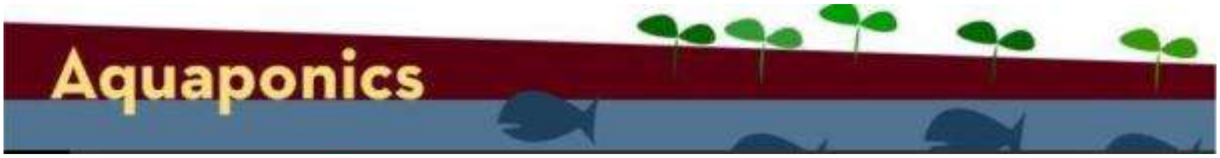

A Comparison of Rockwool, Lava Rock, Expanded Clay Aggregate, and Coconut Coir as Growing Substrate in a Floating Raft Aquaponic System Examining Growth Rates of ‘Improved Amethyst’ Basil and ‘Nancy’ Butterhead Lettuce

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EXECUTIVE SUMMARY

Aquaponics is a rapidly growing field focused on local and sustainable food production. Since aquaponics is a newer field there are no rules about organic certification of the system. This chapter focuses on determining how the substrate affects the productivity of two common raft system plants, lettuce and basil. A current common substrate being used is rockwool, which is not allowed by the Organic Materials Review Institute. The purpose of our research was to see if an organic substrate, such as coconut coir, lava rock or expanded clay aggregate, could produce comparable or more product than rockwool. After growing in a greenhouse aquaponic raft system for 4 weeks, results showed that coconut coir outperforms rockwool when growing lettuce. Similarly expanded clay aggregate produced more basil than rockwool. When growing lettuce and basil within a raft system it would be more beneficial to use coconut coir or expanded clay aggregate, respectfully, rather than rockwool, from both a production and an organic certification viewpoint.

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INTRODUCTION

Issue Description

Aquaponics has significantly grown in popularity over the past decade as a sustainable alternative method of food production. With this increase in popularity comes a need to examine which methods are both the most cost effective and efficient. Though there are many different configurations of aquaponics a commonality they all share is the use of soilless substrates as growing medium. The objective of this experiment is to determine if there is a significant gain in biomass by growing the plants in a particular kind of substrate.

Overview of What is Known

A report by the Food and Agriculture Organization of the United Nations on small scale aquaponic food production provided guidelines for the qualities of a good growing substrate which include a large surface area for bacterial growth, good drainage properties, a neutral pH, does not leach toxins, sufficient space for air and water to flow, easy to work with, lightweight, and available and cost effective (Somerville, Cohen, Pantanella, Stankus, & Lovatelli, 2014). According to the same report, the most popular growing substrates around the world include lava rock, expanded clay aggregate, rockwool, and pumice. These are readily available, relatively inexpensive, and have a majority of the qualities listed above.

A concern among aquaponic producers is the sustainability of the growing substrates used as well as efficiency of the system. The demand from consumers for environmentally friendly products and sustainable production practices keeps growing (Thompson, 1998) and aquaponics producers want to fill that need. In addition, official organic certification specifically prohibits the use of the popular growing substrate rockwool as a growing substrate in organically certified productions because it is a synthetic compound (OMRI, 2015). Though expanded clay aggregate or lava rock is not specifically prohibited growers wanting absolute certainty of organic certification need to use a growing substrate that has already been approved by an organic certifier. For these reasons there has been interest in using certified organic growing substrates such as coconut coir instead of inorganic growing substrates (Inden & Torres, 2001). The negative aspects of using organic growing substrates is that they cannot be used for more than one growing cycle because of natural deterioration and the risk of substrate clogging system pipes (Somerville, et al., 2014).

A study conducted by Lennard and Leonard (2006) found the most effective method of growing lettuce in an aquaponic system is to use a substrate bed setup with continuous flow, however a raft type aquaponic system is often more cost effective than a substrate bed. In order to make this experiment relevant to typical aquaponics users a raft system was chosen for use in this study. An online survey of 809 commercial aquaponic producers found 46% of operations used raft production in their operation (Love et al., 2014).The commercial aquaponics producer survey also found 70% of respondents grew basil and 64% grew salad greens (Love et al., 2014). Due to the popularity of basil and lettuce in aquaponics raft systems these crops were chosen as the experimental plant species of this experiment.

Series Goals

A comprehensive, informational library is compiled through student research as part of an ongoing research project at the University of Minnesota in HORT 4601: Aquaponics: Integrated Fish and Plant Food Systems. The series mission is to supply interested parties with valuable information regarding aquaponics to expand the field and help provide sustainable, safe, healthy, and reliable products. These products may be physical, educational, or value based.

Chapter Goal

The goal of this experiment is to provide aquaponics hobbyists and commercial aquaponics growers with information concerning growing substrate effects on plant biomass produced within an aquaponics system. To this end the crops and system chosen in this study reflect the preferences of typical aquaponics growers.

Objectives

The objectives of this experiment are to:

1. Determine if there is a statistical difference in biomass when lettuce is grown in rockwool, coconut coir, lava rock, or expanded clay aggregate while in a raft aquaponic system.
2. Determine if there is a statistical difference in biomass when basil is grown in rockwool, coconut coir, lava rock, or expanded clay aggregate while in a raft aquaponic system.

Research Questions Tested

Is there a statistical difference in plant biomass among plants grown in rockwool, coconut coir, lava rock, and expanded clay aggregate as the substrate in an aquaponics raft system? Are there significant statistical differences in plant biomass? Which substrate had plants with the highest biomass?

METHODS

Site Description

The following Site Description contains the greenhouse conditions under which the 2015 University of Minnesota HORT 4601 (Aquaponics) Spring Semester class conducted research. The only exception(s) to this are Chapter(s) that indicate(s) otherwise in their Study Design. The location for aquaponics research was in the Minneapolis – Saint Paul Metropolitan area, State of Minnesota, U.S.A., specifically located at the St. Paul Campus of the University of Minnesota (44°59'17.8" N lat., -93°10'51.6" W long.). Plant seeds were sown in 288 plug trays with a pasteurized Berger BM2 Germination Mix (Berger Peat Moss, Saint-Modeste, Quebec, Canada). For seed germination, a mist system in a greenhouse was used from sowing to germination (21±0.8/21±0.7°C, day/night, 16 hrs (0600–2200 HR) lighting at a minimum setpoint of 150 µmol m^{-2} s^{-1}, a mist frequency of 10 min. intervals (mist nozzles, reverse osmosis water) during 0600-2200 HR with a 7 sec. duration (Anderson, et al., 2011). After germination until the true leaf stage, plug trays were moved from the mist greenhouse to capillary mats; the environmental conditions in this greenhouse were 24.4±3.0/18.3±1.5°C day/night and 16 hrs (0600–2200 HR) lighting at a minimum of 150 µmol m^{-2} s^{-1}. Greenhouses used for aquaponics experimentation were located in the Plant Growth Facilities, House Nos. 369-C2 and 369-C4. Greenhouse No. 369-C2 had 23.6±0.8°C (daily integral) whereas No. 369-C4 was at 21.7±0.4°C. Temperature setpoints were 23.5°C and 21.5°C for 369-C2 and 369-C4, respectively, while the photoperiod was long days (0600–220 HR) with supplemental lighting supplied by metal halide high intensity discharge (HID) lamps at a maxima of 1377 µmol m^{-2} s^{-1}; electrical generators served as the electrical power backup system. Both greenhouses were adjacent, A-frame even-span construction sharing one inner wall; the roof, shared inner wall and interior wall adjoining the service walkway were glazed with double-strength float glass whereas the exterior walls had chambered acrylic (Exolite®; Cyro Industries, Mt. Arlington, NJ) glazing. Heating was delivered via hot water in perimeter pipes with galvanized fins for enhanced heat exchange. All environmental settings were controlled via an Argus Control Systems Ltd. computer (Surrey, British Columbia, Canada).

