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Growth and Tissue Elemental Composition Response of Spinach (*Spinacia oleracea*) to Hydroponic and Aquaponic Water Quality Conditions

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Abstract

Spinach (*Spinacia oleracea*, cv. Carmel) was grown in a conventional glass greenhouse under three different nutrient solution treatments. Lighting and temperature conditions were identical. Six growing systems were used to provide a duplicate trough system for each of these three treatments. Six crops (referred to as trials) were harvested from each system over a two month time period. Two treatments received hydroponic nutrient inputs, with one treatment at pH 7.0 (referred to as H7) and the other at pH 5.8 (referred to as H5), and the third treatment was aquaponic (referred to as A7), receiving all of its nutrients from a single fish tank with koi (*Cyprinus carpio*) except for the addition of chelated iron. The pH of the systems were regulated by adding K_2CO_3 to the aquaponic systems, and KOH to the hydroponic systems. Plants were harvested at a marketable size for baby-leaf spinach. Comparisons made between the treatments were total yield (fresh weight and dry weight), leaf surface area, tissue elemental content, and dry weight to fresh weight ratio. Despite some differences in nutrient solution and tissue composition, it was found that dry weight biomass yield values were not different in pairwise comparisons between treatments (A7 vs. H5: p=0.59 fresh weight, p=0.42 dry weight). Similarly, surface area results were not different between treatments. Statistically non-different biomasses were achieved in the A7 and H5 systems for both dry weight and fresh weight. The important comparison is A7 vs. H5, because the H7 treatment is at a pH rarely used in hydroponics, and received slightly more light due to its greenhouse position.

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Introduction

Hydroponics is a method of growing plants using mineral nutrient liquid solution without soil. The method of application of the nutrient solution to the roots varies widely (Jensen & Collins, 1985). Aquaponics is a method of growing plants hydroponically using waste water of an aquaculture system. Aquaponics makes multiple uses of resources such as water and nutrients (Rackocy, 2012; Timmons & Ebeling, 2013). Hydroponics is an increasingly important field as the demand increases for more food and sustainably produced products (Resh, 2012). With urban agriculture on the rise (Mok, 2014), greenhouses are growing food on rooftops and in decaying buildings and abandoned warehouses (Resh, 2012). Hydroponics uses inorganic nutrient fertilizers, while aquaponic treatments rely on fish waste, which has the potential to be at less than ideal concentrations for plants. Aquaponics uses fish waste water to generate the nutrients needed by plants, meaning the nutrient composition is not formulated to exact concentrations and can be less stable. Growing plants in aquaponic waste water provides a sustainable use of the fish waste, which has an added value in marketing to a certain class of consumers. The plants also help filter the fish water and help to reduce nitrate which can be toxic to some fish salmonid species at elevated levels, e.g., greater than 40 mg/L (Timmons and Ebeling, 2013).

The objective of this study is to compare aquaponic and hydroponic spinach yield, and look for differences in leaf surface area and elemental composition between treatments. Two hydroponic treatments were chosen, pH 5.8 and pH 7.0, and one aquaponic treatment was used at pH 7.0. The aquaponic treatment received nutrients from fish waste, with the exception of chelated iron. Fish waste has no iron in it; fish food is iron-free because iron accumulates in the livers of fish to their detriment (Enduta, 2011). Chelated iron is commonly added to the water of an aquaponic system; the amount of iron being absorbed by the fish directly from the water is considered small and non-harmful (Rakocy, 2012).

The solution formula employed for the hydroponic spinach was a formula derived for lettuce by Sonneveld and Straver (1994). In earlier research, the lettuce formula was found to be as effective at half the concentration recommended by Sonneveld and Straver as at full concentration (Both et al., 1997). Half strength Sonneveld and Straver solution has an electroconductivity of 1300 microSiemens/cm. Moderately low pH, around 5.8, keeps most ions available in solution while higher pH, around 6.5 and higher, can cause nutrient deprivation because of nutrient precipitation (Bugbee, 2003). The Sonneveld and Straver solution used in this research was originally designed for use with a pH around 5.8, and both Bugbee (2003) and Both et al. (1997) used this pH for lettuce to maximize nutrient availability.

An added constraint in this research was that our aquaponic treatment needed to be kept at the same low root zone temperature as the two hydroponic treatments to combat Pythium root disease, which is a major hazard in spinach production. *Pythium aphanadermatum* is a devastating organism to which spinach is particularly susceptible. This disease largely has prevented wide scale success in hydroponic spinach production in the world. Diseased plants are characterized by brown roots, upright leaves in the early stages, and slimy black roots and completely wilted tops in the later stages of the disease. The course of the disease is affected by: time of inoculation, concentration of inoculum, and root zone temperature. There are several methods that have been practiced to combat Pythium disease in spinach, and most are only partially successful. In this experiment, the main methods used were maintaining a low root zone temperature, harvesting after 13 days in pond water, and maintaining thorough sanitation by spraying 70% ethanol on all potentially contaminated surfaces during planting and solution

sampling. Harvesting at the baby-leaf stage reduces the risk of Pythium because baby spinach does not grow long enough for the pathogen to complete its full growth cycle (de Villiers & Shelford, 2007).

A major difference of aquaponics compared to hydroponics is its potential for non-uniformity in water nutrient conditions; the system does not have a fixed nutrient solution, and is reliant upon the fish water to provide all nutrients at all times with the exception of chelated iron. This non-uniformity is possibly one of the reasons that aquaponics is sometimes found to have lower yields than hydroponics (Pantanella et al., 2010). Nutrient levels are highly dependent on the fish activity, fish number, and fish species (Endut et al., 2009; Liang & Chien, 2013). Even if the fish waste provides adequate nutrients to successfully grow high yielding plants, the hydrodynamics of the aquaponics system flow can be detrimental to the plant side of the system. For example, the flow rate and rate of recirculation in the system can reduce yield if they are too slow or too fast which causes root stress (Shete et al., 2013). The overall objective of this study was to compare yields in an aquaponics system to yields obtained from a conventional hydroponics system.

