Scientific Research Publishing

Agricultural Sciences, 2024, 15, 1223-1235
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Comparative Analysis of *Lactuca sativa* Growth Using Compost Versus Conventional Soil

Sofia Huber[1], Galen Papkov[2], Xiusheng Yang[3], John Griffis[4], Kathryn Jackson[5], Sarah Bauer[6], Ankit Kumar Singh[7]

[1]Department of Marine and Earth Sciences, The Water School, Florida Gulf Coast University, Fort Myers, FL, USA
[2]Department of Mathematics, Florida Gulf Coast University, Fort Myers, FL, USA
[3]Department of Natural Resources, University of Connecticut, Storrs, CT, USA
[4]Department of Ecology and Environmental Studies, The Water School, Florida Gulf Coast University, Fort Myers, FL, USA
[5]Independent Researcher, Alexandria, VA, USA
[6]Department of Environmental and Civil Engineering, Mercer University, Macon, GA, USA
[7]Department of Cooperative Extension, University of Maine, Orono, ME, USA

Email: schuber2515@eagle.fgcu.edu, gpapkov@fgcu.edu, xiusheng.yang@uconn.edu, jgriffis@fgcu.edu, kgn816@gmail.com, bauer_sk@mercer.edu, ankit.singh@maine.edu

How to cite this paper: Huber, S., Papkov, G., Yang, X.S., Griffis, J., Jackson, K., Bauer, S. and Singh, A.K. (2024) Comparative Analysis of *Lactuca sativa* Growth Using Compost Versus Conventional Soil. *Agricultural Sciences*, **15**, 1223-1235.
https://doi.org/10.4236/as.2024.1511066

Received: April 17, 2024
Accepted: November 16, 2024
Published: November 19, 2024

Abstract

Conventional agricultural techniques have been degrading American soils nationwide since the beginnings of modern-day agriculture through practices such as soil tilling, using nitrogen synthetic fertilizers, and monocultural systems. These techniques contribute to degrading soil health, mass emissions of carbon dioxide into the atmosphere, and decreased biodiversity. Regenerative agriculture techniques include the utilization of cover crops, compost, no-tillage, the integration of livestock, and crop rotation. The APS Laboratory for Sustainable Agriculture focused on the effectiveness of compost by comparing the growth of lettuce in four different treatments: 100% Compost (100%C), 75% Compost 25% Miracle-Gro (75%C - 25%MG), 50% Compost 50% Miracle-Gro (50%C - 50%MG), and finally, 100% Miracle-Gro (100%MG). The lettuce seeds were kept in a growth tent for fifteen days during their period of germination before being transferred to four 1 × 1 × 0.15 m plots in the Food Forest at Florida Gulf Coast University (FGCU) for the 60-day growth period. The lettuce crops grew to full bloom and were ready for harvest. Sampling events took place every six days in which crop growth data including wet weight (g), dry weight (g), nitrogen (mg/g), chlorophyll concentration (mg/cm^2), and leaf area (LA) (cm^2) were collected. Statistical analysis was then conducted from the data. Based on the statistical tests conducted at the 5% significance level using R statistical software, soil treatment type was found to be significant ($p = 0.0002$). Soil treatment type was shown to have significantly impacted wet weight ($p < 0.0001$), dry weight ($p < 0.0001$), and leaf area (p =

0.0011), but not nitrogen (χ^2 [3] = 3.91, p = 0.2717). 100%C and 100%MG of soil treatments produced the most successful lettuce crops. The 100%C soil treatment yielded lettuce crops with the heaviest wet weights and the largest LAs, and the 100% MG soil treatment yielded the heaviest dry weights and the highest nitrogen readings. Results demonstrate the effectiveness and feasibility of using compost as a technique for regenerative agriculture.