The aquaponics system in greenhouse No. 369-C2 consisted of eight aluminum tanks (193x77.5x75 cm, length x width x height; 6.5 cm thick walls) with a floating raft system (2/tank; 60x60x5.5 cm, Owens Corning FOAMULAR 150, R-10 insulation sheathing; Owens Corning Co., Toledo, OH); the water volume in each tank was ~550 L or 0.55 m^3. Two plastic, hemispherical tanks (68x47x26 cm) were connected to each fish tank and served as the biofilters. Each biofilter was filled with 8-10 cm dia. gravel (D-Rock Center, New Brighton, MN). In greenhouse 369-C2, ammonium chloride (1 g/biofilter; Hawkins Chemical Co., Roseville, MN) was used to start the biological filter or biofilter in 8-10 cm dia. lava rock (D-Rock Center, New Brighton, MN) to produce ~1 mg/L ammonia with an initial start of *Carassius auratus* (goldfish) whereas ammonium carbonate was used in 369-C4. Two plastic, hemicylindrical tanks (68x47x26 cm) were mounted above one end of each fish tank and served as the biofilters. Each biofilter was filled with 2 cm dia. granite gravel (Hedberg Aggregates, Stillwater, MN). A low density (approx. 25-30 fish / tank) of *Carassius auratus* was used to start the biological filter in the gravel. Water was lifted to the biofilter tanks by a Danner Supreme 700 GPH mag drive pump. The outflow was valved and split between the two biofilter tanks and a third outlet which discharged directly to the fish tank for added aeration and circulation. Each biofilter received approximately 4 l/min. An automatic bell siphon in each of the biofilter tanks allowed the water level to rise in the gravel from a low point of approximately 2 cm depth to a high of around 15 cm. At the high point the siphon would start and the water would draw down (returning to the fish tank), creating an ebb and flow in the gravel. Potential plant spacing on each raft could be a max. of 16 plants in a 4x4 grid, each plant could be grown in a 12cm dia. Net Cup (Hydrofarm Central, Grand Prairie, TX) filled with Trock rockwool (medium grade, 4CF, 30/PL; Therm-O-Rock East, Inc., New Eagle, PA). Water quality was monitored daily (5/wk excluding weekends) and fish measurements were sampled weekly by students and recorded in an interactive Google Doc® file. The measurements and safety protocols are detailed in Appendix A. Temperature measurements averaged 22.3$\pm$0.9°C and closely approximated the air temperature setpoint. Fish species grown in this house were *Oreochromis* spp. (tilapia) and *Perca flavescens* (yellow perch) at varying densities, depending on age.

Research Techniques

The Float System

This experiment followed the above site description and was conducted in greenhouse No. 369-C2. The raft style of plant production was chosen because of its popularity in commercial aquaponic systems (Love et al., 2014). In a raft system the plants are grown on Styrofoam boards, which float on the water, in the tank. The roots thus grow down into the nutrient rich water. The roots take up the nutrients and filter the water for fish. The water was pumped up into a biofilter, where bacteria break down the solid waste. The tank was aerated to provide adequate oxygen levels for both fish and plant roots. Half the tanks used ambient air temperature water and the other half heated the water to 25 degrees Celsius. Chapter 5 of the HORT 4601 eBook has more details. Worms were introduced into half of the biofilters within the greenhouse. For more details refer to Chapter 6 of the HORT 4601 eBook.

Substrate Tested

Substrates were selected based on their ability to be used in an aquaponics system. The four types of substrate tested were coconut coir, rockwool, lava rock, and expanded clay aggregate. These four substrates were chosen to represent the most commonly used substrates with a raft system. Since substrates come in varying forms, the substrates selected were based on usability within the system. Coconut coir is commonly found in fine particles, approximately the consistency of soil, which wouldn't work well in an aquaponics raft system because the particles would drop into the water. To avoid this problem we purchased coconut coir pots at a local hardware store and filled them with the fine particle coconut coir. The size of the expanded clay aggregate was 1-1.5cm in diameter. The lava rocks were broken into smaller pieces 1-1.5cm in diameter. Rockwool is fairly similar between suppliers and thus the brand stated within site description was used. All substrates were rinsed prior to use within the system limiting the amount of debris introduced to the system. The lava rock and expanded clay aggregate were rinsed several times with water until the substrate was dust free.

Planting Method

In order to test if growing substrate has an effect on plant growth two different plant types were grown in each of four test substrates. The cultivars selected were butterhead lettuce 'Nancy' (*Lactuca sativa*) and basil 'Amethyst Improved' (*Ocimum basilicum*). Plants were chosen based on their time to harvest and known popularity as cash crops in commercial aquaponic systems. The plants were germinated and pre-grown as stated within the above Site Description. Once plants had two to four true leaves they were transplanted into net pots to be put into the raft system. Each plant was carefully removed from the plug tray paying special attention to the roots. The plants were carefully dug out of the plug in order to keep most of the root ball intact. Excess soil was gently knocked from the root ball before being placed in a bowl of water to fully clean off the soil. For the given system set up within the greenhouse, 128 plants of each type were needed.

The plants were placed in a five centimeter net pot so that the roots were within the bottom two centimeters of the pot. When the net pots were placed in the raft only the bottom two centimeters were submerged within the tank water. Plant roots need to be in contact with the tank water to allow the plants to take up nutrients. For expanded clay aggregate, the plant was held between two fingers inside the pot at the desired height. Expanded clay aggregate pellets were then gently placed around the plant roots and stem. The pot was filled enough to provide stability to the current size of plant. Coconut coir planting used pre-formed five centimeter coconut coir planters that were placed inside the net pots and filled with finely shredded coconut coir. Plants were placed in the center of the pot where the coconut coir was packed around them in a style similar to soil planting. Net pots were filled to the top with rockwool and plants were placed in the center of the net pot with rockwool packed around them in a style similar to soil planting. Plants potted into lava rock were held in place in the net pot with the roots just touching the bottom while lava rocks were gently placed around the roots for support. It was not important to fill the net pot with lava rock, only enough to support the plants were needed.

Greenhouse Layout and Randomization

Each of the eight tanks in greenhouse C2 contained two rafts of 16 net pots each. Each tank had one raft of basil and one raft of lettuce. Net pots were assigned identification numbers 1-128 in sequential order throughout the tanks for each plant type. The net pots were equally divided between the four substrates. The identification numbers were then loaded into a randomizer and randomly assigned a substrate; nullifying placement in the room as an effect on the results. Laminated labels with both the identification number and type of substrate were attached to the right of each net pot for easy identification and data collection. A graphic detailing the design process is below.

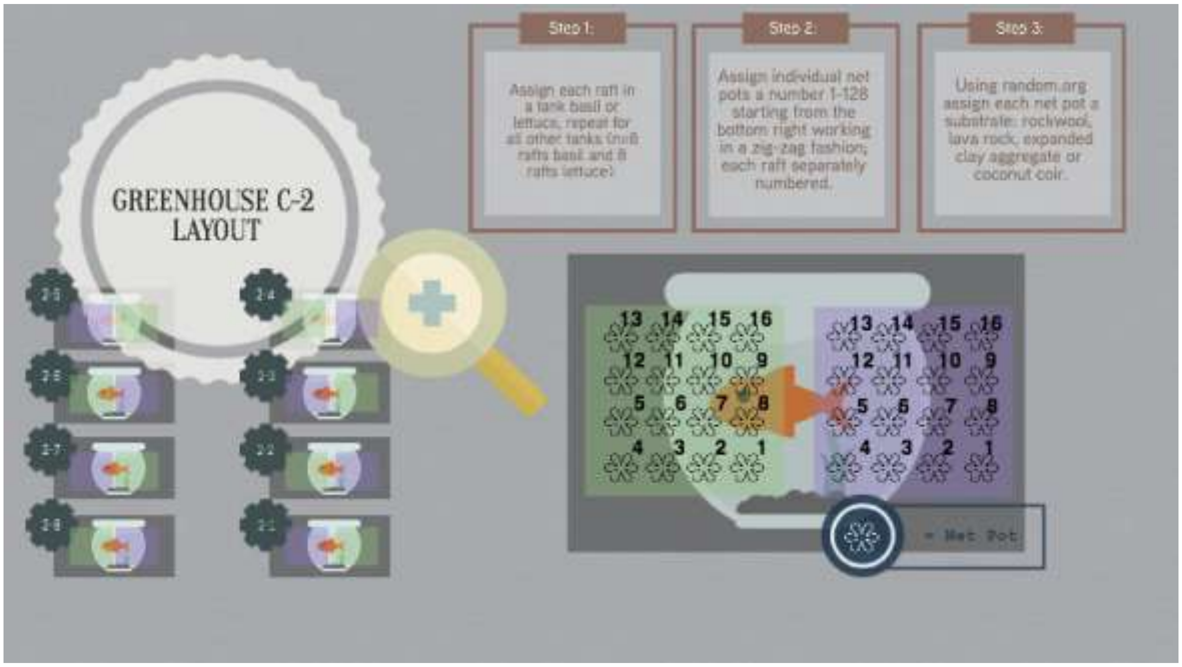

Figure 1. Research design based on eight tanks, with 2 rafts each, with basil or lettuce randomly assigned to one of the rafts in each tank. A total of 128 net pot numbers sequentially arranged throughout the tanks and four substrates randomly assigned.