Materials and Methods

Seeds were germinated at high humidity in a temperature controlled growth chamber for three days (additional details of growth chamber provided below). After germination, flats of spinach were grown for 13 additional days using deep-flow troughs/channels housed in a conventional glass greenhouse under three different treatments which consisted of three different nutrient solution conditions. Two treatments received hydroponic nutrient inputs, with one treatment at pH 5.8 (referred to as H5) and the other at pH 7.0 (referred to as H7). The source water used reverse osmosis (RO) water to make up the nutrient solutions. Growing conditions mimicked conventional deep-flow grow ponds and plants were grown using a modified Sonneveld nutrient solution designed for lettuce (described later). The third treatment was aquaponic (referred to as A7), receiving its nutrients from aquaculture waste with the addition of chelated iron (initial concentration of 3 mg/L). The aquaponic source water was tap water that was initially high in alkalinity. This carbon filtered water had average macro-elemental contents of 50mg/L Ca, 13mg/L Mg, 5.5mg/L S, and an EC of 450µS/cm. The concentrations of the makeup water for Ca, Mg and S varied slightly seasonally. Adjustments in pH were initially made to achieve target values and then made daily using 1 M K_2CO_3 to the aquaponic system or 1 M KOH to the hydroponic systems. Prior to the experiment, initial pH adjustments were made using HNO_3 or the treatment's respective base as just mentioned.

The spinach crops in all treatments were grown in the same greenhouse and aerial space under the same lighting and temperature conditions, with periodic harvest of old and insertion of new trials into hydroponic growing channels (described further below). Six trials were conducted from January 17 to February 19, 2016, with a preliminary trial conducted to validate procedures. Root zone temperature was also matched across all treatments at 18° C. All treatments were grown for 13 days once a flat was floated in its respective channel. Each of the three treatments was applied in a duplicate growing system, resulting in six growing systems. The two hydroponic treatments had four completely independent recirculating systems including their source waters, while the water from the fish tank for the two aquaponic systems was continuously being mixed. An individual trial ended with the harvest of all six flats of spinach from each growing system. Each trial was offset by three days; additional harvest details are

provided below. Styrofoam floats covered all water surfaces to reduce algae growth, minimize evaporation, and block solar radiation, which would have destructed the iron chelator.

The Greenhouse:

The spinach was grown in a middle section of a multi-sectioned glass greenhouse built in 1953, with dimensions 9 m x 11 m x 7 m high to the ridge, oriented east west (Figure 1). An Argus monitoring and control systems logged CO_2, humidity, aerial temperature, and light level, and also controlled aerial temperature and daily light integral (DLI). There was no carbon dioxide supplementation. Two identical water-to-air heat exchangers on opposite sides of the greenhouse rated at 115,000 kJ/h used fans to move air through radiators then across the greenhouse, providing air mixing and rapid adjustment of air temperatures to target values.

A target DLI of 17 mol/m^2/day was used for all trials. The DLI is the quantity of photosynthetically active radiation (PAR, in units of moles/m^2/day) achieved by controlled use of a lighting array to supplement natural light radiation. The environmental parameters were sampled approximately every two seconds and data queues averaged and logged every 2 minutes. DLI was controlled to its target value by supplementing natural light using an array of 20 high pressure sodium (HPS) lights (General Electric, 400 watt clear S51/O, Mogul Base rated ED18 HSP, LU 400/H/ECO). The DLI was reset daily at 6:00 am. The greenhouse had a heating set point of 24°C, and a cooling set point of 25°C. The quantity of both natural and supplemental lighting received at plant level was recorded using a LiCor quantum sensor. The average DLI for trials was consistently between 17.0 and 17.1 mol/m^2/day for all trials. The average natural light integrals were 4.0, 4.1, 4.7, 4.6, 5.4, and 5.4 mol/m^2/day for trials 1-6 respectively. The average supplementary light integrals were 13.0, 12.9, 12.3, 12.4, 11.6, and 11.6 mol/m^2/day for trials 1-6 respectively.

The Channels:

Flats of spinach were floated in six channels raised 1.27 m above the floor. The channels were made of 2x12 lumber, which provided insulation on the sides and ends. They were also insulated at the bottom with 25 mm polystyrene and 12 mm of plywood, and carefully lined with 0.006 in plastic to prevent leakage. The outside dimensions of the channels were 29 cm high x 42.5 cm wide x 244 cm long. Internal dimensions were 26 cm deep x 35 cm wide x 236 cm long. Each channel was accompanied by a reservoir at floor level (volume 50 L). Nutrient solution was cooled in the reservoir, pumped up to the far end of the channel, then drained back to the reservoir by gravity with depth controlled by a standpipe at the downstream end of the channel. The channels were filled to roughly 24 cm with 2 cm of freeboard, which provided a total volume of 200 L per channel plus reservoir volume. The tops of the floating flats were 1.27 m above the floor and 1.30 m below the light fixtures to maximize light uniformity (natural and supplemental) among all channels. At the water level heights maintained, the top of the floats cleared the channel sides. The channels were labeled 1 through 6 and were grouped into three blocks: channels 1 and 2 were block 1 (A7 treatment), channels 3 and 4 were block 2 (H7 treatment), and channels 5 and 6 were block 3 (A7 treatment) (see Figure 1).

