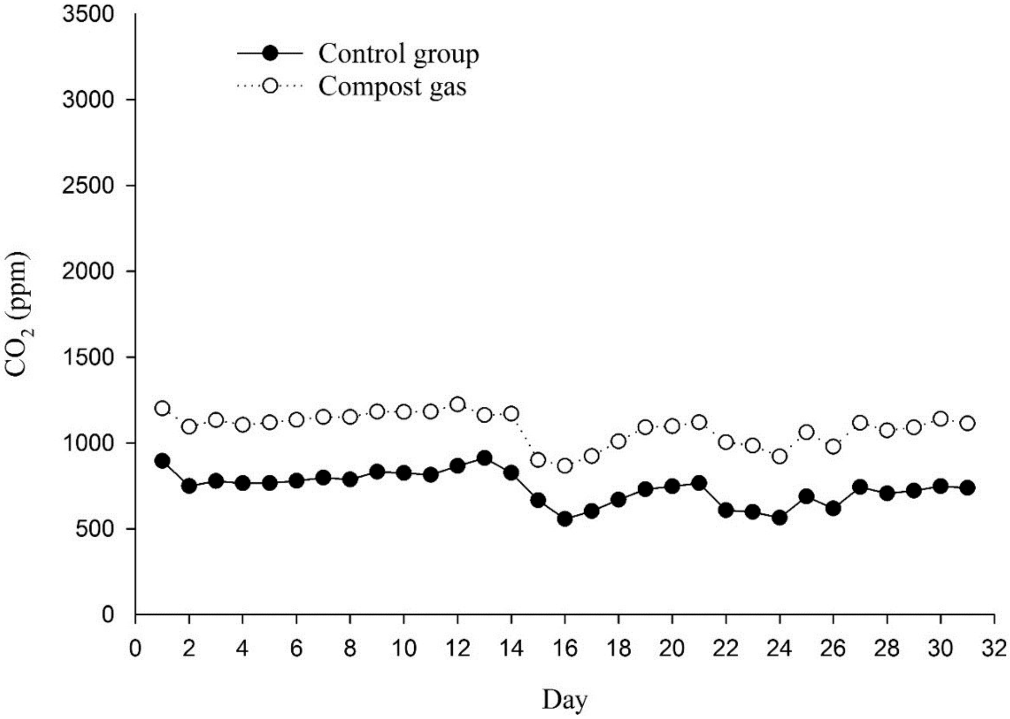

Fig. 12. Daily fluctuations in CO_2 concentration over 31 days in semi-sealed chambers with hydroponic solution and *r*omaine lettuce receiving pure CO_2 gas and in unsealed chambers with hydroponic solution and *r*omaine lettuce under ambient conditions in study 2

In study 3, the retrofitted freight container was modified to include additional ventilation, and a filter was used in compost treatments to remove 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 CO_2 sensor broken, the CO_2 value could not be recorded continuously for 31 days.