Data Collection

Weekly Monitoring

Plant health was observed with any unhealthy plants specifically noted. When unhealthy plants or problems were observed, they were recorded with a photo including the plant’s identifying label. Qualitative observations were also made about the root growth between different substrates. For example, the roots of the expanded clay aggregate were more visible/longer than the roots within the coconut coir. General overall growth was recorded by taking a picture of each tank from the same spot. This gave a generalization of how the plants were doing in comparison to each other.

Wet and Dry Weights

To quantify our results wet and dry weights were taken and recorded in a table. Harvest of lettuce took place on Julian date 97 of 2015. Harvest of basil took place on Julian date 104 of 2015. Wet weights of the foliage, excluding root system and substrate, were taken immediately after harvest. The plants were placed in a small paper bag labeled with that plants identification number. All of the small bags were dried in an oven set at 49 degrees Celsius for 144 hours. Once dried total weights were taken and recorded. All weight data was in the units of grams. The weights were collected as individual plants.

FINDINGS AND DISCUSSION

Data/Results

Average fresh weight of a lettuce plant in coconut coir was 54.5g. This was the highest average fresh weight between the four substrates. Expanded clay aggregate had the next highest fresh weight with 51.2g, followed by lava rock at 49.7g and rockwool with 35.9g. All raw data can be found in Appendix B.

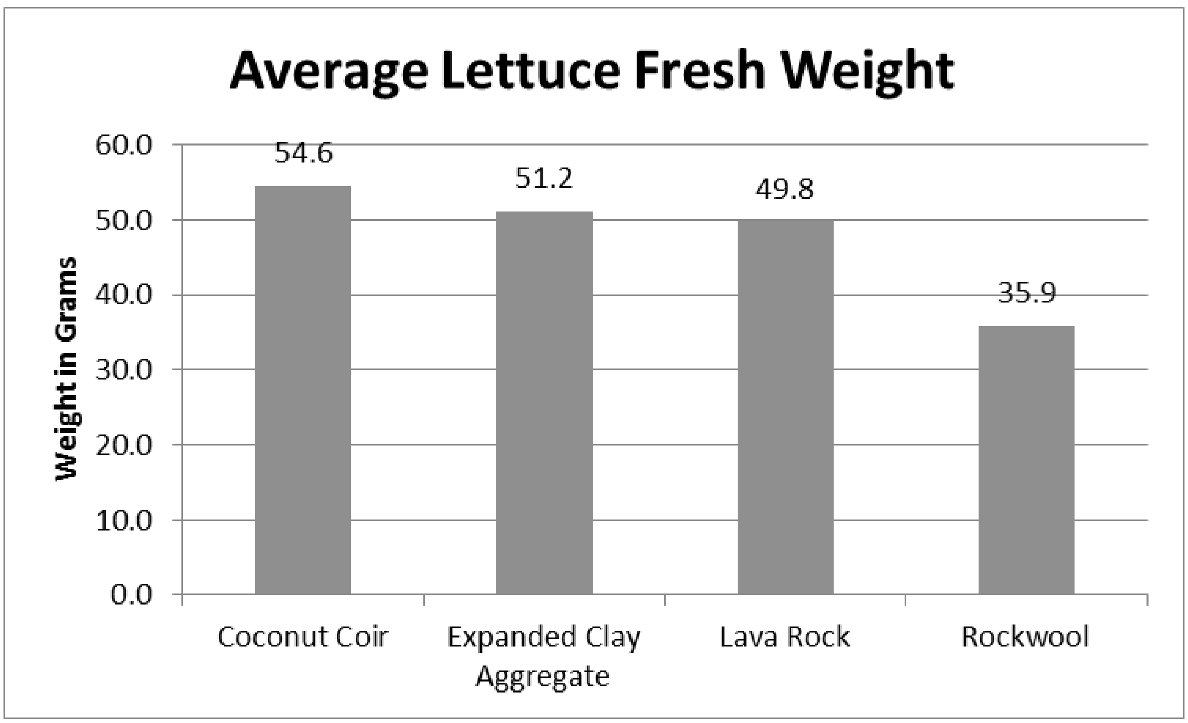

Figure 2. Comparison of average fresh weight of lettuce in different substrates.

Average dry weight of lettuce in coconut coir was 8.24g. As with the fresh weights this was the highest average between the four substrates. Expanded clay aggregate had the next largest average mass for dry lettuce at 6.79g followed by lava rock at 6.65g and rockwool with 4.88g.

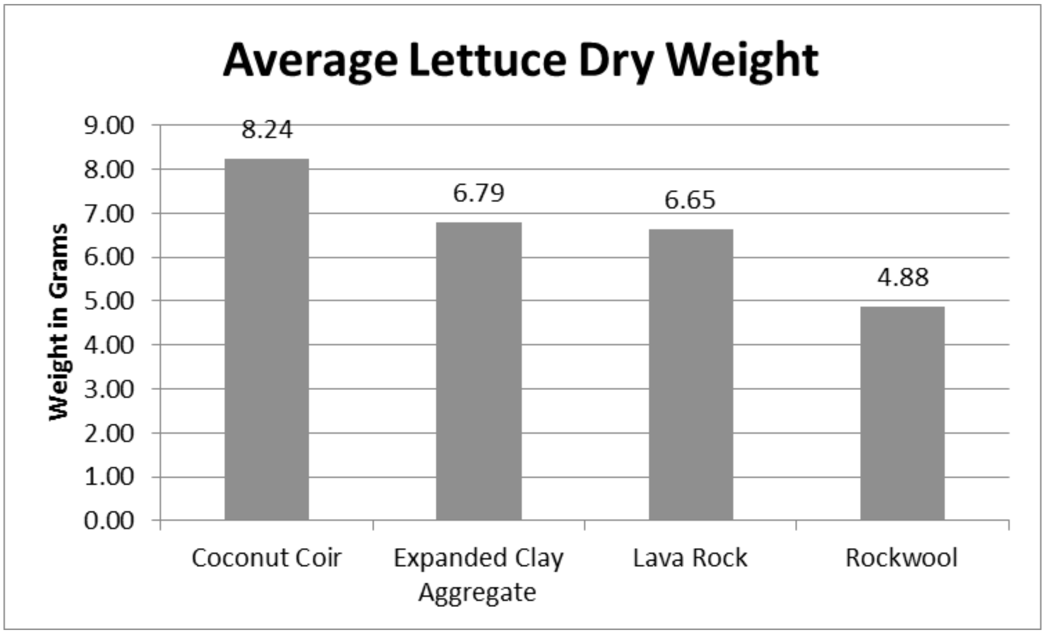

Figure 3. Comparison of average dry weight of lettuce in different substrates.

Average fresh weight of basil in expanded clay aggregate was 8.20g. This was the highest average between the four substrates. Lava rock had the next highest average with 6.34g followed by coconut coir at 5.61g and coconut coir with 5.31g.