Keywords

Compost, Conventional Agriculture, Lettuce, Regenerative Agriculture, Soil Health

1. Introduction

The exponential, global population growth paired with rapidly declining arable land has made the consequences of climate change unavoidable [1]. It is estimated by the Food and Agricultural Organization (FAO) that 33% of global farmland is classified as degraded [2]. Widescale soil degradation is due to the extensive use of conventional agricultural techniques such as smaller farms becoming more extensive, manual labor becoming mechanized, and locally grown markets transforming to export businesses [3], as well as the prioritization of monocultural systems as opposed to biodiversity [4]. Global food supply is projected to become unstable due to extreme weather events brought on by climate change from increased greenhouse gas emissions [5]. Conventional farming practices emit between 21% - 37% of global anthropogenic greenhouse gas emissions [5]. In addition to emitting substantial amounts of greenhouse gases, conventional practices can cause soil desertification and salinization, the spread of farm pests, climate change-induced droughts, and both air and water pollution [6]. Based on current agricultural practices being emission-intensive and degrading the environment, it can be inferred that conventional farming practices are not sustainable, and an alternative is needed to reduce greenhouse gas emissions and repair the global food system.

Regenerative agricultural practices may offer a solution. Regenerative agriculture can be defined by the components of no soil tillage, use of compost, application of cover crops, the integration of livestock, and crop rotation [7] [8]. The goal of regenerative agriculture, contrary to conventional agriculture, is to increase the amount of organic carbon content in farming soil, sequester carbon dioxide from the atmosphere, and increase land productivity [7]. Eliminating soil tillage can positively influence soil fertility and create more stable soil aggregates [9] [10]. Cover crops are planted between growing periods, during which the land would normally be barren [11]. Utilizing cover crops to increase soil cover can in turn increase overall carbon and nitrogen content in the soil, as well as support microbial biomass [12] [13]. Integrating both crop and livestock production into a single farm has been shown to increase overall soluble carbon levels and improve the bacterial concentrations in the soil, by 94.8% and 104% [14]. The rotation of crops over different seasons promotes carbon sequestration in the soil and soil aggregation [15]. The literature demonstrates that the techniques of no soil tilling, cover crops, integrated livestock management, and crop rotation combined, including the use of compost, create the perfect regenerative system to sequester carbon dioxide from the atmosphere, increase ecosystem diversity, and promote soil health.

Compost can be formed through the breakdown from detritus feeders, such as earthworms, and can be comprised of any waste products such as that from yards, agricultural facilities, animal manure, source-separate food waste, or biosolids [16]-[18]. The two main types of organic fertilizers, or compost, are plant organic fertilizers and animal organic fertilizers [19]. Plant fertilizers can be made of plant products, organic kitchens, or both wastes, while animal organic fertilizers consist of animal manure, bone meal, or both [19]. When added to crop soil, both types of compost, including the application of vermicompost, farmyard manure, and enriched compost have all shown to promote soil health and increase long-term crop output [20]. Utilizing compost in farms can enhance plant vegetative growth, increase soil nitrogen and water content, and improve microbial biomass by altering soil chemical properties [21]-[23]. Compost applications can improve the quality of soil structure characteristics such as soil bulk density and soil moisture content [24]. Studies involving compost mulches have revealed an increase in total organic carbon content and electrical conductivity [25]. Compost has also been shown to protect soils and crops from high heat and droughts [26]. Additionally, the benefits compost provides to the soil, such as soil carbon, nitrogen, phosphorus, and potassium as well as plant growth quality have shown to increase over time, along with decreased bulk density [27].

The objective of this study was to evaluate the effectiveness of compost compared to conventional soil systems in lettuce *Lactuca sativa* Rex Butterhead. The data collected in the forms of biomass weight, nitrogen, chlorophyll content, and leaf area were used to determine which soil treatment would best support the growth of the lettuce crops. The results produced from this study may be utilized to inform the public on the effectiveness of compost, mixed, and conventional soil systems in potential crop growth.

2. Materials and Methods

2.1. Location and Experimental Set-Up

The experiment was conducted at Florida Gulf Coast University (FGCU), located in the southwestern region of Florida, specifically Fort Myers, within the United States of America. FGCU is positioned at 26˚27'44"N, 81˚46'54"W (Google Earth). The climate of the southwest region of Florida consists of a wet season with high humidity and rainfall average of 120 cm between April and October, accompanied by temperatures between 23.9˚C - 32.2˚C [28]. The dry season is characterized by an average rainfall of 40 cm between November and March, paired with average temperatures of 23.9˚C - 27.2˚C [28].