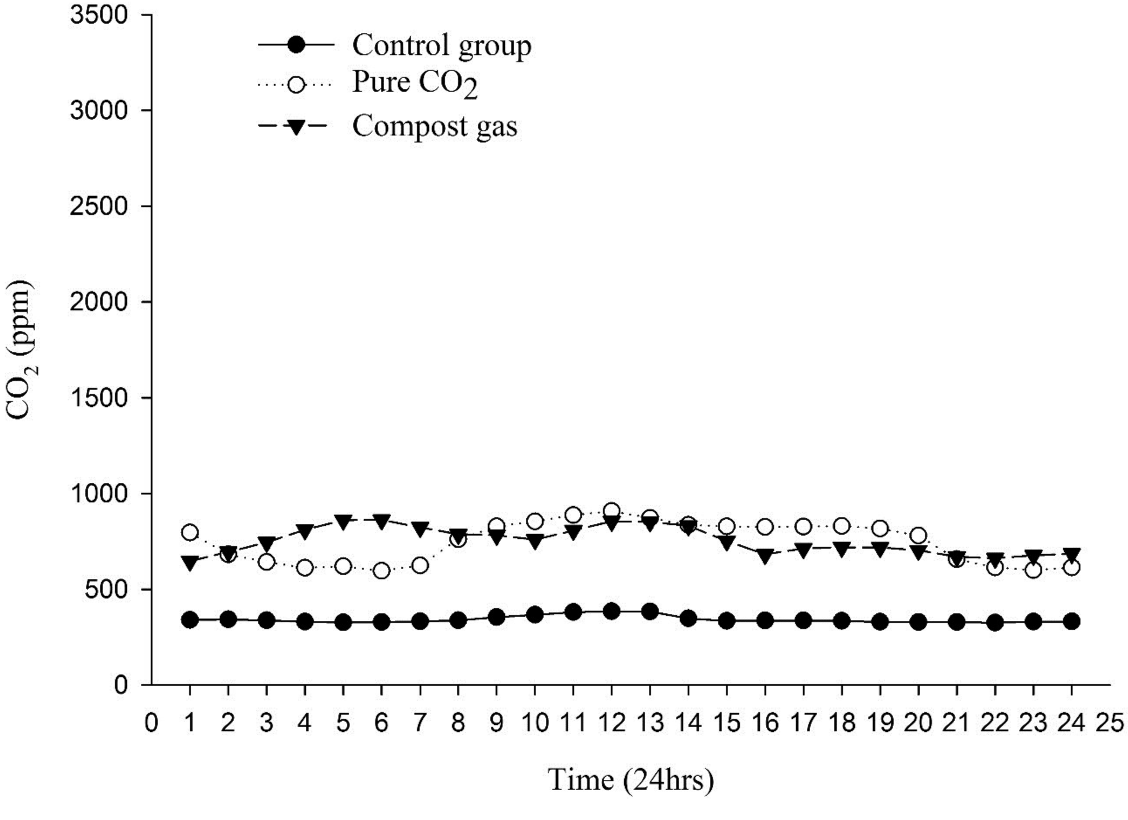

Fig. 13. Hourly fluctuations in CO_2 concentration over 24 hours in semi-sealed chambers with hydroponic solution and romaine lettuce receiving pure CO_2 gas and in unsealed chambers with hydroponic solution and romaine lettuce under ambient conditions in study 3

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_2 environments was higher than the control group. The elevated CO_2 concentrations significantly increased the TC accumulation in both elevated CO_2 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_2 concentrations and various gas sources (from compost emissions or pure CO_2 cylinder)

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

**Values are means (N=4) ±SD. A, ambient condition as a control; C, compost gas condition; P, pure CO_2 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_2 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_2 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_2 enriched treatments and the control group (**Table 12**).

Table 12. The concentration of nitrogen, pH, and EC of the nutrient solution under different CO_2 gas conditions in growth chambers at the end of 31 days.

Study	Treatment (ppm)	pH	EC ($mS \cdot cm^{-1}$)	NH_4^+ ($mg \cdot L^{-1}$)	NO_3^- ($mg \cdot L^{-1}$)
Study 1	A (460)	4.36±0.10 ^{b}	1.34±0.18 $^{(ns)}$	3.43±1.52 $^{(ns)}$	284.4±56.6 $^{(ns)}$
	C	NA	NA	NA	NA
	P (1760)	5.58±0.22 ^{a}	0.97±0.26	2.24±1.48 $^{(ns)}$	288.6±55.1
Study 2	A (736)	4.11±0.14 ^{b}	1.06±0.35 $^{(ns)}$	1.84±1.57 $^{(ns)}$	217.7±46.1 $^{(ns)}$
	C (1085)	5.38±0.43 ^{a}	0.97±0.05 $^{(ns)}$	2.11±1.67 $^{(ns)}$	252.6±48.5 $^{(ns)}$
	P	NA	NA	NA	NA
Study 3	A (342)	4.11±0.17 ^{b}	1.26±0.07 ^{a}	1.13±1.90 $^{(ns)}$	168.5±100.2 $^{(ns)}$
	C (754)	6.01±0.28 ^{a}	0.98±0.07 ^{b}	0.16±0.05 $^{(ns)}$	133.6±56.7 $^{(ns)}$
	P (746)	5.87±0.53 ^{a}	0.99±0.14 ^{b}	0.23±0.20 $^{(ns)}$	121.8±23.1 $^{(ns)}$

**Values are means (N=4) ±SD. A, ambient condition as a control; C, compost gas condition; P, pure CO_2 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 $mS \cdot cm^{-1}$. The pH values from the control group decreased in all three treatments than the initial nutrient solution. However, in elevated CO_2 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_2 treatment was lower than those in the control group. The nitrogen content (both NH_4^+ and NO_3^-) of the nutrient solution collected from higher CO_2 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_2 concentrations on lettuce production