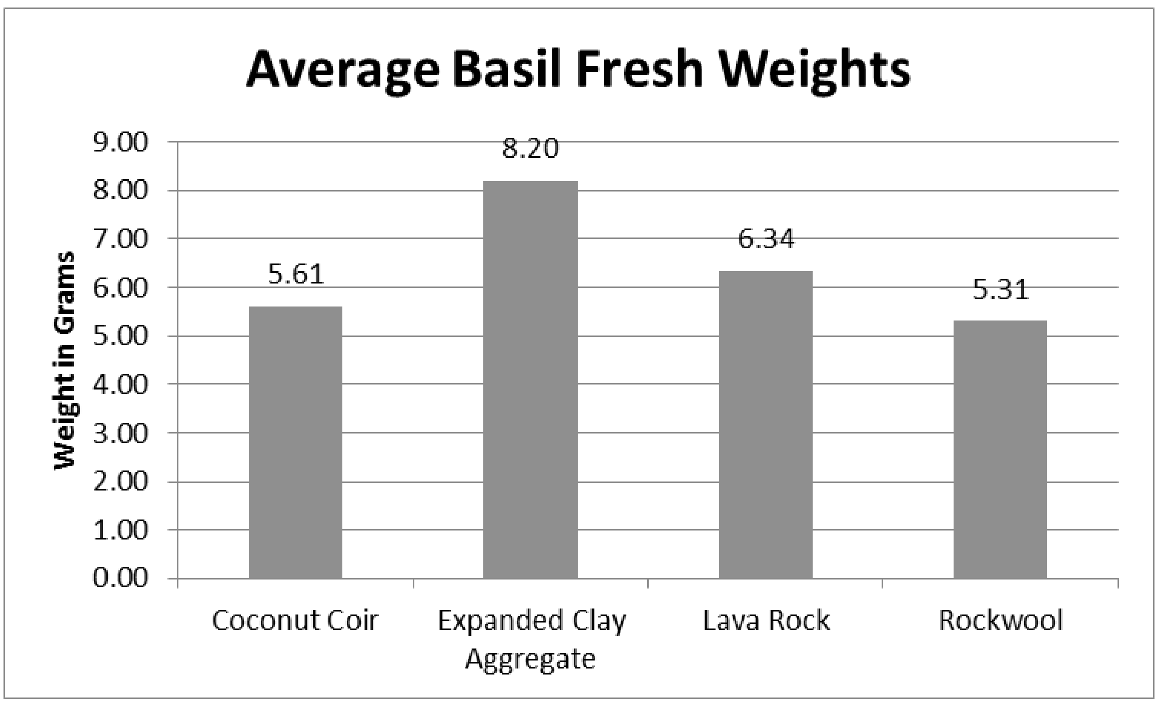

Figure 4. Comparison of average fresh weights for basil in grams in each of the growth substrates.

Average dry weight of basil in expanded clay aggregate was 1.09g. This was the highest average between the four substrates. Lava rock had the next highest average with 0.69g followed by coconut coir at 0.80g and coconut coir with 0.69g.

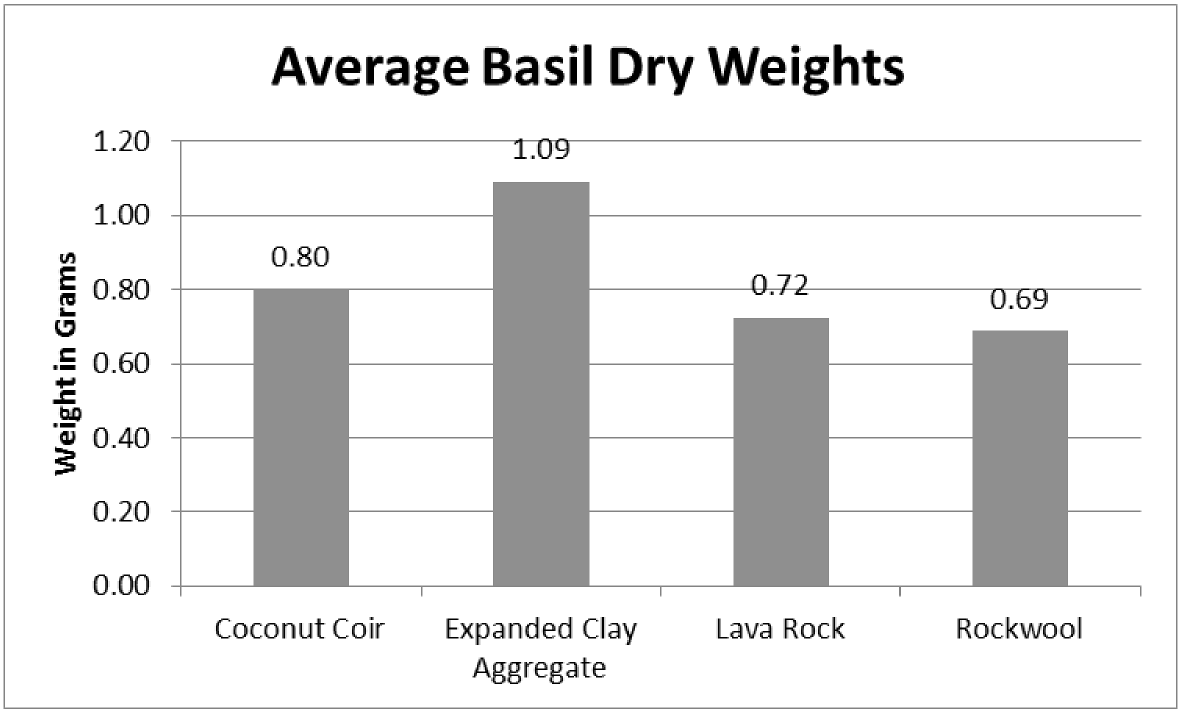

Figure 5. Comparison of average dry weights for basil in grams in each of the growth substrates.

A single factor Analysis of Variance (ANOVA) Test was completed on the fresh weights and dry weights of lettuce and basil using Microsoft Excel 2007 to determine if the differences between fresh weights were due to natural variation or could be correlated to the substrate treatments given to the plants. From the ANOVA P-values it can be said with 90% certainty that there is a statistically significant difference in plant biomass when different growing substrates are used.

Data Tested	ANOVA P-Value
Lettuce Fresh Weight	0.052
Lettuce Dry Weight	0.032
Basil Fresh Weight	0.003
Basil Dry Weight	0.001

Table 1. Table of ANOVA P-values comparing substrates tested.

Furthermore, an ANOVA Mean Separations with a 5% Tukey's Honestly Significant Difference Test (HSD) was run using SPSS (Statistical Package for the Social Sciences, University of Chicago) to determine which of the substrates were statistically different from each other. Table 2 gives the significance between substrates of the fresh and dry weight of lettuce. There is a significant difference in fresh weight of lettuce grown in rockwool vs coconut coir, expanded clay aggregate, and lava rock. There is a significant difference between dry weights of lettuce grown in coconut coir vs lettuce grown in rockwool.

Substrate 1	Substrate 2	P-Value Fresh	P-Value Dry
Coconut Coir	Expanded Clay Aggregate	1	0.767
	Lava Rock	1	0.284
	Rockwool	0.065	0.014
Expanded Clay Aggregate	Coconut Coir	1	0.767
	Lava Rock	1	0.884
	Rockwool	0.065	0.16
Lava Rock	Coconut Coir	1	0.284
	Expanded Clay Aggregate	1	0.884
	Rockwool	0.071	0.577
Rockwool	Coconut Coir	0.065	0.014
	Expanded Clay Aggregate	0.065	0.16
	Lava Rock	0.071	0.577

Table 2. ANOVA results of significance between substrates in fresh and dry weight of lettuce. Results significant at a p-value of 0.1 or below.

There was a significant difference in fresh weight of basil grown in expanded clay aggregate vs coconut coir, rockwool, and lava rock. There is a significant difference in dry weight of basil grown in expanded clay aggregate vs lava rock, rockwool, and coconut coir.

Substrate 1	Substrate 2	P-Value Fresh	P-Value Dry
Coconut Coir	Expanded Clay Aggregate	0.015	0.052
	Lava Rock	0.842	0.969
	Rockwool	0.985	0.607
Expanded Clay Aggregate	Coconut Coir	0.015	0.052
	Lava Rock	0.095	0.011
	Rockwool	0.004	0.001
Lava Rock	Coconut Coir	0.842	0.969
	Expanded Clay Aggregate	0.095	0.011
	Rockwool	0.614	0.844
Rockwool	Coconut Coir	0.985	0.607
	Expanded Clay Aggregate	0.004	0.001
	Lava Rock	0.614	0.844

Table 3. ANOVA results of significance between substrates in fresh and dry weight of basil. Results significant at a p-value of 0.1 and below.