During this process, two university facilities were used, including the grow tent in the Aquarium Room in the Water School (AB-9, Room 114), and space in the FGCU Food Forest (FGCU-FF). The FGCU-FF is a student-run, sustainable, polyculture food system. There were four 1 × 1 m plots of land in the FGCU-FF for the plugs to be transplanted into after a 15-day growth period. Before the plugs were transplanted to the FGCU-FF, they were grown in the Aquarium room of the Water School in the grow tents. The air temperature was kept between 20.0-23.3˚C throughout all seasons by the FGCU maintenance facility.

Rock-wool (Rockwool Starter Plugs, 12.09” × 5.63” × 4.76”, BIOMAN, Amazon) and coconut coir (Premium Coco Coir Brick for Plants, 3.94” × 3.94” × 3.94”, Legigo, China) plug mediums were used to grow the plugs saturated in a black tray with Reverse Osmosis (RO) water. The tray was covered in newspaper and placed in the dark grow tent for 48 h during the first period of the germination stage. After the 48 h rest period, the LED lights were turned on to a 16 h on and 8 h off life cycle (06:00-22:00) for the first 15 days. During this 15-day period, the plugs were given starter solution daily, starting with 200 mL and increasing as time progressed over the growing period. The starter solution was a mix of 3.6 g of “Jack’s hydroponic 15.5-0-0” (calcium nitrate) in addition to 3.8 g of “Jack’s hydroponic 5-12-26”, which was added for every 10 L of RO water. Once transferred to the FGCU-FF, the lettuce plugs were watered daily, using RO water stored in a 55 gal water drum (Blue 55 Gal Water Storage Tank 28” × 22” × 30”, WaterPrepared LLC, USA) outside in the FGCU-FF. Approximately 200 mL of RO water was added to each lettuce plug every morning during the 60-day growth period before sunrise to ensure absorption before evaporation.

The grow tents (The Original Gorilla Grow Tent® 5 × 5, Gorilla Inc., Santa Rosa, California) employ the use of lighting elements and environmental monitoring to establish plug growth before transplanting them into the FGCU-FF plots. The size of the grow tent is 1.5 × 1.5 × 2.1 m with a weight of 30 kg. The tent stays thermally insulated with a reflective 1680D canvas covering, openings for extended attachments, and three large access points to track the plug growth. Temperature was regulated through ventilation with a duct fan (CLOUDLINET T6 Inline Duct Fan, AC Infinity Inc, Los Angeles, California) at the top of the grow tent. The duct fan has dimensions of 0.2 × 0.3 × 0.2 m, weighs 3.3 kg, and has a duct size of 0.15 m. With a current of 1.6 A, voltage of 100 - 240 V, and a total airflow of 11.4 m^3 per minute, the fan had a life expectancy of a total of 67,000 h, or about seven and a half years. This experimental set-up of the grow tents is similar to that in experiments performed by the Yang Laboratory at the University of Connecticut [29]-[33].

After the plugs were grown in the grow tent for 15 days, they were then transplanted into the four designated plots in the FGCU-FF. There were four plots of 1 × 1 × 0.15 m for four soil treatments. Each soil treatment consisted of a total of approximately 30 gal of soil. Before the soil treatments were added, the plots were cleared of any weeds or existing vegetation. A weed-prevention tarp was laid down with holes cut for the lettuce plugs. Cardboard was placed between plots, and pest-prevention nets were installed around the soil treatments, as shown in Figure 1. The four treatments included varying ratios of Blue Ribbon Organics Organic Compost (Blue Ribbon Organics OMRI Certified Organic Compost, 7.9 gal, Blue Ribbon Organics, USA) and Miracle-Gro Potting Soil (Miracle-Gro Potting Mix, 16 qt., The Scotts Company LLC, USA). Miracle-Gro potting soil was used to represent the most general soil conditions one may encounter nationwide at conventional farms. The compost from Blue Ribbon may be considered a plant organic fertilizer, as it was made of food and yard waste. According to the Miracle-Gro packaging, the soil mixture is made up of one or more products, including forest products, peat, perlite, peat moss, fertilizer (total nitrogen of

