Evaluation of a Novel Poultry-Derived Fertilizer

by

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Abstract

Poultry litter, a common soil amendment, can be applied to soils as a plant nutrient source. Due to a balanced N: P ratio, applying poultry liter based on N rates may result in an overapplication of phosphorous which leads to eutrophication within aquatic environments. To reduce contamination risks, poultry litter can be altered through several different processes, such as anaerobic or aerobic digestion, and can be pelletized for a more uniform product distribution. In a novel fertilizer produced through this method, assessments were conducted to determine both chemical and physical product quality and application results when applied to a variety of crops. Product assessments were made of a proprietary process which combines aerobic digestion and ammonification to physically and chemically alter poultry litter. Through this process, standard poultry litter is transformed from a 1.5-1-1.5 N-P-K chemical formulation to a 11.5-1-1.5 N-P-K granulated product (C&G fertilizer). Nutrient release rates were investigated using a soil incubation test and a rapid water incubation test. Nutrient release rates in soil were evaluated at a 0.89 kg m^3 rate (C&G, Synthetic, or Poultry Litter) with soil maintained at 0.3 cm^3/cm^3 volumetric water content at 30 C over a 55-day period. Rapid water incubation was conducted by adding one gram of fertilizer (C&G, Synthetic, or Osmocote) to 100 mL of water for 24-hour period. Electric conductivity was monitored to evaluate nutrient release over time. In soil, significant and increasing quantities of potassium, ammonium, and nitrate were released in the first six days of incubation for C&G. After six days, nitrate and potassium continue to increase while phosphorus and ammonium plateaued in release. In plant assays, three crops were grown using four fertilizer treatments at four rates. The treatments were Synthetic uncoated fertilizer, C&G, a nutrient even blend (C&G + Synthetic), and Poultry litter. Fertilizers were applied on a N basis of at the rates 0 kg m^3, 0.44 kg m^3, 0.89 kg m^3, and 1.78 kg m^3. C&G

preformed similarly to a synthetic fertilizer across pH & EC sampling, growth indices and nutrient tissue analysis. Final results suggest the C&G fertilizer may be utilized similarly to synthetic, uncoated fertilizers for quick nutrient release.

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List of Abbreviations

PL	Poultry Litter
C&G	Cleaned & Green
SYN	Synthetic
CRF	Controlled Release Fertilizer
SRF	Slow Release Fertilizer
N	Nitrogen
P	Phosphorus
K	Potassium

Chapter 1: Literature Review

1.1 Introduction

This paper shall focus on fertilization methods, with an emphasis on both poultry litter (PL) products, including extended and rapid-release fertilizers. Extended-release fertilizers are categorized into two types: Slow-release and controlled-release. Controlled-release fertilizers often consist of synthetic, plastic-coated prills and depend on environmental controls for breakdown. Slow-release fertilizers lack the synthetic coating and depend on microbial breakdown for nutrient release. Poultry litter is considered a rapid-release fertilizer, where nutrient release and availability begin upon incorporation. However, the rapid-release of high nutrient volumes may cause environmental concerns and require multiple applications for desired plant nutrition throughout the growing season. Thus, this paper will primarily focus on processes that result in slow-release products.

This review will first provide a historical background and appeal behind PL usage is provided, followed by an examination of the nutrient and physical composition of both PL fertilizers derived from it. Processes for utilizing PL products as fertilizers and for energy production will be discussed, as each process can contribute to nutrient usage in agriculture. Chemical and physical properties, as well as testing evaluations will follow, with a focus on physical testing solely for slow release products. Environmental consequences will address interactions with plants, soil, and aquatic habitats. While PL application has the potential to enhance plant growth, it may raise concerns about water quality due to excess nutrient runoff, especially P and N. The relationship with soils is generally positive, especially with treated products compared to raw litter. Finally, the paper will acknowledge the potential for disease spread associated with PL and attempt to alleviate concerns regarding PL-derived products carrying pathogens that may cause humans illness.

1.2 Historic Background

Poultry production covered 71,580 x 10^6 m^2 a in the US as of 2010 with Arkansas, Georgia, and Alabama ranking as the top three states for broiler production (Cadet et al. 2012a, Putman et al. 2017). By 2021, the United States Department of Agriculture estimated a total production value of $46 billion with Georgia (1.3 billion birds) and Alabama (1.2 billion birds) surpassing Arkansas (1 Billion birds) in total production population (USDA National Agricultural Statistics Service Poultry-Production and Value 2022). Globally, poultry consumption is on the rise, generating excess litter creation, as acknowledged by Shakya's for outlook on India (Shakya and Agarwal 2017a) . While the southern regions dominate US broiler production, the midwestern states of Iowa, Indiana, and Ohio have the largest industries in egg production, with total production increasing by 17% between 2008 and 2017 (Hoover et al. 2019).

Poultry litter is composed of manure, bedding material (usually wood shavings), liquid waste, excess food, and animal portions (i.e. feathers) of the animals housed (Shakya and Agarwal 2017b). The United States alone produces 10 million metric tons of PL, with Alabama contributing 1.6 million tons (Kpomblekou-A et al. 2002). Elevated levels of δ^{15}N can trace possible manure use in Northern Europe back 8000 years (Bogaard et al. 2013). The application of PL and its components have sparked legal battles between the government and industry. Oklahoma has had a decades-long litigious history with both the neighboring state of Arkansas and the state's poultry industry, primarily concerning excess nutrients in the Illinois River upon entering Oklahoma from Arkansas (Panach et al. 2007).

Current anxieties surrounding P stem from projected international increases in demand outpacing P supplies. Phosphogypsum use and manure recycling as fertilizer present methods to alleviate the demand for mining (Nedelciu et al. 2020). Despite the US being a major producer of both N and P, the US Department of Agriculture, reported fertilizer prices near record highs (USDA Foreign Agricultural Service 2022). Transforming PL, abundant in the American South and Midwest, into marketable material, holds promise for reducing costs of agricultural commodities.

1.3 Composition

Essential nutrients for plant growth present in PL include the macronutrients N, P, and K, and the micronutrients calcium (Ca), sulfur (S), iron (Fe), magnesium (Mg), and manganese (Mn) (Shakya and Agarwal 2017b). Concentrations of N and P are greater in PL compared to other livestock, and therefore has a potential as fertilizer. Nitrogen and P can be up to five and three times greater than other manure sources (Gollehon et al. 2001). Phosphorous has particular importance as it is considered a non-renewable resource, with the majority of reserves limited to five countries, with Morocco holding the most significant percentage (Brownlie et al. 2021). Slow-release derivatives from PL offer an opportunity to "recycle" excess P offering potential economic and environmental benefits.

Poultry litter also contains levels of potentially hazardous elements including Arsenic (As), Cadmium (Cd), and Lead (Pb), and potentially contain excess Ca, Mn, zinc (Zn), and copper (Cu). Elevated environmental levels of these elements can bioaccumulate in organisms, including humans, potentially leading to adverse health effects (Cadet et al. 2012b). The nutrient composition of PL changes when transitioned to a slow-release product. The nutrients N, P, K, Ca, Mg, Na, Al, and Cl undergo enrichment under combustion or pyrolysis. Carbon remains essentially stable under the same conditions (Bergfeldt et al.). Raw litter typically contains near-even percentages of N, P, and K (3-2-2) which can cause an over-application of P and K (Table. 1.1; Gaskin et al. 2013).

1.3.1 Testing Methodology

1.3.1.1 Chemical

Laboratory tests are essential for determining the nutrient composition of PL byproducts. Soil incubation tests involve applying controlled environment parameters, including constant temperature and moisture levels, to leach nutrients into the soil over a specified period. Increasing the duration of soil incubation quantifies the characteristics of nutrient release the soil. Prior to setup, drying and soil sieving to 2mm must be conducted for proper sampling (Gelderman and Mallarino 2011). Various soil extractions can be conducted after incubation depending on the nutrient of interest. For N analysis, tests may be performed for mineralizable N, ammonium, and nitrate (Anderson et al. 2010). Utilization of 2M KCl via extraction and centrifugation allows nitrate and ammonium measurements with Spectro-photography (OSU Soil Fertility Lab 2022). The process focuses on the readings of infrared wavelengths to determine nutrient concentrations (Nocita et al. 2015). For P and K analysis, Mehlich-1, or Mehlich-3 (for non-acidic soils), tests are performed, using the acids 0.05M HCl and 0.0125M H_2SO_4 (Zhang and Wang 2014). Soil incubation durations vary to measure fertilizer nutrient release over time, often focusing on N mineralization. The tests involve mixing a nutrient source within a previously dried and remoistened soil and regularly checking moisture levels for consistency (Calderón et al. 2005).

Plant assays record nutrient uptake, recognizing that not all elements, beneficial or detrimental, will be completely absorbed by the plants. Foliar analysis measures nutrient levels in mature leaf growth, highlighting whether a nutrient is at an acceptable level, deficient, or toxic (Viveros 2000). Chlorophyll measurements obtained from Soil Plant Analysis Development (SPAD) meters offer indirect nutrient levels, as chlorophyll production requires specific elements. Variations in chlorophyll levels can be recorded to indirectly assess nutrient uptake providing indications of plant health (Guo et al. 2020).

1.3.1.2 Physical

Physical testing of slow-release products includes various qualities: such as hardness, friability, particle size analysis, storage, caking, and shape characteristics. Hardness ensures that the product has sufficient durability to withstand handling before application. Hardness quantification use machine compression tests, with the initial break point signifying stress limits (Walker et al. 1997). Friability tests involve placing fertilizer in rotating drums with an inside surface for repeated scrapping; any lost material indicates friability (Cotabarren et al. 2019).

1.4 Creation Process

To avoid excess nutrient leaching, PL can be transformed into a slow-release fertilizer, either in granular or char, by either "dry" or "wet" methods. The slow-release form has the additional benefit of being decontaminated of pathogens that may cause illness in humans. Dry methods, including methods include pyrolysis, combustion, and gasification, do not rely on liquids to digest the material (Bergfeldt et al.; Manogaran et al. 2022). Conversely anaerobic digestion is the "wet" method (Kelleher et al. 2002). An innovative method of transformation, aerobic digestion, occurs through microbial and thermophilic processes (Table 1.2; Zhang et al. 2022).

Currently, the usage of PL is limited due to challenges such as poor transportability due to weight against product value, environmental (composition) concerns, and economic viability. In Alabama, the profitability of using PL on corn and cotton crops is constrained by transportation distance, reaching a maximum viability distance of 164 miles after five years of continual use, and approximately 130 miles for a single year (Paudel et al. 2004). In Oklahoma, decreasing litter mass through methods like composting can improve transportability, however not all operations have the resources to do so (Penn et al. 2011). In Alabama, a PL utilization faces logistical challenges as the locations of areas for poultry and crop production overlap seldomly. By transforming PL into a slow-release and concentrated form, the material becomes more transportable across greater distances, thereby improving its marketability as an organic fertilizer.

1.4.1 Dry Methods

Combustion can be completed two methods: mass burn and fluidized bed combustion (Kelleher et al. 2002). According to Kelleher (2002), combustion allows the ability to work with materials of higher moisture content and is relatively cost-effective. In fluidized bed combustion (FBC) the process begins by drying the litter, or other desired material, followed by a rapid combustion of carbon and excess heating, occurring in a matter of seconds (Ravelli et al. 2008). Mass burn is the combustion process within a single-stage chamber (Abelha et al. 2003). The process further includes the separation of combustible material via sensor controlled scrapers, then followed by the blowing of hot air to suspend fuel. After the combustion of material occurs, the heat produced can be used to warm a structure, while the leftover char can be fertilizer (Billen et al. 2015).

Pyrolysis involves a thermochemically alters PL without the presence of a gasification medium. Pyrolysis occurs at three temperature levels of increasing heat, each requiring particles of decreasing size due to the speed of the reaction. Slow pyrolysis, a method in practice for thousands of years, primarily produces solid products. In contrast, fast pyrolysis, popularized in recent decades, primarily produces gaseous components. The newest method, flash pyrolysis, can burn to temperatures of 1000 C and finish the process in a matter of seconds, producing condensed vapors and organic solid matter (Bahng et al. 2009; Bergfeldt et al.). The solid matter, considered a biochar, constitutes a minor product of the reaction, roughly 15%, and the majority are gaseous forms including bio-oil and fuel gas. Since pyrolysis produces higher volumes of gas, energy generation is the main production focus rather than fertilizer (Bridgwater 2012).

Gasification is a distinctive version of pyrolysis involving a complex thermochemical conversion comprising of four stages: drying, devolatilization (pyrolysis), combustion, and reduction (Manogaran et al. 2022). Gasification occurs at high temperatures with an oxidizing agent including air, steam, CO_2, or oxygen. The process begins with a low temperature dehydration followed by pyrolysis intensifying upwards of 500 C, eventually inducing an oxidizing event. Gasification reduces larger particles within the PL into smaller particles, creating gaseous products and solids (ash, char, leftover contaminants) resulting from incomplete conversions (Kumar et al. 2009). The entire process occurs in seconds to minutes, with gases being the main product desired (Shakya and Agarwal 2017b).

Dry process, dry digestion, often referred to as anaerobic or aerobic digestion (AD), may be considered a misnomer as water is included in the process, but not to a level that allows for fluidity of the matter. No consensus regarding the upper water content limit exists for AD, with thresholds between 15% and 40%. Regardless, all forms require four to ten times less water than "wet" methods (Shapovalov et al. 2020). Aerobic Digestion relies on bacteria to complete the break down process (Manogaran et al. 2022). The optimum biomass pH for bacterial populations in AD is between 6 and 7; if the environment becomes too acidic and bacterial populations will experience inhibition (Matheri et al. 2017).

Continuous batch AD occurs within a singular vertical or horizontal drum, relying on plug flow to move older material through the digestive drum. These drums do not mix the material; rather, pumps and outside mixers induce the thermophilic or mesophilic temperature in the new material by inoculating a portion of the exiting incoming material (Rapport et al. 2008). Batch systems differ from continuous systems as all material is loaded within a concrete box at the beginning, under mesophilic conditions, and percolates through the porous bottom. The batch method requires a larger area, about ten times the size, and has an increased likelihood of clogging than the continuous version, but it is less expensive (Shapovalov et al. 2020). The higher temperature of thermophilic reactions reduces pathogen populations of *Salmonella* and *Enterococcus* spp. more effectively than mesophilic reactions, but thermophilic reactions produce lower amounts of biogas than mesophilic reactions (Bi et al. 2019; Chen et al. 2015; Fatoba et al. 2021). Anaerobic digestion comprises of four stages. The process begins with hydrolysis, or the breakdown of the biomass into smaller particles. Acidogenesis follows hydrolysis, in which bacterial populations create an acidic environment, often requiring hydrogen carbonate to avoid to an acidic pH. Acetogenesis follows, forming acetate, being used in the final step of methanogenesis creating methane (Matheri et al. 2017).

Aerobic digestion represents a new autothermal thermophilic process, with relatively little research conducted in comparison to anaerobic digestion (**Fig. 1.1**). The process is dependent on microorganisms for product production synthesis (Zhang et al. 2022). While the initial modeling was proposed in 1969, most research investigation into aerobic digestion has been conducted in recent years (Kambhu and Andrews 1969). The process occurs in two steps: the first drum digests the material, and the second undergoes a thermophilic reaction for sanitization due to heat. Mixing and adequate oxygen must occur for proper sanitation to occur.

The process exhibits potential for optimization allowing for large quantities to be produced by minimal staffing (Martín et al. 2018). Successful testing on pig waste demonstrated that the process capable of reducing waste into a more compact product (Lee and Han 2016).

1.4.2 Wet Methods

Anaerobic Digestion is the creation of biogases within an anaerobic environment using specific groups of bacteria such as methane forming, homoacetogenic, and acetotrophic bacteria (Szuhaj et al. 2016; Kremp et al. 2018; Mutungwazi et al. 2020). Similar to the dry method, the wet method requires a neutral pH level to complete the process. The method requires an oxidation-reduction potential below 200 mV for the conversion to take place. Similar to gasification, anaerobic digestion consists of four stages: fermentation or hydrolysis, acidogenesis, acetogenesis and methanogenesis (Manogaran et al. 2022). Similar to dry anaerobic digestion, bacteria are required to form the product. Employment of chemical pretreatments are common for both “dry” and “wet” to mitigate ammonia inhibition and toxicity to anaerobic organisms. The propensity for inhibition is a result of urea and amino acids degrading into ammonia (Zahan and Othman 2019). Similar to anaerobic digestion, an aerobic process may be completed with the general difference being exposure to oxygen.

1.5 Environmental Consequences

Both fresh litter and slow-release products offer nutritional benefits to plants and soil, acting as an important and economical P source (Oliveira Nascimento et al. 2021). The moisture content of PL influences whether P or N is more available for plant uptake. Higher moisture contents (~70%) enhances P is availability, while lower moisture percentages allow increased N uptake (Higgins et al. 2021). In another experiment, a variety of fertilizers including synthetic, granular, and a mixed formula, all containing the same total P and N to plants, were tested.

Granulized PL fertilizer slowed the release of P, more similarly matching plant demand. Furthermore, granulized PL encouraged colonization and growth of arbuscular mycorrhizal fungi, which aided in P uptake (Ngo et al. 2022).

1.5.1 Plants Relationships

1.5.1.1Vegetables

When compared to cow and goat manure, PL provided the most significant response in plant growth. Both PL and granular, slow-release fertilizer, when applied to tomatoes, have increased growth, and yields in tomatoes. (Usman 2015). Applying a blend of synthetically-derived and PL nutrient sources improved plant growth over singular nutrient sources (Usman 2015; Henry et al. 2017). However, PL biochar applications to tomato, pepper, and lettuce provided mixed results(Akça and Namlı 2015). In lettuce no significant differences were observed between the control and experimental variables (biochar, inorganic 15-15-15, and mixture) (Akça and Namlı 2015). In peppers, treatments varied but were all higher than the control. Tomato growth correlated with application rates rather than nutrient source (Akça and Namlı 2015). Applications of PL biochar prior to transplanting recorded the highest values in height, leaf area, and fruit yield, with the second highest values observed during the week of planting (Adekiya and Agbede 2017). However, PL biochar applications three and six weeks after transplanting trended negatively in all categories, suggesting timing of applications is important. Similar results regarding application timing have been observed, with additional acknowledgement that soil texture and pH also have an effect on the success of PL application (Lin et al. 2018). Greater crop yields were produced when applying 6 tons of granular PL per acre crop yield, while increasing to 9 tons/acre decreased yield (Adekiya and Agbede 2017). Additionally, the application of 6 tons per acre may shorten the time required for fruit ripening to 82 days from 90 days while improving the nutritional content (Stepantsova et al. 2021).

1.5.1.2 Monocots

Litter has been long investigated with large acreage crops such as corn, wheat, and other grains. However, when deficiency occurred, urea applications produced higher yields than the PL alone (Singh et al. 2022). When growing spring wheat and barley, granular application of 2 tons per acre produced similar results to the control of no fertilizer. However, upon increasing application to 4 tons per acre increases in vitality and yield were noted. Increasing PL applications to a rate of 6 tons per acre barely improved development but negatively impacted wheat. Soil nutrient levels at the 2 tons per acre were comparable to control levels, but increasing application led to a doubling of macro and micronutrients studied, signifying the attainment of maximum nutrient uptake (Stepantsova et al. 2021).

Sistani (2014) reported no improvements or differences in growth or yield of corn grown in Brazil using PL in comparison to synthetic fertilizer. However, the investigators attributed weather and management practices for the similarities in corn yields across nutrient sources (Sistani et al. 2014). In a study conducted by the University of Arkansas comparing pelleted PL, PL, and urea in corn yield, urea applications resulted in greater yields than either PL material. As a result, the authors recommended a combination of urea and PL source material for farmers looking for economical ways to cut fertilizer costs (Slaton and Sabbe 2008).

1.5.1.3 Ornamentals

Composted PL has been shown to produce bedding plants such as 'Celebrity Red' petunia and 'Quartz Scarlet' verbena of similar quality to those grown using commercially used inorganic fertilizers. Applications of PL resulted in the higher chlorophyl content in petunia beyond 12 weeks (Marble et al. 2011). Final project conclusions indicated that composted PL may successfully be applied at higher N rates than inorganic fertilizers for bedding plants (Marble et al. 2011). In two separate experiments, non-composted litter produced plants of similar quality to inorganic sources (Altland et al. 2003). However, the two experiments diverged with excess N. The divergence could be the result of quality differences between the PL applied as the plants were noted as superior.

While PL offers benefits, PL-derived biochar has been found to produce larger plants in *Gima kalmi*. The biochar created contained elevated levels of micronutrients and decomposed at a slower rate (Sikder and Joardar 2019). In container production, composted PL can increase water holding capacity, supplement fertilizer, and replace lime for increasing pH when using a pine-bark-based fertilizer (Barbosa et al. 2023). Furthermore, when PL composes 20-40% of the substrate, most nutrient requirements are fulfilled, except N. The study concluded PL can reduce reliance on non-renewable peat moss. Another study conducted by Auburn University, similarly, concluded that PL could help replace peat moss in wood-based potting media, specifically plants that can handle high electrical conductivity and pH variability (Marble et al. 2010). Furthermore, the plants grown in PL produced plants of similar size to peat moss. However, the study raised concerns about increased shrinkage as the use of PL increased the bacterial population which breaks down cellulose. Interestingly, dicots grown in pasteurized PL outperformed the monocot areca palm (Broschat 2008). The plants were grown in container production had excess nutrient release in the early weeks of production. Poultry litter can produce petunias of similar quality to those grown with commercial liquid fertilizer, ready for sale within forty days (Owen et al. 2011). While crop-dependent, PL has reason to be considered in ornamental container production.

1.5.2 Soils

Soil benefits vary depending on the method of soil amendment. Soils amended with PL exhibited increased stability of soil aggregates within the > 0.25, 0.5, and 1 mm diameter ranges, while biochar produced decreases in aggregates greater than 2mm in range (Li et al. 2021). Another study, which did not discriminate on size ranges, did report an increase in aggregation and clods within soils amended with pelletized PL. The application produced continued improvement after four years of continual incorporation (Feng et al. 2019). Furthermore, Feng (2019) reported no differences in aggregation between the fall of 2014 and the spring of 2015, suggesting conservation tillage practices did not break up the aggregates within the humid Mississippi location. In Mississippi, cotton cultivation produced pronounced differences between PL and commercial fertilizer. The pH increased with the litter application and decreased with the commercial-grade product. Litter decreased soil bulk density, while commercial fertilizer produced no change and increased the stability of soil aggregates (Adeli et al. 2010).

In West Virginia, increases in PL application produce a positive linear relationship with total water content within the soil (Mandal et al. 2013). Mandal and Feng suggest that increased amounts of organic matter led to increased water content because organic matter has greater water-holding capacity. Conducting soil incubation tests revealed the leachate capacity of fertilizer nutrients within a soil with the Dumas method conducted for C and N analysis, and Spectroscopy for all other Macro and Micronutrients (Ngo et al. 2022). Nutritional benefits similarly varied depending on the application method and the nutrient of focus. Granular litter can offer a nutritional advantage over traditional fertilizer as the organic makeup of the elements breaks down slower within the soil better matching plant uptake. Additionally, reductions in nutritional waste, especially of P, have been recorded (N N Apaeva et al 2020).

Soil type and nutrient form can significantly impact nutrient levels within different soils when comparing PL to synthetic fertilizer. Frazao (2019) applied granular PL fertilizer and synthetic fertilizer to Oxisols and Entisols in Brazil. At project initiation, the Oxisol recorded higher levels of non-labile P (87%) than Entisols (53%). Non-labile P experienced no differences post-P fertilization, but labile and moderately labile experienced increases in both soils, with a greater increase observed in the Entisol (Frazão et al. 2019). Frazao theorized that repeated applications may impact the non-labile P pool within soil over time. Over twenty years of PL application to a field in Ohio, P accumulation occurred in the topsoil, but further mobilization within the lower layers did not occur (Hoover et al. 2019). Soil acidification may occur from long-term application of fertilizers as the N sources ammonia and urea have an affinity for producing acidic conditions. Similar to inorganic fertilizer, raw manure may establish conditions encouraging soil acidification (Van der Stelt et al. 2007). However, biochar application from pyrolysis, including from animal waste, may increase soil pH and increase nutrient availability (Dai et al. 2017; Bolan et al. 2023).

1.5.3 Aquatic Environment

When improperly used, traditional applications of PL potentially pose negative effects, such as eutrophication, to aquatic ecosystems; however, slow-release products derived from PL can mitigate the negative consequences. The presence of excess N and P in the aquatic environment produces negative environmental and economic consequences (Schindler 1974; Finlay et al. 2013). Agricultural operations are a major source of excess nutrients, although not solely responsible. Fertilizer applications generally produce a degree of nutrient leaching which is undesired for water contamination and financial losses from wasted material. In the Vouga catchment of Portugal, the use of multiple smaller applications (or slow-release fertilizer) lessened the movement of excess nutrients to the environment (Rocha et al. 2015). In the Ohio Hoover study, higher levels of PO_4-P were recorded from PL-amended plots; but the total P recorded did not exceed the levels for enhanced eutrophication, contrary to the expected results (Hoover et al. 2019).

Green roofs, often utilized in urban agriculture systems and private recreation spaces, play a role in lessening total runoff and act in nutrient dynamics. Acting both as a sink and source for nutrients in runoff. Plants and growing media on green roofs retain NH_3-N, total N, lead, and zinc. However, green roofs act as a source of total P, dissolved copper, and more soluble forms of P and N in the environment (Gregoire and Clausen 2011).

Poultry litter often contains highly soluble P. In the event of precipitation, increased P concentrations can occur within aquatic habitats. While all treatments produced higher concentrations than the controls, the alum-treated manure produced a greater capacity to lower P solubility (Delaune et al. 1995). Delaune's (1995) project used a computer model incorporating desired parameters, environmental statistics, and historical information for the results produced. Similar results were produced in the Blackland prairie near Waco, Texas under expanded studied parameters (Harmel et al. 2004). Incorporating litter fertilizer into soil decreased watershed nutrient concentrations compared to top dressing. Chicken litter lessened concentrations more effectively than synthetic. Nutrient levels of P and N decreased following the second year of application. However, all fertilizer methods recorded increased nutrient levels than the control.

1.6 Disease Concerns

There is concern of the potential for disease transmission to humans, associated with PL and products created from litter. However, the processes to create fertilizer, char, biogas, etc. generally produce enough heat to eliminate possible contagions present in raw litter. While populations of *Enterococcus* spp. may have existed in soil before litter application, the total populations, species diversity, and resistance to antibiotics and multidrug resistance increased after application. Some species of *Enterococcus* spp., which naturally reside in animal intestines (including humans), appeared only after litter application. Certain species of *Enterococcus* spp. have the potential to cause infection in humans, causing concern in the scientific community (Fatoba et al. 2021).

Salmonella is responsible for foodborne illness outbreaks in poultry products 30% of the time and in eggs 24% of the time and is the predominant reservoir (Gould et al. 2013). As moisture and ammonia levels decrease over increasing periods of storage increase populations of heat-resilient *Salmonella* spp. may increase, requiring longer thermal periods to eradicate the populations. The desiccation of litter provides a habitat for *Salmonella*, allowing population increases for heat-resistant variants (Chen et al. 2013; Chen et al. 2015). Ammonia can inactivate *Salmonella*; but, as levels decrease during mineralization, the degree of inactivation decreases. For example, 9-month-old litter required a minimum of an hour at 150 C to exterminate populations, while 75 C could significantly lower populations at 0 and 3 months (Chen et al. 2015). Infection typically occurs via fecal matter or the reproductive system. However, the uniform heat and chemical treatment common in pelletized PL contains the ability to exterminate harmful pathogens (Chen and Jiang 2014).