In a previous investigation of effect of channel position within the greenhouse, plants were found to grow non-differently in the outer pairs of channels (blocks 1 and 3; de Villiers and Anderson, 2016). In that six-trial study, yields per flat were almost identical in given trials, and, as a consequence a paired two-tailed t-test gave a p value of 0.83 in comparing the pairs of outer channels (channel 1&2 versus channel 5&6). Due to the central placement of the 6 channels under the lighting array, the lighting array's central placement in the greenhouse, and the

greenhouse's bilateral symmetry, we expected no location effect on growth between our outside channel locations (1&2 and 5&6), but block 2 (channel 3&4) showed a 7% increase in growth compared to the outer blocks (de Villiers and Anderson, 2016). On a fresh weight basis, p=0.016 in a one-tailed paired t-test of 3&4 with 1&2, and p=0.013 in a similar test of 3&4 with 5&6 (see Table 1). This increase correlated with a higher light level measured in the middle of the greenhouse in mapping the supplemental lighting array, and increased natural light in the middle due to geometry (see Figure 2). In view of the advantage in light intensity in the middle block position, we chose the two outside blocks for the A7 and H5 treatments, since this was our primary comparison of interest for commercial application and there was no yield advantage between these channels. Based upon these considerations, we conducted all trials without reversal of channel positions for treatment assignments. A general overall photo of the experimental arrangement is shown in Figure 3.

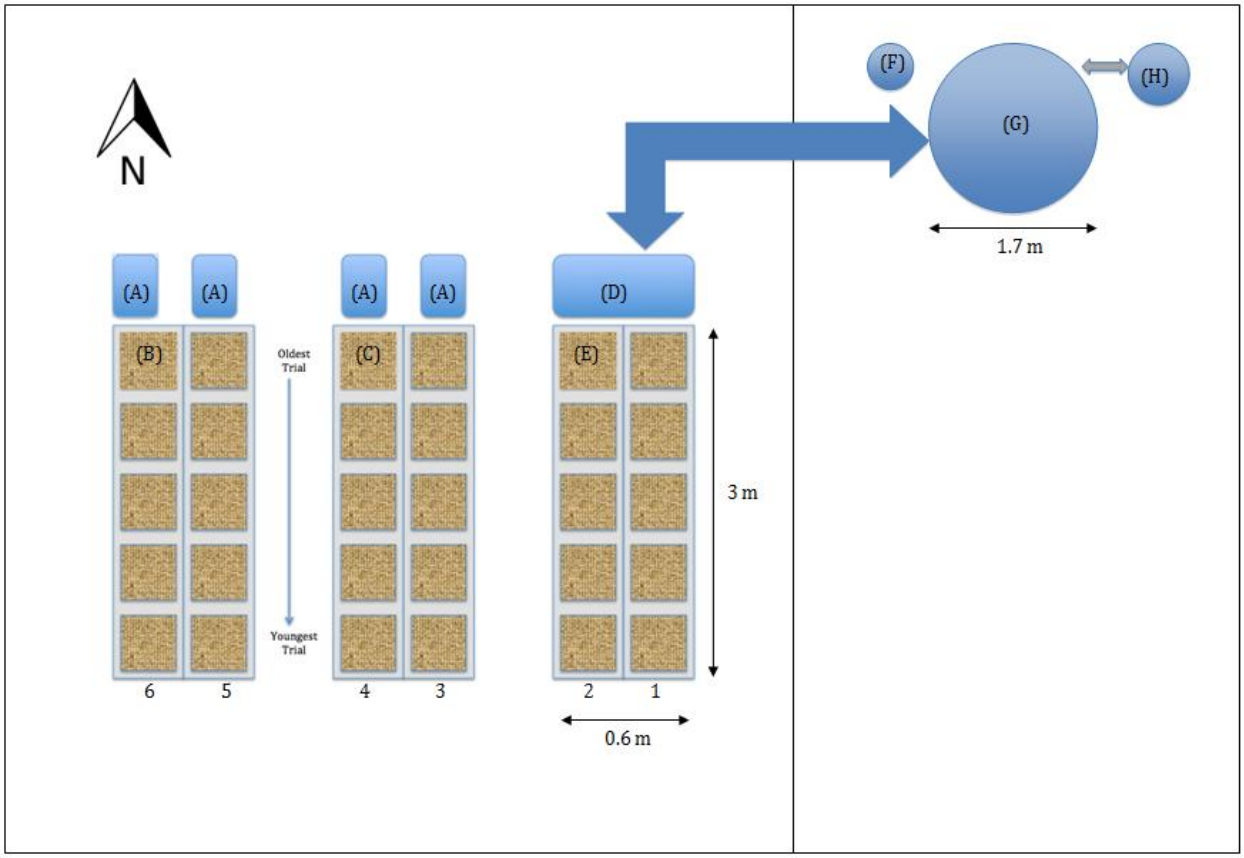

Figure 1: Floor plan of greenhouse. Fish tank is separated from spinach channels by a thin greenhouse partition wall. Channels are numbered 1 through 6.
[A] Heat exchangers used to cool and filter individual hydroponic channels.
[B] Duplicate channels of H5 treatment
[C] Duplicate channels of H7 treatment
[D] Shared aquaponic reservoir water, with pumps to returnwater to the fish system
[E] Block 3: Channels of A7 treatment, not duplicate because of shared water
[F] Bead filter to filter fish waste
[G] Fish tank
[H] Sump pump and settling tank for recirculting fish system

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
12	140	143	159	175	180	177	185	194	188	178	181	173	157	143	138
11	160	169	183	200	204	200	200	217	196	195	205	197	179	166	158
10	176	183	196	211	214	209	217	223	219	210	215	208	190	182	173
9	182	191	199	212	215	210	215	221	213	211	217	211	194	187	177
8	185	197	199	208	212	207	211	217	212	209	214	209	194	190	183
7	186	198	194	200	207	201	204	212	209	205	209	202	189	188	180
6	188	202	194	200	207	201	202	211	207	203	207	200	189	189	180
5	189	206	198	203	211	205	205	214	209	206	209	202	191	189	179
4	184	204	198	205	214	209	208	215	210	207	210	201	191	187	176
3	179	195	190	201	210	206	206	214	209	206	208	199	189	183	172
2	162	177	172	183	193	193	193	202	198	194	196	187	176	170	160
1	141	151	150	162	169	171	172	180	178	174	174	165	160	155	141

Figure 2. Light map of all greenhouse, in units of lux. Colors and numbers both represent light intensity, from green to red, showing light symmetry about the center. North is pointing up, making orientation the same as Figure 1. Position units are arbitrary locations in the greenhouse, with channel 1 at the right, and channel 6 at the left. The important result is the similarity and comparibility of the exterior channels, and advantage of the center, as described in *Biomass Results*.