Interpretation

The substrate that produced the highest harvestable weight of lettuce was coconut coir. Using coconut coir as a substrate is the most beneficial to lettuce growth between the substrates tested. Similarly, using rockwool is the least productive means of growing lettuce compared to the other substrates tested and switching to any other tested method would have benefits.

For growers who produce lettuce in a similar raft system using expanded clay aggregate or lava rock switching substrate to coconut coir could increase production on average up to 24%. Growers who switch from rockwool to coconut coir could increase production on average up to 59%.

The substrate that produced the highest harvestable weight of basil was expanded clay aggregate. Growers producing basil should consider using expanded clay aggregate as a substrate if they are not already. Basil biomass had a 54% difference between the most productive expanded clay aggregate and the least productive rockwool. Plant biomass increased 46% from coconut coir to expanded clay aggregate. Expanded clay aggregate plant biomass was 29% higher than that of lava rock.

Limitations of Study

A limitation of this study is that there were two other research projects using the same equipment at the same time, manipulating the water temperature and nutrient levels. Therefore it was possible that plant growth was not solely based on substrate used. Randomization of substrate location was used to mitigate the effects of environmental factors.

Another limitation of this study is the small sample size for each treatment. Eight of the basil plants died within the first two weeks after transplantation making that sample size even smaller. A larger sample size would minimize the effect of those deaths on our data. Five of the eight plants that died were planted in coconut coir which may have skewed the results.

Recommendation

Growers who produce lettuce in a raft aquaponics setup should use coconut coir as a substrate to increase yield. Rockwool is not recommended as a growing substrate for lettuce as it produces significantly less biomass compared to plants grown in coconut coir, lava rock, and expanded clay aggregate. Growers who produce basil should use expanded clay aggregate as this substrate produced plants with the most biomass on average.

Results from statistical analysis show that growing lettuce in rockwool produces significantly less biomass than every other substrate tested. Producers would see a significant increase in harvestable lettuce when grown in coconut coir. In addition coconut coir grows less algae in the substrate which reduces smell and increases aesthetics. It is theorized that rockwool holds too much moisture in a raft aquaponic system which could suffocate plants.

Basil produced significantly more biomass when grown in expanded clay aggregate as compared to every other substrate tested. Though it had the highest biomass it should be noted that the basil lacked support from the expanded clay aggregate and often fell over. Rafts were often moved and jostled to gain access to the fish which may be the cause of fallen basil. A possible solution to this problem would be using deeper pots, adding substrate as the plant grows, or using a shorter cultivar of basil, such as 'Pluto'.

Future Directions

In future studies experiments should be done in a more controlled environment that mimics producers growing conditions to eliminate most limitations of this study. The same methods and setup can also be used to compare other organic substrates such as shredded palm, mycelium, recycled expanded glass, or rice husk. This would give growers more OMRI approved substrates to work with besides the coconut coir that was tested in this experiment.

Acknowledgements

Funding source

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Inspiration

Michelle Menken, Organic Certifier, MN Crop Improvement Association for inspiration.

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APPENDIX A: GREENHOUSE MAINTENANCE INSTRUCTIONS, HORT 4601, SPRING SEMESTER 2015

Greenhouse Maintenance Instructions

- Wash your hands thoroughly prior to entering and after leaving each Aquaponics greenhouse (in accordance with the posting on the greenhouse doors).
- No food or drink in the greenhouses as well as no eating or drinking therein! Greenhouse water is NOT potable.
- Make sure that the water is flowing and being aerated. Water is pumped from each tank into the biofilters flows back into the tank via gravity. Aeration is via an air pump next to the window (one per room). Water should be flowing and aerated 24/7. If not, call Jay Maher IMMEDIATELY (his number is at the end of this document and posted outside of C2)! Stop feeding! Until help arrives, monitor the fish/water quality and mechanically aerate or exchange the water if necessary.
- Check the plants for visible growth issues. The plants will NOT require watering, but any pale green leaves may signal a lack of N. Watch for these signs or anything else that may seem abnormal. Report them to an instructor when these are noted.
- Measure water quality in half of the tanks each morning (even tanks on even days, odd tanks on odd days). Take these measurements PRIOR to feeding or measuring fish. We are interested in temperature, oxygen, pH, nitrite, ammonia, and alkalinity. Take all measurements/water samples from the front of each tank (i.e., the end of the tank that is closest to the center aisle). If a result is puzzling, take another measurement. If the result persists then record it and alert an instructor. If you break a glass sample tube, sweep up the area and put all glass in the sharps container.
    - Temperature should be close to ambient (23^{o}C) unless the water is being experimentally heated: Turn on the temperature/pH probe and submerge the sensor end in the water (avoid getting the plastic housing wet). Record the equilibrium temperature in ^{o}C. When you are done, rinse the probe off in tap water, top up the storage solution inside of the cap well, and place the cap on the probe. Store upright.
    - Oxygen (should be >6 ppm): Turn on the probe, remove the protective cap, and submerge the probe into the water up to the wire. Move the probe in a small circle (approximately the diameter of a penny) until the reading equilibrates. Record the oxygen concentration, rinse the probe off in tap water, and replace the cap.
    - pH (should be 6.5-7.5): As per temperature.
    - Nitrite (should be <0.75 ppm): Collect 5 ml of tank water in a small sample tube. Add 5 drops of the appropriate solution to the beaker. Cap/stopper and shake. After 5 minutes, use the color card to determine the approximate nitrite level (it is okay to sub-divide a category). Dispose of the solution in the sink in hall C and rinse both the cap/stopper and tube with tap water.

- Ammonia (should be <0.75 ppm): As per nitrite except that you add 8 drops from one bottle and 8 drops from another.
  - Alkalinity (should be 3-4 drops): Collect 5 ml of tank water in a small sample tube. Add one drop of the appropriate solution, cap/stopper and shake for ~2 seconds. Repeat until the blue solution turns yellow. Record the number of drops that you added. Dispose of the solution and clean the tube as per the nitrite protocol.
- Clean the pump filters in each of your tanks after sampling water quality. Lift the pump out of the water (don't be alarmed by the gurgling), slide the sponge filter off of the pump, and put the pump back into the water (so that it doesn't burn out). Then wring the filter out under running water in the hallway sink until clean and replace the filter. Do not swap filters among tanks.
- Feed the fish and check for pain/distress. Feed the fish in all tanks each morning (~8-9 a.m.) and afternoon (~3-4 p.m.) according to the rates and instructions from Jay Maher. There is fish feed in each greenhouse. Take this opportunity to check for pain/distress. Clinical signs are reduced or increased breathing (movement of the mouth and gill covering), darkening of the skin, altered swimming behavior (listlessness, surface breathing, loss of equilibrium), aggression, reduced feeding, and (in the case of an infection) sores. Report any dead fish to Jay Maher. Place them in a Ziplock bag and place the bag on the utility table; Jay will take care of disposal.
- Measure fish growth (and check for pain/distress) in four tanks once per week. Add tank water to a Green bucket and place the bucket on a scale. Zero the scale. Obtain 5 fish from a given tank as follows. First, gain access to the tank by placing the floating rafts on the biofilter and removing the air stones. If there is a tank heater attached to one of the air stones then turn off the heater and wait 10-15 minutes for the element to cool before removing the last air stone (the element can overheat very quickly if exposed to air). Then unplug the pump and place both the pump and weighted bucket on the floor. Second, use the PVC gate to concentrate fish at either the front or back of the tank. Be careful not to injure any fish during this procedure. Use the dip net for that tank to remove 5 fish from a given tank. Place these fish in the tared bucket and report mean mass in grams (mass of the fish in the bucket divided by number of fish in the bucket). Then transfer individual fish to the measuring board so that you can measure total length in mm. Return the fish to its original tank. When all fish have been processed, return the water to the tank and report mean fish length. Handle the fish gently and watch for any signs of pain/distress (see previous bullet). Before moving on to the next tank, wash the PVC gate, buckets, and measuring board.
- Clean up at the end of each day. Place any large plant parts (e.g., leaves/stems) that may have fallen onto the floors into the Orange bucket and then transfer them to the compost wheelbarrow. Sweep the floors clean. Properly store all equipment related to fish feeding/measuring and water quality.
- Complete the Maintenance Checklist and then report results via the Google sheet '4601.15 Aquaponics data record'.