1.7 Conclusions

Commercial poultry production generates excessive amounts of litter, that when burned or digested, can provide fertilizer benefits with fewer complications than regular litter. The transformation of litter into fuel or soil amendment gives new use to a formerly potentially pathogenic product. Current literature focuses on anaerobic digestion with little research on aerobic digestive processes. Current aerobic digestion work shall focus on slow-release fertilizer for testing in tomato, rye, and ornamental annuals. Incubation tests will be conducted to measure nutrient leachate. The product “Cleaned & Green”, will be studied for application comparison with synthetic fertilizers and PL.

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Table 1.1: Differences of N-P-K between raw chicken litter and a slow release undergone digestive processes.

Comparison of Raw Litter to Slow Release				
Fertilizer Type	Nitrogen (%)	Phosphorous (%)	Potassium (%)	Source
Raw Chicken Litter	3	2	2	(Gaskin et al. 2013)
Cleaned & Green Granular	11.59	1.5	1.5	Auburn Soil Testing Lab

Table 1.2: A comparison between methods for processing chicken litter into secondary products.

Methods of Transforming Poultry Litter					
Process	Wet or Dry	Temperature (C)	Main Product	Additional Factors	Source
Pyrolysis	Dry	Slow: 277-677 Fast:577-977 Flash:777-1027	Bio-oil Biochar Fuelgas	Each method produces different ratios of each product	Manogaran
Combustion	Dry	Rapid High Temperatures	Ash and Electricity	Volumes can get up to 50 tons per time. Heat recovery can be a product	Kelleher
Gasification	Dry	100-900 depending on stage	Combustible Gas Char Ash		Manogaran
Dry Anaerobic Digestion	Dry	Thermophilic Or Mesophilic	Soil amendment		Manogaran
Wet Anaerobic Digestion	Wet	Thermophilic Or Mesophilic	Soil amendment		Manogaran
Aerobic Digestion	Either	Autothermal Thermophilic	Soil Amendment	Highly Dependent on Microorganisms	(Zhang et al. 2022)

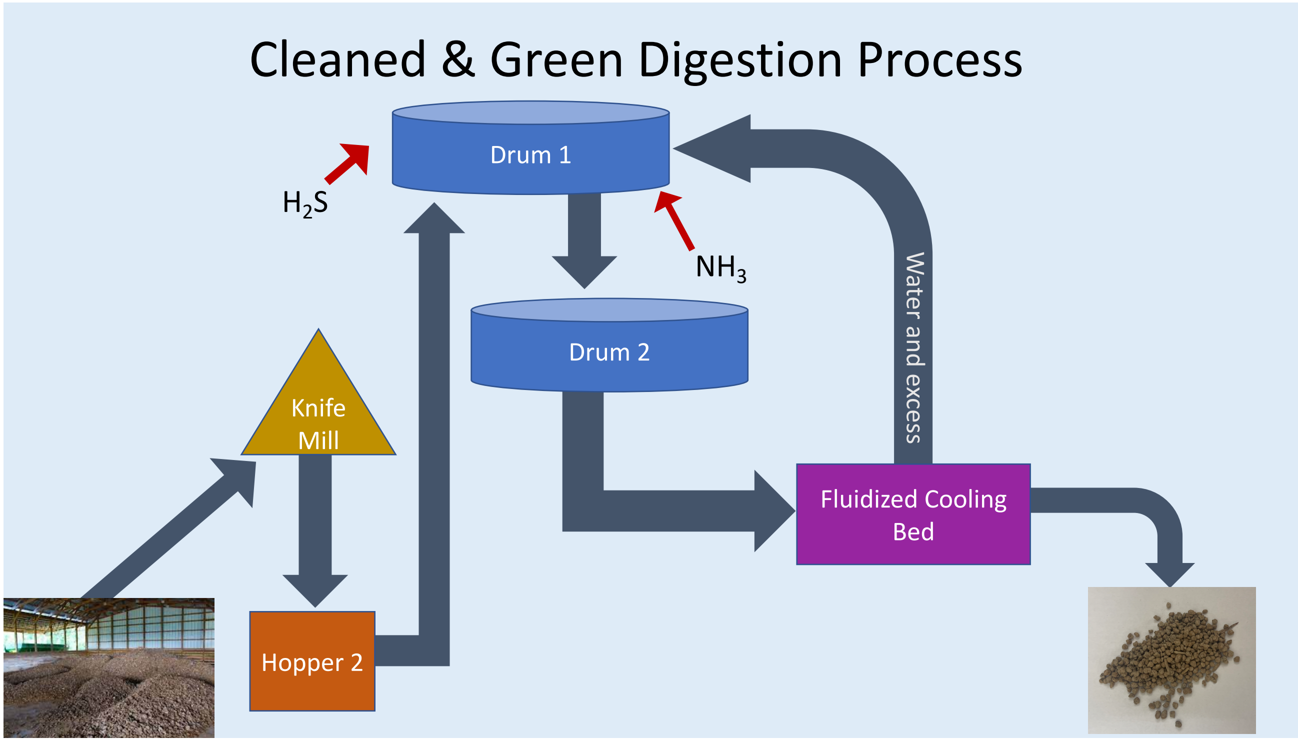

Figure 1.1. Simplified process of aerobic digestion proposed by Alabama company Cleaned and Green. Image created by Austin Lindquist

Chapter 2: Evaluation of Physical and Chemical Characteristics of a Novel Poultry-derived Fertilizer

Abstract

Poultry litter, a common soil amendment, can be applied to soils as a plant nutrient source. Due to a balanced N: P ratio, applying poultry liter based on N rates may result in overapplication of P, leading to eutrophication within aquatic environments. To reduce contamination risks, PL can be altered through several different processes, such as anaerobic or aerobic digestion, and can be pelletized for a more uniform product distribution. Product assessments were made of a proprietary process that combines aerobic digestion and ammonification to physically and chemically alter PL. Through this process, standard PL is transformed from a 1.5-1-1.5 N-P-K chemical formulation to a 11.5-1-1.5 N-P-K granulated product (C&G fertilizer). Nutrient release rates were investigated using a soil incubation test and a rapid water incubation test. Nutrient release rates in soil were evaluated at a 0.89 kg m^3 rate (C&G, Synthetic, or PL) with soil maintained at 0.3 cm^3/cm^3 volumetric water content at 30 C over 55 days. Rapid water incubation was conducted by adding one gram of fertilizer (C&G, Synthetic, or Osmocote) to 100 mL of water for 24 hours. Electric conductivity was monitored to evaluate nutrient release over time. Within the first 10 minutes, 82% of C&G and 95% of Synthetic nutrient release had occurred in the water-based method. Osmocote had the slowest and most variable release rate. In soil, significant and increasing quantities of P, K, ammonium (NH_4^+), and nitrate (NO_3^-) were released in the first six days of incubation for C&G. After six days, nitrate and potassium continued to increase while P and (NH_4^+) plateaued in release. Initial results suggest the C&G fertilizer may be utilized similarly to synthetic, uncoated fertilizers.

2.1 Introduction

Improper application of traditional PL can lead to significant adverse effects, such as eutrophication. Excess N and P in aquatic environments result in detrimental environmental and economic consequences from eutrophication (Schindler 1974; Finlay et al. 2013). However, slow-release products derived from PL can mitigate these harmful environmental effects. Eutrophication is particularly exacerbated in shallow water conditions where light and temperature significantly influence aquatic habitats. Algal and cyanobacterial blooms thrive in nutrient-rich water, which leads to mass die-offs of aquatic life as decomposers thrive and consume the proliferated dead bacteria and algae. Agricultural operations are a significant source of excess nutrients, but the industry is not solely to blame. Fertilizer applications generally result in some nutrient leaching, contributing to water contamination and financial losses from wasted material. However, adopting improved management practices can mitigate these issues. In the Vouga catchment of Portugal, using multiple smaller applications or slow-release fertilizers reduced the movement of excess nutrients into the environment (Rocha et al. 2015). A study conducted in Ohio demonstrated higher levels of PO_4-P were recorded from PL-amended plots; however, the total phosphorous levels did not exceed those that cause enhanced eutrophication, contrary to the expected results (Hoover et al. 2019).

Poultry litter often contains highly soluble P, which can lead to increased P concentrations in aquatic habitats following precipitation. However, Al-treated PL has demonstrated an ability to lessen the degree of P leaching. While all treatments produced higher concentrations than the controls, alum-treated manure exhibited a greater capacity to lower P solubility than raw litter or triple superphosphate (Delaune et al. 1995). The project used a computer model incorporating desired parameters, environmental statistics, and historical information for the results produced. Similar results were produced in the Blackland prairie near Waco, Texas under expanded parameters (Harmel et al. 2004). Incorporating litter as fertilizer into soil decreased watershed nutrient concentrations compared to top dressing, with PL being more effective than synthetic fertilizers. Nutrient levels of N and P decreased following the second year of application, although all fertilizer methods recorded increased nutrient levels than the control (Harmel et al. 2004).

Poultry litter is composed of manure, bedding material, liquid waste, excess food, and animal portions (e.g., feathers) from housed poultry (Shakya and Agarwal 2017). The United States alone produces 10 million metric tons of PL, with Alabama contributing 1.6 million tons (Kpomblekou-A et al. 2002). The application of PL has sparked legal disputes between the government and industry. For example, a decades-long legal battle between Oklahoma and Arkansas focuses on excess poultry industry nutrients entering the Illinois River and polluting Oklahoma (Panach et al. 2007). Poultry litter contains essential nutrients for plant growth, including macronutrients such as N, P, K, calcium (Ca), sulfur (S), and magnesium (Mg), and micronutrients like iron (Fe) and manganese (Mn) (Shakya and Agarwal 2017). Compared to other livestock manures, PL has higher levels of N and P, making it a valuable fertilizer; its N can be up to five times greater and P can be up to three times greater relative to other manure sources (Gollehon et al. 2001). However, raw litter typically contains near-even percentages of N, P, and K, which can cause an over-application of P and complete K requirements (Table 2.1; Gaskin et al. 2013). Poultry litter may also contain potentially hazardous elements, including Arsenic (As), Cadmium (Cd), and Lead (Pb), and potentially contain excess Ca, Mn, Zn, and Cu. Elevated environmental levels of these elements can bioaccumulate in organisms, including humans, potentially leading to adverse health effects (Cadet et al. 2012).

Concerns regarding disease transmission associated with PL and its products have garnered attention in recent years. Populations of heat-resilient *Salmonella* spp. increase as litter is stored and has a decrease in moisture and ammonia. This increase necessitates longer thermal treatments to eradicate these populations. Furthermore, the desiccation of PL provides an ideal habitat for *Salmonella*, facilitating population growth for heat-resistant variants(Chen et al. 2013; Chen et al. 2015). While NH_4^+ can inactivate *Salmonella*, the effectiveness diminishes as levels decrease during mineralization. For instance, studies have shown that 9-month-old litter required a minimum of an hour at 150 °C to exterminate *Salmonella* populations, whereas 75 °C could achieve similar results in fresher litter at 0 and 3 months (Chen et al. 2015). *Salmonella* poses a significant risk in poultry products, contributing to foodborne illness outbreaks in poultry products 30% of the time and in eggs 24% of the time, and is the predominant *Salmonella* reservoir (Gould et al. 2013). Typically, the infection occurs through exposure to fecal matter or within the reproductive system of infected animals. However, processes involved in converting PL into fertilizer, char, biogas, and other products often entail sufficient heat to eliminate potential pathogens present in raw litter. This uniform heat and chemical treatment, commonly employed in pelletized PL, possesses the capability to exterminate harmful pathogens (Chen and Jiang 2014). Studies have indicated that while populations of *Enterococcus* spp. may have existed in the soil prior to litter application, their total populations, species diversity, and resistance to antibiotics and multidrug resistance increased after application. Previously absent in soils, the application of PL has introduced *Enterococcus* spp., which is of particular concern. These species, which naturally reside in animal and human intestines, have the potential to cause infection in humans, prompting concern within the scientific community (Fatoba et al. 2021).

Digestion systems for PL management can be broadly categorized into dry and wet methods, with further subdivisions into aerobic and anaerobic processes. Each method has unique characteristics, advantages, and limitations, making them suitable for different applications (Shammas and Wang 2007a; Shapovalov et al. 2020). Dry digestion, often referred to as anaerobic or aerobic digestion, may be a misnomer in its terminology because water is included in the process, but not to a level that allows for fluidity of the matter. No consensus exists regarding the upper water content limit, and, as a result, thresholds are between 15% and 40%. Regardless, all forms of dry digestion require four to ten times less water than "wet" methods (Shapovalov et al. 2020). The aerobic digestion process relies on bacterial populations to complete the breakdown process (Manogaran et al. 2022). The optimal biomass pH for bacterial populations in digestion is between 6 and 7; if the environment becomes too acidic, bacterial activity is inhibited, potentially halting the digestion process (Matheri et al. 2017).

Anaerobic digestion comprises four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The process begins with hydrolysis or the breakdown of complex biomass into smaller particles. Acidogenesis follows hydrolysis, in which bacterial populations induce an acidic environment, often requiring hydrogen carbonate to mitigate acidic pH levels. Acetogenesis is next, leading to the formation of acetate, which is used in the final step of the process, methanogenesis. Methanogenesis creates methane from acetate, producing biogas (Matheri et al. 2017).

Continuous batch dry digestion operates within a singular vertical or horizontal drum, relying on plug flow to move older material through the digestive drum. Although these drums do not agitate the material, pumps and external mixers are used to induce the thermophilic (high-temperature) or mesophilic (medium-temperature) conditions in new material. This occurs by transferring part of the existing digestate to inoculate the new material (Rapport et al. 2008). In contrast, batch systems differ from the continuous method, as all material is loaded within a concrete box at onset under mesophilic conditions. The material then percolates through the porous bottom. Although the batch method requires a larger area, about ten times the size, and has an increased likelihood of clogging compared to the continuous version, but it is more cost-effective (Shapovalov et al. 2020). The higher temperatures of thermophilic reactions are more effective at reducing pathogen populations than mesophilic reactions, but at the cost of producing lower amounts of biogas (Bi et al. 2019).

Wet anaerobic digestion involves the generation of biogases within an anaerobic environment using the activity of specific groups of bacteria, such as methane-forming homoacetogenic and acetotrophic bacteria (Szuhaj et al. 2016; Kremp et al. 2018; Mutungwazi et al. 2020). The wet method requires a similar pH level to the dry method to facilitate the process. The setup requires an oxidation-reduction potential below 200 mV for the conversion to occur. Anaerobic digestion consists of four stages: fermentation or hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Manogaran et al. 2022). The steps for dry anaerobic digestion are described in greater detail in the above section. As in dry anaerobic digestion, bacteria are required to form the product. The employment of chemical pretreatments is common for both "dry" and "wet" to alleviate ammonia inhibition and toxicity to anaerobic organisms. The propensity for inhibition arises from the degradation of urea and amino acids into ammonia (Zahan and Othman 2019). Aerobic digestion is a process that operates in the same manner as anaerobic digestion, only in the presence of oxygen.

Aerobic digestion represents a relatively novel autothermal thermophilic process, with less research conducted than anaerobic digestion. The process is similar to anaerobic digestion, even dependent on microorganisms, but works in the presence of oxygen, unlike anaerobic synthesis (Zhang et al. 2022a). While the initial modeling was proposed in 1969, most research investigation aerobic digestion has been conducted in recent years (Kambhu and Andrews 1969; Shammas and Wang 2007b; Sridevi Dhanarani et al. 2016; Pugazhendhi et al. 2020; Zhang et al. 2022b; Rubežius et al.). The aerobic digestion process focused on in the study occurs in two steps: Acidification and ammonification. Initially, the first drum digests the litter via microbiology and acidic conditions into a cellular material (Fig. 2.1). Following this, the second drum undergoes a thermophilic reaction sanitizing and stabilizing the product into a digested sludge (Shammas and Wang 2007a). Proper mixing and adequate oxygen are imperative for proper sanitation to occur. The process exhibits potential for optimization, enabling large quantities to be produced by minimal staffing (Martín et al. 2018). The final product of aerobic digestion is considered an easier system to operate, producing a generally stable, odor-free final product. Similar to other processes discussed, aerobic digestion reaches the necessary temperatures to eliminate pathogens. Successful testing on pig waste demonstrated the process capable of reducing waste into a more compact product (Lee and Han 2016).

Cleaned & Green (C&G; Patent No: US 10,723,665 B1) is a novel poultry-derived granular fertilizer produced via an aerobic digestion process. In summary, raw litter is cleaned of metals and shredded in a knife mill, prior to being acidified with H_2S in a large drum. Following this, an ammonification step occurs to stabilize the product and increase the N ratio. Then, the product enters a fluidized cooling bed and becomes granularized. All water and excess material are recycled into the initial acidification drum.

The primary objective of this project is to thoroughly characterize the novel C&G fertilizer produced through aerobic digestion, assessing its potential for future commercialization. This comprehensive characterization involves evaluating both the physical properties and nutrient release profile of the fertilizer. Physical testing will include assessments of particle size distribution and hardness, with comparisons made against existing fertilizers to establish benchmarks and identify any unique advantages or limitations. Additionally, we will conduct detailed studies to monitor the nutrient release, involving laboratory tests, to compare its performance with current market-available products. By integrating these analyses, our aim is to evaluate the nutrient release characteristics and compare it with raw PL and commercially available rapid-release fertilizers.

2.2 Materials and Methods

Fertilizers of synthetic and poultry-derived origin underwent a variety of physical and chemical testing. The fertilizers utilized included: a synthetic, commercial-grade, fast-release fertilizer (Syn), poly-coated fertilizer, novel poultry-derived granular fertilizer produced by the company Cleaned & Green, and raw PL. Nutrient characterization of raw PL and C&G products from 2019 and 2023 are contained in Table 2.1. To measure nutrient release, soil and rapid water incubations characterize the discharge of nutrients over increasing time intervals, with both tests providing insight into whether a fertilizer is similar to a rapid or extended-release. The C&G fertilizer underwent chemical and physical analysis to further characterize the fertilizer product. Raw PL was supplied by the Charles C. Miller Jr. Poultry Research & Education Center. The synthetic fertilizer is a rapid-release fertilizer (Piedmont Fertilizer Company, Opelika, AL, USA). The final product undergoing testing is a polymer-coated, controlled-release fertilizer produced by Osmocote (ScottsMiracle-Grow, Marysville, Ohio, USA).

2.2.1 Physical testing

Physical testing was conducted to measure particle size distribution (PSD) and prill hardness on C&G 2019 & 2023, C&G 2023 Overs, and C&G 2023 Unders. While some particles can still pass through, the 2019 & 2023 particles were between the sieve sizes of 2 mm and 3.35 mm. The Unders were particles smaller than 2 mm and the Overs were greater than 3.35 mm. Particle size distribution and shape characterization utilizing a Camsizer P4. The Camsizer P4 relies on two cameras, one for larger particles and a zoomed lens for smaller ones, to capture and count the number of particles between 0.02 to 30 mm to gauge the PSD. The resulting images determine prill shape, size, and number (Dawson and Bureau 2021). For the scope of this work, PSD characteristics, such as median particle diameter and particle distribution breadth, which is measured as the difference between the 90th and 10th quartiles of the distribution, and particle shape characterization using sphericity as the metric to determine the prill's resemblance to a perfect sphere. Prill hardness was characterized utilizing a Chatillon MT Series manual tester equipped with a DFZ II force gauge following previously created methods (Wang et al. 1996; Felton et al. 1997). The Chatillon MT Series manual tester measured tensile strength by lowering a metal plate onto the force gauge and towards the prill at a speed of 2.5 mm per minute.

2.2.2 Chemical Analysis

Chemical analysis was conducted on PL, C&G 2019, and C&G 2023. Products from 2019 and 2023 were relatively consistent across major nutrient elements, 11.6-1-1.5 and 11.2-1-1.7 (N-P-K), respectively. Poultry litter had an even N-P-K ratio, 1.5-1-1.5, and similar levels of micronutrients. However, the PL had a pH of 6.8 compared to C&G's pH of 4.08, indicating C&G had a greater capacity to acidify substrate. Given the organic source of the fertilizer, testing protocols were followed to measure colony-forming units for C&G and PL (Gutierrez and Schneider 2022). In summary, samples were passed through a brass sieve to remove large clumps and feathers. The samples' microflora were enumerated, and portions were sterilized through irradiation. The samples were nutrient enriched and adjusted for pH and water activity, and sampled over six days to monitor bacterial populations, pH, and total ammonia N (TAN). Additionally, separate testing for the presence of *Salmonella* occurred (Dunn et al. 2022) In summary, thirty grams of litter from each sample were combined with 270 mL of buffered peptone water (BPW) in sterile Whirl-Pak® bags, shaken for one minute, and incubated at 37°C. For the polymerase chain reaction (PCR), a positive (*Salmonella enterica*) and negative (*Escherichia coli*) were used. After incubation, samples were transferred to Rappaport-Vassiliadis (RV) and Tetrathionate (TT) broths, incubated, and then streaked onto Xylose Lysine Tergitol-4 (XLT-4) plates for further incubation and PCR confirmation of *Salmonella*.

2.2.3 Incubation Test

Water-based incubation assessments were conducted to determine the nutrient release patterns of a novel, PL-derived fertilizer, C&G (11N–1P–1K–15S), a polymeric resin-coated fertilizer (17-5-11; Osmocote, Scotts Miracle-Gro, Marysville, OH), and a synthetic fertilizer (herein referred to as "Syn") blended to contain similar nutrient values (Table 2.2). One gram of each fertilizer product was added to 100 mL DI water (n=3). Solutions were stirred at 60 rpm and maintained a temp of 20 C. Testing procedures were conducted using Cancellier et al. (2018) methodology; electrical conductivity measurements were taken with a calibrated meter at room temperature. For the release test, three replicates of each fertilizer at the same rate were incubated in deionized water at room temperature. Samples underwent constant mixing and were periodically tested for EC after equilibration and homogenization. The measurements were taken at 1, 3, 5, 10, 15, 20, 30, 60, 120, 240, 360, and 1440 minutes to monitor the dissolution and ion release dynamics of the fertilizers.

Soil-based incubation tests were performed with clay soil, from Auburn University, AL with a pH ranging from 6.3 to 6.5. The soil was homogenized by drying, pulverizing, and sieving to exclude particles >2 mm. Soil preparation was conducted in a similar manner to Keeney and Bremner (1966). Incubation jars (n=80) were filled with 100 g of dry soil. Cleaned & Green, Syn, and PL were incorporated at a rate of 0.89 kg m^3 in half of the jars. Soil samples were maintained at a volumetric water content of 0.3 cm^3/cm^3 and 30 C. On each sample date, four replications per treatment (C&G, PL, Syn, and Control) were removed, air-dried, homogenized, and partitioned for nutrient extraction. Ammonium and nitrate concentrations were determined using 2M KCL extraction, fluorimetry, and colorimetry on a multimode plate reader (Whitehead 1981). Total P and K were determined using a Mehlich 1 extraction (Mehlich 1953).

Soil & water-based nutrient release data were analyzed via PROC Reg procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Effects of fertilizer, rate, and the fertilzer*rate interaction on dry weight and foliar nutrient concentrations were analyzed via ANOVA with the PROC Glimmix procedure. Means were separated using Tukey's honest significant difference (HSD) at a 5% alpha level.

2.3 Results & Discussion

2.3.1 Physical Characteristics

Particle size distributions between the 2019 and 2023 batches did not differ significantly at 2.0 mm and 2.8 mm median particle size, respectively (P = 0.3076). These differences are likely more reflective of the post-production screen processes, which can be easily altered. The process yielded little dust and highly spherical particles (0.737-0.775). For C&G 2019, the median particle size was 2 mm, the size distribution ranged between 1.5 – 2.6 mm, and the mean sphericity was 0.752 (Fig. 2.2a). For C&G 2023, the median particle size was 2.8 mm, the size distribution ranged between 2 – 3.5 mm, and the mean sphericity was 0.731 (Fig. 2.2b). The Overs had a median particle size of 5.2 mm, the size distribution range was 3.8 – 6.3 mm, and the mean sphericity was 0.737 (Fig. 2.2c). The unders had a median particle size of 1.8 mm with a size distribution range of 1.4 – 2.2 mm, and a mean sphericity of 0.775 (Fig. 2.2d).

The prill hardness for the 2019 and 2023 products was not significantly different; however, the 2019 product was numerically higher ($P = 0.3216$). The Overs recorded the greatest hardness and were considerably harder than other C&G products ($P = 0.0057$). The unders were the weakest material and were significantly weaker than C&G 2023 and 2019 products ($P = 0.0042$). The difference in hardness between years may be attributed to different feedstocks for the products. Crush strength results demonstrated crush forces were low. For reference, a typical fertilizer prill will crush under 3.3-5.5 lbs of pressure. Monoammonium phosphate and ammonium sulfate may withstand pressures of 3.3-6.6 lbs (Fulton and Port 2016). In its current state, the material would likely not withstand normal plant handling. Different manufacturing processes may be deployed to improve prill stability.

2.3.2 Nutrient Release Tests

2.3.2.1 Rapid Water

In rapid water release incubation, Syn released nutrients both rapidly and in the greatest quantities compared to other fertilizers (Fig. 2.3). All three fertilizers were significantly different from each other ($P < 0.0001$). Electric conductivity was affected by a fertilizer*time interaction ($P < 0.0001$). Within 10 minutes, Syn released 95% of the recorded nutrients, and the resulting final EC was 8.83 ± 0.61 mS/cm. Beyond 3 minutes, no significant increase in EC occurred for Syn ($P = 0.1849$). In comparison, C&G released 82% of recorded nutrients within the same timeframe, and C&G recorded a final EC of 7.80 ± 0.21 mS/cm. Beyond 20 minutes, no difference in EC occurred for C&G (*P* = 0.6829). Little to no change in EC was recorded in the resin-coated product after 24 hrs. This was to be expected given that the polymer coating slows the release of the product over time. The poly-coated fertilizer recorded a final EC of 0.88 ± 0.72 mS/cm. Despite soaking for 24 hours, the ECs of the poly-coated fertilizer were not different (*P* = 0.0547). These release characteristics suggest C&G performs similarly to the Syn product, however, C&G's release does occur over a more extended period of time.

The results indicate distinct nutrient release patterns among the three fertilizers tested. The Syn fertilizer exhibited a rapid nutrient release, with a majority of nutrient release occurring within the first 10 minutes. The Syn fertilizer behaved in a similar manner to "rapid-release" fertilizers, which are designed to be highly soluble in water. However, such rapid solubility may pose undesired risks, including nutrient burning of plant tissues or environmental pollution in eutrophication if misused (Schindler 1974; Finlay et al. 2013; Landschoot 2016). In contrast, the poly-coated displayed a significantly slower nutrient release and had a minimal release, which was observed after 24 hours. The slowed release is consistent with the known mechanisms behind poly-coated fertilizers, where water must penetrate the coating to initiate the release of nutrients. This "burst" effect occurs from the breakdown of the polymer prills due to diffusive capabilities (Shavit et al. 1997a). For the mechanism to work, the coating must partially degrade to initiate the process, significantly limiting initial release and ensuring a more gradual nutrient release over time (Shaviv et al. 2003). Interestingly, the C&G fertilizer displayed nutrient release in a manner intermediate to a rapid-release fertilizer. While C&G released nutrients more rapidly than a poly-coated fertilizer, C&G did not match the same rapidity or final concentration of the commercial-grade Syn fertilizer. Such behavior could prove advantageous for providing a quick nutrient supply with lessened risks commonly associated with rapid-release fertilizer. Continued analysis of C&G chemical breakdown and nutrient release within soils is necessary to understand the practical utilization of the product.