Figure 3. Picture of channels and general experimental setup. The two channels on the left are the hydroponic pH 5.8 treatment. The two channels in the middle are the hydroponic pH 7.0 treatment. The two channels on the right are the aquaponic treatment. The insulated PVC pipe coming in from the top right is the inflow and the outflow from the fish tank in a greenhouse to the right, off screen.

Table 1. Fresh weight biomass results (grams/plant) from experiment conducted to measure and test any potential differences between channels, in order to justify the decision not to reverse treatment positions; the major factor of concern was light distribution throughout the greenhouse (de Villiers and Anderson, 2016).

	Channel						
Trial	1	2	3	4	5	6	Average
1	3.64	3.48	3.98	3.88	3.97	3.61	3.76
2	2.82	2.80	3.36	2.88	3.12	3.20	3.03
3	3.88	3.69	4.00	3.77	3.64	3.47	3.74
4	3.24	3.08	3.63	3.49	2.87	3.53	3.31
5	2.19	2.20	2.14	2.19	1.94	2.15	2.14
6	2.53	2.75	2.74	2.79	2.53	2.54	2.65
Average	3.05	3.00	3.31	3.17	3.01	3.08	3.10

Seeding:

The seeding protocol involved hand-seeding spinach (*Spinacia oleracea*, cv. Carmel) seeds into each cell of each flat. During trials 1 and 2, seeds were double seeded in each cell, but due to somewhat low germination in the flats, trials 3 to 6 were triple seeded. After germination, stands were thinned to only one plant per cell prior to floating the plants in the channels (see *Stand correction* below for more details).

We have found the notorious and historical difficulty in geminating spinach largely disappears if seeding is into pre-moistened medium and the moisture content of the medium is controlled. Potting media used was LM-1 germination mix, by Lambert. Pre-moistened medium was created by adding 1 L of reverse osmosis water for every 1 kg of LM-1 potting media, to achieve a moisture content of roughly 3 parts water to one part dry matter (the potting media is roughly half water as received). Potting media was screened through a 6 mm sieve into multi-cell Styrofoam flats. Depth of seeding was controlled by a wooden dibbler, that compacted the medium to a set depth. Germination success is improved by discarding any seeds that looked unhealthy, e.g. misshapen, discolored, or oddly sized, which we practiced. The goal of seed selection was to create the most uniform plant stand possible.

After seeding, the trays were sealed in 15 liter white plastic bags, to control humidity to near saturation, and germinated in a growth chamber shielded from light, for eighty hours. The germination chambers were maintained at 24° C. We found that eighty hours was the best time to float the flats, as that was the time when most plants had recently emerged but minimal growth or stretching had occurred.

After floating, the plants were grown in the channels for an additional 14 days under full lighting. The seeding, floating, and growing procedures were the same for all trials and treatments except the increased seeding as previously described. Trials overlapped with each other in their channels, since flats were added every 3 days as a new trial, so it took five successive trial placements (trials) before the first trial was harvested; thus trial 1 and 6 were completely independent in all ways, including their water environments.

Stand Correction:

An attempt was made to control for the variation in germination among flats. The procedure entailed adjusting plant stands to a set number of healthy, equivalent, plants in the

interior of each of the flats for each particular trial. The number was determined by the worst germination of the six flats in a given trial after having removed any deformed or very late emerging plants. From the remaining plants, additional healthy plants were removed so that each trial had the same number of plants per flat. Once the number of plants to remove from each flat was determined, these plants were randomly selected and removed.

Even though trials were stand corrected to be the same within trial, there were small differences between trials since each trial had a different number of healthy plants at the time of floating. The number of plants varied from 44 to 51 out of the 56 potential guarded cells that were to be harvested for data analysis.

The Hydroponic Systems:

The hydroponic channels were closed-loop recirculating water systems, with each channel independent and unconnected to others. Circulating pumps (24 L/min) mixed the water in the channels at a rate equal to a hydraulic retention time (HRT) of 8 minutes. This flow rate maintained good mixing in the channels and roots appeared to be kept under gentle movement, which we did to avoid possible problems of root stress previously mentioned by Shete et al., 2013. A crucial part of the hydroponic channels was the nutrient solution reservoir (made with modifications to a cooler chest). Each channel's flow went through its reservoir, in which temperature was computer-controlled by activation of cooling coils in which water cooled to 7° C was passed when necessary. Two hundred liters of nutrient solution were added to each channel to begin the experiment. Nutrient solution was added on an as-needed basis to replace water loss by evaporation or transpiration. For each given trial, one flat was floated in each channel at the same time to commence that particular trial. The flats were 6.3 cm thick and comprised 132 cells in an 11 by 12 matrix. Cell density in the flats was 1250 cells/m^2. Only plants from the central 56, doubly-guarded cells, were harvested.