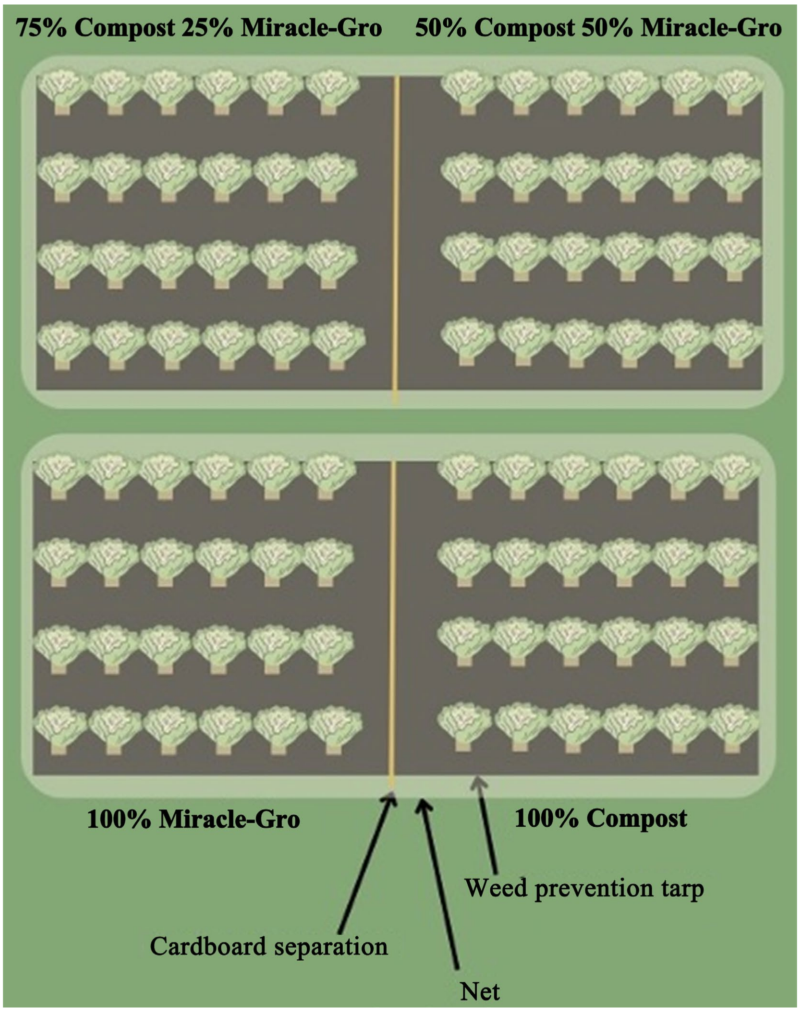

Figure 1. Set-up of the experiment at the FGCU-FF. The four plots can be seen, each 1 × 1 × 0.15 m, with 24 lettuce crops in each. The plots are labeled with the 75% Compost, 25% Miracle-Gro and 50% Compost, 50% Miracle-Gro soil treatments at the top and the 100% Miracle-Gro and 100% Compost soil treatments at the bottom. The paired plots each have a weed prevention tarp with holes cut for the plugs, pest prevention nets, and cardboard separators between the two treatments. Between the two set-ups, there is a 0.3-m space between the center to allow for a walking path to tend to the crops.

0.21%, available phosphate of 0.11%, and soluble potash of 0.16%). The 100% Miracle-Gro (100%MG) treatment had 30 gal of Miracle-Gro Potting soil, the 100% Compost (100%C) treatment had 30 gal of Blue Ribbon compost, the 50% Compost and 50% Miracle-Gro (50%C - 50%MG) treatment had 15 gal of Blue Ribbon compost and 15 gal of Miracle-Gro Potting soil, and the 75% Compost and 25% Miracle-Gro (75%C - 25%MG) treatment had 22.5 gal of Blue Ribbon compost and 7.5 gal of Miracle-Gro Potting soil.