2.3.2.2 Soil Incubation

Differences were recorded in most additional major and minor elements between C&G 2019 and C&G 2023, potentially resulting from varying feedstock sources. Depending on the feedstock, C&G could have significant added value as a source of micronutrients. A major discrepancy was observed with S concentrations between the 2019 and 2023 samples. However, the low S content in 2019 C&G is believed to be a clinical error. The C&G process increased nutrient concentrations of the source material (AU PL), except for Ca. Both sources recorded similar bacterial population levels, which are essential for N mineralization (Cabello et al. 2019). Both C&G products and raw PL tested negative for the presence of *Salmonella* **(**Table 2.3**)**.

2.3.2.2.1 Nitrogen

Soil incubation data revealed distinct trends in the concentrations of ammonium (NH_4^+), nitrate (NO_3^-), and total N by fertilizer type (Fig. 2.4). Soil ammonium concentrations were affected by treatment, day, and a treatment*day interaction (Table 2.4). In the background levels, C&G, and PL treatments quadratic trends were observed over time. The background levels and PL experienced a negative, decreasing trend. The C&G trend experienced a positive, increasing trend. The Syn treatment did not experience any trends over time and had a daily mean of 85 ± 0.25 ppm, with a range of 84 ppm to 85 ppm. Background NH_4^+ concentrations were initially 14 ± 2.04 ppm, peaking on Day 6 with a mean of 16 ± 2.83 ppm. After Day 6, background NH_4^+ began to slowly decline in untreated soils, with a final mean concentration of 6 ± 0.41 ppm by Day 55, a 63% decline. Initially, C&G released NH_4^+ at 65 ± 5.97 ppm, peaking on Day 45 at

132 ± 10.01 ppm, suggesting active mineralization of organic N into ammonium. Between Day 35 and Day 45, NH_4^+ mean concentrations rose 40% in soils treated with C&G. Following C&G peak NH_4^+ concentrations, NH_4^+ gradually declined, reaching a mean of 119 ± 9 ppm by Day 55. In contrast, PL released an initial NH_4^+ concentration of 81 ± 2.34 ppm, a continuously declined through Day 55. By Day 55, NH_4 concentrations had dropped to a mean of 6 ± 0.36 ppm, representing a 92% decline. Synthetic had an initial release of NH_4^+ at 85 ± 0.25 ppm, with mean NH_4^+ concentration levels remaining at 85 ± 0.15 ppm through Day 55. Cleaned & Green was the only nutrient source that had a positive trend in NH_4^+ concentrations through Day 55. Unlike C&G, neither Syn nor PL experienced a doubling of $NH4^+$.

In concurrence with the rapid water release test, the soil-based incubation tests indicate C&G fertilizer exhibits similar release characteristics to a synthetic fertilizer, a quick-release fertilizer. However, C&G exhibited a delayed release of NH_4^+, which is typically associated with slow-release fertilizers. Although there was an initial release of NH_4^+, a significant rise in mean concentrations 40% occurred between Day 35 and Day 45. Neither Syn nor PL increased ammonium levels over 55 Days. Poultry litter NH_4^+ concentrations decreased over 55 days possibly due to volatilization or consumption from microorganisms (Calderón et al. 2005; Feng et al. 2023). The extended-release of NH_4^+ C&G could be attributed to microbial activity, but this notion requires further investigation given the diverging results from PL. Investigations into field applications of raw PL have concluded that a significant portion of N may volatize, leading to nutritional deficiencies in crops (Sharpe et al. 2004). Volatilization is more likely to occur in soils with a higher pH. In practice, injecting PL into the soil rather than surface application can reduce volatilization rates by limiting PL interactions with the atmosphere (Kulesza et al. 2014). Such methods have shown increased N concentrations in soils. As expected from an inorganic rapid-release fertilizer, the Syn product experienced no change over time, indicating no reliance on bacterial populations for nutrient release (Landschoot 2016).

Nitrate was affected by fertilizer type, day, and a fertilizer*day interaction ($P < 0.0001$). All fertilizers released a significant concentration of NO_3^- from the background levels ($P < 0.0001$). The C&G fertilizer significantly differed from all other fertilizer treatments ($P = 0.0051$). Synthetic and PL applications affected NO_3^- concentrations similarly ($P = 0.0521$). Synthetic and PL demonstrated quadratic trends in NO_3^- availability over time (Fig. 2.5). Soils supplied with C&G treatment increased NO_3^- concentrations linearly over time. In contrast to trends in NH_4^+, NO_3^- concentrations for all treatments exhibited a delayed response, increasing notably between Day 15 and Day 55 (Fig. 2.5). Background NO_3^- concentrations on Day 0 were 13 ± 3 ppm, with mean concentrations peaking on Day 55 at 92 ± 8.23 ppm, an 86% increase in NO_3^- concentrations. Initially, NO_3^- concentrations in soils supplied with C&G were 9 ± 1.78 ppm, peaking on Day 55 with a mean concentration of 189 ± 20.76 ppm, a 2000% increase. Between Day 15 and Day 25, NO_3^- concentrations from C&G rose from 29 ± 3.44 ppm to 89 ± 7.33 ppm on average, an increase of 207%. Similarly, PL released NO_3^-at a mean concentration of 9 ± 1.85 ppm on Day 0, peaking on Day 55 with a mean concentration of 189 ± 31.16 ppm. Between Day 15 and Day 25, NO_3^- concentrations rose from a mean of 46 ± 2.91 ppm to 130 ± 7.05 ppm, an increase of 183%. In contrast, NO_3^- levels in soils supplied with Syn had a mean concentration of 11 ± 1.67 ppm on Day 0, peaking on Day 55 with a mean concentration of 264 ± 32.72 ppm, a 2300% increase. Between Day 15 and Day 25, NO_3^- concentrations rose from a mean of 35 ± 4.21 ppm to 86 ± 3.18 ppm, an increase of 146%. Poultry litter and C&G had similar release rates, nearly matching, over 55 days. Rather than the fertilizers simply “releasing” NO_3^-, bacteria-induced nitrification may be partially responsible for the decrease in NH_4^+ in PL as it is converted into NO_3^-. While only PL experienced a decrease in NH_4^+, the increase in NO_3^- may occur within soils all four fertilization treatments, causing an increase of NO_3^- even in the control soil (Arp and Stein 2003).

Similar to trends observed in NH_4^+, C&G NO_3^- concentrations climbed rapidly after Day 15. The C&G product released NO_3^- consistent with a slow-release fertilizer, exhibiting a linear increase over 55 days. This contrasts with studies in biochar that reported a decline over time in nitrate release, both had continued increases in concentrations (Gwenzi et al. 2017). Both PL and Syn released NO_3^- in a similar manner to C&G but at higher concentrations. Microbial comminutes can be inactive in the presence of low soil pH, resulting in decreased N fixation. However, N-fixing bacteria involved with nitrification can successfully fix N under acidic conditions. For example, archaebacteria can carry out N fixation with a soil pH as low as 3 (Mintie et al. 2003; Booth et al. 2005). Although the soil pH for C&G-incorporated soils was lower than PL-incorporated soils, pH difference alone would likely not inhibit bacterial processes affecting nitrification. The linear increase in NO_3^- concentrations from C&G suggests a gradual and sustained release of N resembling a slow or controlled-release fertilizer. An extended-release fertilizer reduces leaching capacity, avoiding the waste of valuable nutrients (Paramasivam and Alva 1997). Further research is needed to understand why C&G acts differently than PL and what implications it has for growing crops.

All fertilizer products significantly increased total N relative to background levels ($P < 0.0001$) and showed a positive trend through the 55-day trial (Fig. 2.6). Background N concentrations were 26 ± 4.2 ppm on Day 0, with mean concentrations peaking on Day 55 at 98 ± 8.47 ppm, a 277% increase in N concentrations. By Day 55, NO_3^- accounted for 94% of total N in untreated soils. Total N levels in C&G were 74 ± 6.37 ppm at Day 0, peaking on Day 55 with a mean concentration of 308 ± 26.85 ppm. Between Day 15 and Day 25, C&G total N concentrations rose from a mean of 114 ± 4.1 ppm to 175 ± 6.78 ppm, a 54% increase. By Day 55, NO_3^- accounted for 61% of total N from C&G applied soils. In contrast, total N concentrations from PL were relatively higher at 90 ± 3.63 ppm on Day 0, peaking on Day 55 with a mean concentration of 194 ± 31.41 ppm. Between Day 15 and Day 25, total N concentrations rose from 87 ± 5.31 ppm to 155 ± 7.21 ppm, an increase of 78%. By Day 55, NO_3^- accounted for 97% of the total N released from PL. Total N concentrations from Syn were 95 ± 1.52 ppm on average at Day 0, peaking on Day 55 with a mean concentration of 348 ± 32.74 ppm. Between Day 15 and Day 25, total N concentrations rose from a mean of 120 ± 4.23 ppm to 170 ± 3.27 ppm, an increase of 42%. From Day 25 to Day 55, total N levels in Syn-applied soils steadily increased, reaching a mean concentration of 348 ± 32.74 ppm, a 266% increase from Day 0. By Day 55, NO_3^- accounted for 76% of the total N released by Syn.

All forms of N were affected by fertilizer type, day, and a fertilizer*day interaction (P = 0.0001). Over the course of the incubation period, a pattern developed indicating a transition from ammonium-dominated release to an accumulation of nitrate due to nitrification, where NH_4^+ is converted to NO_3^-. Although differences in speciation were present, total N concentrations continued a relatively consistent positive trend throughout the incubation period. For PL, net mineralization was significantly less than C&G and Syn, indicating nitrification processes were balanced by N losses or immobilization. For C&G and Syn, positive quadratic trends in total N were observed over time (Fig. 2.6). A quadratic trend was observed in background levels of total N,, which reached a maximum at Day 45 before decreasing through Day 55. Synthetic, C&G, and PL had mineralization rates of 42%, 35%, and 25, respectively.

Ammonium dominated the initial N release from C&G, while nitrate gradually became the main form of N as time progressed. Poultry litter exhibited rapid release of N in the form of NH_4^+ followed by a continued decrease in NH_4^+. This decrease was not fully compensated by nitrate release, resulting in PL having the lowest final TN among fertilizer treatments, excluding the control. Decreased TN for PL has been similarly reported in other studies where N was not released to the expected levels (Abbasi et al. 2007). The Syn fertilizer also followed a similar trend of releasing a significant amount of NH_4^+ species at the onset. However, NO_3^- steadily increased in total release. By the conclusion of the incubation period, all fertilizer treatments resulted in higher total N soil levels compared to the control. The initial dominance of NH_4^+ for C&G and PL suggests N undergoing a rapid mineralization process for organic N, which is quickly followed by rapid nitrification, converting NH_4^+ to NO_3^-. Similar results have been reported following PL applications between 45 to 60 days, often followed by a decrease in N concentration (Azeez and Van Averbeke 2010; Baitilwake et al. 2012). However, the prolonged presence of NH_4^+ in C&G-incorporated soils hints at a more complex relationship with N.

Additionally, it is essential to emphasize the differing release mechanisms for each fertilizer. The Syn fertilizer, characterized by rapid-release properties and a lack of microbial dependence, demonstrated an immediate release and supply of N in both forms evaluated before stabilizing in NH_4^+ (Shavit et al. 1997b; Catanzaro et al. 1998; Li et al. 2022). In contrast, PL total N release was dominated byNH_4^+, followed by a gradual loss of total N, potentially from volatilization. Cleaned & Green exhibited the unique characteristic of prolonged increased release of both NH_4^+ and NO_3^-. The intermediate behavior of C&G, providing both an initial burst and a sustained release, could be particularly advantageous in managing nutrient availability and minimizing losses due to volatilization or leaching. Further studies are needed to explore the interactions between soil properties, microbial activity, and fertilizer formulations to optimize their effectiveness in different agricultural settings.

3.2.3.2.2 Phosphorus

Phosphorus was affected by fertilizer type ($P < 0.0001$), time ($P < 0.0045$), and a treatment*day interaction ($P < 0.0001$). Background levels of P exhibited a minima quadratic trend over time (Fig. 2.7). In untreated soils, concentrations of P decreased, reaching a minimum at Day 15, before increasing through Day 55. The PL, C&G, or Syn treatments had no observed P release trends over time (Fig. 2.7). Background P concentrations were highest at Day 0 with a mean of 60 ± 2.04 ppm. After Day 0, background P concentrations began a slow decline of 15% to 50 ± 16.49 ppm by Day 55. Cleaned & Green P levels were 49 ± 7.19 ppm on Day 0, peaking on Day 6 with a concentration mean of 59 ± 7.18 ppm. By Day 55, P had slightly declined to 57 ± 9.61 ppm. Poultry litter P concentrations were 164 ± 25.32 ppm on Day 0, peaking on Day 25 with a mean P concentration of 178 ± 22.09 before declining to 143 ± 14.34 ppm by Day 55. Poultry litter was the only fertilizer product to increase mean soil-extractable P. Synthetic P concentrations were 55 ± 8.85 ppm at Day 0, with mean P concentration levels declining to 49 ± 2.34 ppm by Day 55, a 9% decline.

Mineralization rates of P were low from these nutrient sources. Only 9% of the P applied with C&G was recovered. Synthetic and PL P concentrations indicated a recovery of only 1% and 23% of P applied, respectively. Due to the differences in nutrient ratios, PL was expected to result in higher P concentrations than Syn or C&G since application rates were determined by lbs. of N. Across all recorded nutrients, P concentrations were the lowest. While soil pH can affect P readings in Mehlich 1 tests, soil pH testing concluded the test soils were within an acceptable range for accurate readings. Throughout the incubation period, no sample recorded a pH greater than 6.5, with PL-treated soils recording the highest pH among treatments. Testing in calcareous soils can cause decreased P recovery by reducing reactivity by the Mehlich 1 extract (Novais et al. 2015; Gamble and Mitchell 2018; Medeiros et al. 2021). Phosphorous recovery may be limited by excess aluminum and iron ions from metal precipitation, affecting P reactivity to testing (Prasad and Chakraborty 2019; Yi et al. 2023). Despite being lower than concentrations of K, soil P concentrations were still rated "high" to "very high" by the Alabama Cooperative Extension System (Prasad and Chakraborty 2022). The risk of eutrophication is greater with orthophosphate species due to their high bioavailability. Ideally, fertilizers would produce inorganic particulate P to minimize runoff and eutrophication risks (Carpenter 2008; Kovar and Pierzynski 2009; Daniel et al.). However, most common fertilizer sources rely on orthophosphate forms. Slow-release fertilizers are preferred for gradual P release, reducing the immediate risk of runoff (Shaviv 2001). Poultry litter contains orthophosphates, condensed phosphates that can hydrolyze into orthophosphate, and organic P such as phytate (Sims and Wolf 1994). The total P concentrations recovered from the soil samples were deemed insufficient against background levels, and speciation was not conducted.

Similar studies investigating raw PL and poultry-derived products have exhibited comparable release trends (Gwenzi et al. 2017; Piash et al. 2022; Feng et al. 2023). Studies on poultry-biochar have demonstrated a gradual release of P over a 12-day period, with C&G performing similarly to raw PL (Piash et al. 2022). However, C&G is produced under aerobic respiration rather than the pyrolysis processes utilized in these studies, which may account for the differences in release patterns. Another study investigating non-poultry-based biochar reported similar results where P was released rapidly during the initial 12 days of the incubation (Gwenzi et al. 2017). Both soil sorption capacity and the ability of P to form complexes with other soil nutrients have been proposed for observed stable conditions (Peng et al. 2020; Feng et al. 2023). In conclusion, the P release characteristics of C&G did not align with the properties of PL.

3.2.3.2.3 Potassium

Potassium concentrations were affected by fertilizer type, time, and a treatment*day interaction ($P < 0.001$). Background levels exhibited no release trends over time with a mean concentration of K of 89 ± 3.1 ppm (Fig. 2.8). Potassium was immediately released for all treatments with concentrations remaining generally stable for the duration of the study. Cleaned & Green exhibited a maxima quadratic trend for P release over 55 days. C&G K concentrations were 129 ± 7.67 ppm on Day 0, peaking on Day 25 with a concentration mean of 163 ± 9.30 ppm. By Day 55, C&G K concentrations experienced minor declines to 152 ± 0.22 ppm, an 18% increase from Day 0. In PL, a negative linear trend in soil extractable K was observed over time. PL quickly released K at a mean of 591 ± 85.78 ppm on Day 0. By Day 55, K concentrations had declined to a mean of 451 ± 14.38 ppm, a 24% decline from the peak. A negative linear trend in soil extractable K was also observed from applications of Syn. Synthetic released K at a mean concentration of 135 ± 12.34 ppm on Day 0, which quickly peaked to 157 ± 23.52 by Day 2. Syn mean K concentrations declined slowly to 139 ± 12.14 ppm by Day 55, a 3% increase from Day 0. Ninety-one percent of K supplied by C&G was recovered, the highest of all three fertilizer products. Of the K supplied by Syn and PL, 67% and 81% was recovered, respectively. Applications of PL recorded higher K concentration than N concentration despite both being applied in similar quantities.

While P and K were applied at similar rates within their respective products, their releases and availability in products like C&G differed significantly. Trends in K availability from C&G are similar to investigations of PL release characteristics for K (Hirzel et al. 2010). For C&G and Syn, soil K concentrations ranged from "medium" to "high", dependent on crop requirements. In comparison, K concentrations for PL were "very high" (Patterson 2020). These findings are consistent with similar incubation tests where K concentrations remained relatively stable throughout the study (Eckhardt et al. 2018; Feng et al. 2023). Despite this stability, Syn treatments consistently recorded the lowest ppm levels for both P and K, while PL recorded the highest release concentrations for both nutrients. Poultry litter, on the other hand, naturally contains elevated levels of both P and K, given the N-P-K ratio in PL is more balanced than most commercial fertilizers. However, such P and K potency can lead to over-application for both nutrients when applied at N rates for crops. In conclusion, the K release characteristics closely resemble P release behavior for C&G, offering a more nutrient-rich option than traditional Syn fertilizers. Further research should include application and nutrient management strategies optimization across varying soil types.

3.4 Conclusions

Under rapid-release water incubation tests, C&G behaved similarly to a commercial synthetic fertilizer. However, during extended soil incubation, C&G exhibited properties resembling both rapid and slow-release fertilizers. While a majority of P and K were released at initiation, N continued to release over the 55-day incubation period, with both NH_4^+ and NO_3^- concentrations increasing over time. Different soil tests of C&G applied may record differing P concentrations as the soil had a high P retention capacity, however such additions should remain less than PL. Ammonium had a greater initial release while NO_3^- was initially lower and increased linearly over time. The similar increase in NO^- experienced in PL and C&G may have been the result of bacteria-induced nitrification.

Cleaned & Green fertilizer lessens the potential environmental consequences of N release by extending the release of total N in comparison to raw PL. The C&G product released similar levels of total N, P, and K to a commercial Syn fertilizer blended at a similar nutrient ratio. Both PL and Syn did release higher levels of NO_3^- than C&G but had lower concentrations in NH_4^+ by Day 55. Additionally, C&G could serve as a replacement for raw PL, providing similar micronutrient levels and higher N release, without the over-application of P. This dual functionality makes C&G a versatile and environmentally friendly option for agricultural applications.

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Table 2.1. Chemical analysis on poultry derived fertilizers 1) raw poultry litter sourced from the Auburn University poultry facility and 2) aerobically digested Cleaned and Green (C&G) fertilizer products from 2019 & 2023.

Parameter	**Content on Dry Matter Basis**		
Source Material	**Raw Poultry Litter**	**C&G 2019**	**C&G 2023**
Ash (%)	54.3	23.0	
Carbon (%)	45.67	13.4	
Sulfur (%)	7.56	1.85	15.0
Nitrogen (%)	1.43	11.6	11.42
Phosphorus (%)	0.72	0.70	0.91
Potassium (%)	1.32	1.25	1.46
Calcium (%)	11.63	1.47	5.25
Magnesium (%)	0.31	0.33	0.35
Aluminum (ppm)	531	1190	216
Boron (ppm)	20	9	32
Copper (ppm)	32	77	50
Iron (ppm)	460	600	842
Manganese (ppm)	262	258	384
Sodium (ppm)	7490	8327	6825
Zinc (ppm)	219	3949	286
pH	6.8	4.08	
Per cent (%) multiplied by 20 equals pounds per ton. Parts per million (ppm) multiplied by 0.002 equals pounds per ton.			

Table 2.2. Nutrient comparison chart for fertilizers utilized in during nutrient release tests.

Fertilizer Nutrient Comparison				
Fertilizer Type	Nitrogen (%)	Phosphorous (%)	Potassium (%)	Source
Raw Chicken Litter	1.3	1	1.5	Auburn Soil Testing Lab
Cleaned & Green Granular	11.59	1	1.5	Auburn Soil Testing Lab
[1]Synthetic Fertilizer	21	20	20	Peafowl Fertilizer
Poly-Coated Fertilizer	17	5	11	Osmocote

[1] Synthetic fertilizer was weighed out to match the Cleaned & Green nutrient ratio prior to study.

Table 2.3. Bacterial colony forming units for poultry-derived fertilizer products. Testing followed methods set by Gutierrez and Schneider (2022).

	Sample	
	AU Poultry Litter	**C&G 2023**
Mean[1]	5.83	5.94
Std Dev.	±0.25	±0.10

[1]All means reported are the log colony forming units per gram of material

Table 2.4 Analysis of variance (ANOVA) for the effects of fertilizer treatments on soil nutrient concentrations for the studied nutrients.[z]

		P-values				
Source of variability	df[y]	NH_4^+	NO_3^-	Total N	Total P	Total K
A: Fertilizer	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
B: Time	9	<0.0001	<0.0001	<0.0001	0.0045	<0.0001
A × B	26	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[z]Treatment effects were analyzed using PROC Glimmix in SAS 9.4 (SAS Institute, Cary, NC, USA).
[y]df = degrees of freedom

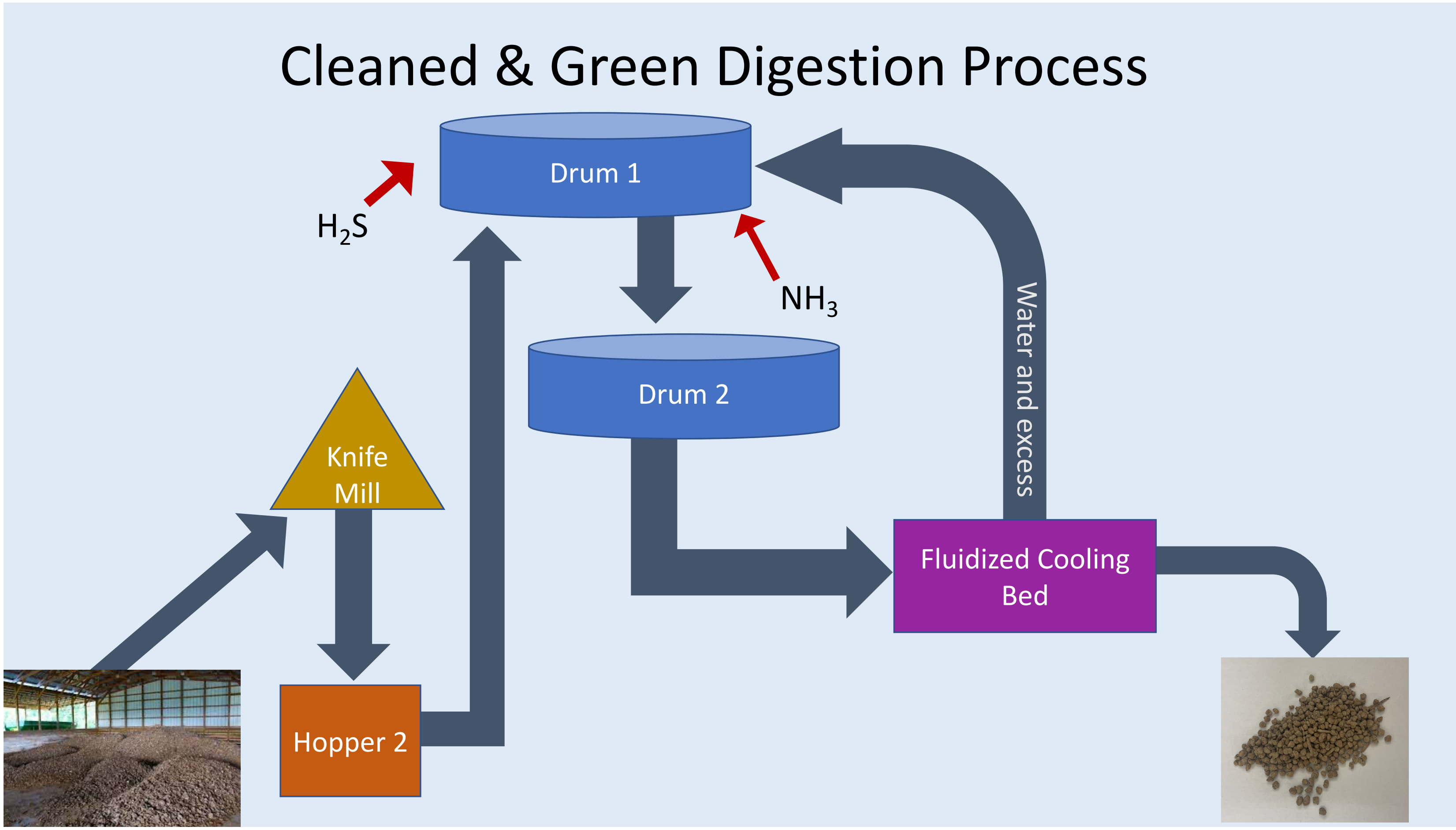

Fig. 2.1. Simplified process of aerobic digestion proposed by Alabama company Cleaned and Green (C&G). Raw poultry litter undergoes multiple steps of acidification and ammonification prior to being granularized. The final product being an odor-free, pathogen-free product. Image created by Austin Lindquist.