The Aquaponic System:

The aquaponic system contained koi (*Cyprinus carpio*) separately housed in an adjacent section of the climate-controlled greenhouse range, as shown in Figure 1. Koi were chosen as the fish because the common carp is a hardy fish and can tolerate a wide range of water temperature, and koi retain that resilience. The fish tank was 1000 L and had been in continuous operation for two years. The system continually recirculated water with minimal water discharge and used a bead filter to capture and mineralize solids. The bead filter was back-flushed once per week to remove retained solids, which allowed significant time for solids mineralization. The fish system was plumbed to flow water from the bead filter to the two aquaponics channels before returning the water to the fish tank. The flow from the aquaponics channels was calibrated to be 5 L/min per channel. It was controlled by a sump pump in a common reservoir, similar to that in the hydroponic systems, connected to both aquaponic channels. The resulting aquaponic HRT value was 20 minutes per channel, over twice the hydroponic HRT, because higher flow rates seemed to disturb the fish. However, the aquaponic HRT was only higher in the reservoir, because the internal HRT remained uniform throughout all treatments due to each channel volume being the same.

The pH of the system was regulated daily by adding 1M K_2CO_3. Due to fish nitrification and fish respiration, the pH was stable, so there was no need for pH lowering chemicals such as acid. Fish were fed a synthetic manufactured Koi feed (Blackwater Creek's Max Growth Koi Food "Premium Koi and Goldfish Food"). The fish were fed 90 g on weekdays, and 60 g on weekends, which was approximately 1% body weight per day on weekdays; the koi were

approximately 200 - 400 g in size. See Table 2 for the elemental analysis of the fish feed used as determined by the Cornell Nutrient Analysis Lab (CNAL).

Table 2. Blackwater creek farm Max Growth formula elemental contents via acid digestion.

Element	Content	Units
Total C	40	%
Total N	6.3	%
Total H	6.3	%
Ca	4.1	%
P	2.2	%
K	1.2	%
S	5623	mg/kg
Na	4137	mg/kg
Mg	3545	mg/kg
Fe	789	mg/kg
Al	283	mg/kg
Sr	269	mg/kg
Zn	226	mg/kg
Mn	88	mg/kg
B	28	mg/kg
Cu	16	mg/kg
Ba	15	mg/kg
As	4.2	mg/kg
Cr	1.7	mg/kg
V	1.2	mg/kg
Pb	1.1	mg/kg
Ni	1.0	mg/kg
Cd	0.7	mg/kg
Mo	0.5	mg/kg
Co	0.5	mg/kg
Ti	0.0	mg/kg

The aquaponic treatment channels were not independent water systems from one another. Water from both systems came from and was returned to the fish tank, and the systems shared a reservoir which was cooled in the same way as the hydroponic channels.

The fish tank was separated from the spinach channels by a glass greenhouse partition wall, which had the advantage of allowing the fish tank to be kept at a colder air temperature. Since spinach root zone temperatures needed to be kept at 18° C, we maintained the fish water near 20° C and the rest of the chilling was accomplished by the chiller described above.

Measurements:

Alkalinity was measured by titrating to an endpoint of pH 4.5 using 0.01 M (0.02 N) sulfuric acid. The resulting equilibrium alkalinities were approximately 20 mg/L $CaCO_3$ for the H5 treatments and 40 mg/L $CaCO_3$ for both the H7 treatment and the aquaponic treatment. The alkalinity measurement had an accuracy of +/-4 mg/L as $CaCO_3$.

Electroconductivity (EC) was measured between 1200 to 1500 µS/cm. EC was not controlled in the treatments, but due to the differences among treatments, slightly different EC ranges resulted. In the aquaponic treatment, the average EC was 1400 - 1500 µS/cm. In the H5

and H7 treatments, the average EC was 1200 - 1300 µS/cm and 1300 - 1400 µS/cm, respectively. Typically, there was very low variability in the nutrient solution EC in the H5 treatment. The additional nutrient ions in the A7 treatment resulted in the EC stabilizing at the highest values. The pH control partially resulted in the H7 treatment having a higher EC values than the H5 treatment.

Nutrients:

The hydroponic treatments received a custom-made inorganic hydroponic fertilizer. To set up the hydroponic experiment, each of the four hydroponic channels was filled with 200 L of the modified Sonneveld and Straver lettuce solution (Sonneveld and Straver, 1994). To create 200 L, one liter each of two concentrates known as Stock A and Stock B, were mixed. The nutrients contained in each Stock solution are shown in Table 3.

Table 3: Nutrient contents of Stock solutions.

Stock	A	B
Nutrients	Calcium nitrate ($Ca(NO_3)_2 \cdot 3H_2O$ Chelated iron (Sprint 330, Fe-DTPA) Ammonium nitrate (NH_4NO_3) Potassium nitrate (KNO_3) (33% of N)	Potassium nitrate (KNO_3) (67% of N) Epsom salts ($MgSO_4 \cdot 7H_2O$) Manganese sulfate ($MnSO_4 \cdot 1H_2O$) Boric acid (H_3BO_3) Ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) Copper sulfate ($CuSO_4 \cdot 5H_2O$) Potassium sulfate ($K_2SO_4$)

The tubs were first filled with water, then Stock A and B concentrates were added on a 1:1 ratio sequentially while vigorously stirring between additions. Additional modified Sonneveld and Straver solutions were created in 200 L quantities in barrels, using RO water and then used for replenishment of lost tub water over the course of each trial.

To set up the aquaponic experiment, each of the two aquaponic channels was filled with 200 L of fish water, during a time when the fish tank was routinely replenished with tap water (to replace water lost during solids filter cleaning). The aquaponic channels required the addition of iron, since iron is a required element for plants so we added chelated iron in the form of Fe-DTPA (Sprint-330) to the aquaponic system. The chelated iron is in a form which is not taken up by fish but is available to the spinach.

Upon completion of the experiment, tissue analysis data were run by the Cornell Nutrient Analysis Laboratory using hot plate acid digestion plus ICP-AES metal analysis and combustion ash analysis for carbon and nitrogen. Nutrient solution analysis was done immediately before and after the entire experiment. One sample was taken immediately prior to the addition of the first plants , and the other sample was taken after trial 6 was harvested. The nutrient solution analysis was also run by the Cornell Nutrient Analysis Laboratory. Elemental analysis results are shown in Tables 5 and 6.