A comprehensive crop control natural fungicide, insecticide, pesticide, and miticide was used as needed to deter pests and diseases (Trifecta Crop Control Ready to Use Maximum Strength Natural Pesticide, Fungicide, Miticide, Insecticide, 32 oz, Trifecta, Amazon Inc., Seattle, Washington). After the first week of growth in the FGCU-FF, the plots experienced an infestation of armyworm caterpillars. The crops were sprayed every three days after sundown, first with water and then with natural crop control, to mitigate this problem and mechanically remove them as seen while sampling.

2.2. Data Acquisition

Average temperature (˚C) and precipitation levels (cm) were recorded using environmental sensors. The FGCU weather monitoring station website recorded daily average temperatures and precipitation. A lettuce crop was randomly selected every six days to obtain the required biomass to run tests.

Four types of soil treatments were used in this study: 100% *Compost*, 100% *Miracle-Gro*, 50% *Compost*, 50% *Miracle-Gro*, and 75% *Compost*, 25% *Miracle-Gro*. Fourteen samples were collected at harvest for each 100%C and 100%MG treatment. Thirteen samples were obtained for the 50%C - 50%MG and twelve samples were taken for 75%C - 25%MG at harvest.

The Leafscan app [34] was downloaded via the app store on a mobile device (iPhone 12 Pro, Apple Inc., Cupertino, California) to calculate the total leaf area (LA). To calibrate the app, a reference sheet with four black dots in a square 10.5 cm away from each other. The leaves were separated from the node and then placed on the reference sheet for a picture to be taken by the app measured in cm^2. The Leafscan app measures to the nearest 0.01 cm^2 by differentiating between the colors of the white paper and the green leaf. After each recording, the data was exported as a CSV (Comma Separated Value) file.

To collect wet weight, each sample was washed to remove dirt particles and dried to prevent water weight additions. The roots were then removed from the samples to limit the amount of excess soil-skewing data. To obtain dry weight, after collecting all other data, the sampled crops were placed in individual brown paper bags to be left in a drying oven for six days at 65˚C. After six days, the samples were removed and weighed at the next sampling event.

A chlorophyll meter (AMTAST Chlorophyll Meter for Testing Plant Chlorophyll Content Unit SPAD, Amazon Inc., Seattle, Washington) was used to calculate each sample's overall chlorophyll and nitrogen content. For each sample, in

Soil Plant Analysis Development (SPAD) units, three chlorophyll readings and nitrogen concentrations (mg/g), were recorded and averaged. The chlorophyll recordings were then translated from SPAD to chlorophyll concentrations (mg/cm^2) through conversion calculations [35]. SPAD is used to calculate the difference between the levels of red light (640 nm) and infrared light (700 - 900 nm).

2.3. Statistical Analysis

For this study, statistical tests were conducted at the 5% significance level using R statistical software [36]. Due to a strong correlation of r = 0.99 between nitrogen and chlorophyll, chlorophyll was left out of subsequent analyses. Since multivariate normality was not satisfied, a permutation multivariate analysis of variance (PERMANOVA) was conducted. The *ggstatsplot* package [37] was used to perform Kruskal-Wallis tests and pairwise comparisons via Dunn's test for post-hoc analysis. Where appropriate, p-values were adjusted by the Holm method.

3. Results

Our results demonstrated that there was viable lettuce crop production to full bloom at harvest. On the 60th day of growth, all the lettuce crops were harvested from all four plots. At harvest, 14 crops were sampled from the 100%C and 100%MG plots, 13 crops from the 50%C - 50%MG, and 12 crops from the 75%C - 25%MG plot. The missing crops from the last two plots are due to the armyworm caterpillars' infestation. The median values for wet weight, dry weight, LA, nitrogen, are presented in **Table 1**, along with the mean values of chlorophyll. The average temperature outside in the FGCU-FF during the length of this experiment conducted between October 15, 2023, and December 14, 2023, was 21.29˚C and an average rainfall of 0.0826 cm each day, with a total rainfall of 5.54 cm [38]. **Table 1** provides a summary of all listed data.