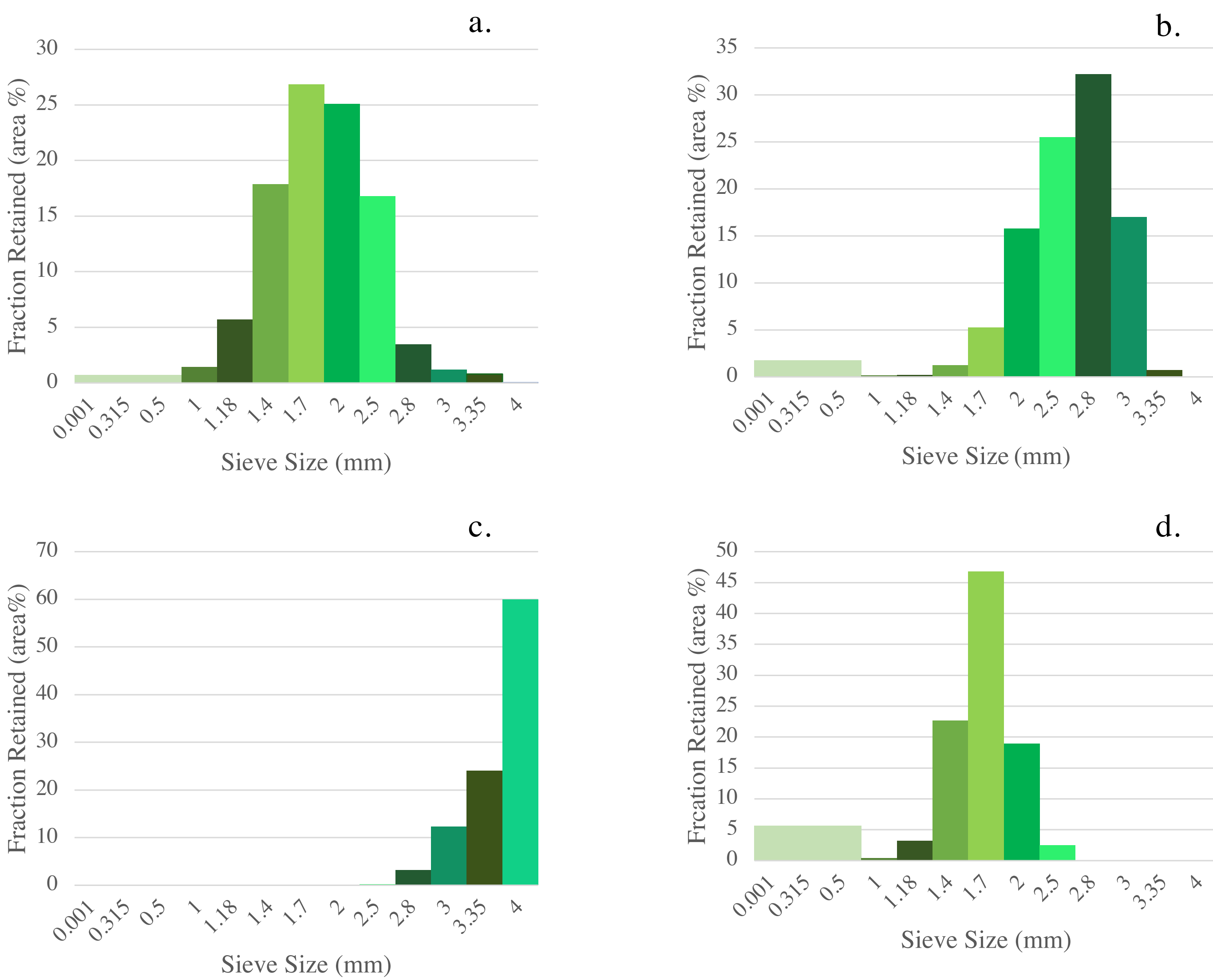

Fig. 2.2. Comparison of the particle size distribution (mm) between years and Cleaned & Green (C&G) source material: (a) C&G 2019, (b) C&G 2023, (c) C&G 2023 Overs, (d) C&G 2023 Unders.

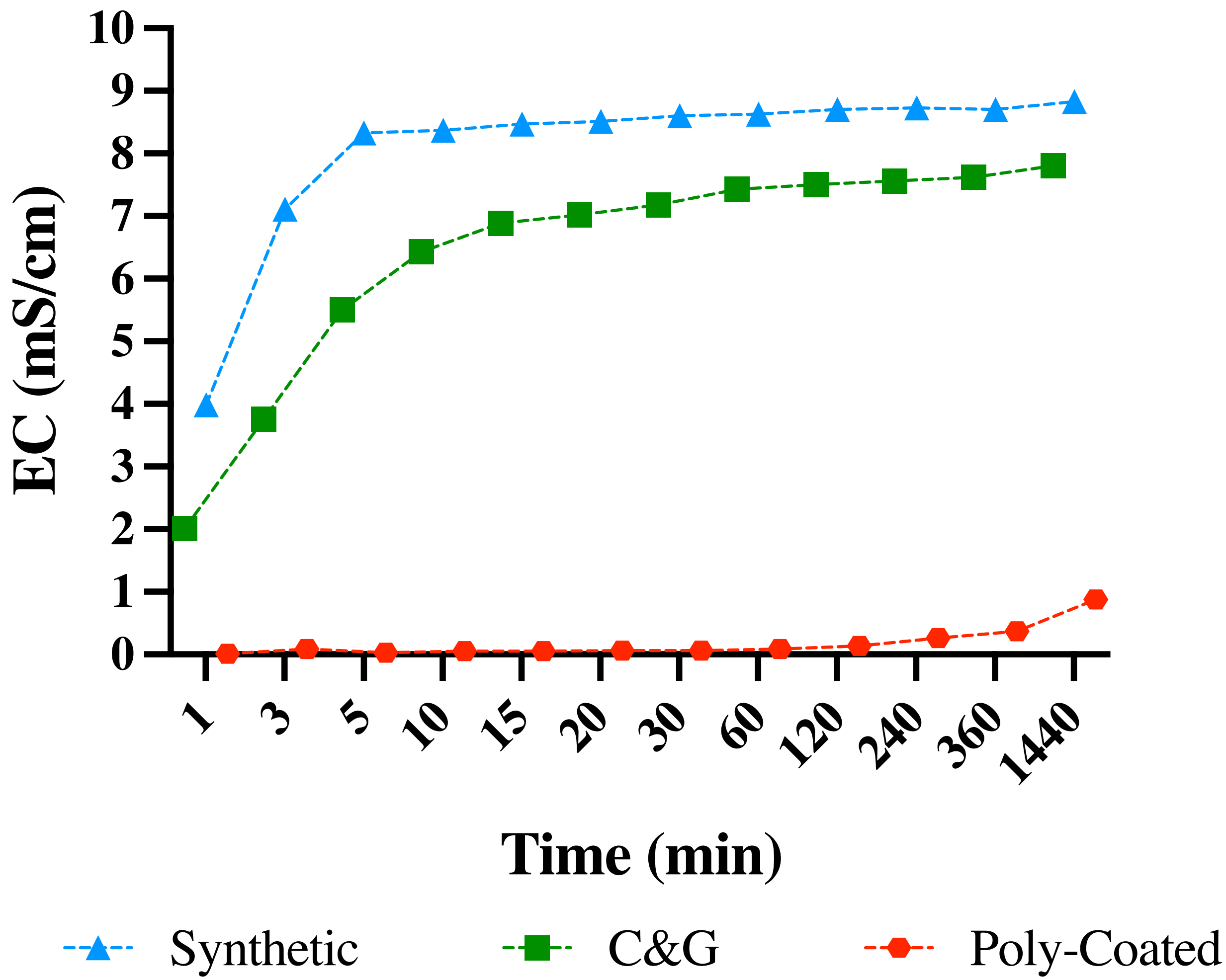

Fig. 2.3. Nutrient release rates from water incubation, determined by electrical conductivity (EC) of fertilizers over a 24 hour period. Fertilizers were applied at a nitrogen rate of 1 gram per 100 ml of deionized water and underwent constant mixing. Fertilizer sources include a) novel poultry litter-derived fertilizer (C&G), b) synthetic granular fertilizer (Synthetic), and c) resin-coated fertilizer (Coated).

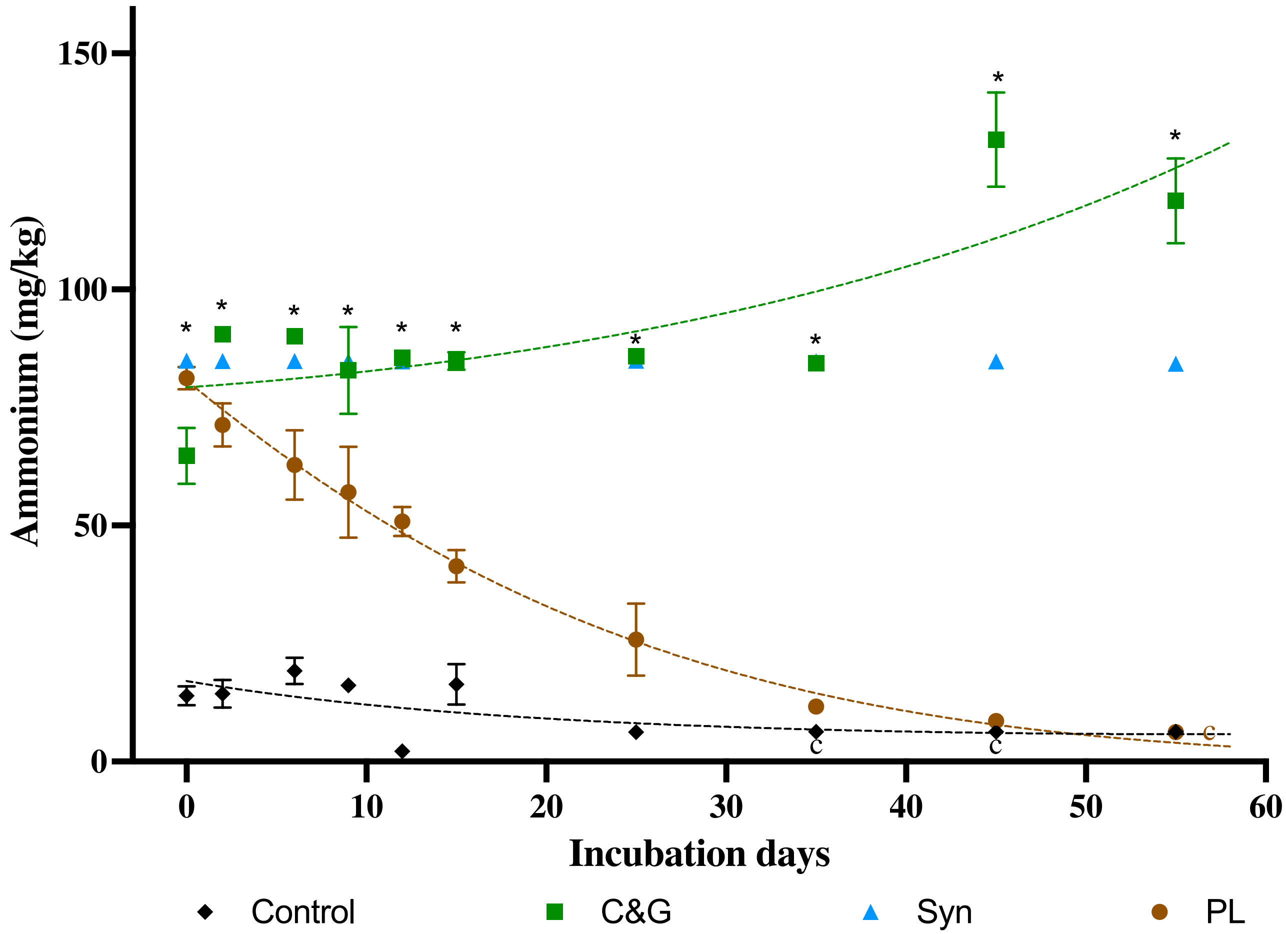

Fig. 2.4. Ammonium concentration within soil over the course of 55 days. The fertilizer nitrogen rate is 0.89 kg m^3 added to the soil. Fertilizer sources include a) control (soil), b) novel poultry litter-derived fertilizer (C&G), c) poultry litter (PL), and d) commercial synthetic fertilizer (Syn). The control did not receive any fertilizer application. The ammonium release followed either a linear (L) or quadratic (Q) response as C&G ($Y = 80.238 + 0.09248\ x + 0.01327\ x^2$, Trend: Q***, R^2: 0.61), the control ($Y = 16.296 – 0.444\ x - 0.0004\ x^2$), and PL ($20.714 + 3.458\ x -0.04\ x^2$, Trend: Q***, R^2: 0.97). * represent significant difference between the treatments for a given date at P<0.05.

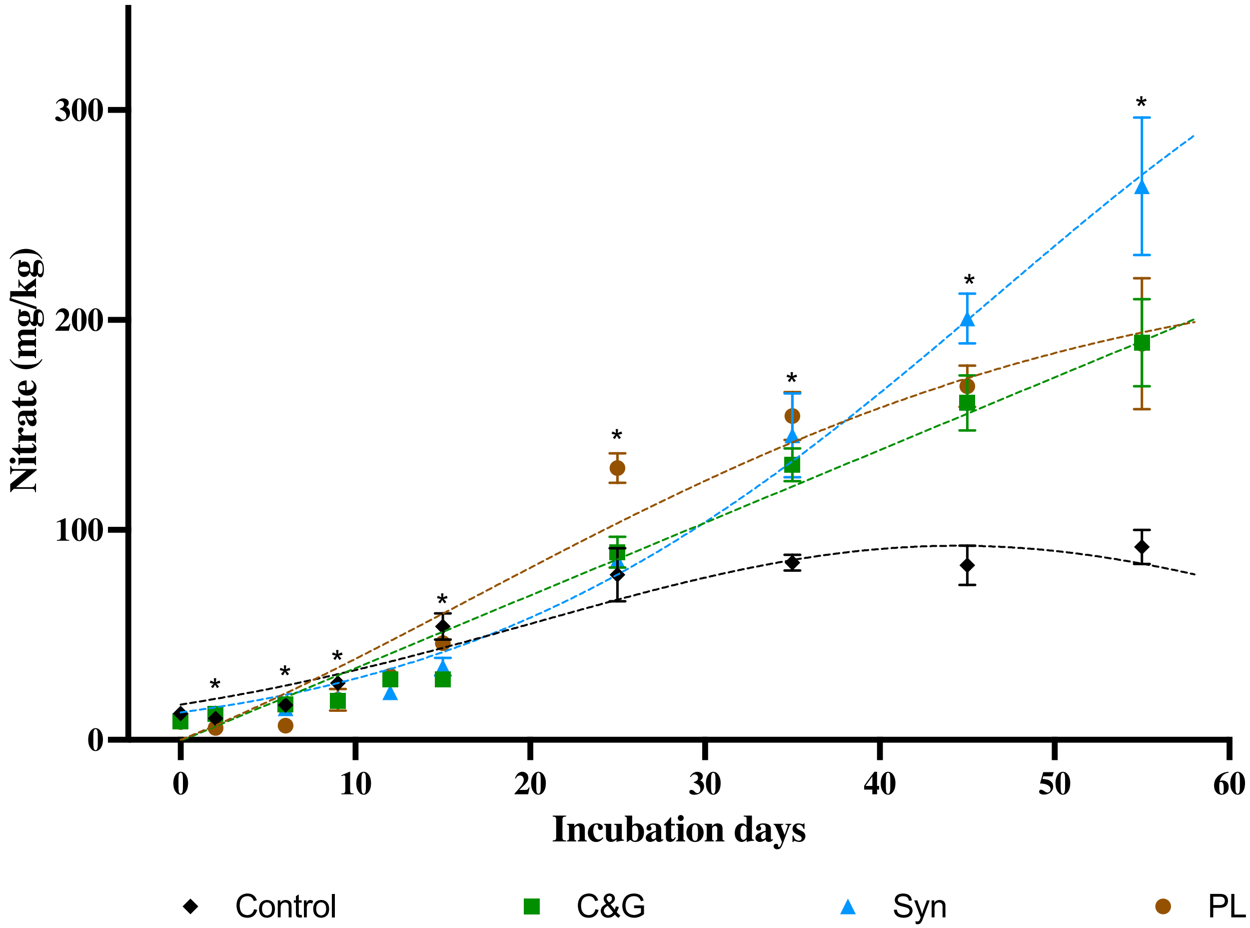

Fig. 2.5. Nitrate concentration within soil over the course of 55 days. The fertilizer nitrogen rate is 0.89 kg m^3 added to the soil. Fertilizer sources include a) control (soil), b) novel poultry litter-derived fertilizer (C&G), c) poultry litter (PL), and d) commercial synthetic fertilizer (Syn). The control did not receive any fertilizer application. The nitrate release followed either a linear (L) or quadratic (Q) response as Syn ($Y = 3.7416 + 1.758\ x + 0.05616\ x^2$, Trend: Q***, R^2: 0.98), C&G ($Y = 3.462^*\ x - 0.4657$, Trend: L***, R^2: 0.97), PL ($Y = -13.248 + 5.473\ x - 0.0306\ x^2$, Trend: Q***, R^2: 0.94), and the control ($Y = 4.518 + 3.5473\ x - 0.0371\ x^2$, Trend: Q***, R^2). * represent significant difference between the treatments for a given date at $P<0.05$.

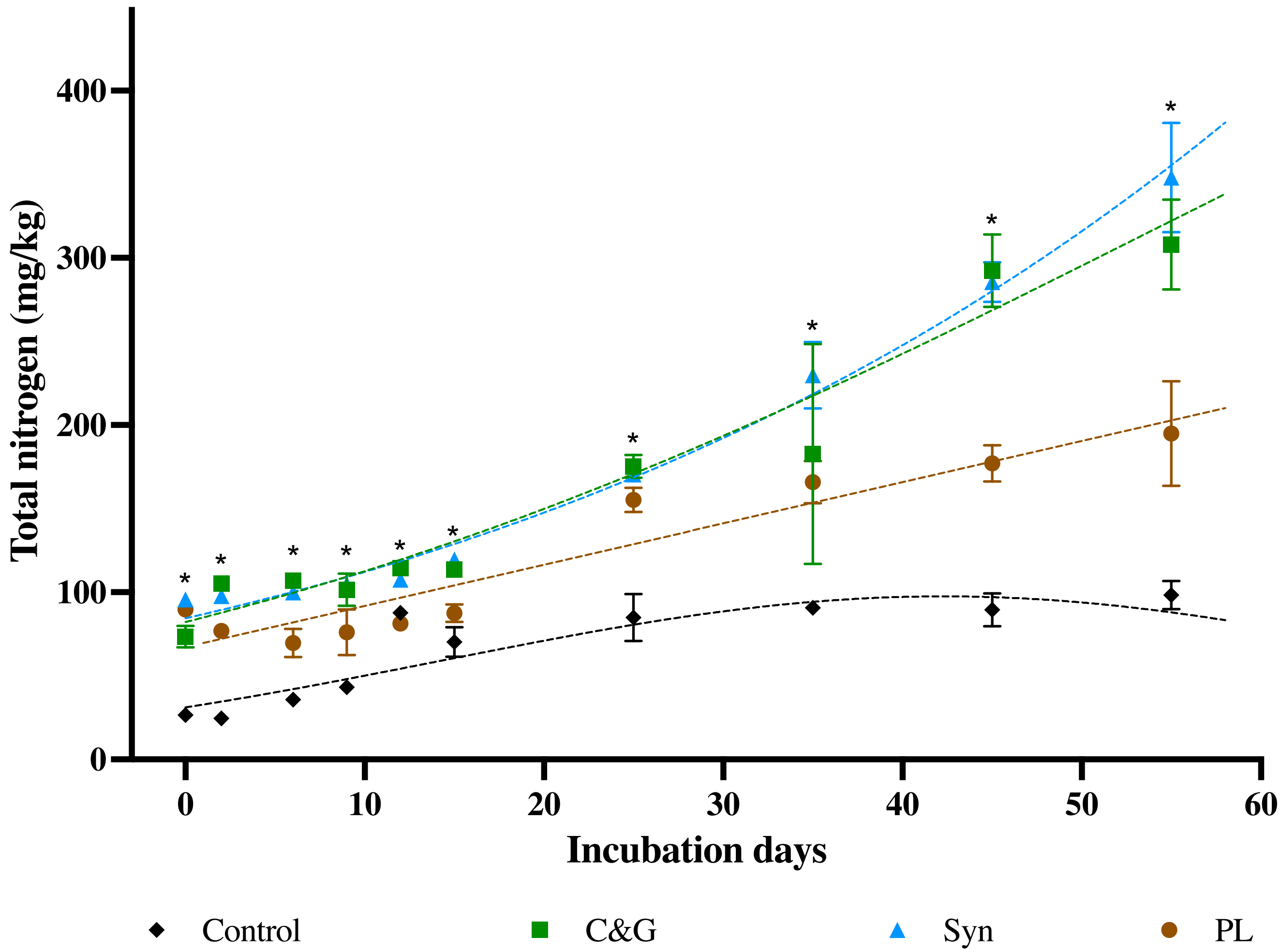

Fig. 2.6. Total Nitrogen concentration within soil over the course of 55 days. The fertilizer nitrogen rate is 0.89 kg m³ added to the soil. Fertilizer sources include a) control (soil), b) novel poultry litter-derived fertilizer (C&G), c) poultry litter (PL), and d) commercial synthetic fertilizer (Syn). The control did not receive any fertilizer application. The total N release followed either a linear (L) or quadratic (Q) response as Syn ($Y = 88.584 + 1.7654\ x + 0.0559\ x^2$, Trend: Q***, R^2: 0.98), C&G ($Y = 79.333 + 1.3\ x + 0.0241\ x^2$, Trend: Q***, R^2: 0.96), PL ($Y = 2.468^*\ x + 67.12$, Trend: L***, R^2: 0.85), and the control ($Y = 20.714 + 3.458\ x – 0.04\ x^2$, Trend: Q***, R^2: 0.94). * represent significant difference between the treatments for a given date at $P<0.05$.

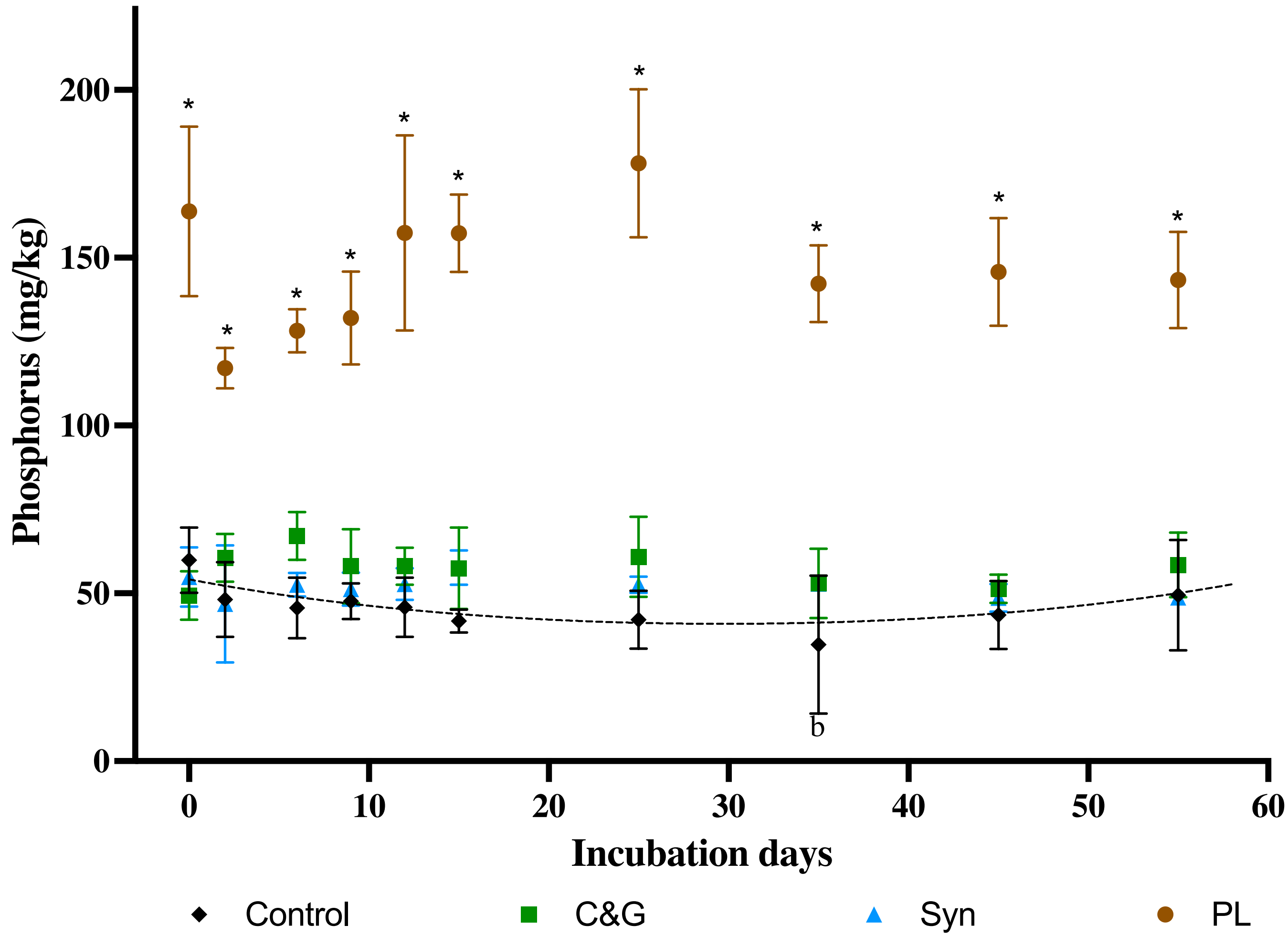

Fig. 2.7 Phosphorous concentration in the soil over the course of 55 days. The fertilizer nitrogen rate is 0.89 kg m^3 added to the soil. Fertilizer sources include a) control (soil), b) novel poultry litter-derived fertilizer (C&G), c) poultry litter (PL), and d) commercial synthetic fertilizer (Syn). The control did not receive any fertilizer application. The P release followed a quadratic (Q) response to the control ($Y = 53.706 – 0.869\ x + 0.0146\ x^2$, Trend: Q*, R^2: 0.187). * represent significant difference between the treatments for a given date at $P<0.05$.

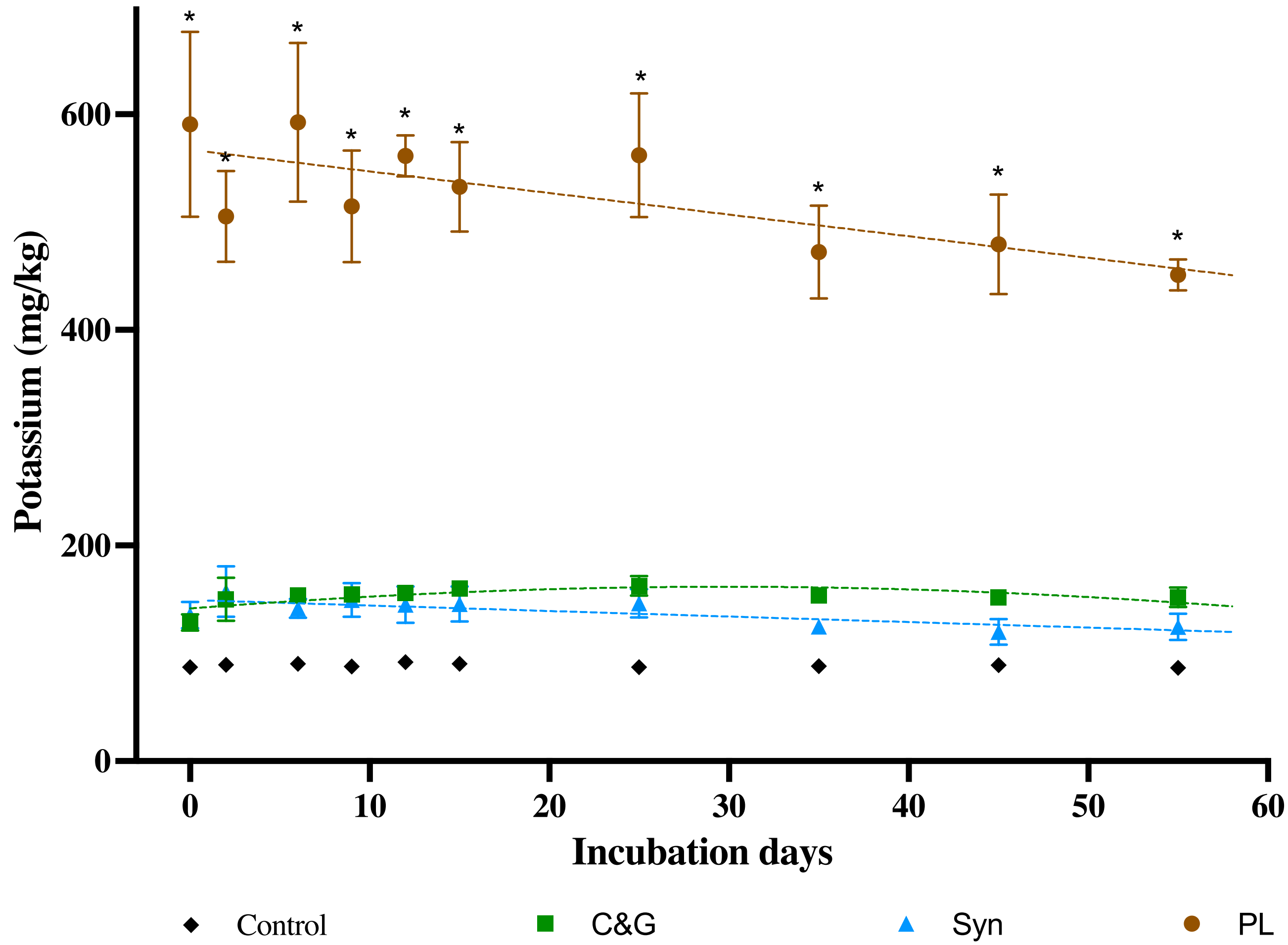

Fig. 2.8. Potassium concentration in the soil over the course of 55 days. The fertilizer nitrogen rate is 0.89 kg m^3 added to the soil. Fertilizer sources include a) control (soil), b) novel poultry litter-derived fertilizer (C&G), c) poultry litter (PL), and d) commercial synthetic fertilizer (Syn). The control did not receive any fertilizer application. The K release followed either a linear (L) or quadratic (Q) response as PL (Y= -2.004*x + 567.1, Trend: L***, R^2: 0.30), C&G (Y= 141.289 +1.3685x – 0.02297x^2, Trend: Q***, R^2: 0.32), and Syn (Y= -0.5123*x +149.5, Trend: L***, R^2: 0.29). * represent significant difference between the treatments for a given date at *P*<0.05.