- Color-coded buckets in the houses: Green buckets are for water/fish use only; Orange buckets are for compost plant materials; White buckets are for cleaning and disinfecting. Clear plastic bins are for plant harvest. Ziplock plastic bags are for any dead fish.

Appendix B: Raw Data Input From Harvesting of Lettuce and Basil

Basil Raw Data

Unique ID #	Tank	Wet Weight [g]	Dry Weight [g]
1-rw	2.1	6.25	0.85
2-rw	2.1	2.05	0.44
3-lv	2.1	4.82	0.77
4-rw	2.1	6.32	1.03
5-rw	2.1	5.5	0.75
6-cc	2.1	4.11	0.99
7-cc	2.1	6.79	0.96
8-rw	2.1	10.07	0.97
9-lv	2.1	4.33	0.66
10-rw	2.1	4.85	0.77
11-rw	2.1	2.74	0.44
12-ca	2.1	5.78	0.8
13-ca	2.1	1.46	0.5
14-cc	2.1	2.96	0.45
15-rw	2.1	0	0
16-ca	2.1	4.28	0.63
17-cc	2.2	4.33	0.54
18-ca	2.2	9.19	1.61
19-lv	2.2	8.91	0.9
20-ca	2.2	6.94	0.75
21-ca	2.2	10.48	1.05
22-cc	2.2	9.26	1.15
23-ca	2.2	7.94	1.28
24-ca	2.2	10.32	1.29
25-lv	2.2	7.37	1.07
26-rw	2.2	6.59	1.45
27-ca	2.2	10.13	1.22
28-rw	2.2	3.38	0.59
29-cc	2.2	0	0
30-cc	2.2	5.74	0.99
31-lv	2.2	6.2	0.32
32-lv	2.2	3.88	0.37
33-lv	2.3	3.05	0.52
34-ca	2.3	5.52	0.93
35-lv	2.3	2.52	0.6
36-rw	2.3	3.29	0.69
37-rw	2.3	1.53	0.36
38-rw	2.3	1.08	0.33
39-ca	2.3	6.9	0.83
40-cc	2.3	1.57	0.5

41-rw	2.3	0.51	0.23
42-ca	2.3	3.74	0.82
43-ca	2.3	8.51	1.49
44-cc	2.3	2.3	0.51
45-ca	2.3	0	0
46-cc	2.3	1.5	0.38
47-lv	2.3	3.75	0.92
48-lv	2.3	2.7	0.42
49-ca	2.4	10.49	1.12
50-cc	2.4	0	0
51-ca	2.4	15.64	1.34
52-rw	2.4	13.5	1.25
53-lv	2.4	14	1.25
54-lv	2.4	9.54	0.9
55-rw	2.4	2.08	0.16
56-lv	2.4	9.51	0.83
57-rw	2.4	7.74	0.69
58-rw	2.4	7.26	0.72
59-lv	2.4	8.06	0.59
60-lv	2.4	9.01	0.87
61-rw	2.4	5.69	0.68
62-cc	2.4	7.19	0.7
63-lv	2.4	3.94	0.47
64-lv	2.4	4.42	0.46
65-rw	2.5	3.22	0.53
66-ca	2.5	5.01	0.46
67-lv	2.5	3.85	0.29
68-cc	2.5	4.87	0.34
69-cc	2.5	7.12	0.66
70-lv	2.5	6.12	0.52
71-cc	2.5	9.58	1.27
72-rw	2.5	7.67	0.6
73-lv	2.5	4.63	0.26
74-ca	2.5	7.21	0.78
75-rw	2.5	8.99	0.92
76-cc	2.5	6.68	0.86
77-lv	2.5	5.18	0.38
78-ca	2.5	8.04	0.46
79-cc	2.5	4.4	0.57
80-cc	2.5	8.92	1.41
81-cc	2.6	0	0
82-lv	2.6	1.15	0.24
83-cc	2.6	0	0

84-rw	2.6	3.65	0.53
85-ca	2.6	5.87	0.95
86-cc	2.6	7.47	0.96
87-ca	2.6	12.12	1.08
88-rw	2.6	6.67	0.57
89-cc	2.6	9.61	1.01
90-rw	2.6	7.02	0.86
91-rw	2.6	0.46	0.23
92-ca	2.6	8.39	0.72
93-lv	2.6	17.1	1.44
94-ca	2.6	7.1	2.28
95-cc	2.6	0.56	0.22
96-rw	2.6	9.58	0.91
97-cc	2.7	1.43	0.01
98-lv	2.7	6.19	0.85
99-cc	2.7	0.77	0.22
100-ca	2.7	5.7	0.47
101-rw	2.7	2.98	0.15
102-ca	2.7	11.42	0.74
103-rw	2.7	11.79	1.02
104-cc	2.7	6.55	0.75
105-lv	2.7	19.89	1.82
106-ca	2.7	9.19	0.88
107-ca	2.7	14.69	2.29
108-ca	2.7	11.63	1.82
109-lv	2.7	8.34	0.98
110-cc	2.7	13.98	2.17
111-ca	2.7	14.58	1.99
112-cc	2.7	13.04	1.69
113-lv	2.8	1.87	0.62
114-lv	2.8	2.57	0.65
115-ca	2.8	4.63	0.94
116-lv	2.8	2.24	0.53
117-lv	2.8	4.28	0.9
118-rw	2.8	1.44	0.42
119-cc	2.8	3.61	0.7
120-ca	2.8	6.59	1.12
121-lv	2.8	9.09	1.2
122-lv	2.8	4.59	0.97
123-cc	2.8	2.48	0.74
124-cc	2.8	0	0
125-cc	2.8	4.74	0.8
126-lv	2.8	4.7	0.77

127-rw	2.8	0	0
128-rw	2.8	5.46	1
Total Weights [g]		359.51	47.15

Lettuce Raw Data

Unique ID #	Tank	Wet Weight [g]	Dry Weight [g]
1-cc	2.1	66.3	6.3
2-lv	2.1	98.5	10.31
3-lv	2.1	79.1	8.14
4-cc	2.1	106.6	14.41
5-rw	2.1	104.1	16.28
6-cc	2.1	77.9	12.22
7-lv	2.1	46.8	5.16
8-ca	2.1	91.5	10.67
9-rw	2.1	49.7	6.24
10-cc	2.1	107.3	24.59
11-lv	2.1	80.5	10.73
12-ca	2.1	99.5	12.69
13-ca	2.1	65.5	5.94
14-lv	2.1	46.6	4.53
15-lv	2.1	71.8	7.09
16-rw	2.1	60.8	5.88
17-rw	2.2	43.9	6
18-lv	2.2	5.1	2.4
19-ca	2.2	24	4.02
20-cc	2.2	10.8	2.63
21-rw	2.2	12.5	2.69
22-lv	2.2	19.3	3.19
23-ca	2.2	45.8	5.43
24-cc	2.2	88.5	17.49
25-lv	2.2	57.5	5.77
26-ca	2.2	58.9	3.48
27-lv	2.2	17.1	3.19
28-cc	2.2	28.8	3.85
29-rw	2.2	14.6	2.82
30-ca	2.2	37	4.92
31-lv	2.2	23.8	3.19
32-ca	2.2	74.3	10.28
33-ca	2.3	9.7	3.26
34-lv	2.3	15.3	3.8
35-rw	2.3	10.7	3.33
36-cc	2.3	21.9	5.95
37-rw	2.3	21.1	4.83