Harvest:

Plants were harvested after 14 days of grow-out in the channels, at a typical marketable size for baby-leaf spinach. During harvest, flats of plants were removed from the channels one at a time and taken out of the greenhouse. Plants were harvested at night because they are growing

very fast in the day, so night harvesting minimized any advantage a particular flat could have received by its order of harvest. If harvested during the day, flats harvested later would have a relative yield advantage.

Each flat had 132 cells for possible plants, but the two outer perimeter rows of plants were perimeter guarding plants. After removing the two perimeter rows, each flat had the plants from interior/guarded cells for analysis. During harvest, plants were snipped where the stem emerges from the medium and the number of plants per row were counted. All plants within the same row were grouped, and stems and leaves were separated in each row. Finally, total stems and leaves per row were weighed with an accuracy of 0.01 grams. This data was used to calculate both total flat mass and average mass per plant.

After separation, stems and leaves were placed in labeled brown paper bags and dried in ovens maintained at 70° C for three days. We consider dry weight to be a pure representation of biomass yield, since water concentration can make interpretation difficult. We also present fresh weight data as being equally important because fresh weight is marketable weight.

Leaf Surface Area

In order to determine and calculate surface area, leaves were cut after weighing and pressed flat for measurement with a clear scratch-resistant acrylic polycarbonate plexiglass. Each row would all fit underneath a 25 cm x 50 cm piece of the plexiglass. Images of the pressed leaves were then used to determine total leaf surface area.

Surface area was measured using the computer software called ImageJ. ImageJ is an open-source image processing program, with the intent of being used for scientific multidimensional images. To use the software, a picture was loaded, and the brightness and hue threshold of the image was changed to the point where only green color was recognized. Surface areas were extracted using a reference length within the picture.

Statistical Analysis

Three treatment conditions (H5, H7, and A7) were evaluated based primarily upon fresh and dry matter responses. We collected data for six trials from January 13 to February 19, 2016, corresponding to a total day length increase of 84 minutes (9 hr 19 min to 10 hr 43 min, or a 15% increase in natural day length). We first evaluated if there was a trial effect on biomass response across all trials, which may have been impacted by changing day length. We also evaluated trial effect on individual plant response since our stand correction procedure gave us the same number of plants for each flat but resulted in a different number of plants per flat by trial and the natural daylenth increased for each trial. After evaluating for a trial effect, data was combined among trials to provide increased degrees of freedom for statistical analysis of treatment effects. After combining data by treatment, treatments were compared using paired t-tests.

Results and Discussion

Trial Effect

We found no statistical difference in biomass responses for wet and dry weights within each treatment between trials ($p=0.29$). Inspection of the data for plant size (Table 4a) shows a trend of slightly decreasing individual plant size as trials progress. This may have been due to the increasing natural light as part of a fixed DLI target, but it may have been also related to the number of plants per flat increasing slightly over the trials conducted. While the DLI was

controlled to a constant value of 17 mol/m^2/day, successive spinach trials received an increasing percentage of natural light for each successive trial (see *The Greenhouse* section). Obviously, a much larger number of plants in an individual flat would result in smaller individual plants. However, probably the most important response variable for commercial interest is the total fresh weight (biomass) produced from an individual flat. Table 4c shows this variable. Here we also saw no significant effect (p=0.25) by trial when treatments were grouped. Note that this last analysis meant that our sample number was only 6, compared to a sample number of 42, when we used the flat row as an individual data point, which then meant we obtained 42 as our sample number when combining across treatments because each flat had 7 rows and there were 6 flats per trial. The remainder of the analysis results are from grouping trial response by treatment (12 responses for each treatment) and then comparing treatment effect.

Biomass Results

Our biomass results, which were normally distributed, as shown in Tables 4 and 5, showed no statistical differences between the A7 and H5 treatments, (A7 vs. H5: p=0.59 fresh weight, p=0.42 dry weight). Similarly, leaf surface area results were not different between treatments.

The H7 treatment obtained higher yields than the other two treatments for fresh weight (H7 vs. A7: p=0.03 fresh weight, p=0.42 dry weight, H7 vs. H5: p=0.01 fresh weight, p=0.84 dry weight). However, research reported by Anderson et al. (submitted) for butterleaf lettuce showed that a H7 treatment reduced growth by 24% compared to the H5 treatment. If we reduce the yields of H7 by 7% to account for the possible benefit of additional light in the center two channels, the response of the H7 is statistically not different from the other two treatments. The more important result is the similar yield of A7 vs. H5, implying that fish waste may provide everything spinach needs to grow other than iron. This result is especially significant given the exterior position symmetry of these two treatments, and the symmetry of yield in the channel equivalency experiment (de Villiers and Anderson, 2016).

Table 4. Average plant fresh weight (a) and dry weight (b) data for all channels and trials, in grams per plant. Standard deviations given for average values in parentheses. Total flat biomass (c) is shown for fresh weight data, in grams. Channel 1 and 2 is aquaponics (A7), Channel 3 and 4 is hydroponics pH 7 (H7), Channel 5 and 6 is hydroponics pH 5.8 (H5).