Chapter 3: Evaluation of Growth Characteristics in Response to Application of a Novel Poultry-Derived Fertilizer

Abstract

Multiple processes have been developed to reduce the negative effects of raw litter application by altering the physical and chemical nature into a more suitable plant nutrient product. This investigation focused on a novel aerobic digestion process utilizing Cleaned & Green's (C&G) proprietary method to extend the nutrient release time of fertilizer while eliminating potential pathogens. Chemical and physical characteristics were assessed on C&G fertilizer by conducting plant assays and physical testing. Three plant species, tomatoes, rye, and petunias were grown to evaluate plant growth responses. Substrate pH and electrical conductivity (EC) were evaluated, and plant growth index, dry weight, and foliar analysis were recorded. Increasing rates of fertilizer application resulted in increased EC rates and decreased plant growth two weeks after planting. In tomatoes, electrical Conductivity two weeks after planting was 0.84, 4.56, 5.52, and 7.56 mS/cm for 0 kg N m^{-3}, 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, and 1.78 kg N m^{-3}. Similar trends were recorded in petunias. Growth indices indicated the control had the lowest size, with diverging results by species on the largest plants by rate. Similar results were recorded between tomatoes and Petunia. Rye, with different nutrition requirements, experienced few differences between treatments. Initial results suggest the C&G fertilizer may be utilized

similarly to a rapid-release synthetic fertilizer without the potential environmental burdens imposed by raw PL applications.

3.1 Introduction

The application of controlled-release fertilizers (CRFs) is a common nutrient management strategy in North American container production of ornamental plants (Alam et al. 2009). Controlled-release fertilizers consist of a synthetic polymer coating that encases water-soluble nutrients, slowly releasing as water and temperature break down the product. Dependent on coating thickness and environmental parameters, CRF's can last from 3 to 14 months (Pasian 2013). Before the popularization of CRFs, the industry relied on conventional water-soluble fertilizers which often have low nutrient use efficiency (Wu 2011; Lawrencia et al. 2021). These conventional fertilizers were problematic due to nutrient leaching, volatilization, and toxicity damage (Gil-Ortiz et al. 2020; Lawrencia et al. 2021). The reliance on CRFs has led to the development of species-specific recommendations to supply adequate nutrition to commonly grown crops like hydrangeas and boxwood (Clark and Zheng 2015).

The use of CRFs is not limited to the nursery industry; they are also recommended within the landscaping industry to follow best management practices (Chen et al. 2011). Even with species-specific CRFs plants have complex nutritional requirements that change with age and season. To meet such requirements, growers can mix CRFs of different release periods or use CRFs in conjunction with a water-soluble rapid-release fertilizer (Yeager et al. 2010). Dependence on CRFs has increased due to environmental protection legislation introduced over previous decades, particularly focused on decreasing water eutrophication. The widespread adoption of CRFs is considered a best management practice that limits environmental pollution, by reducing excess nutrients within aquatic habitats (Mack et al. 2019).

Despite the benefits of CRFs, recent legislation focusing on single-use plastics has the potential to impact the industry. Recently, the European Union passed new fertilizing products legislation to reduce reliance on mineral fertilizers in favor of organic-based ones (European Parliament and of the Council of 5 2019). The far-reaching law allows for the use of synthetic polymers during a transitional period, after which non-biodegradable products shall be banned and water retention (European Parliament and of the Council of 5 2019). The German fertilizer ordinance takes this a step further, limiting the size and duration of use for synthetic polymers in fertilizers (Till 2017). In the United States, no federal single-use plastic bans exist, and the states that have passed such bands have not focused on fertilizers or agriculture (Usman et al. 2022; NCSL 2021).

Slow-release fertilizers (SRFs) are an alternative to CRFs, lacking a synthetic coating and dependent on microbial breakdown for nutrient release (Morgan et al. 2009). Polymer products derived from cellulose have already begun to replace some single-use plastic products, and there is growing interest in the expanded use of bio-plastics (Ramesh Kumar et al. 2020). However, in areas dense in animal production, research into alternative sources lies in animal wastes (Hossain et al. 2021; Dadrasnia et al. 2021; Li et al. 2021; Mironiuk et al. 2023; Steiger et al. 2024). Recently, one product composed of recycled eggshells, wheat, and chitosan was developed as a granular product that can adsorb orthophosphate when wet and released at a later time, acting like a never-ending source of fertilizer (Steiger et al. 2024). Biochar, a carbon-rich, solid product produced from organic material which has undergone a pyrogenic process (primarily cow or poultry), has been heavily researched for slow-release characteristics (Yu et al. 2019; Bhatt et al. 2023).

Through the process of aerobic digestion, raw PL is converted into granular fertilizer through repeated processes of acidification and ammonification. The final product of the process is a generally stable, odor-free product (Shammas and Wang 2007; Martín et al. 2018). One product is the novel PL-derived fertilizer produced by Clean & Green (C&G), whose physical and chemical characteristics were examined in the previous chapter. Unlike polymer-coated CRFs, the C&G product is carbon-based and produced from excess poultry waste. Based on nutrient release tests, C&G is an intermediate between water-soluble synthetic fertilizers and SRFs: C&G functions like a water-soluble synthetic with an extended nutrient release, similar to an SRF, relying on microbial activity to help initiate release. Furthermore, C&G contains a blend of micronutrients rarely included in general fertilizer mixes. However, the suitability of C&G for crop production requires quantification. Therefore, the objective of this study is to grow a variety of crops using both C&G and potential market-available products to determine suitable uses for this novel fertilizer.

3.2 Materials and Methods

3.2.1 Tomatoes

On May 3rd, 2023, tomatoes (*Solanum lycopersicum* ‘Celebrity’) were transplanted into 1 gal containers (Classic 400; Nursery Supplies Inc., Fairless Hills, PA) filled with a pine bark: peat (3:1 v:v) amended substrate. Fertilizer treatments included the following: PL-derived fertilizer, C&G (11N–1P–1 K–15 S), a synthetic fertilizer (herein referred to as “Syn”) blended to contain similar nutrient values to C&G, an even nutrient Blend of C&G and Syn, and PL (1.3-1-1.5), and a control receiving no fertilization. Fertilizer treatments were applied at three rates:

0.44 kg N m^{-3}, 0.89 kg N m^{-3}, and 1.78 kg N m^{-3}. Each treatment-rate combination included six replicants. All non-C&G treatments received micronutrients (Tracer Micronutrient Mix; Harrell's, Lakeland, FL) for equitable growing conditions. Daily irrigation of 500ml water was applied at the plant base, with plants requiring an additional 500 ml following week 6. Since yield was not included in the study, flower structures were removed daily to preserve nutrients within the non-reproductive portions of the plant.

Substrate pH and EC were monitored weekly on the control and 0.89 kg N m^{-3} replicants and every other week for 0.44 kg N m^{-3} and 1.78 kg N m^{-3} using the Pour-Through method (Wright 1986a). Growth indices were measured every second week utilizing a wooden yardstick. Representative plants were photographed at weeks 4 and 8 for visual comparison. In week eight, replicants were cut at the crown and weighed for fresh weight. Dry weights were recorded the following week after oven drying at 140 F. Approximately, 20 grams of dry tissue was reserved for foliar tissue analysis for nitrogen, phosphorus, and potassium. Chlorophyll contents were measured using Spad-502 Plus (Konica Minolta, Tokyo, Japan) in week 8. Nutrient sufficiency standards follow those set by Clemson extension (Clemson University Regulatory Services 2013).

3.2.2 Rye

Rye (*Lolium multiflorum)* were seeded for germination into 1 gal containers (Classic 400; Nursery Supplies Inc., Fairless Hills, PA) filled with a pine bark: peat (3:1 v:v) amended substrate. Prior to germination, we initiated a paper towel test and substrate sowing to determine the germination rate. Following fertilizer incorporation, we applied seeds to the substrate surface and covered it with minimal peat. Ten days post-initiation containers were seeded again if germination failed to take place. Fertilizer treatments were applied at three rates: 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, and 1.78 kg N m^{-3}. Each treatment-rate combination included six replicants. All non-C&G treatments received micronutrients (Tracer Micronutrient Mix; Harrell's, Lakeland, FL) for equitable growing conditions. Daily irrigation of 500ml water was applied at the plant base, with plants requiring an additional 500 ml following week 6.

Growth indices were measured biweekly. Representative plants were photographed at weeks 4 and 8 for visual comparison. In week eight, replicants were cut to the roots and weighed for fresh weight. Dry weights were recorded the following week after oven drying at 140 F. In week 8, chlorophyll contents were measured using a Spad-502 Plus (Konica Minolta, Tokyo, Japan).

3.2.3 Petunias

On March 20th, 2024, petunias (*Petunia x hybrida* 'Supertunia Vista Bubblegum') were transplanted into 1.98 l containers (6" standard; Dillen Products., Middlefield, OH) filled with a pine bark: peat (3:1 v:v) amended substrate. Fertilizer treatments included the following: Synthetic, C&G, Blend (Synthetic+C&G), PL, and a control receiving no fertilization. Fertilizer treatments were applied at three rates: 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, and 1.78 kg N m^{-3}. Each treatment-rate combination included six replicants. All non-C&G treatments received micronutrients and lime (Tracer Micronutrient Mix; Harrell's, Lakeland, FL) for equitable growing conditions. Replicants were grown inside a greenhouse, receiving daily irrigation of 125ml water applied at the plant base. Petunias were grown in two locations: Auburn, AL, and Mobile, AL. Previous testing of Auburn irrigation water concluded the water contained approximately 0.16 ppm P, 2.34 ppm K, 5.99 ppm S, 0.15 ppm Zn, 0.14 ppm Cu, 14.21 ppm Ca, 4.02 ppm Mg, 6.68 ppm Na, and <0.02 ppm B, Mn, Fe, and Al (Bartley et al. 2023). Climate conditions were monitored in both locations. Average outdoor temperatures in Auburn were 17.22 ± 4.13 °C with greenhouse temperatures set to maintain a minimum of 18.3 °C and a maximum of 25.6 °C. Average greenhouse temperatures in Mobile were 22 ± 6.04 °C. Flower structures were preserved and submitted along with foliar structures for nutrient concentration analysis.

Electrical conductivity (EC) and pH were collected once a week, beginning on Day 0, using the Pour Thru method (Wright 1986b). Plant volumes were recorded at weeks 2 & 4. Plant volumes were calculated by multiplying plant height and two canopy widths. Representative plants were photographed at weeks 2 and 4 for visual comparison. Chlorophyll contents were measured using a Spad-502 Plus (Konica Minolta, Tokyo, Japan) in week 4. Fresh weight, dry weight, and foliar nutrient concentrations were recorded post-destructive harvest during week 4. Plants were cut at the substrate level, and fresh weights were recorded. Plant material was air-dried for one week at 60 C before recording dry weights. Five (5) g of leaf tissue from each replicant was reserved for tissue nutrient analysis. For treatments that failed to produce 5g individually, single representative samples were submitted for the entire treatment. For tissue analysis, all vegetative and floral growth was submitted.

Foliar testing was conducted by the Auburn Soil, Water, and Forage lab (Auburn, AL, USA) for foliar concentrations of nitrogen (N), phosphorus (P), and potassium (K). Nutrient concentrations were considered deficient at the following: N (3.85%), P (0.47%), and K (3.13%) concentrations (Gibson et al. 2008). All other micronutrients followed standards set by the Plant Analysis Handbook III (Bryson et al. 2014).

3.2.4 Statistical Analysis

Plant tissue nutrition data were analyzed via PROC Reg procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Effects of fertilizer, rate, and the fertilizer*rate interaction on dry weight and foliar nutrient concentrations were analyzed via ANOVA with the PROC Glimmix procedure. Means were separated using Tukey's honest significant difference (HSD) at a 5% alpha level.

3.3 Results

3.3.1 Electrical conductivity and pH.

This section will focus EC and pH observations from the 0.89 kg N m^{-3} rate (Fig. 3.1). Notably, the EC levels of leachate rapidly dropped from a peak of 7.91 ± 0.1 mS/cm for all fertilizer applications to 2.46 ± 0.17 within the first three weeks after transplant (Fig 3.1). The initial EC of Control containers was 0.49 ± 0.02 and decreased to 0.22 ± 0.01 by week 8. Containers treated with C&G and PL fertilizers maintained higher EC levels by the conclusion of the study. Poultry litter was the only treatment to maintain EC levels >1± 0.25 mS/cm for eight weeks. Plants treated with C&G had leachate EC levels of 0.56 ± 0.15 mS/cm at week 8. At week 8, Synthetic and Control had similar EC levels at 0.18 ± 0.02 mS/cm and 0.21 ± 0.01 mS/cm, respectively. Fertilizer applications at the 0.89 kg N m^{-3} rate had initial EC levels nearly twice the upper limit for plant growth in container production but fell to acceptable levels over time before ending below desired EC levels (Nelson 2011).

Across all fertilizer treatments, substrate pH was reduced by approximately 1 unit but increased steadily to 6.4-8 (Fig. 3.2). Notably, the pH levels of leachate increased from a low of

5.8 ± 0.47 for all fertilizer applications to 6.6 ± 0.2.6 six weeks after transplant and remained steady. The Control recorded an initial pH of 6.67 ± 0.16 and increased to 7 ± 0 by week 8. Containers with C&G and PL applied maintained lower pH levels throughout the study, concluding at 6.43 ± 0.06 and 6.43 ± 0.15, respectively. Synthetic and Blend recorded similar pH readings of 6.63 ± 0.06 and 6.76 ± 0.12, respectively, at week 8. From initiation to the study's conclusion, all treatment pH levels fell within the acceptable range of 5.2-6.8 for optimum nutrient uptake (Van Iersel, 2024).

3.3.2 *Tomato*

3.3.2.1 Fresh & Dry Weights.

Tomato fresh weight was significantly influenced by rate, fertilizer treatment, and the interaction rate*fertilizer (Table 3.1, $P < 0.0001$). Poultry litter applied at rates of 0.89 kg N m^{-3} and 1.78 kg N m^{-3} produced the largest tomato plants (Table 3.2**).** Conversely, PL applied at a rate of 0.44 kg N m^{-3} produced tomato plants comparable in size to C&G or Blend applied at rates of 0.44 kg N m^{-3} and 0.89 kg N m^{-3}. Increased mortality rates were observed in all fertilizers applied at 1.78 kg N m^{-3} except for PL. Tomatoes receiving Syn at 1.78 kg N m^{-3} had an 83% mortality rate, prohibiting their inclusion in many analyses. Tomatoes receiving C&G or Blend at 1.78 kg N m^{-3} only lost one specimen each. At a rate of 0.44 kg N m^{-3}, tomatoes receiving Syn fertilizer had the lowest fresh weights across all treatments. Of all fertilized tomato plants, Blend applied at 1.78 kg N m^{-3} produced tomato plants with the lowest fresh weights after eight weeks.

A similar trend was noted in dry weights where weights of tomato plants were significantly affected by rate, fertilizer type, and the interaction between rate and fertilizer type ($P < 0.0001$). Again, PL at the rates 0.89 kg N m^{-3} and 1.78 kg N m^{-3} produced the heaviest dry weights across all treatment and rate combinations (Table 3.2**)**. The C&G and Blend treatments had comparable wights to Syn at 0.89 kg N m^{-3}. The Syn at the 0.44 kg N m^{-3} rate had the lowest weight among treatments Visually, tomatoes fertilized with PL exhibited greater nutrient deficiencies compared with other treatments. However, these plants were among the heaviest suggesting a higher nutrient demand than other treatments. Treatments with PL more closely resembled weights in similar experiments, while all other treatments fell below average dry weights, suggesting similar growth limitations may be occurring for both C&G and Syn.

3.3.2.2 Plant height.

At week 4, tomato plant heights were significantly affected by a rate*treatment interactions ($P < 0.0001$). Fertilizer type and rate also influenced tomato height ($P < 0.0001$). Poultry litter applied at the rate of 0.44 kg N m^{-3} produced the tallest tomato plants (Table 3.3**).** Conversely, PL applied at a rate of 0.89 kg N m^{-3} produced tomatoes comparable in size to C&G or Blend applied at rates of 0.44 kg N m^{-3} (Fig. 3.3). Tomatoes receiving Syn at 1.78 kg N m^{-3} had an 83% mortality rate and were excluded from height analysis. Furthermore, tomatoes receiving Syn consistently produced smaller plants, with the 0.44 kg N m^{-3} rate producing tomatoes smaller than all treatments except Blend and C&G at 1.78 kg N m^{-3}. Tomatoes receiving C&G or Blend at 1.78 kg N m^{-3} demonstrated improved vitality from Syn, only losing one specimen each. However, Blend applied at 1.78 kg N m-3 produced the smallest tomatoes after four weeks, excluding the control.

By week 8, tomato plant heights were significantly affected by rate*treatment (P = 0.0009) respectively. Individually, tomato volumes were significantly influenced by rate and fertilizer type ($P < 0.0001$). Poultry litter applied at all rates nominally produced the tallest tomato plants, particularly at the 1.78 kg N m^{-3} rate (Table 3.3**).** All treatments at the 0.44 kg N m^{-3} and 0.89 kg N m^{-3} rates produced statistically comparable tomatoes (Fig. 3.4). Excluding PL, the 1.78 kg m^{-3} underperformed across treatments, with Blend and C&G being comparable to one another and only being taller than the control. Except for Poultry litter at the 1.78 kg N m^{-3} rate, all tomato plants grew at comparable rates between weeks 4 and 8 around 40 cm in height. For example, plants receiving the Blend at 1.78 kg N m^{-3} and 0.44 kg N m^{-3} both grew 37.9 cm between week 4 and week 8. With the tomato plants from 1.78 kg N m^{-3} growing from 13.1cm to 51cm and the 0.44 kg N m^{-3} rate tomato plants growing from 42.5 cm to 80.4 cm. However, the initial stunting in the 1.78 kg N m^{-3} rate resulted in plants remaining smaller by project conclusion. Plants receiving PL at the 1.78 kg N m^{-3} rate grew 125.7 cm from week 4 to week 8.

3.3.2.3 Foliar macronutrient concentrations.

Foliar N concentrations were affected by a treatment*rate interaction ($P < 0.0001$). By the study's conclusion, tomatoes produced with C&G and Blend supplied at 1.78 kg N m^{-3} had the highest foliar N concentrations 2.47% ± 0.26% and 2.56% ± 0.17%, respectively (Table 3.4). At 1.78 kg N m^{-3}, C&G and Blend fertilized tomatoes had foliar N concentrations 24% and 36% higher than Syn, respectively. However, tomatoes, regardless of nutrient source, fertilized at 1.78 kg N m^{-3} were N deficient (Clemson University Regulatory Services 2013). Synthetic applied at 0.44 kg N m^{-3} produced tomatoes with foliar N of 1.97% ± 0.65%, comparable to 1.78 kg N m^{-3} C&G and Blend. Fertilizers Syn, Blend, and C&G at the 0.89 kg N m^{-3} rate and Blend and C&G

at the 0.44 kg N m^{-3} rate produced tomatoes of comparable foliar N concentrations between 1.49% - 1.94%. Although PL produced the largest tomato plants, their foliar N was lowest at 0.86% ± 0.13%, 0.96% ± 0.01%, and 1.39% ± 0.15% for rates of 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, 1.78 kg N m^{-3}, respectively. Neither the control nor Syn at 1.78 kg N m^{-3} were included in the analysis due to insufficient plant material for analysis.

Given all treatments were N deficient by eight weeks, the addition of CRFs or frequent fertigation events would be necessary in order to produce a fruit crop (Clemson University Regulatory Services 2013). By week 8, all treatments experienced yellowing, purpling, or a combination of nutrient deficiency symptoms. Tomato plants receiving applications of PL produced the largest plants and exhibited the greatest N deficiency. Such discrepancy can be explained by the higher nutrient requirements required by PL-supplied plants and the mobility of N to easily move from old to new growth (Marschner 2011). However, if tomatoes are being grown for retail sale, the shorter growth period may be sufficient until the point of sale. While Syn fertilized plants had higher foliar N than C&G and Blend at the 0.44 kg N m^{-3} rate after eight weeks, all were comparable at the 0.89 kg N m^{-3} rate, and Syn was outperformed by all fertilized treatments at the 1.78 kg N m^{-3} rate. However, the Syn treatments still required the addition of micronutrients present in C&G. Use of C&G can prevent financial expenses from buying separate micronutrient fertilizers. Therefore, tomato mortality and damage from ammonium sulfate synthetic fertilizers can be decreased by using a 1:1 N blend with C&G and producing tomatoes of comparable quality at the recommended rate.

Foliar P concentrations were affected by a treatment*rate interaction ($P < 0.0001$). Individually, P concentrations were affected by nutrient source and rate, ($P < 0.0001$) and ($P =$

0.0069) respectively. By study conclusion, tomatoes produced with PL contained the highest foliar concentrations of P at 0.44% ± 0.05%, 0.46% ± 0.05%, and 0.38% ± 0.13% for application rates of 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, 1.78 kg N m^{-3}, respectively (Table 3.4). Tomatoes treated with PL had P concentrations 124%, 86%, and 18% higher than respective treatments as the rate increased. Tomatoes fertilized with Blend at 1.78 kg N m^{-3} contained comparable foliar P levels at 0.37% ± 0.1%. No other treatment combinations reached the 0.3% sufficiency level for foliar P concentrations recommended for greenhouse tomatoes (Clemson Regulatory Services 2013). Tomatoes supplied with 1.78 kg N m^{-3} C&G contained the foliar P concentrations at 0.26% ± 0.03%. The treatments of Syn, Blend, and C&G at 0.44 kg N m^{-3} rate and 0.89 kg N m^{3} rate produced tomatoes of comparable foliar P concentrations between 0.07% - 0.19%. Nominally, foliar P was lowest for Syn at the rate the 0.44 kg N m^{-3} at 0.07% ± 0.01%.

In comparison, PL treatments at all rates and Blend at 1.78 kg N m^{-3} produced tomato plants sufficient in P after eight weeks. Treatments with PL recording elevated foliar P concentrations are in line with literature regarding the even N-P-K ratio within the material (Gollehon et al. 2001; Gaskin et al. 2013). However, if tomatoes are being grown for retail sale, a single fertilizer application of C&G, Blend, or Syn may be sufficient for a short growth period. Despite only nominal differences in P concentration, plant mortality and damage by Syn can be decreased by using a 1:1 blend with C&G and produing plants of comparable quality at the recommended rate. Given excess P concentrations can cause significant water quality issues, future studies should quantify leachate P concentrations to better understand how efficiently P is used (Finlay et al. 2013).

Foliar K concentrations were affected by a fertilizer treatment*rate interaction ($P < 0.0001$). No fertilizer and rate combinations produced tomato plants sufficient in K foliar concentrations. By the study conclusion, tomatoes produced with PL contained the highest concentrations of K at 0.79% ± 0.21%, 0.71% ± 0.81%, and 1.32% ± 0.53% for rates of 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, 1.78 kg N m^{-3}, respectively (Table 3.4). Tomatoes supplied with 0.44 kg N m^{-3} of Syn had comparable foliar K levels at 0.74% ± 0.46%. No discernable differences in foliar K concentration were observed for other treatments with concentrations ranging from 0.34% - 0.63%. Nominally, foliar K was lowest for C&G at the rate the 0.89 kg N m^{-3} at 0.34% ± 0.17%.

All treatments were K deficient by eight weeks. Therefore, the addition of CRFs or frequent fertigation events would be necessary in order to produce a fruit crop (Clemson University, South Carolina 2013). Tomato plants receiving applications of PL again recorded the highest nutrient concentration with K content increasing by rate being 35%, 51%, and 88% higher. Tomato plants receiving PL had higher foliar K concentrations, with concentrations similar to foliar N concentrations. The results are in line with prior analysis which recorded a near-even N-P-K ratio in PL. Synthetic, C&G, and Blend treatments at all rates performed in a similar manner, suggesting equitable value for K as fertilizer. There were no discernable advantages of blending C&G with Syn in regard to K foliar concentration.

Calcium, magnesium, and micronutrients contained few differences. All treatments met the 1% sufficiency level in calcium ranging in foliar concentrations from 1.49% - 2.71%. All treatments met the minimum sufficiency requirement of 0.35% in Magnesium. Treatments ranged Mg in foliar concentrations from 0.55% - 1.04%. In boron, all treatments met the sufficiency requirement of 30 ppm and ranged from 32-49 ppm. All treatments met the

Manganese sufficiency level of 25 ppm, ranging from 135 ppm to 364 ppm. All treatments, except for C&G & Blend at the 0.44 kg N m^{-3} rate, fell below the zinc sufficiency level of 18 ppm, ranging from 0.45 ppm to 32 ppm. However, no clear trend occurred between treatment or rate. All treatments, except for PL & Syn at the 0.89 kg N m^{-3} rate and PL at the 1.78 kg N m^{-3} rate, met the Iron sufficiency level of 50 ppm, ranging from 42 ppm to 95 ppm. Additional nutrients supplied to non-C&G resulted in comparable ranges with C&G-treated tomatoes. All treatments would benefit from the addition of CRFs or frequent fertigation events to prevent potential yield loss (Clemson University Regulatory Services 2013). Since no visual micronutrient deficiencies occurred, future research could focus use of C&G as a starter fertilizer charge for polymer coated fertilizers and fertigation practices.