The heaviest lettuce crops, regarding wet weight, were produced by the 100%C soil treatment at a median weight of 79.24 g, with the heaviest crop at 113.14 g. The smallest wet weight was produced by the 50%C - 50%MG soil mixture with a median weight of 34.14 g, with the heaviest crop recorded at 65.83 g. Wet weights demonstrate a large disparity in the effectiveness of the soil treatments in lettuce growth, with the 100%C soil treatment producing over double the median wet weight in the 50%C - 50%MG solution, or 45.10 g more. Within wet weights, the harvest weights showed a large variation in mass for all treatments. While the soil treatments of 100%-C and 100%-MG produced the heaviest lettuce crops, they also produced the most variable harvests.

Regarding dry weight, the heaviest crops were produced by the 100%-MG soil treatment, with a median weight of 3.57 g and the heaviest crop weighing 4.89 g. The smallest dry weight was produced by the 50%C - 50%MG soil composition with a median weight of 1.59 g, and the heaviest crop was recorded at 3.07 g. The dry weights demonstrate a noticeable disparity in the effectiveness of the soil treatments in lettuce growth, with the 100%MG soil treatment producing a little over double the median wet weight of that in the 50%C - 50%MG solution, or 1.98 g. Within the dry weights, the harvest weights showed a variation in mass for all treatments. Comparative to the wet weight results, the 100%MG yielded the overall largest crops, however, the 100%C soil showed the greatest variability in mass. The variability in compost composition can lead to fluctuations in crop biomass, as inconsistent nutrient levels and microbial activity may affect plant growth and yield unpredictably.

The crops with the overall highest leaf areas were produced by the 100%C soil treatment at a median LA of 1289.58 cm^2, with the largest LA at 1763.72 cm^2. The lowest leaf area was produced by the 50%C - 50%MG soil composition with a median leaf area of 704.12 cm^2, and the highest crop recorded at 1296.66 cm^2. The LAs show a disparity in the effectiveness of the soil treatments in lettuce growth, with the 100%C soil treatment yielding a substantial amount greater median leaf area of that in the 50%C - 50%MG solution, 585.46 cm^2 more. Within the LAs, the crops showed a large variation in mass for all treatments.

Measuring nitrogen levels in leaves is crucial for assessing plant health and diagnosing nutrient deficiencies or excesses. Additionally, it helps optimize fertilizer application, ensuring efficient use of resources while maximizing crop yield and quality. Lettuce crops with 100%MG soil yielded the highest nitrogen content, with a median of 9.75 mg/g and a peak reading of 11.23 mg/g. The second highest nitrogen content was produced by the 100%C and 50%C - 50%MG, with both medians at 9.67 mg/g. However, the highest reading for the 100%C soil treatment was 10.13 mg/g, and the highest reading for the 50%C - 50%MG was 10.27 mg/g. The lowest nitrogen reading was produced by the 75%C - 25%MG soil composition with a median reading of 9.43 mg/g, with the highest reading recorded at 9.75 mg/g. The median nitrogen readings do not demonstrate a large disparity, with the difference from the 100%MG readings and 75%C - 25%MG being only 0.32 mg/g. The medians again do not demonstrate a large disparity within the nitrogen readings for each soil treatment.

The difference between the highest and lowest nitrogen readings for each treatment was 1.36 mg/g in the 100%C treatment, 2.9 mg/g in the 100%MG treatment, 1.32 mg/g in the 75%C - 25%MG treatment, and 1.77 mg/g in the 50%C - 50%MG. While the soil treatments of 100%MG produced the highest nitrogen reading, they also produced the harvests with the greatest amount of variability. Due to the previously mentioned strong correlation between the nitrogen and chlorophyll variables, it can be inferred that similar results would also be produced upon evaluating chlorophyll readings as well. The 100%C soil treatment produced the highest mean chlorophyll concentrations at 0.0161 mg/cm^2, and the 75%C - 25%MG produced the lowest mean at 0.0144 mg/cm^2. There was not a large visible disparity between each of the soil treatments and their produced chlorophyll concentrations.