(a)	Channel						
Trial	1	2	3	4	5	6	Average
1	3.01	3.09	3.21	3.18	3.16	3.11	3.13 (0.07)
2	2.85	2.99	3.08	2.90	3.00	2.94	2.96 (0.08)
3	2.76	3.23	2.92	2.99	2.62	2.87	2.90 (0.21)
4	2.59	2.62	2.93	3.03	2.87	2.82	2.81 (0.17)
5	2.96	2.86	3.10	3.07	2.96	3.02	2.99 (0.09)
6	2.72	2.73	2.71	2.82	2.68	2.84	2.75 (0.06)
Average	2.82 (0.16)	2.92 (0.23)	2.99 (0.18)	3.00 (0.13)	2.88 (0.20)	2.93 (0.11)	

(b)	Channel						
Trial	1	2	3	4	5	6	Average
1	0.152	0.157	0.161	0.163	0.163	0.161	0.159 (0.004)
2	0.144	0.160	0.156	0.154	0.160	0.152	0.154 (0.006)
3	0.162	0.170	0.156	0.160	0.147	0.154	0.158 (0.008)
4	0.145	0.154	0.158	0.161	0.170	0.149	0.156 (0.009)
5	0.159	0.156	0.163	0.154	0.165	0.157	0.159 (0.004)
6	0.155	0.150	0.147	0.154	0.157	0.157	0.153 (0.004)
Average	0.153 (0.007)	0.158 (0.007)	0.157 (0.006)	0.158 (0.004)	0.160 (0.008)	0.155 (0.004)	

(c)	Channel						
Trial	1	2	3	4	5	6	Average
1	108	111	110	118	109	115	111 (3.8)
2	124	120	120	130	128	120	123 (4.4)
3	121	117	116	120	115	100	114 (7.5)
4	105	103	125	114	120	106	112 (7.6)
5	126	141	140	143	130	144	137 (6.2)
6	130	142	134	142	136	140	137 (3.0)
Average	119 (11.9)	122 (13.0)	124 (11.2)	127 (11.9)	123 (10.3)	120 (17.0)	

Table 5. Treatment-averaged fresh weight leaves + stems biomass data for each treatment for all trials. This table also shows the number of plants to which each trial was stand corrected (potential maximum of 56), determined by the lowest germination number.

	Block FW, g/plant			Number of plants per flat (post stand correction)	
Trial	A7	H7	H5		Average
1	3.05	3.19	3.14	44	3.14
2	2.92	2.99	2.97	47	2.95
3	2.99	2.95	2.74	46	2.92
4	2.60	2.98	2.84	44	2.86
5	2.91	3.08	2.99	51	3.01
6	2.72	2.76	2.76	48	2.77
Average	2.87	2.99	2.91	47	

Elemental Composition Results

Generally, many plant tissue elements were lower in the aquaponic trials, but many were similar. Specifically, notable elements that were significantly lower (alpha value 0.05) in aquaponic tissue were: calcium (Ca), cobalt (Co), manganese (Mn), molybdenum (Mo), and lead (Pb), potassium (K), sulfur (S), and strontium (Sr). Surprisingly, zinc (Zn) was significantly higher in aquaponic tissue. The standard error is the standard deviation divided by the square root of n (n=6 in this case).

Tissue elements in Table 6 are summed. Not included in the elemental analysis are oxygen and hydrogen which have been reported in plant tissue to be 45% for oxygen and 6% for hydrogen (Curtis, 2008). Adding this 51% or 510,000 mg/kg to our other elements results in a cumulative tissue elemental mass of 97 to 98% of the possible 1 million mg/kg; the small difference between our summation and 1 million could be attributed to variations in carbohydrate content for our particular cultivar compared to the reference data or laboratory errors in measurements of the macro elements, but are certainly within reasonable analytical accuracy.

For nutrient solution comparisons between treatments, A7 nutrient solutions (Table 7) that were significantly lower (before and after) were: sodium (Na), Ca, Mn, Mo, and Pb. Elements that were significantly higher in the aquaponic nutrient solution (both before and after) were: iron (Fe), magnesium (Mg), Co, Na, and Zn. Two of these, Co, Na, were significantly lower in the tissue analysis comparison even though they were elevated above H5 and H7 concentrations in the nutrient solutions. Additionally, Pb was higher in aquaponic nutrient solution before the experiment, but lower at the end of the experiment, which implies that Pb was potentially taken up by the fish system (fish or biological filter).

The nutrient solution composition at the start and end of the experiment gives insight regarding spinach uptake and requirement of elements. Percent differences and concentration differences were calculated to show relative increase or decrease of elements with time (see Table 8). If there was a large increase in the nutrient solution, plants did not utilize the available elements, and if there was a decrease, they potentially could have used more if available. Since many of the elements increased but not largely, using the Sonneveld lettuce formula seems to work well for spinach. The source of Pb in the channels was due to contamination in the water due to leaching from garden hose that was used as parts of the water recirculating systems; the

higher initial values in A7 were because of a longer exposure time before starting the actual experiment.

We performed a mass balance to double check the methods used to determine elemental increase or decrease with time. The tissue analysis data was used to determine the amount of a given element extracted by plants from the water. Forty liters of nutrient solution was added to each of the hydroponic channels (channels 3-6) over the duration of the experiment. The total amount of a given element added to the channels was then calculated using the Sonneveld and Straver formula. Using Ca as an example, the amount extracted was 907 mg/channel for the H5 treatment, which was calculated using the value 6250 mg/kg (Table 6) and using the number of plants per flat and average weight of H5 plants. The amount added was 3600 mg/channel, using the Sonneveld target of 90 mg/L times the 40 L addition. The net excess of Ca was calculated to be 14.1 mg/L, using 200 L as the volume of the channel. The measured net addition of calcium was 13.2 mg/L, confirming the accuracy of the calculations.

Table 6. Tissue analysis results comparing the three treatments averaged over all six trials. Variability between trials of each treatment was not significant (p=0.29), which allowed averaging over all trials. Superscript letters identify that significant differences occur between values with different superscript letters for a given element.