3.3.2.4 Chlorophyll Concentration.

A significant difference in chlorophyll content was observed in a treatment*rate interaction (P < 0.0410). Treatments 0.44 kg N m^{-3} Syn and 0.89 kg N m^{-3} rate for Syn and Blend contained the highest chlorophyll contents (Table 3.3). The lowest chlorophyll content was observed in the Control. None was recorded in Syn at 1.78 kg N m^{-3} due to the mortality of all but one plant. There were no statistical differences among other treatments (P = 0.8650). Previous studies have observed a correlation between N concentrations and chlorophyll content with a SPAD critical value sufficiency range of 40.2 – 44.3 suggesting SPAD readings as an alternative to destructive sampling (Fontes and Ronchi 2002; Ulissi et al. 2011; Jiang et al. 2017). However, chlorophyll content means observed in several treatments fell within this range despite recording a nutritional deficiency in all treatments. Chlorophyll contents in greenhouse tomatoes receiving adequate fertilization have recorded levels between 50.9 to 154.0 (Li et al.

2017). Sampling from newer growth could explain such discrepancy as new growth will contain higher N concentrations than the plant as a whole, from which foliar testing occurred.

3.3.3 *Rye*

3.3.3.1 Fresh & Dry Weights.

The fresh weight of rye plants had few differences by rate, fertilizer type, and the interaction between rate and fertilizer type, but all were significant (Table 3.5, $P < 0.0001$). Notably, PL at the rate of 1.78 kg N m^{-3} produced the heaviest rye plant weights (Table 3.6**).** Blend and C&G applied at 0.44 kg N m^{-3} produced rye comparable in size to C&G, Blend, Synthetic, and PL applied at rates of 0.89 kg N m^{-3}. In comparison to the tomatoes, no mortality was observed in any fertilizer application; however, germination inhibition was observed at rates of 0.89 kg N m^{-3} and 1.78 kg N m^{-3} for all fertilizer types. Rye receiving Syn and PL produced the smallest plants when supplied at 0.44 kg N m^{-3}. Visually, no rye plants appeared nutritionally deficient in comparison to tomatoes. Additionally, visual differences in plant size diminished between weeks 4 and 8. Notably, rye supplied with 1.78 kg N m^{-3} PL produced fresh weights 67% and 53% higher than Syn and C&G, respectively, at the same rate.

The dry weight rye plants were significantly affected by fertilizer type and the interaction between rate and fertilizer type ($P < 0.0001$). Dry weights were significantly affected by rate ($P = 0.0254$). Dry weights trended in a similar manner to fresh weights, with few differences being recorded between treatments and rates. The main difference being rye plants treated with Blend at 1.78 kg N m^{-3} produced the lowest dry weights after eight weeks. Visually, no deficiency was prevalent among treatments.

3.3.3.2 Plant Height.

Week four rye heights were significantly affected by a rate*treatment interaction ($P < 0.0001$). Poultry litter applied at 0.44 kg N m^{-3} produced the tallest rye plants nominally, with PL at 0.89 kg N m^{-3} and Syn at 1.78 kg N m^{-3} comparable in size (Table 3.7**).** Conversely, all other treatments at rates 0.44 kg N m^{-3} and 0.89 kg N m^{-3} performed similarly and produced shorter rye. Similar to tomatoes, treatments at the 1.78 kg N m^{-3} continued the trend of producing the smallest rye, excluding the control, with PL larger than Blend and C&G (Fig. 3.5). Visually, no deficiencies were noted.

Rye height was influenced by rate, fertilizer treatment, and a treatment*rate interaction ($P < 0.0001$) at week 8. However, when excluding the control only rate was significant ($P = 0.0079$). The tallest plants on average were supplied PL at the rate of 0.89 kg N m^{-3} produced the tallest rye plants. The shortest plants on average were supplied Syn at the 1.78 kg N m^{-3} nominally, excluding the Control (Fig. 3.6). Nutritionally speaking, rye has different nutritional requirements and would be expected to behave differently than a vegetable crop like tomatoes (Franzen 2023). While no foliar nutrient concentrations were conducted for Rye, visual deficiency was not prevalent among any treatment. Additionally, rye being a grass has significantly different nutrient requirements from horticultural crops, demanding higher rates of N but significantly less P and K for optimal growth. While rye is a common row crop traditionally grown in a field setting, this study focused on growing plants within a greenhouse to better monitor individual replicants.

3.3.3.3 Chlorophyll Concentration.

A significant difference in chlorophyll content was observed by treatment ($P < 0.0001$), rate ($P = 0.00245$), and a treatment*rate interaction ($P < 0.0001$). Chlorophyll content was unable to be recorded for Control plants due to insufficient plant material or Syn at the 1.78 kg N m^{-3} rate. The Syn treatment at the 0.89 kg N m^{-3} rate recorded the nominally highest chlorophyll content (Table 3.7). Optimal SPAD readings in a similar monocot species, winter wheat, range between 40 and 45 for maximum grain yield (Mehrabi and Sepaskhah 2022). No treatment reached the minimum threshold for maximum grain yield. However, several treatments did have recorded SPAD readings above 39, near the optimum SPAD range. The following treatments had readings above 39: C&G and Blend at the 1.78 kg N m^{-3} rate and Syn and Blend at the 0.89 kg N m^{-3} rates.

3.3.4 *Petunias*

3.3.4.1 Electrical conductivity and pH.

Both locations, Auburn and Mobile, exhibited a similar downward trend of electrical conductivity over time. The section will focus on the 0.89 kg N m^{-3} and the Control. In Auburn, initial mean EC levels recorded a high of 6.12 ± 0.76 mS/cm for all fertilizer applications and dramatically dropped to 2.78 ± 4.6 mS/cm within the first two weeks (Fig. 3.7a). By the conclusion of the study, treatments recorded mean EC readings at 1.22 ± 0.21 mS/cm. Notably, Syn was the only fertilizer to record a final mean EC level below 1 mS/cm. In the Control, initial EC levels were 0.61 ± 0.05 mS/cm and dropped to 0.18 ± 0.01 mS/cm. Initial EC levels were above the desired level and fell to an acceptable level within two weeks. In Mobile, initial mean EC levels were 4.72 ± 3.22 mS/cm for all fertilizer applications and dramatically dropped to 0.9 ± 0.05 mS/cm within the first two weeks (Fig. 3.7b). By the conclusion of the study, treatments recorded mean EC readings at 0.32 ± 0.05 mS/cm. Notably, all treatments recorded a final mean EC level below 1 mS/cm. In the control, initial EC levels were 0.28 ± 0.05 mS/cm and dropped to 0.14 ± 0.01 mS/cm. Initial EC levels for Mobile plants were similarly above desirable levels, but experienced a more rapid drop off, falling below desired levels within the same time.

No significant differences in leachate pH were observed between locations, so pH will be discussed together. Over four weeks, pH exhibited an increasing trend over time. For continuity purposes, the remainder of the discussion on pH shall focus on the 0.89 kg N m^{-3} rate similar to electrical conductivity. At Auburn, the initial mean pH for all fertilizer applications was 5.7 ± 0.3 and increased to 6.1 ± 0.06 by week 2 (Fig. 3.8a). By the conclusion of the study, treatments recorded a mean pH of 6.0 ± 0.3. Notably, Syn and PL increased to respective pH levels of 6.5 ± 0.42 and 6.0 ± 0.2 while C&G and Blend began at 5.6 ± 0.15 and 5.8 ± 0.05, increased and returned to their original pH levels. In the Control, initial pH levels were 6.3 ± 0.09 and increased to a final pH of 6.5 ± 0.06. From initiation to conclusion, all treatment pH levels fell within an acceptable range of 5.2-6.8 for optimum nutrient uptake. In Mobile, the initial mean pH for all fertilizer applications was 4.9 ± 0.38 and increased to 6.5 ± 0.17 by week 2 (Fig. 3.8b). By the conclusion of the study, treatments recorded a mean pH of 6.9 ± 0.09. In comparison to the Auburn study, all fertilizer treatments increased from their original pH levels by ~2 units. In the Control, initial pH levels were 5.8 ± 0.05 and increased to a final pH of 6.9 ± 0.04. In comparison to Auburn, initial pH levels at Mobile were below acceptable ranges and rose into the recommended range before finishing the study higher than the optimum maximum pH (Van Iersel 2020).

3.3.4.2 Fresh & Dry Weights.

A significant difference in dry weight was observed between the two locations and by treatment*location interactions (Table 3.8). Petunia dry weight was affected by rate, fertilizer type, and the interaction between rate and fertilizer type ($P < 0.0001$). Locations will be discussed separately due to differing trends. Fresh weights exhibited similar trends to dry weights (Table 3.9). In Auburn, C&G and PL at 0.44 kg N m^{-3} produced the heaviest petunia plants. Across all treatments, application rates of 0.44 kg N m^{-3} produced plants of greater size than 0.89 kg N m^{-3} or 1.78 kg N m^{-3}. Despite this, Syn at 0.44 kg N m^{-3} produced plants only marginally heavier than Blend or C&G and 0.89 kg N m^{-3}, and smaller than PL at 0.89 kg N m^{-3}. Increased mortality was observed in Syn and Blend fertilizers applied at 1.78 kg N m^{-3}, with Syn experiencing a 50% mortality rate. Petunias receiving PL at 1.78 kg N m^{-3} did not have increased mortality but did experience stunting in growth. Petunias receiving 1.78 kg N m^{-3} C&G demonstrated improved vitality, not losing any specimens. However, treatments applied at 1.78 kg N m^{-3} consistently produced the smallest plants after four weeks, with Syn producing the smallest plants, excluding the Control. Visually, petunias fertilized with 1.78 kg N m^{-3} PL exhibited over-fertilization and stunting beginning at two weeks and remained stunted through the study conclusion. No other treatments exhibited visual nutritional deficiency.

In Mobile, Blend, closely followed by C&G, at 0.44 kg N m^{-3} produced the heaviest petunia plants (Table 3.10). Across all treatments, applications at 0.44 kg N m^{-3} produced plants of greater size than 0.89 kg N m^{-3} or 1.78 kg N m^{-3}. Despite this, Syn at 0.44 kg N m^{-3} produced plants only marginally heavier than Blend and PL and smaller than C&G at 0.89 kg N m^{-3}. No increased mortality was observed in treatments applied at 1.78 kg N m^{-3}, but treatments, excluding C&G, did experience a general stunting. Unlike petunia produced at Auburn, petunias supplied with PL resulted in 31% and 67% heavier plants than Syn and Blend, respectively. Petunias receiving 1.78 kg N m^{-3} C&G demonstrated improved vitality, performing comparably to C&G at 0.89 kg N m^{-3}. Except for Blend at 0.44 kg N m^{-3}, C&G consistently outperformed other treatments, regardless of rate. Visually, all petunias receiving fertilization appeared nutritionally sufficient through the study conclusion.

Except for PL applied at 0.44 kg N m^{-3}, petunias produced in Mobile were consistently heavier than petunias produced at Auburn. However, in both Auburn and Mobile, the PL 1.78 kg N m^{-3} rate produced the smallest plants 121% and 40%, respectively, smaller than the 0.44 kg N m^{-3} rate. In Auburn, C&G and Blend produced final plants of comparable weight, while Syn consistently underperformed, suggesting C&G nutrient release is preferential for growing petunias. In Mobile, all fertilizer types performed similarly at rates of 0.44 kg N m^{-3} and 0.89 kg N m^{-3}. However, C&G produced significantly heavier petunias than other fertilizer types at 1.78 kg N m^{-3}. These results mirror results in tomatoes where C&G outperformed Synthetic products at higher N rates. However, comparisons between tomato and petunia diverge when comparing responses to PL. In tomatoes, PL and 1.78 kg N m^{-3} produced the heaviest tomato plants, whereas, in petunias, PL produced smaller plants, and the heaviest plants were supplied 0.44 kg N m^{-3}.

3.3.4.3 Growth Indices.

A significant differences in growth indices at week 2 were observed between the two locations. There were no significant interactions observed between location*treatment or location*rate, (P = .3149) and (P = 0.2681). Location (P = 0.0003) and rate (P = 0.0010) affected petunia growth indices but not fertilizer type (*P* = 0.0556). In Auburn, there were significant differences observed between fertilizer and rates (*P* < 0.0001). The Blend at the 0.44 kg N m^{-3} rate produced the largest average volume of 1172.4 cm^3, followed by PL at the same rate, 1097.9 cm^3 (Table 3.11, Fig. 3.9a). Following this, the 0.44 kg N m^{-3} C&G and all treatments grown at 0.89 kg N m^{-3} produced comparable volumes. The Syn at the 0.44 kg N m^{-3} rate produced plants only larger than the control and 1.78 kg N m^{-3} rate. The control and treatments at the 1.78 kg N m^{-3} rate were the smallest, all with comparable-sized plants. In Mobile, petunia growth indices were affected by fertilizer type (*P* < 0.0001) and rate (*P* = 0.0001). Excluding the control, the only difference reported was between the 0.44 kg N m^{-3} rate and 1.78 kg N m^{-3} rates for Syn and Blend. The average size of 0.44 kg N m^{-3} treated plants, regardless of fertilizer type, was 1786.9 cm^3 and the average size of Syn and Blend were 801.7 cm^3 and 774.5 cm^3, respectively (Table 3.12, Fig. 3.9b).

Week 4 growth indices trended similarly to petunia weights. Volumes experienced a significant difference by location (*P* < 0.0001). Petunias were affected by fertilizer type (*P* < 0.0001), rate (*P* < 0.0001), treatment*location interaction (*P* < 0.0001), and a rate*location interaction (*P* < 0.0085). In Auburn, there were significant differences observed by fertilizer type and rate (*P* < 0.0001). The 0.44 kg N m^{-3} rate produced the largest plants, with Blend producing the overall largest plants, followed by PL and C&G (Fig. 3.9c). The 0.44 kg N m^{-3} of Syn produced plants comparable to 0.89 kg N m^{-3} fertilizer applications of Blend and C&G. However, Syn at 0.89 kg N m^{-3} produced the smallest petunias at that application rate. C&G petunias were larger than other treatments at application rates of 0.44 kg N m^{-3} and 0.89 kg N m^{-3}. The Control produced larger plants than Blend or Syn at the 1.78 kg N m^{-3} rate. In Mobile, there were significant differences observed between treatments and rates (*P* < 0.0001). The 0.44 kg N m^{-3} rate with C&G produced the overall largest plants (Fig. 3.9d). The smallest plants were produced by the Control and 1.78 kg N m^{-3} of Blend or Syn. There were few differences among all other treatments.

Petunias produced in Mobile consistently produced larger plants than Auburn at both week 2 and week 4. Discrepancies in plant growth at 1.78 kg N m^{-3} PL can be attributed to the age of the PL (Figs. 3.10 & 3.11). The PL utilized at Auburn had been recently collected, while the PL utilized in Mobile was significantly older material. The age of composted material has been observed to stunt plant growth (Gouin 1998). Greater degrees of stunting have been observed in 30-day-old compost compared to 90-day-old compost in other floriculture crops (Purman and Gouin 1992). However, in both Auburn and Mobile, the 1.78 kg N m^{-3} PL rate produced the smallest plants 67.8% and 71.2%, respectively, the size of the 0.44 kg N m^{-3} rate. In Auburn, C&G and Blend produced petunias of comparable size. Syn fertilizer consistently underperformed Blend and C&G, suggesting C&G nutrient release is preferential for growing petunias. In Mobile, all three treatments performed similarly at the rates of 0.44 kg N m^{-3} and 0.89 kg N m^{-3}. However, at 1.78 kg N m^{-3}, C&G produced significantly larger petunias than Blend or Syn, 464% and 1224%, respectively.

These results mirror results in tomatoes where C&G outperformed Syn and Blend products. In both petunias and tomatoes, stunting occurred at fertilizer applications of 1.78 kg N m^{-3}. However, over the eight-week tomato trial, C&G and Blend treatments were afforded the time to recover from early-stage stunting. The four-week petunia trial did not allow stunted plants an opportunity to recover. The trials also differed in which fertilizer type and rate produced the largest plants. Poultry litter supplied at 1.78 kg N m^{-3} produced the largest tomato plants but the smallest petunias. In Auburn, the application of fresh PL may have attributed to greater stunting compared to Mobile (Kithome et al. 1999; Steiner et al. 2010). Finally, optimum growing conditions for petunias occur around 26 °C (Warner 2010). The elevated temperatures experienced for the Mobile location were, on average, closer to the optimum temperature than in Auburn, further promoting growth across treatments.

Nursery production for many species, especially woody ornamentals often occurs outside on nursery pads where environmental factors can affect growth (Shreckhise et al. 2019). A similar trial was initiated on *Hydrangea paniculata* 'Little Lime' to investigate C&G's suitability in such settings. Due to frequent rainfall and irrigation, EC readings signified total nutrient leaching within two weeks. As a result, the study concluded early and C&G was deemed not suitable for such growing conditions (data not shown).

3.3.4.5. Macronutrient Concentration.

Foliar N concentrations were affected by a fertilizer*location interaction (P = < 0.0001) and a rate*location interaction ($P < 0.0001$). Foliar N concentrations were affected by fertilizer type ($P < 0.0001$) and rate (< 0.0001) but not location ($P = 0.9084$). In Auburn, all fertilizer types supplied at 0.89 kg N m^{-3} and 1.78 kg N m^{-3} produced plants sufficient in N. The highest concentrations of foliar N resulted from applications of 1.78 kg N m^{-3} PL, 1.78 kg N m^{-3} C&G, and 0.89 kg N m^{-3} Syn at 6.49%, 6.17% ± 0.53%, and 6.17%, respectively (Table 3.13). Synthetic applied at 0.44 kg N m^{-3} produced plants with foliar N of 3.84% ± 0.30%. Blend, PL, and C&G applied at 0.44 kg N m^{-3} produced plants of comparable foliar N concentrations between 2.37% - 2.9%. All fertilized petunias had nominally higher foliar N than the Control.

Although PL applied at 0.44 kg N m^{-3} produced the heaviest petunias, its foliar N content was the lowest of any fertilizer and rate combination.

In Mobile, all treatment combinations produced petunias with sufficient foliar N with the exception of Control, 0.44 kg N m^{-3} Blend, 0.44 kg N m^{-3}, and 0.89 kg N m^{-3} PL (Table 3.14). Petunias fertilized with C&G and Syn at the 1.78 kg N m^{-3} had the highest foliar concentrations of N, 6.13% ± 0.4% and 5.85% ± 0.35%, respectively (Table 3.9). All other treatment combinations produced plants of comparable foliar N concentrations between 5.74% - 4.01%. Although PL at the 0.44 kg N m^{-3} rate produced the heaviest plants, foliar N was the lowest. Petunias supplied with C&G at 0.44 kg N m^{-3} had higher foliar N concentrations than PL at 0.44 kg N m^{-3} and 0.89 kg N m^{-3} by 12% and 50%, respectively. Poultry litter applied at 0.44 kg N m^{-3} had 59% higher foliar N than the Control.

Petunias produced in Auburn recorded higher foliar N concentrations than those produced in Mobile. While both locations used litter from the same source, the petunias produced in Mobile utilized leftover material from 2023, while petunias produced in Auburn received fresh material collected in March 2024. The average PL-fertilized petunia grown in Auburn had foliar N concentrations 63% higher than those grown in Mobile. As PL ages nitrogen is lost through ammonia volatilization reducing N availability (Kithome et al. 1999; Steiner et al. 2010). Nitrogen application has a greater effect on plant growth than P or K. However insufficient application of either P or K may affect N uptake (Kim and Li 2016; Alvarado-Camarillo et al. 2018).

Foliar P concentrations were affected by fertilizer*location and rate*location interactions ($P < 0.0001$) and ($P < 0.0001$). Foliar P concentrations were affected by nutrient source ($P <$

0.0001) and rate ($P < 0.0001$) but not by location, ($P = 0.6877$). In Auburn, petunias given PL applied at 0.89 kg N m^{-3} contained the highest foliar concentrations of P at 0.58% ± 0.1% (Table 3.8). With few exceptions, fertilizers applied at 0.89 kg N m^{-3} and 1.78 kg N m^{-3} contained comparable foliar P at 0.45% ± 0.04%. The only fertilizer and rate combinations that resulted in sufficient foliar P concentrations were 0.44 kg N m^{-3} PL, 0.89 kg N m^{-3} Syn, 1.78 kg N m^{-3} C&G, and 1.78 kg N m^{-3} Blend. Petunias fertilized at a rate of 0.44 kg N m^{-3} contained the next lowest foliar P concentrations, averaging 0.19% ± 0.03% across all fertilizer types. Though not statistically significant, 0.44 kg N m^{-3} C&G foliar P concentrations were 23% and 33% higher than Syn and Blend, respectively.

In Mobile, petunias fertilized with PL at 1.78 kg N m^{-3} contained the highest concentrations of P at 0.56% ± 0.2%. Petunias fertilized with 0.89 kg N m^{-3} and 1.78 kg N m^{-3} C&G, 0.44 kg N m^{-3} and 0.89 kg N m^{-3} PL, 1.78 kg N m^{-3} Syn, and 1.78 kg N m^{-3} Blend contained comparable foliar P levels at ranging from 0.51% ± 0.08% to 0.8 ± %. However, PL at the 0.89 kg N m^{-3} rate and C&G at the 1.78 kg N m^{-3} rate were the only treatment combinations that resulted in sufficient foliar P concentrations for greenhouse petunias. With few exceptions, petunias fertilized at the 0.44 kg N m^{-3} rate contained the lowest foliar P concentrations ranging from 0.17% ± 0.02% to 0.33% ± 0.14%. Blend at the 0.89 kg N m^{-3} rate had similar foliar P concentrations to petunias fertilized at 0.44 kg N m^{-3}, but was nominally higher. Though similar, foliar P concentrations in petunias receiving 0.44 kg N m^{-3} C&G were nominally higher than Syn and Blend at similar rates, 23% and 25%, respectively. Soilless substrates have a limited capacity for retaining P, with liming agents further reducing soluble P in peat and pine bark (Argo and Biernbaum 1996; Whipker 2014; Bartley et al. 2023). In addition to insufficient application, substrate absorption characteristics may have contributed to deficient foliar P concentrations. However, only the Control appeared visibly deficient, suggesting that the recommended foliar P concentrations may be higher than what is required for market-quality plant health. Previous studies have produced quality crops using low-P fertilizers (Winsor 1968). Furthermore, plants receiving lower P rates have exhibited higher rates of P efficiency with limited effects on flowering (Kim and Li 2016).

Foliar K concentrations were affected by fertilizer*location and fertilizer*rate interactions ($P < 0.0001$) and ($P < 0.0001$). Concentrations were affected by fertilizer type ($P < 0.0001$) and location ($P = 0.0196$), but not rate ($P = 0.9158$). Foliar K concentrations between Auburn and Mobile locations trended similarly, with petunias fertilized with PL containing the highest concentrations of P, and only PL applications resulted in sufficient foliar K concentrations. In Auburn, few differences in foliar K concentrations were recorded between Syn, Blend, and C&G fertilizers. However, petunias receiving 0.89 kg N m^{-3} contained 47.8% more foliar K than petunias receiving 0.44 kg N m^{-3}. Although similar to other fertilizer combinations, foliar K concentrations were nominally lowest for C&G applied at 0.44 kg N m^{-3} at 1.42% ± 0.12%.

In Mobile, few statistical differences in foliar K concentrations were recorded across Syn, Blend, and C&G treatments. Foliar K concentrations were highest in petunias fertilized with PL at 0.44 kg N m^{-3} and 0.89 kg N m^{-3}. Following these treatments, C&G at the 1.78 kg N m^{-3} rate and the Control. There were no differences in remaining treatments with foliar K being the lowest for Syn at the rate the 0.89 kg N m^{-3} at 1.99% ± 0.51%. Notably, between locations, petunias produced in Mobile contained, on average, 25% more K than petunias produced in

Auburn. Despite general deficiencies observed in this study, prior research has similarly produced marketable petunias and other bedding plants with foliar K concentrations below recommended levels (Burnett et al. 2016; Alvarado-Camarillo et al. 2018). While K was deficient in many petunias, foliar Mg was sufficient. Furthermore, while K has an antagonistic relationship with Mg, the effect is not mutual and is not a limiting factor in plant uptake of K (Xie et al. 2021). Rather than competitive effects, the low foliar K concentrations observed in this study were likely the result of the underapplication of K in the fertilizer treatments.

Few differences were observed in foliar concentrations of Ca, Mg, and other micronutrients (Bryson et al. 2014). All treatments were deficient in Ca, ranging in foliar concentrations from 0.56% - 0.91%. All treatments, except PL for the 0.44 kg N m^{-3} and 0.89 kg N m^{-3} rates, satisfied the minimum sufficiency requirement of 0.33% in Mg (Bryson et al. 2014). All treatment combinations had Mg foliar concentrations ranging from 0.31% to 0.52%. For Boron (B), only PL and Syn applied at rates of 0.89 kg N m^{-3} and 1.78 kg N m^{-3}, and Blend applied at rates of 1.78 kg N m^{-3} met the foliar tissue analysis sufficiency requirement of 18 ppm for petunias. All other treatment combinations resulted in B foliar concentrations ranging from 12-17 ppm. All fertilizer and rate applications resulted in Manganese sufficiency levels of 44 ppm, ranging from 68 ppm to 169 ppm. Except for C&G at 0.44 kg N m^{-3}, all fertilizer treatments resulted in foliar concentrations exceeding the Zinc sufficiency level of 33 ppm and ranged from 27 ppm to 104 ppm. All fertilizer treatments, except for C&G and Blend applied at 0.44 kg N m^{-3}, met the Iron sufficiency level of 84 ppm, ranging from 71 ppm to 130 ppm. Additional micronutrients applied to petunias receiving Syn, Blend, and PL resulted in comparable foliar micronutrient ranges to petunias given C&G and no visual deficiency was prevalent for the nutrients. Fertilizing with C&G produced petunias and tomatoes with similar foliar micronutrient concentrations as applying Syn with a micronutrient fertilizer. These results suggest that C&G, applied alone or incorporated in a blend, has the potential to displace micronutrient fertilizers for short-term, container-grown crops.

3.3.4.6. Chlorophyll Concentration.

Chlorophyll content was different between location ($P < 0.0001$) and treatment*location ($P < 0.0001$). Between Auburn and Mobile replications, petunia chlorophyll concentrations were affected by treatment ($P < 0.0001$) and rate ($P < 0.0001$). Petunia chlorophyll content in Auburn was, on average, 9.4% higher than in Mobile. Such discrepancy could be the result of differences in time or weather during data collection. Despite these differences, both locations exhibited similar trends in response to fertilizer treatments. The highest chlorophyll content resulted from applications of 0.89 kg N m^{-3} C&G at both locations. In petunias, SPAD readings below 40 indicate nutrient deficiency (Smith et al., 2004). At both locations, Syn and Blend applied at 1.78 kg N m^{-3} and the Control fell below this threshold. Additionally, in Auburn, PL at 1.78 kg N m^{-3} and, in Mobile, the PL at 0.89 kg N m^{-3} also fell below this threshold (Tables 3.11 & 3.12). All other treatments can be considered sufficient with few differences recorded. As previously mentioned with tomatoes, correlations between chlorophyll content and N concentration have been recorded (Fontes and Ronchi 2002; Ulissi et al. 2011; Jiang et al. 2017). In Auburn, the high levels of N, particularly in PL, applied may have induced toxicity, limiting growth, and causing yellowing leaves. In Mobile, petunias lacked the degree of damage observed in Auburn, though both treatments at 1.78 kg N m^{-3} experienced similar declines in volume and weight, suggesting similar trends (Table 3.9).