Wet weight, dry weight, LA, nitrogen, and chlorophyll were recorded for each sample. One LA, nitrogen, and chlorophyll observation were missing due to damaged sample. Linear interpolation was used to approximate the missing values in the data analysis. Table 1 provides a summary of all listed data.

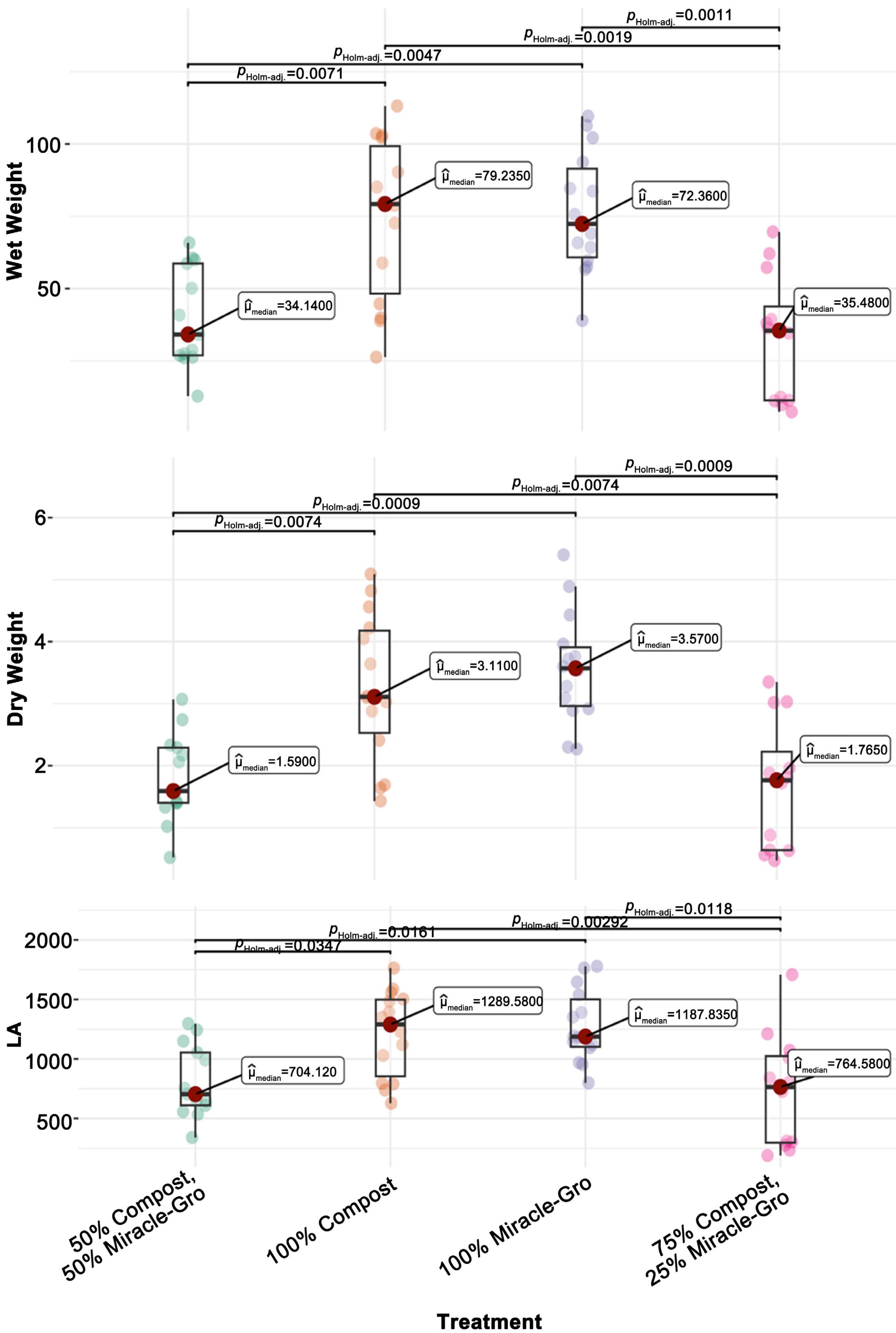

Figure 2. Boxplots for wet weight, dry weight, and leaf area by soil condition are displayed. The boxplots connected by a line are significantly different ($p < 0.05$).