In all three studies, both leaf and root biomass production were higher in the elevated CO_2 treatments, including compost and pure gas (**Table 10**). This study had similar trends to an enriched CO_2 environment study conducted by Pérez-López et al. (2013). Their study examined lettuce (cv. Paris Batavia (PB); cv. Oak Leaf (OL)) under elevated CO_2 at different light intensities and salt stress grown in plastic pots (containing a mixture of perlite and vermiculite). Under a high CO_2 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_2 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 NH_3 volatilization and cause ammonia toxicity. Previous studies have shown that H_3PO_4 effectively removed ammonia gas released from the litter in broiler houses (Reece et al., 1979). In studies 2 and 3, a phosphoric acid (H_3PO_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 CO_2 in the chambers for increased lettuce production. As observed in study 1 relative to studies 2 and 3, the filter traps containing H_3PO_4 mixed with glycerol used to remove 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 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* ssp., have positive effects by generating plant-promoting substances (Ordog, 1999; Schwarz & Gross, 2004). *Chlorella* ssp. is very sensitive to the CO_2 changes and very common to be used for carbon dioxide removing results of the high photosynthetic efficiency to convert CO_2 to 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 (C_2H_5OH) was used as a disinfectant and sterilizing agent, especially in elevated 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 CO_2 delivery to the growth chambers and maintain the control group CO_2 concentrations closer to outdoor conditions.

3.5.2. Effects of elevated CO_2 concentrations on lettuce

Elevated CO_2 promotes CO_2 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_2 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_2 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_2 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_2 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_2 conditions but with lower protein content. Under elevated CO_2 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_2 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_2 conditions (Pérez-López et al., 2015). The accumulation of carbon also differs between cultivars under elevated CO_2 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 $\text{umol}\cdot\text{s}^{-1}\cdot\text{m}^{-2,}$ which was much lower than the lettuce recommendation light intensity of 250 $\text{umol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ (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_2 environment (Dong et al., 2018).

3.5.3. Effects of elevated CO_2 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_2 and the control group. The EC was 22% lower in elevated CO_2 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_4^+ uptake by the roots results in electrochemical compensation enhancing the release of protons, which results in a lower pH. In contrast, higher NO_3^- 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 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 (NO_3^- 14:1 NH_4^+), which indicated that the 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 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 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 NH_4^+ and NO_3^- per milligram in the nutrient solution increased lettuce biomass by 4.21 and 0.15 grams, respectively. In contrast, under elevated CO_2 environments, the average consumption of NH_4^+ and 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 NH_3 gas in a controlled environment results in toxic damage to plants (Zandvakili et al., 2019). For N volatilization, like NH_3, can be significant during the composting process and the decomposition of organic matter. Based on the incubation experimental results, 99.58 mg NH_3 gas was generated from the compost treatments (based on a 0.92 mg $NH_3\text{-N}\cdot kg^{-1}$ $DM\cdot hr^{-1}$ emission rate). Many researchers have shown that 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 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 H_3PO_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 H_3PO_4-gas filter, but growth was stunted in study 1 without the filter. The lettuce harvested from the compost gas elevated 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 NH_3, such as methane (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 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 CO_2 conditions, lettuce biomass production was higher than that in the control group. Elevated 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 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_2 conditions and caused damage to plant growth due to high concentrations of NH_3 gas generation. H_3PO_4 filters made significant contributions to removing NH_3 gas while not affecting CO_2 gas emissions from the decomposing compost materials to promote lettuce growth. Lettuce under elevated CO_2 conditions, enhanced by H_3PO_4-filtered compost gas, was not significantly different from those under pure CO_2 treated conditions. As a result of this study, it is evident that CO_2 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_2 conditions.

The compost source CO_2 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_2 enrichment methods (burning carbon-based fuels such as nature gas, propane, or directly from the CO_2 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_2 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_2 conditions to achieve the optimum production yields and quality.

5. Overall conclusion

Elevated CO_2 promoted romaine lettuce (cv. Parris Island) growth, similar to other lettuce cultivars under similar conditions. Direct use of compost material as the CO_2 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_3 gas generated from CF material and allow CO_2 gas to emit and accumulate in the controlled environment. Positive effects on lettuce biomass production resulted from using the compost gas as a CO_2 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_2 enrichment environment treatments in our study.

More nutrient absorption into lettuce under the high CO_2 conditions was inferred from changes in solution pH. The incubation study results suggest that nitrogen may have not only been lost as NH_3 but also potentially as N_2O.

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_2 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_2 to elevate the concentrations in a 600 m^2 or 2400 m^3 greenhouse up to 1000 ppm CO_2. Similar CO_2 enrichment in a greenhouse requires 40.32 to 48.96 m^3 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_2 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_2, and possibly heat, into CEA systems will demonstrate the true potential for circularization of urban economies.

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