3.4 Conclusion

Overall, C&G, both as a stand-alone and blended nutrient source, has shown positive effects on plant growth. It was less volatile than the ammonium sulfate blend of synthetic fertilizer utilized in these assays, resulting in fewer plant fatalities. In the event of fertilizer overapplication, plants supplied with C&G rebounded quicker than synthetic fertilizers. Fertilizing with C&G resulted in petunias and tomatoes having similar foliar micronutrient concentrations as those treated with Syn and a micronutrient fertilizer, suggesting that C&G, whether applied alone or blended, has the potential to replace micronutrient fertilizers for short-term, container-grown crops. Plant assays complemented the nutrient release tests from Chapter 2 as the product behaved in a similar manner to synthetic fertilizers.

The Cleaned & Green product has the potential to be a commercial alternative to current synthetic fertilizers in greenhouse production. This novel, litter-based product has the greatest potential in short-duration production systems where irrigation and leachate fractions can be carefully controlled. The fertilizer has the potential as a starter fertilizer for longer-duration crops. At this time, use as the primary fertilizer source for outdoor container production is not recommended. In concurrence with Chapter 2, C&G is a versatile and environmentally friendly option for agricultural applications.

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Table 3.1 Analysis of variance (ANOVA) for the effects of fertilizer treatments on the development of *Lycopersicum* x 'Celebrity' for the studied traits.[z]

		P-values						
Source of variation	df[y]	Fresh weight	Dry weight	Height	Chlorophyl content	Foliar N	Foliar P	Foliar K
A: Fertilizer	11	<0.0001	<0.0001	<0.0001	0.0410	<0.0001	<0.0001	<0.0001
B: Rate	3	<0.0001	<0.0001	<0.0001	0.0010	0.0010	0.0069	0.0327
A×B	11	<0.0001	<0.0001	<0.0001	0.0410	<0.0001	<0.0001	<0.0001

[z]Treatment effects were analyzed using PROC Glimmix in SAS 9.4 (SAS Institute, Cary, NC, USA).
[y]df = degrees of freedom

Table 3.2 Fertilizer type and rate effects on tomatoes (*Solanum lycopersicum* 'Celebrity') fresh and dry weights produced in Auburn, AL.

Fertilizer type	Rate (kg N m^{-3})	Fresh weight (%)	Dry weight (%)
Synthetic[y]	0.44	111.3de[z]	19.5cd
	0.89	162.8cd	29.9bc
	1.78	--	--
Blend[x]	0.44	171.9c	30.4b
	0.89	181.4c	32.5b
	1.78	89.4e	12.6d
C&G[w]	0.44	195.9c	33.7b
	0.89	191.7c	32.7b
	1.78	167.6cd	24.9bc
Poultry litter[v]	0.44	201.8c	34.8b
	0.89	294.4b	49.9a
	1.78	372.4a	58.2a
Control[u]	--	1.4f	0.2e

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.

[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triplesuperphosphate P, potash. The amount of N, P, and K applied to each plant was 16.0 g, 1.09 g, and 1.09 g, respectively.

[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.

[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.

[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.

[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 1.84 mg per container.

Table 3.3 Fertilizer type and rate effects on tomatoes (*Solanum lycopersicum* 'Celebrity') heights and chlorophyl content in Auburn, AL.

Fertilizer type	Rate (kg N m^{-3})	Heights (cm) Week 4	Heights (cm) Week 8	Chlorophyl content
Synthetic[y]	0.44	37.9c[z]	75.6abc	43.2a
	0.89	40.0bc	74.7abc	44.0a
	1.78	--	--	--
Blend	0.44	42.5abc	80.4ab	40.1ab
	0.89	37.9c	75.8abc	43.1a
	1.78	13.1d	51.0c	39.6ab
C&G	0.44	43.4abc	83.8ab	39.6ab
	0.89	40.6bc	80.8ab	37.8ab
	1.78	19.5d	61.8bc	41.6ab
Poultry litter	0.44	54.2a	88.3ab	34.9ab
	0.89	50.6ab	86.4ab	38.4ab
	1.78	40.9bc	90.4a	40.6ab
Control	--	10.4d	12.3d	24.1b

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triplesuperphosphate P, potash. The amount of N, P, and K applied to each plant was 16.0 g, 1.09 g, and 1.09 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 1.84 mg per container.

Table 3.4 Fertilizer type and rate effects on tomatoes (*Solanum lycopersicum* 'Celebrity') tissue macronutrient concentrations produced in Auburn, AL.

Fertilizer type	Rate (kg N m^{-3})	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Synthetic[y]	0.44	1.97abc[z]	0.07d	0.74ab
	0.89	1.53dc	0.19cd	0.53b
	1.78	--	--	--
Blend	0.44	1.55cd	0.12cd	0.46b
	0.89	1.78cd	0.17cd	039b
	1.78	2.56a	0.37ab	0.63b
C&G	0.44	1.49cde	0.12cd	0.47b
	0.89	1.94bcd	0.19cd	0.34b
	1.78	2.47ab	0.26bc	0.40b
Poultry litter	0.44	0.86f	0.44a	0.79ab
	0.89	0.96ef	0.46a	0.71b
	1.78	1.39def	0.38ab	1.32a
Control	--	--	--	--

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triplesuperphosphate P, potash. The amount of N, P, and K applied to each plant was 16.0 g, 1.09 g, and 1.09 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 1.84 mg per container.

Table 3.5 Analysis of variance (ANOVA) for the effects of fertilizer treatments on the development of *Lollium multiflorum* for the studied traits.[z]

		P-values			
Source of variation	df[y]	Fresh weight	Dry weight	Height	Chlorophyl content
A: Fertilizer	12	<0.0001	<0.0001	<0.0001	<0.0001
B: Rate	3	<0.0001	0.0254	<0.0001	0.0245
A×B	12	<0.0001	<0.0001	<0.0001	<0.0001

[z]Treatment effects were analyzed using PROC Glimmix in SAS 9.4 (SAS Institute, Cary, NC, USA).
[y]df = degrees of freedom

Table 3.6 Fertilizer type and rate effects on rye (*Lolium multiflorum*) fresh and dry weights produced in Auburn, AL.

Fertilizer type	Rate (kg N m^{-3})	Fresh weight (%)	Dry weight (%)
Synthetic[y]	0.44	50.2b[z]	12.6a-d
	0.89	67.9ab	12.2a-d
	1.78	61.9ab	9.6cd
Blend[x]	0.44	67.2ab	15.8ab
	0.89	77.0ab	13.4ab
	1.78	50.8b	8.5d
C&G[w]	0.44	61.0ab	14.5a-d
	0.89	78.6ab	17.2a
	1.78	72.7ab	12.4a-d
Poultry litter[v]	0.44	51.5b	11.3bcd
	0.89	79.8ab	15.8ab
	1.78	97.0a	17.1a
Control[u]	--	0.9c	0.1e

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triplesuperphosphate P, potash. The amount of N, P, and K applied to each plant was 16.0 g, 1.09 g, and 1.09 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 1.84 mg per container.

Table 3.7 Fertilizer type and rate effects on rye (*Lolium multiflorum*) heights and chlorophyl content produced in Auburn, AL.

Fertilizer type	Rate (kg N m^{-3})	Heights (cm) Week 4	Heights (cm) Week 8	Chlorophyl content
Synthetic[y]	0.44	37.8ab[z]	87.8ab	34.2ab
	0.89	40.0ab	72.2ab	39.9a
	1.78	51.1a	63.7b	36.6ab
Blend	0.44	42.5ab	83.2ab	37.0a
	0.89	37.8ab	84.0ab	39.3a
	1.78	15.7cd	69.9ab	39.7a
C&G	0.44	43.4ab	75.8ab	38.6a
	0.89	40.6ab	80.2ab	37.5a
	1.78	28.8bc	66.9ab	39.7a
Poultry litter	0.44	52.3a	78.3ab	33.0ab
	0.89	51.2a	92.3a	29.5b
	1.78	34.3b	69.9ab	37.0a
Control	--	8.9d	15.2c	--

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triplesuperphosphate P, potash. The amount of N, P, and K applied to each plant was 16.0 g, 1.09 g, and 1.09 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 1.84 mg per container.

Table 3.8 Analysis of variance (ANOVA) for the effects of fertilizer treatments on the development of *Petunia* x *hybrida* 'Supertunia Vista Bubblegum' for the studied traits.[z]

		P-values						
Source of variation	df[y]	Fresh weight	Dry weight	Growth index	Chlorophyl content	Foliar N	Foliar P	Foliar K
A: Fertilizer	12	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
B: Rate	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS[x]
C: Location	1	<0.0001	<0.0001	<0.0001	0.0076	NS	NS	0.0196
A×B	12	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A×C	12	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
B×C	3	0.0002	0.0085	NS	0.0201	<0.0001	<0.0001	NS

[z]Treatment effects were analyzed using PROC Glimmix in SAS 9.4 (SAS Institute, Cary, NC, USA).
[y]df = degrees of freedom
[x]NS = Not Significant

Table 3.9 Fertilizer type and rate effects on petunia (*Petunia x hybrida* 'Supertunia Vista Bubblegum') fresh and dry weights produced in Auburn, AL.

Fertilizer type	Rate ($kg\ N\ m^{-3}$)	Fresh weight (%)	Dry weight (%)
Synthetic[y]	0.44	22.9bcd[z]	2.9bcd
	0.89	15.3def	1.7def
	1.78	3.8fg	0.5f
Blend[x]	0.44	30.5abc	4.2ab
	0.89	19.5cd	2.6cde
	1.78	4.5fg	0.7f
C&G[w]	0.44	31.6ab	4.4a
	0.89	17.4de	2.5cde
	1.78	8.3efg	1.2ef
Poultry litter[v]	0.44	39.7a	4.7a
	0.89	30.4abc	3.3abc
	1.78	5.7fg	0.8f
Control[u]	--	3.1g	0.5f

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triple superphosphate P, potash. The amount of N, P, and K applied to each plant was 4.22 g, 0.28 g, and 0.28 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 0.48 mg per container.

Table 3.10 Fertilizer type and rate effects on petunia (*Petunia x hybrida* 'Supertunia Vista Bubblegum') fresh and dry weights produced in Mobile, AL.

Fertilizer type	Rate ($kg\ N\ m^{-3}$)	Fresh weight (%)	Dry weight (%)
Synthetic[y]	0.44	29.5bc[z]	4.1bc
	0.89	30.2abc	3.6bcd
	1.78	6.1d	1.9efg
Blend[x]	0.44	36.7abc	5.6a
	0.89	33.9abc	4.0bc
	1.78	12.0d	1.3fg
C&G[w]	0.44	38.5ab	5.0ab
	0.89	39.2ab	4.4abc
	1.78	37.7ab	4.3abc
Poultry litter[v]	0.44	26.5c	3.3cde
	0.89	41.1a	3.6bcd
	1.78	26.5c	2.4def
Control[u]	--	4.8d	0.6f

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triple superphosphate P, potash. The amount of N, P, and K applied to each plant was 4.22 g, 0.28 g, and 0.28 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 0.48 mg per container.

Table 3.11 Fertilizer type and rate effects on petunia (*Petunia x hybrida* 'Supertunia Vista Bubblegum') volumes and chlorophyll content produced in Auburn, AL.

Fertilizer type	Rate (kg N m^{-3})	Growth indices (cm^3) Week 2	Week 4	Chlorophyl content
Synthetic[y]	0.44	590cde[z]	6795bc	53.3ab
	0.89	657b-e	3655cde	45.4bcd
	1.78	479e	317e	24.6e
Blend	0.44	1172a	12605a	54.5ab
	0.89	897a-e	6287bcd	55.0ab
	1.78	540e	508e	38.4cde
C&G	0.44	1087abc	10559ab	55.3ab
	0.89	874a-e	6837bc	58.2a
	1.78	579de	1717de	50.4abc
Poultry litter	0.44	1097ab	10747ab	47.0bcd
	0.89	1055a-d	8354ab	50.0abc
	1.78	744a-e	839e	36.5de
Control	--	469e	597e	24.8e

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triple superphosphate P, potash. The amount of N, P, and K applied to each plant was 4.22 g, 0.28 g, and 0.28 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 0.48 mg per container.

Table 3.12 Fertilizer type and rate effects on petunia (*Petunia x hybrida* 'Supertunia Vista Bubblegum') volumes and chlorophyll content produced in Mobile, AL.

Fertilizer type	Rate (kg N m^{-3})	Growth indices (cm^3) Week 2	Week 4	Chlorophyl content
Synthetic[y]	0.44	1577abc[z]	10755abc	45.2ab
	0.89	1234a-d	7892be	45.4ab
	1.78	801bcd	657e	28.5de
Blend	0.44	1774ab	14710ab	48.0ab
	0.89	1417abc	9898a-d	48.4ab
	1.78	774cd	1736de	35.5cd
C&G	0.44	1954a	16238a	45.7ab
	0.89	1836a	13237abc	49.4a
	1.78	1626abc	8042b-e	46.5ab
Poultry litter	0.44	1840a	11315abc	35.8cd
	0.89	1660abc	12950abc	40.7bc
	1.78	1319a-d	6557cde	45.5ab
Control	--	460d	1827de	25.4e

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triple superphosphate P, potash. The amount of N, P, and K applied to each plant was 4.22 g, 0.28 g, and 0.28 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 0.48 mg per container.

Table 3.13 Fertilizer type and rate effects on petunia (*Petunia x hybrida* 'Supertunia Vista Bubblegum') tissue macronutrient concentrations produced in Auburn, AL.

Fertilizer type	Rate (kg N m^{-3})	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Synthetic[y]	0.44	3.84cd[z]	0.18d	1.76d
	0.89	6.17a	0.48abc	1.87d
	1.78	5.71ab	0.45abc	2.29d
Blend	0.44	2.90de	0.16d	1.46d
	0.89	4.93abc	0.30dc	1.82d
	1.78	5.76ab	0.48abc	2.28d
C&G	0.44	2.87de	0.23d	1.42d
	0.89	5.09abc	0.37bc	1.67d
	1.78	6.17a	0.49ab	2.26d
Poultry litter	0.44	2.37e	0.46abc	3.95bc
	0.89	4.05bcd	0.58a	4.79ab
	1.78	6.49a	0.46abc	5.60a
Control	--	1.25e	0.16d	2.62cd

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triple superphosphate P, potash. The amount of N, P, and K applied to each plant was 4.22 g, 0.28 g, and 0.28 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 0.48 mg per container.

Table 3.14 Fertilizer type and rate effects on petunia (*Petunia x hybrida* 'Supertunia Vista Bubblegum') tissue macronutrient concentrations produced in Mobile, AL.

Fertilizer type	Rate (kg N m^{-3})	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Synthetic[y]	0.44	4.01cde[z]	0.18e	2.46c
	0.89	5.74ab	0.33cde	2.93bc
	1.78	5.85a	0.45abc	2.00c
Blend	0.44	4.02cde	0.35b-d	2.71c
	0.89	5.66ab	0.18e	2.72c
	1.78	5.33abc	0.42a-d	2.43c
C&G	0.44	3.18def	0.23de	2.39c
	0.89	5.06abc	0.41a-d	2.44c
	1.78	6.13a	0.52ab	3.11abc
Poultry litter	0.44	1.93fg	0.4a-d	6.15ab
	0.89	2.81efg	0.49abc	6.19a
	1.78	4.45bcd	0.56a	5.01abc
Control	--	1.05g	0.20de	2.98abc

[z]Data were analyzed using a one-way anova and subsequent means were compared using the Tukey honest significant difference ($P \leq 0.05$). Means within a column with the same letter do not significantly differ from each other.
[y]Synthetic fertilizer was a custom blend comprised of ammonium sulfate, triple superphosphate P, potash. The amount of N, P, and K applied to each plant was 4.22 g, 0.28 g, and 0.28 g, respectively.
[x]Blend fertilizer was comprised of Synthetic and C&G blended together at a N ratio of 1:1.
[w]Cleaned & Green (C&G) is a poultry litter-based fertilizer product enhanced through a proprietary process. Its composition is 11.5-1-1.5 14-S.
[v]Poultry litter was collected at the Miller Poultry Science Center in Auburn, AL on Apr 17, 2023. Its composition is 1.3-1-1.5.
[u]Control plants received only the nutrients Mg, S, Cu, Fe, Mn, Mo, and Zn (Harrell's profertilizer) at a rate of 0.48 mg per container.

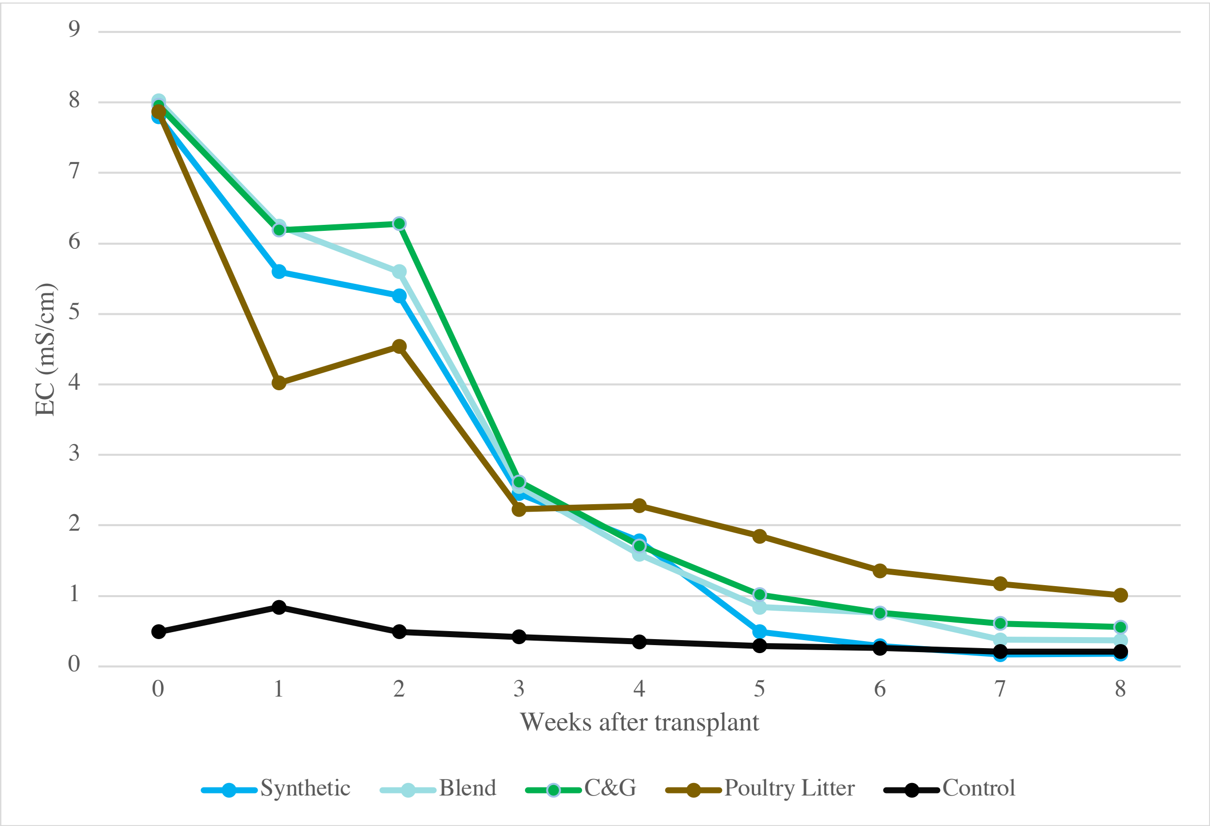

Figure 3.1. Electrical conductivity measurements for tomatoes applied at 0.89 kg N m^{-3} . Lines present averages of three measurements collected per treatment on a weekly basis. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

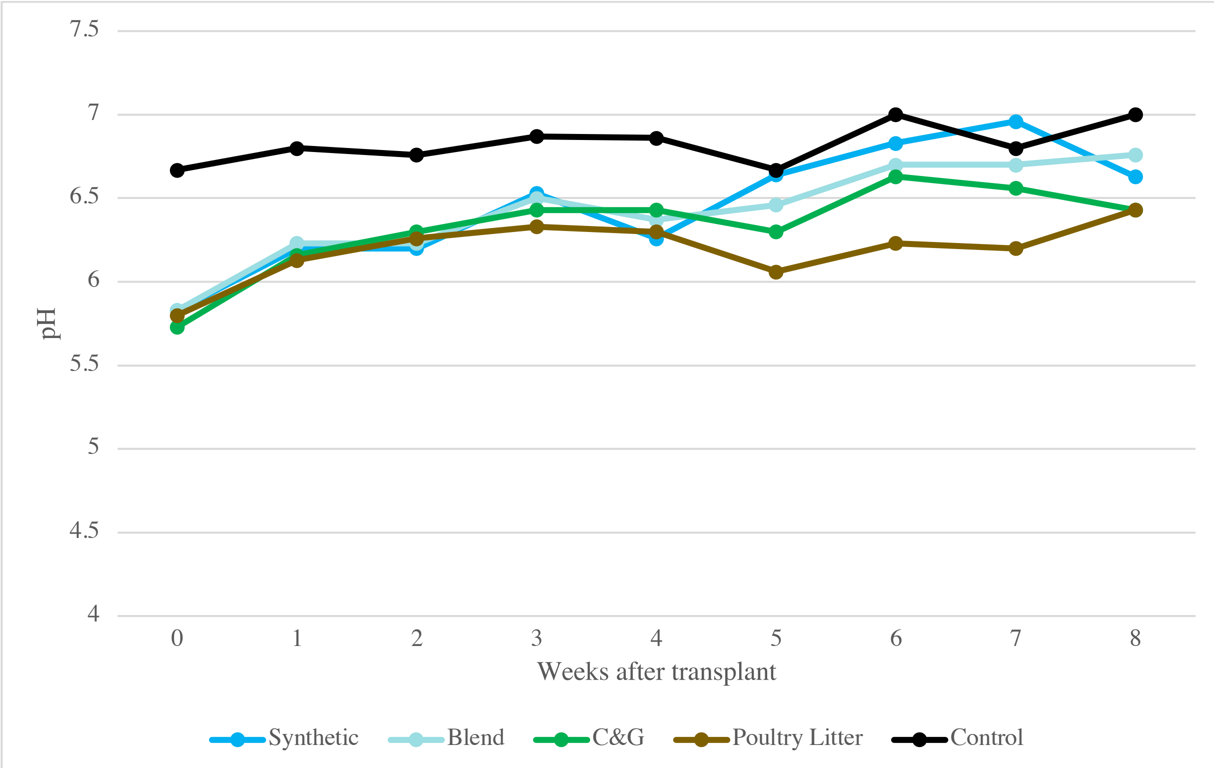

Figure 3.2. pH measurements for tomatoes applied at 0.89 kg N m^{-3} . Lines present the averages of three measurements collected per treatment on a weekly basis. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

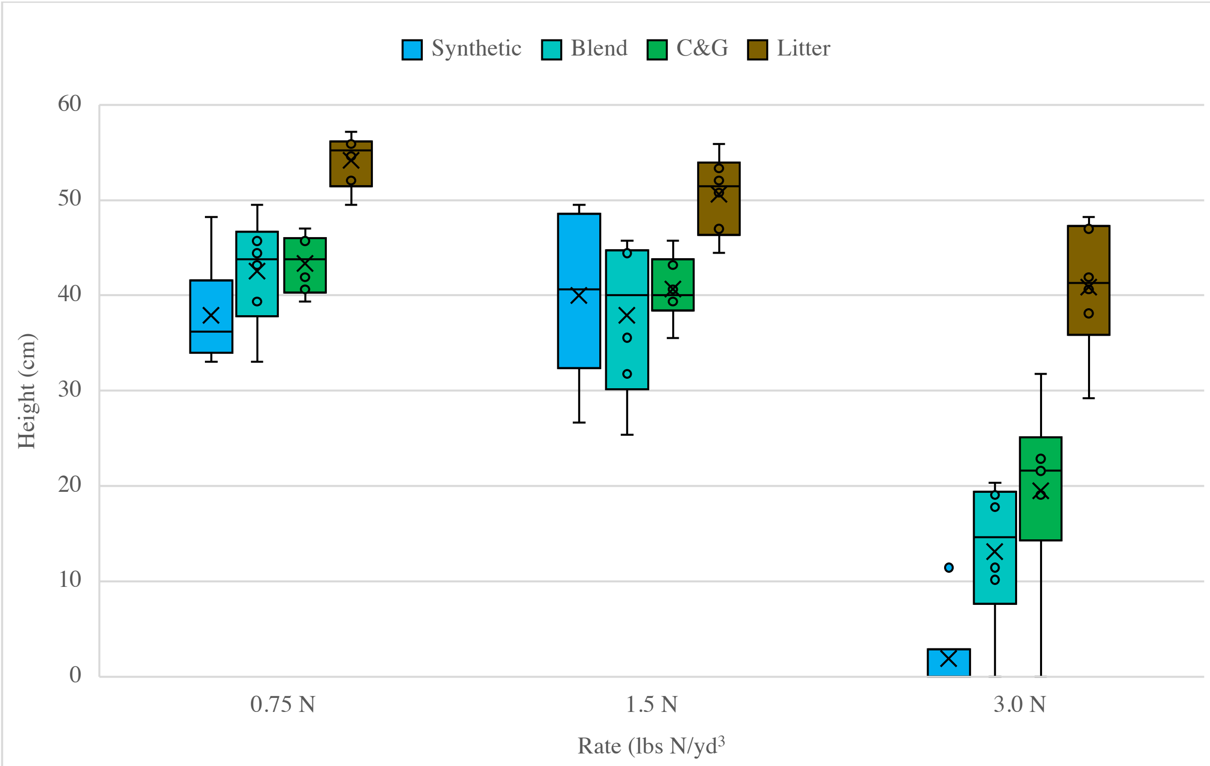

Figure 3.3. Four-week tomato heights for 0.89 kg N m^{-3} . Plants are grouped according to rate of application. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

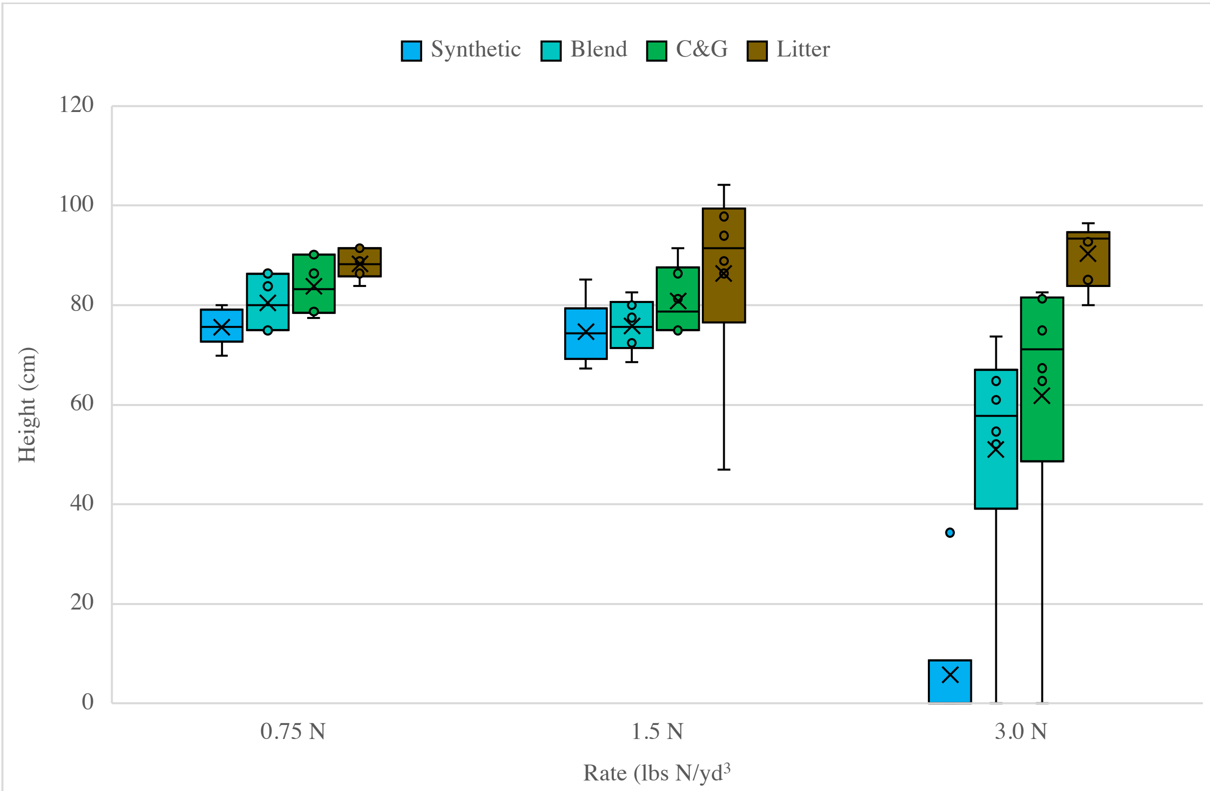

Figure 3.4. Eight week tomato heights for 0.89 kg N m^{-3}. Plants are grouped according to rate of application. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}**)** and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