Table 1. The median measurements for wet weight, dry weight, leaf area, and nitrogen for each soil condition are recorded below as used for statistical analysis, along with the mean chlorophyll measurements. Interquartile ranges are provided in parentheses. Based on the values, there may be some significant differences in wet weight, dry weight, and leaf area, but not in nitrogen (and thus chlorophyll).

	100% Compost	75% Compost, 25% Miracle-Gro	50% Compost, 50% Miracle-Gro	100% Miracle-Gro
Wet Weight (g)	79.24 (50.99)	35.48 (32.47)	34.14 (31.77)	72.36 (30.65)
Dry Weight (g)	3.11 (1.65)	1.77 (1.59)	1.59 (0.89)	3.57 (0.95)
LA (cm^2)	1289.58 (644.16)	764.58 (727.8)	704.12 (422.99)	1187.84 (398.57)
Nitrogen (mg/g)	9.67 (0.58)	9.43 (0.39)	9.67 (1)	9.75 (0.95)
Chlorophyll (mg/cm^2)	0.0161	0.0144	0.0150	0.0149

Statistical Analysis

Soil type was found to be important ($F[3, 49] = 7.34$, $p = 0.0002$). In particular, it significantly impacted wet weight (χ^2 [3] = 23.34, $p < 0.0001$), dry weight (χ^2 [3] = 24.15, $p < 0.0001$), and leaf area (χ^2 [3] = 16.01, $p = 0.0011$), but not nitrogen (χ^2 [3] = 3.91, $p = 0.2717$). Figure 2 highlights the impact of soil composition. Specifically, 100%C and 100%MG had the same effect on wet weight, dry weight, and LA. However, they both produced significantly greater wet weights, dry weights, and leaf areas compared to the 75%C - 25%MG and 50%C - 50%MG compositions.

4. Conclusions

In conclusion, the 100%C and 100% MG soil treatments produced the most successful lettuce crops. The 100%C soil treatment yielded lettuce crops with the heaviest wet weights and the largest LAs, and the 100% MG soil treatment yielded the heaviest dry weights and the highest nitrogen readings.

It is not unexpected for the 100%C and 100%MG soil treatments to produce data results so closely related, as the research previously mentioned demonstrates that compost benefits overall crop growth, and conventional soils, such as Miracle-Gro, promote crop growth. Miracle-Gro and other conventional soils are used on such a large scale because they allow farmers to produce bountiful crops for a period. However, the soil eventually becomes degraded and cannot be used any longer. Due to the 100%C treatment yielding crops of overall similar success to the 100%MG soil treatment, the conclusion may be drawn that compost-based soil systems could compete directly with conventional soils. Should this experiment be repeated, it would be recommended that the experiment be performed in a more controlled environment to eliminate other environmental factors, such as the armyworm caterpillars, and the experiment be conducted with more lettuce crops to have a wider spread of data.

The conclusions from this experiment can be used to ascertain if encouraging farmers to apply compost to their farmland is a wise solution to the problems associated with conventional practices, or if more components of regenerative agriculture are needed to make a noticeable impact on plant growth. The benefits of protecting soil, crops, and promoting crop growth make compost a promising alternative compared to fertilizers applied in conventional agricultural practices.

Acknowledgements

Thank you to Dr. Minh Nguyen and the Honors College at FGCU for providing financial support, and Dr. Melodie Eichbauer of The Office of Scholarly Innovation & Student Research (SISR) at FGCU for providing financial support to complete this project. Special thanks to Rory Dunn, Madison Alberts, George Buss, Paige Carroll, and Mya Griffith for their guidance, volunteer time, and emotional support.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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