38-rw	2.3	12.8	3.51
39-cc	2.3	16.8	4.93
40-cc	2.3	11.8	3.13
41-lv	2.3	8.7	2.97
42-lv	2.3	13.1	3.62
43-cc	2.3	21.4	5.81
44-ca	2.3	22	5
45-rw	2.3	27.9	4.76
46-lv	2.3	29.5	6.61
47-ca	2.3	20.8	4.91
48-ca	2.3	12.9	3.04
49-lv	2.4	72.7	13.87
50-ca	2.4	59.7	7.13
51-cc	2.4	56.2	4.26
52-ca	2.4	24.7	2.62
53-cc	2.4	25.9	3.22
54-cc	2.4	83.7	13.79
55-cc	2.4	65.4	9.16
56-lv	2.4	96.7	12.66
57-ca	2.4	90.4	3.85
58-ca	2.4	73.3	4.3
59-cc	2.4	80.3	12.36
60-rw	2.4	33.9	3.8
61-rw	2.4	11.2	0.32
62-cc	2.4	60	6.25
63-cc	2.4	58.2	5.46
64-rw	2.4	83.4	8.73
65-rw	2.5	32	5.81
66-ca	2.5	31.4	4.88
67-lv	2.5	30.8	3.21
68-cc	2.5	60.4	9.11
69-lv	2.5	36.5	3.84
70-cc	2.5	57.7	9.71
71-cc	2.5	40.4	5.32
72-ca	2.5	24.6	3.31
73-rw	2.5	24.4	4.55
74-ca	2.5	33.9	9.07
75-lv	2.5	28.2	5.98
76-lv	2.5	49.3	10.3
77-lv	2.5	47.2	5.98
78-rw	2.5	38	6.76
79-rw	2.5	31.1	4.82
80-cc	2.5	28.1	4.53

81-cc	2.6	68.7	7.75
82-ca	2.6	29.9	3.48
83-lv	2.6	30.6	3.75
84-cc	2.6	16.5	2.75
85-ca	2.6	17.3	2.84
86-rw	2.6	38.3	4.45
87-rw	2.6	39.5	3.75
88-lv	2.6	56.7	7.89
89-lv	2.6	86.3	15.91
90-rw	2.6	47.6	5.51
91-cc	2.6	38.9	5.31
92-rw	2.6	11.5	2.65
93-rw	2.6	6.8	2.48
94-ca	2.6	30.1	3.58
95-ca	2.6	62.9	7.63
96-cc	2.6	34.5	3.81
97-rw	2.7	75	8.27
98-rw	2.7	48	4.41
99-cc	2.7	49.9	5.5
100-ca	2.7	67.1	6.78
101-lv	2.7	43	3.73
102-cc	2.7	132	22.86
103-lv	2.7	51.5	3.98
104-lv	2.7	85.8	11.01
105-rw	2.7	88.9	8.89
106-lv	2.7	98.5	17.17
107-lv	2.7	55.4	4.54
108-ca	2.7	122	23.65
109-ca	2.7	80.1	15.11
110-cc	2.7	109.8	14.9
111-lv	2.7	78.2	4.62
112-ca	2.7	98.4	18.67
113-ca	2.8	13.8	2.73
114-lv	2.8	32.6	3.52
115-rw	2.8	16.8	2.98
116-rw	2.8	13	2.99
117-rw	2.8	13.6	2.54
118-cc	2.8	31	3.38
119-rw	2.8	39.8	3.79
120-cc	2.8	42.2	4.78
121-rw	2.8	32.5	3.58
122-cc	2.8	48.6	8.23
123-rw	2.8	49.3	6.64

124-rw	2.8	16.2	2.25
125-ca	2.8	38.4	4.91
126-ca	2.8	58.3	5.3
127-ca	2.8	64.2	7.84
128-ca	2.8	56.9	5.98
Total Weights [g]		3066.5	423.77

SPSS Raw Data

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  /SHEET=name 'Basil'
  /CELLRANGE=full
  /READNAMES=on
  /ASSUMEDSTRWIDTH=32767.
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  /INTERCEPT=INCLUDE
  /POSTHOC=Temp Worms Fish Substrate(TUKEY)
  /CRITERIA=ALPHA(0.05)
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*Fish Worms*Substrate Fish*Substrate Temp*Worms*Fish Temp*Worms*Substrate Tem
p*Fish*Substrate Worms*Fish*Substrate Temp*Worms*Fish*Substrate.
```

Univariate Analysis of Variance

[DataSet1]

Warnings

Post hoc tests are not performed for Temp because there are fewer than three groups. Post hoc tests are not performed for Worms because there are fewer than three groups. Post hoc tests are not performed for Fish because there are fewer than three groups.

Between-Subjects Factors

		N
Temp	1	58
	2	62
Worms	1	60
	2	60
Fish	1	60
	2	60
Substrate	1.0	27
	2.0	30
	3.0	32
	4.0	31

Tests of Between-Subjects Effects

Dependent Variable: Basil Fresh Wt (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	756.054[a]	31	24.389	2.206	.002
Intercept	3867.311	1	3867.311	349.846	.000
Temp	222.183	1	222.183	20.099	.000
Worms	17.812	1	17.812	1.611	.208
Fish	143.988	1	143.988	13.025	.001
Substrate	121.091	3	40.364	3.651	.016
Temp * Worms	23.520	1	23.520	2.128	.148
Temp * Fish	.986	1	.986	.089	.766
Temp * Substrate	9.739	3	3.246	.294	.830
Worms * Fish	9.205	1	9.205	.833	.364
Worms * Substrate	11.173	3	3.724	.337	.799
Fish * Substrate	15.609	3	5.203	.471	.703
Temp * Worms * Fish	.547	1	.547	.049	.824
Temp * Worms * Substrate	12.662	3	4.221	.382	.766
Temp * Fish * Substrate	32.724	3	10.908	.987	.403
Worms * Fish * Substrate	32.936	3	10.979	.993	.400
Temp * Worms * Fish * Substrate	8.682	3	2.894	.262	.853
Error	972.780	88	11.054		
Total	6646.723	120			
Corrected Total	1728.834	119			

a. R Squared = .437 (Adjusted R Squared = .239)

Post Hoc Tests

Substrate

Multiple Comparisons

Dependent Variable: Basil Fresh Wt (g)

Tukey HSD

(I) Substrate	(J) Substrate	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	-2.7030*	.88198	.015	-5.0127	-.3933
	3.0	-.7185	.86883	.842	-2.9938	1.5568
	4.0	.3056	.87522	.985	-1.9864	2.5976
2.0	1.0	2.7030*	.88198	.015	.3933	5.0127
	3.0	1.9845	.84494	.095	-.2283	4.1972
	4.0	3.0086*	.85151	.004	.7787	5.2385
3.0	1.0	.7185	.86883	.842	-1.5568	2.9938
	2.0	-1.9845	.84494	.095	-4.1972	.2283
	4.0	1.0241	.83788	.614	-1.1701	3.2184
4.0	1.0	-.3056	.87522	.985	-2.5976	1.9864
	2.0	-3.0086*	.85151	.004	-5.2385	-.7787
	3.0	-1.0241	.83788	.614	-3.2184	1.1701

Based on observed means.

The error term is Mean Square(Error) = 11.054.

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Basil Fresh Wt (g)

Tukey HSD[a,b,c]

Substrate	N	Subset 1	Subset 2
4.0	31	5.3077	
1.0	27	5.6133	
3.0	32	6.3319	6.3319
2.0	30		8.3163
Sig.		.634	.104

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 11.054.

a. Uses Harmonic Mean Sample Size = 29.878.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = 0.05.