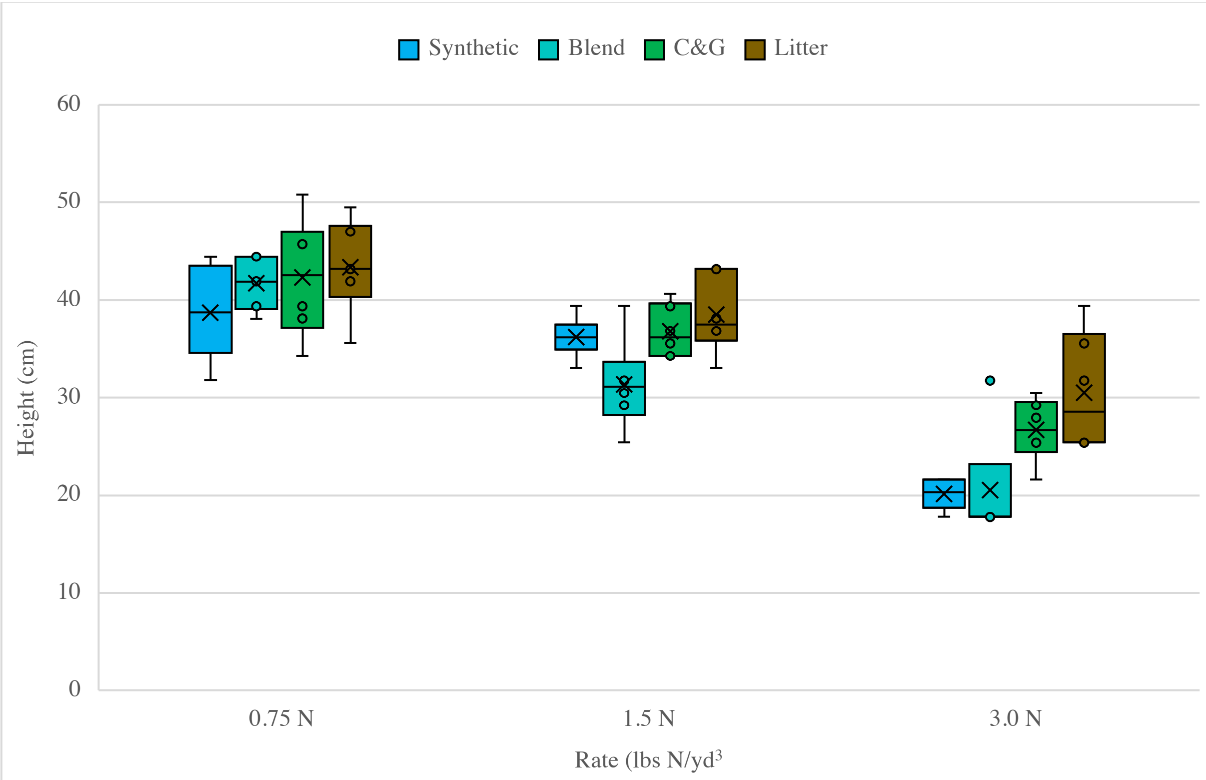

Figure 3.5. Four-week rye heights for 0.89 kg N m^{-3} . Plants are grouped according to rate of application. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

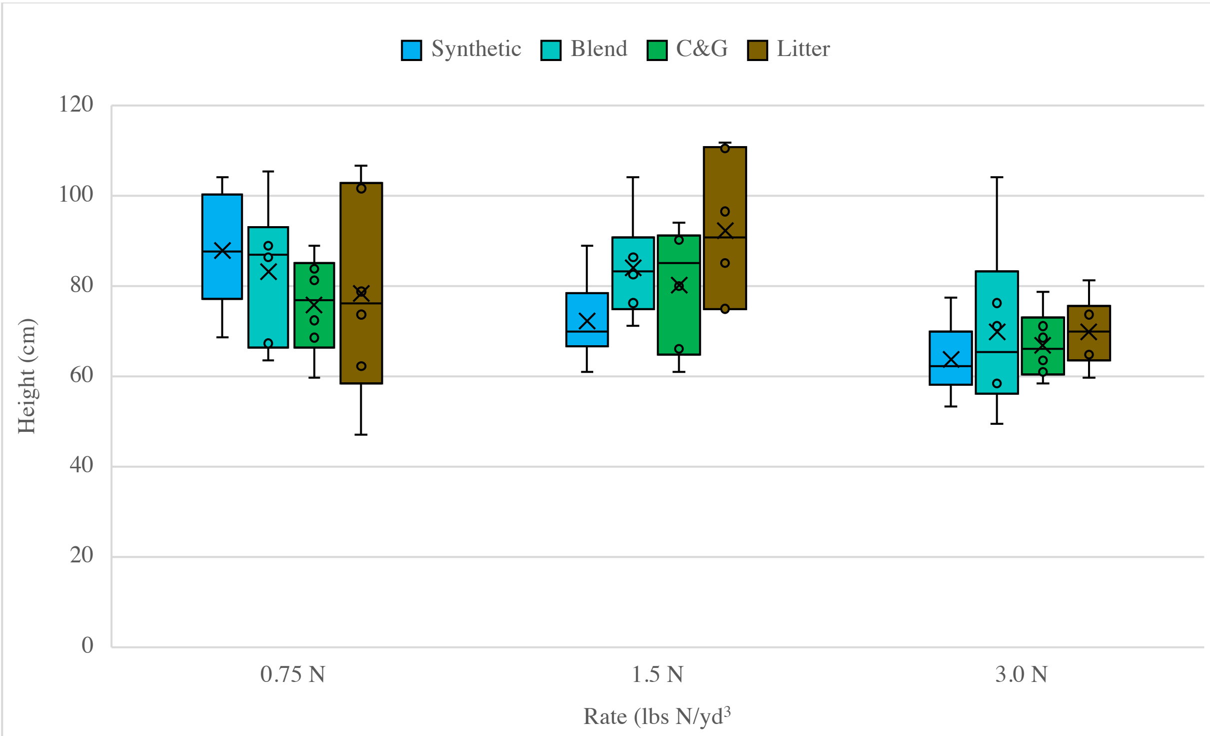

Figure 3.6. Eight-week rye heights for 0.89 kg N m^{-3} . Plants are grouped according to rate of application. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

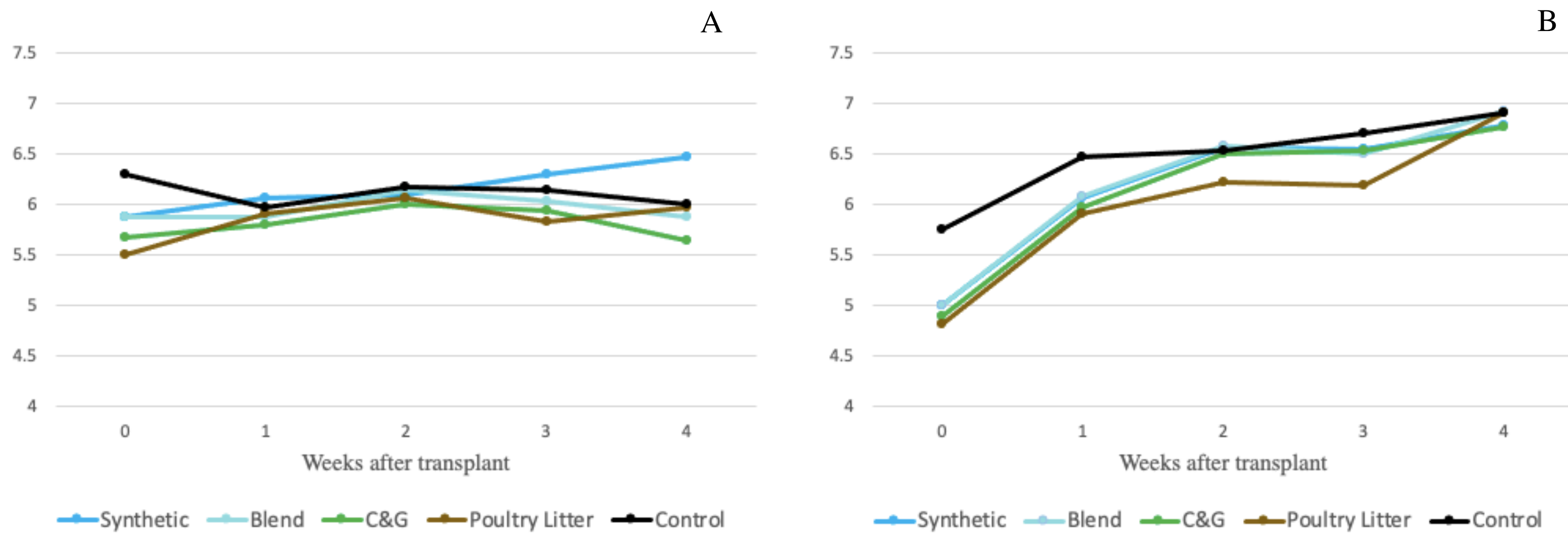

Figure 3.7. pH measurements for petunias grown at the 0.89 kg N m^{-3} rate A) Auburn measurements and B) Mobile measurements. Lines present the averages of three measurements collected per treatment on a weekly basis. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

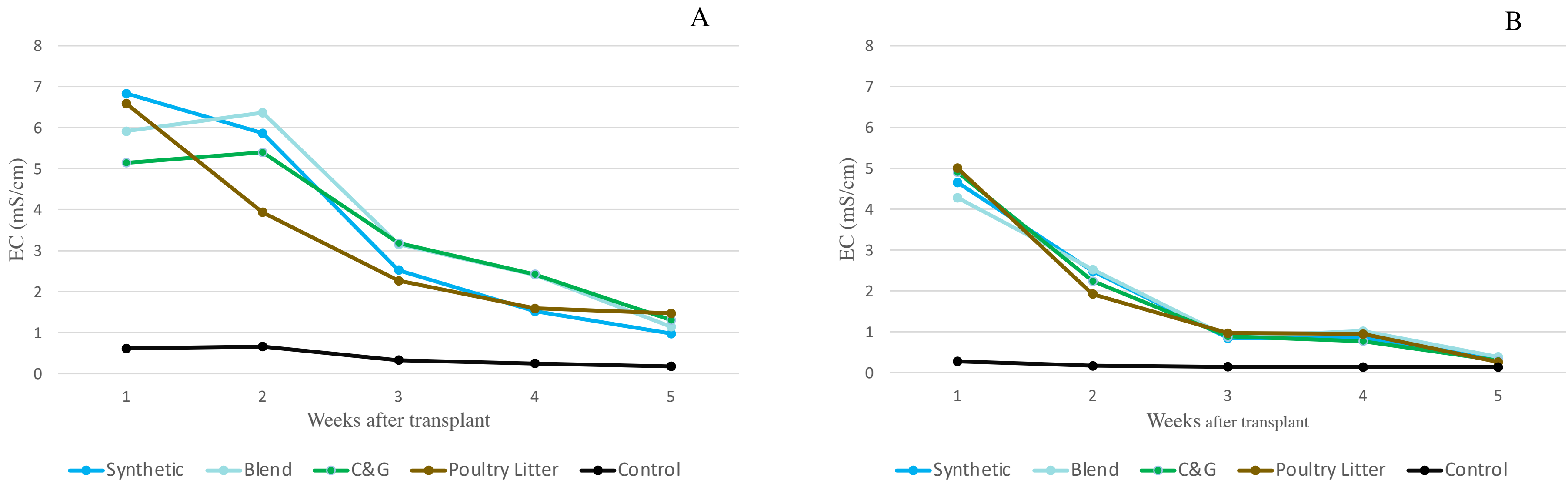

Figure 3.8. Electrical conductivity measurements for petunias which had an application of 0.89 kg N m^{-3} A) Auburn measurements and B) Mobile measurements. Lines present averages of three measurements collected per treatment on a weekly basis. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

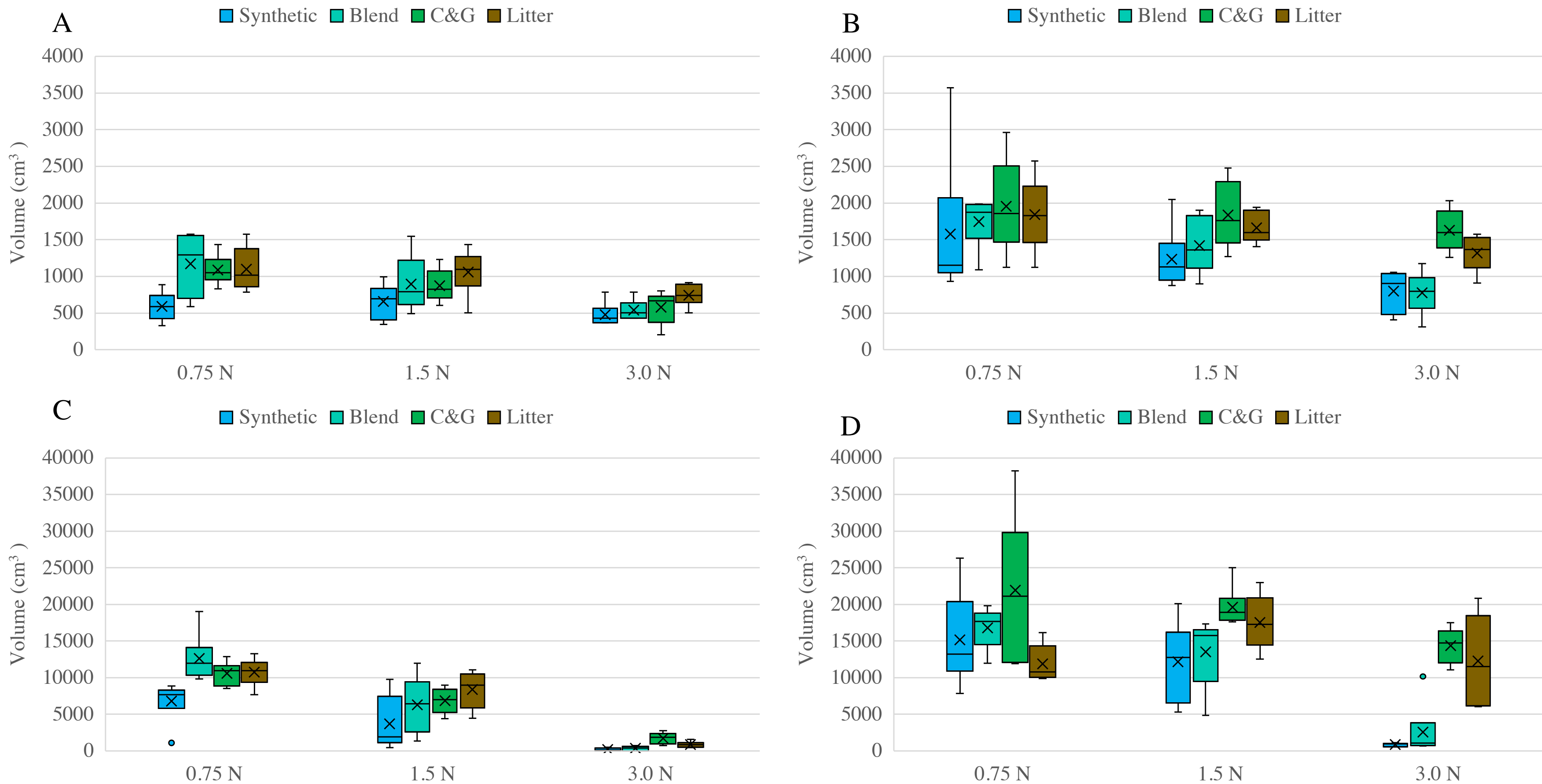

Figure 3.9. Two week volumes for 0.89 kg N m^{-3} A) Auburn measurements and B) Mobile measurements. Four weeks volumes for 0.89 kg N m^{-3} C) Auburn measurements and D) Mobile measurements. Plants are grouped according to rate of application. Treatments include: Synthetic, Blend (Cleaned and Green + Synthetic, Cleaned and Green (C&G) a poultry litter derived product, poultry litter, and a control receiving no additional fertilizer. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

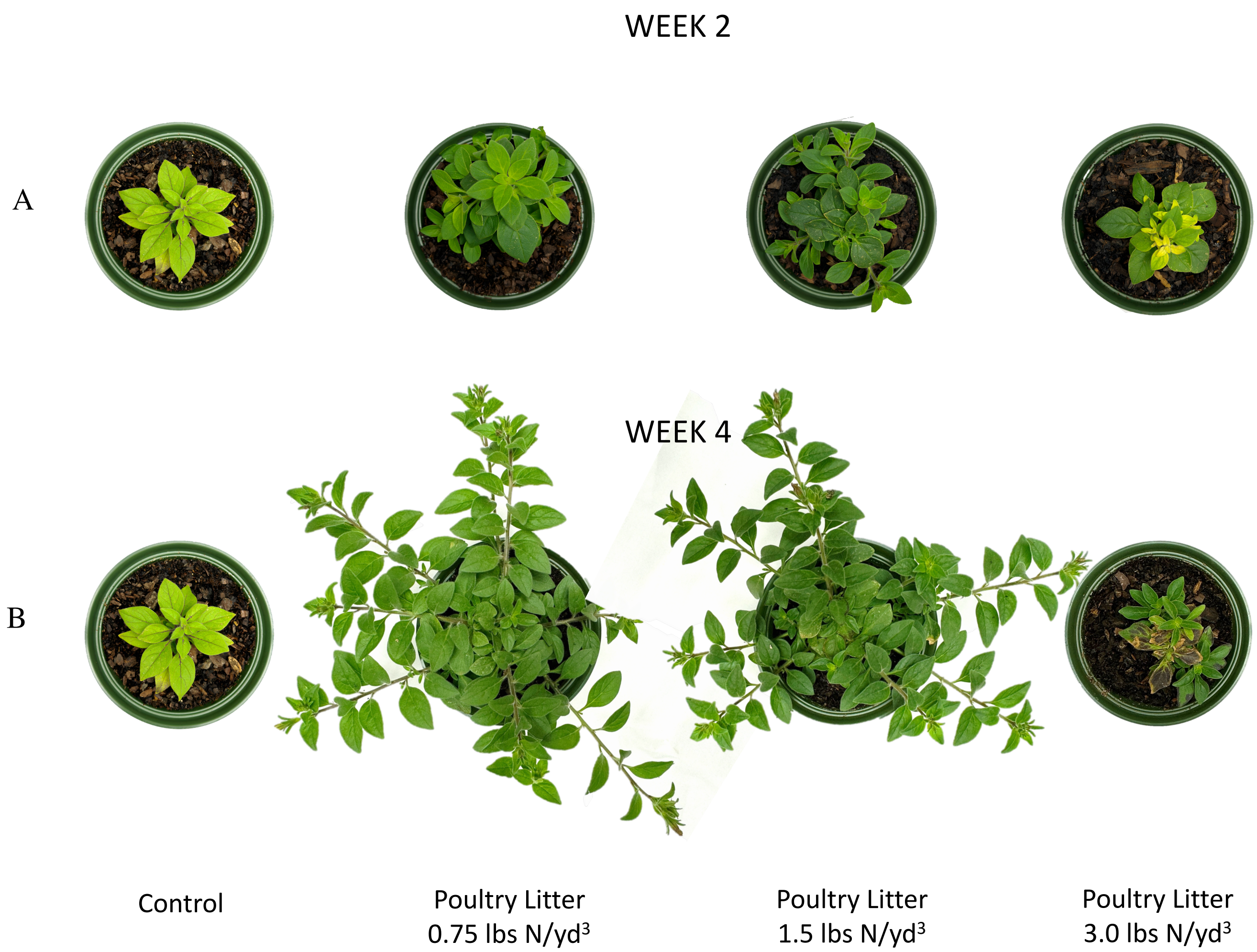

Figure 3.10. Overhead view of petunias receiving poultry litter at the rates 0 kg N m^{-3}, 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, 1.78 kg N m^{-3} nitrogen grown in Auburn A) Two week overhead view B) Four week overhead view. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

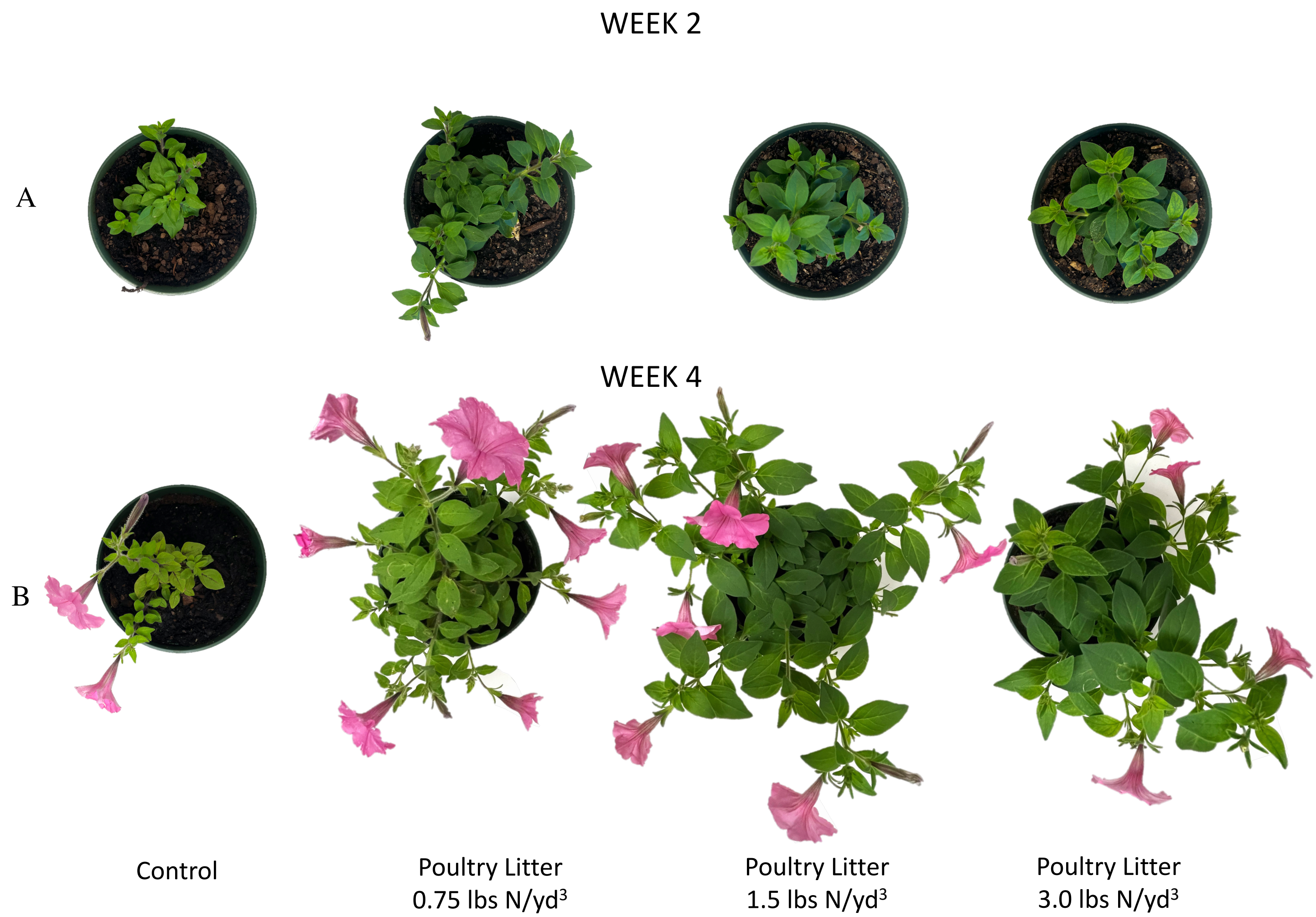

Figure 3.11. Overhead view of petunias receiving poultry litter at the rates 0 kg N m^{-3}, 0.44 kg N m^{-3}, 0.89 kg N m^{-3}, 1.78 kg N m^{-3} grown in Mobile A) Two week overhead view B) Four week overhead view. Micronutrients (Mg, S, Cu, Fe, Mn, Mo, and Zn, rate: 0.89 kg m^{-3}) and lime (rate: 2.96 kg m^{-3}) were applied to all treatments.

Chapter 4: Final Conclusions

The novel poultry-derived Cleaned & Green fertilizer acts in a similar manner to traditional synthetic fertilizers both in terms of total macronutrient release and plant growth parameters. While releasing similar levels of nitrogen, C&G, and the Synthetic diverged nitrogen speciation upon release. The C&G fertilizer has a continual increase in release of nitrate where the Synthetic had an initial release of nitrate and remained flat after, relying on ammonium release to perform similarly. Furthermore, K exhibited a similar initial release behavior concentrations for C&G similar to the rapid-release fertilizer. Only the raw PL had significant concentrations of P recorded. Poultry litter exhibited release habits similar to other studies where phosphorus and potassium were overapplied due to a near even nutrient ratio.

The C&G product performed comparable to Synthetic at low rates of nitrogen. However, plants grown with Clean & Green's product produced similar results at higher rates where both Synthetic and Poultry Litter decreased plant quality. In tomatoes, the high nitrogen rate of Synthetic resulted in an elevated mortality rate and stunting in the lone surviving replicant. While plants treated with C&G did have initial stunting, by Week 8 the plants experienced similar rates of growth as other rates. In Petunias, variability in poultry litter quality produced stunting in the Auburn replicants and uniform growth in Mobile replicants. However, the C&G treated plants had similar trends as tomatoes, suggesting a uniform product lacking the volatility experienced in both Synthetic and Poultry Litter.

The Cleaned and Green fertilizer is recommended for use in settings where traditional rapid-release synthetic fertilizers are used, particularly in crops with short growing seasons. In industry, the fertilizer may be used as a starter fertilizer, applied in conjunction with fertigation or CRF's to produced market quality products. The additional blend of Ca, Mg, and micronutrients provided adequate nutrition, saving growers to apply all nutrients at a single point in the growing season. The product may have additional appeal within the residential setting. The stable nature and natural origin of the product may entice residential consumers searching for an alternative product. The C&G fertilizer is not recommended for outdoor container production due to rapid nutrient leaching. For both industry and residential, the C&G fertilizer provides an additional market fertilizer option which can be locally sourced.