```
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  /INTERCEPT=INCLUDE
  /POSTHOC=Temp Worms Fish Substrate(TUKEY)
  /CRITERIA=ALPHA(0.05)
  /DESIGN=Temp Worms Fish Substrate Temp*Worms Temp*Fish Temp*Substra
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p*Fish*Substrate Worms*Fish*Substrate Temp*Worms*Fish*Substrate.
```

Univariate Analysis of Variance

Warnings

Post hoc tests are not performed for Temp because there are fewer than three groups.
Post hoc tests are not performed for Worms because there are fewer than three groups.
Post hoc tests are not performed for Fish because there are fewer than three groups.

Between-Subjects Factors

		N
Temp	1	58
	2	62
Worms	1	60
	2	60
Fish	1	60
	2	60
Substrate	1.0	27
	2.0	30
	3.0	32
	4.0	31

Tests of Between-Subjects Effects

Dependent Variable: Basil Dry Wt (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.672[a]	31	.247	1.408	.109
Intercept	63.414	1	63.414	360.778	.000
Temp	.227	1	.227	1.292	.259
Worms	.043	1	.043	.246	.621
Fish	.356	1	.356	2.026	.158
Substrate	1.939	3	.646	3.677	.015
Temp * Worms	.377	1	.377	2.143	.147
Temp * Fish	.062	1	.062	.351	.555
Temp * Substrate	.080	3	.027	.151	.929
Worms * Fish	.335	1	.335	1.907	.171
Worms * Substrate	.164	3	.055	.312	.817
Fish * Substrate	.315	3	.105	.597	.619
Temp * Worms * Fish	.542	1	.542	3.082	.083
Temp * Worms * Substrate	.302	3	.101	.573	.634
Temp * Fish * Substrate	.768	3	.256	1.457	.232
Worms * Fish * Substrate	.576	3	.192	1.093	.356
Temp * Worms * Fish * Substrate	.104	3	.035	.197	.898
Error	15.468	88	.176		
Total	104.272	120			
Corrected Total	23.140	119			

a. R Squared = .332 (Adjusted R Squared = .096)

Post Hoc Tests

Substrate

Multiple Comparisons

Dependent Variable: Basil Dry Wt (g)

Tukey HSD

(I) Substrate	(J) Substrate	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	-.2899	.11122	.052	-.5811	.0014
	3.0	.0494	.10956	.969	-.2375	.3363
	4.0	.1362	.11036	.607	-.1528	.4252
2.0	1.0	.2899	.11122	.052	-.0014	.5811
	3.0	.3392*	.10655	.011	.0602	.6183
	4.0	.4261*	.10737	.001	.1449	.7073
3.0	1.0	-.0494	.10956	.969	-.3363	.2375
	2.0	-.3392*	.10655	.011	-.6183	-.0602
	4.0	.0868	.10565	.844	-.1899	.3635
4.0	1.0	-.1362	.11036	.607	-.4252	.1528
	2.0	-.4261*	.10737	.001	-.7073	-.1449
	3.0	-.0868	.10565	.844	-.3635	.1899

Based on observed means.

The error term is Mean Square(Error) = .176.

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Basil Dry Wt (g)

Tukey HSD[a,b,c]

Substrate	N	Subset	
		1	2
4.0	31	.6619	
3.0	32	.7488	
1.0	27	.7981	
2.0	30		1.0880
Sig.		.593	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .176.

a. Uses Harmonic Mean Sample Size = 29.878.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = 0.05.

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UNIANOVA lettuceFreshWtg BY Temp Worms Fish Substrate
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  /INTERCEPT=INCLUDE
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  /CRITERIA=ALPHA(0.05)
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*Fish Worms*Substrate Fish*Substrate Temp*Worms*Fish Temp*Worms*Substrate Tem
p*Fish*Substrate Worms*Fish*Substrate Temp*Worms*Fish*Substrate.
```

Univariate Analysis of Variance

Warnings

Post hoc tests are not performed for Temp because there are fewer than three groups. Post hoc tests are not performed for Worms because there are fewer than three groups. Post hoc tests are not performed for Fish because there are fewer than three groups.

Between-Subjects Factors

		N
Temp	1	64
	2	64
Worms	1	64
	2	64
Fish	1	64
	2	64
Substrate	1.0	32
	2.0	32
	3.0	32
	4.0	32

Tests of Between-Subjects Effects

Dependent Variable: lettuce Fresh Wt (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	75404.145[a]	31	2432.392	7.288	.000
Intercept	204609.683	1	204609.683	613.020	.000
Temp	20.261	1	20.261	.061	.806
Worms	3965.190	1	3965.190	11.880	.001
Fish	15081.422	1	15081.422	45.185	.000
Substrate	2908.418	3	969.473	2.905	.039
Temp * Worms	13312.787	1	13312.787	39.886	.000
Temp * Fish	2620.178	1	2620.178	7.850	.006
Temp * Substrate	589.204	3	196.401	.588	.624
Worms * Fish	59.910	1	59.910	.179	.673
Worms * Substrate	620.252	3	206.751	.619	.604
Fish * Substrate	32.063	3	10.688	.032	.992
Temp * Worms * Fish	25846.157	1	25846.157	77.436	.000
Temp * Worms * Substrate	390.898	3	130.299	.390	.760
Temp * Fish * Substrate	420.664	3	140.221	.420	.739
Worms * Fish * Substrate	174.445	3	58.148	.174	.914
Temp * Worms * Fish * Substrate	2911.660	3	970.553	2.908	.039
Error	32042.219	96	333.773		
Total	337635.970	128			
Corrected Total	107446.364	127			

a. R Squared = .702 (Adjusted R Squared = .605)

Post Hoc Tests

Substrate

Multiple Comparisons

Dependent Variable: lettuce Fresh Wt (g)

Tukey HSD

(I) Substrate	(J) Substrate	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	-.009	4.5674	1.000	-11.951	11.932
	3.0	.159	4.5674	1.000	-11.782	12.101
	4.0	11.459	4.5674	.065	-.482	23.401
2.0	1.0	.009	4.5674	1.000	-11.932	11.951
	3.0	.169	4.5674	1.000	-11.773	12.111
	4.0	11.469	4.5674	.065	-.473	23.411
3.0	1.0	-.159	4.5674	1.000	-12.101	11.782
	2.0	-.169	4.5674	1.000	-12.111	11.773
	4.0	11.300	4.5674	.071	-.642	23.242
4.0	1.0	-11.459	4.5674	.065	-23.401	.482
	2.0	-11.469	4.5674	.065	-23.411	.473
	3.0	-11.300	4.5674	.071	-23.242	.642

Based on observed means.
The error term is Mean Square(Error) = 333.773.

Homogeneous Subsets

lettuce Fresh Wt (g)

Tukey HSD[a,b]

Substrate	N	Subset
		1
4.0	32	33.850
3.0	32	45.150
1.0	32	45.309
2.0	32	45.319
Sig.		.065

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = 333.773.

a. Uses Harmonic Mean Sample Size = 32.000.

b. Alpha = 0.05.

```
UNIANOVA lettuceDryWtg BY Temp Worms Fish Substrate
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  /METHOD=SSTYPE(3)
  /INTERCEPT=INCLUDE
  /POSTHOC=Temp Worms Fish Substrate(TUKEY)
  /CRITERIA=ALPHA(0.05)
  /DESIGN=Temp Worms Fish Substrate Temp*Worms Temp*Fish Temp*Substrate Worms
*Fish Worms*Substrate Fish*Substrate Temp*Worms*Fish Temp*Worms*Substrate Tem
p*Fish*Substrate Worms*Fish*Substrate Temp*Worms*Fish*Substrate.
```

Univariate Analysis of Variance

Warnings

Post hoc tests are not performed for Temp because there are fewer than three groups.
Post hoc tests are not performed for Worms because there are fewer than three groups.
Post hoc tests are not performed for Fish because there are fewer than three groups.

Between-Subjects Factors

		N
Temp	1	64
	2	64
Worms	1	64
	2	64
Fish	1	64
	2	64
Substrate	1.0	32
	2.0	32
	3.0	32
	4.0	32

Tests of Between-Subjects Effects

Dependent Variable: lettuce Dry Wt (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1278.939[a]	31	41.256	3.300	.000
Intercept	4592.819	1	4592.819	367.423	.000
Temp	7.098	1	7.098	.568	.453
Worms	19.859	1	19.859	1.589	.211
Fish	215.861	1	215.861	17.269	.000
Substrate	141.103	3	47.034	3.763	.013
Temp * Worms	277.896	1	277.896	22.232	.000
Temp * Fish	15.589	1	15.589	1.247	.267
Temp * Substrate	37.253	3	12.418	.993	.399
Worms * Fish	3.868	1	3.868	.309	.579
Worms * Substrate	33.981	3	11.327	.906	.441
Fish * Substrate	9.837	3	3.279	.262	.852
Temp * Worms * Fish	220.573	1	220.573	17.646	.000
Temp * Worms * Substrate	35.628	3	11.876	.950	.420
Temp * Fish * Substrate	33.195	3	11.065	.885	.452
Worms * Fish * Substrate	22.860	3	7.620	.610	.610
Temp * Worms * Fish * Substrate	121.041	3	40.347	3.228	.026
Error	1200.008	96	12.500		
Total	7575.660	128			
Corrected Total	2478.946	127			

a. R Squared = .516 (Adjusted R Squared = .360)

Post Hoc Tests

Substrate