Element [mg/kg]	A7	H5	H7	Standard Error
Macronutrients				
C	333264	331684	335368	755
N	57213	57312	57545	69.5
K	41574[a]	41623[a]	42903[b]	308
Mg	12180	11772	12208	100
P	10063	9659	10957	271
Ca	5389[a]	6250[b]	6380[c]	220
S	3485[a]	3782[b]	3934[c]	93.3
Micronutrients				
Na	868[b]	747[a]	892[c]	31.7
Fe	89.0	91.1	98.1	1.94
Zn	80.1[c]	67.5[b]	59.8[a]	4.17
Sr	28.6[a]	29.4[b]	30.8[c]	0.47
Al	26.0[b]	24.7[a]	28.0[c]	0.68
Mn	22.0[a]	39.4[b]	42.6[b]	4.54
Cu	5.39	4.58	3.67	0.35
Ba	1.89	2.00	1.77	0.05
Mo	1.46[a]	2.39[b]	2.50[c]	0.23
Cr	1.21	1.20	1.25	0.011
Ti	1.11[b]	1.06[a]	1.16[c]	0.020
Pb	1.03[a]	1.32[b]	1.35[b]	0.074
As	0.87	0.87	0.85	0.004
Cd	0.84	0.86	0.87	0.007
Co	0.70[a]	0.74[b]	0.73[b]	0.007
V	0.68	0.73	0.73	0.010
Total	464330	463139	470504	1614

Table 7. Nutrient solution analysis results comparing the three treatments averaged over the channel duplicates. Data is from samples taken at the start of Trial 1after pH stabilization and at the conclusion of Trial 6; expressed in mg/L. Superscript letters identify that significant differences occur between values with different superscript letters, which applies both to treatment differences and start/end differences.

	Start			End			
Element [mg/L]	A7	H5	H7	A7	H5	H7	Standard Error
Macronutrients							
K	214	216	219	255	205	246	8.12
Ca	87.6^{a}	93.8^{b}	94.0^{b}	94.5^{b}	107.0^{c}	111.2^{c}	3.70
Mg	22.6^{c}	12.8^{a}	13.1^{a}	23.7^{c}	15.9^{b}	15.7^{b}	1.92
S	21.0	19.3	19.6	23.7	23.4	23.5	0.835
Na	19.34^{c}	4.73^{a}	5.59^{a}	17.74^{c}	6.38^{b}	7.00^{b}	2.69
P	12.4	30.5	31.1	16.9	32.2	34.0	3.73
N: NO_3-N	135.1	149.7	154.7	165.7	141.1	142.7	5.12
TAN	1.1	8.9	9.5	1.1	9.0	8.6	1.1
Micronutrients							
Fe	2.891^{c}	0.868^{a}	0.870^{a}	1.882^{b}	1.011^{a}	1.006^{a}	0.3328
Sr	0.552	0.586	0.590	0.632	0.628	0.663	0.0163
Zn	0.517^{b}	0.182^{a}	0.184^{a}	0.627^{c}	0.209^{a}	0.215^{a}	0.0805
Al	0.100	0.090	0.090	0.089	0.091	0.090	0.0017
Cu	0.045	0.050	0.050	0.039	0.052	0.050	0.0020
Mn	0.033^{a}	0.162^{c}	0.157^{c}	0.020^{a}	0.070^{a}	0.047^{a}	0.0255
Ba	0.018	0.016	0.011	0.018	0.016	0.008	0.0016
As	0.010	0.006	0.006	0.009	0.006	0.007	0.0008
Pb	0.010^{c}	0.000^{a}	0.000^{a}	0.002^{a}	0.005^{b}	0.005^{b}	0.0015
Cd	0.004	0.003	0.003	0.004	0.003	0.003	0.0001
Co	0.003^{b}	0.001^{a}	0.001^{a}	0.003^{b}	0.001^{a}	0.001^{a}	0.0004
V	0.0029	0.0025	0.0025	0.0025	0.0024	0.0024	0.0001
Mo	0.002	0.025	0.026	0.002	0.023	0.027	0.0050

Table 8. Relative change of nutrient solution elements in A7 and H5 treatments. Percent change shows the magnitude of the element increase or decrease (depending on whether the percent is greater or less than 100%). Calculations were performed using the nutrient solution element data from start of experiment to end.

	Percent Change (start to end)		**Net Concentration Change (mg/L)**	
Element	A7	H5	A7	H5
Macroelements				
K	119.1	94.8	40.926	-11.133
Ca	107.8	114.0	6.890	13.209
Mg	104.9	123.8	1.119	3.055
S	112.7	121.2	2.685	4.095
Na	91.7	134.9	-1.598	1.652
P	136.6	105.5	4.531	1.678
Microelements				
Fe	65.1	116.5	-1.009	0.143
Sr	114.4	107.1	0.080	0.042
Zn	121.3	114.8	0.110	0.027
Cu	87.8	104.5	-0.005	0.002
Mn	60.9	43.4	-0.013	-0.092
Ba	99.8	99.2	0.000	0.000
Cd	103.7	108.4	0.000	0.000
Al	88.7	101.2	-0.011	0.001
Co	99.6	156.0	0.000	0.000
Cr	72.1	79.3	0.000	0.000
As	89.3	112.5	-0.001	0.001
B	77.4	105.6	-0.005	0.008
Ni	96.4	291.6	0.000	0.002
Mo	70.6	92.3	-0.001	-0.002
Pb	22.5	3496.3	-0.008	0.005
V	89.1	99.3	0.000	0.000

Applicability

This study brought to light the idea that non-optimal nutrient conditions for spinach growth (fish waste water) can still produce the quality product that the inorganic hydroponic nutrient combinations produce. Although it was concluded that aquaponic spinach grew to a non-different yield than hydroponic spinach, these results may not be repeatable with different crops or greenhouse environments. Every crop responds differently to different nutrient components and ratios, but the hydroponic solution formulated specifically for lettuce is typically used for many leafy greens. An important question we must ask is what would have happened if a formulation for a different crop were used, or if there existed a nutrient formulation specifically for spinach.

This study does not go into the sustainability of one treatment over the other, nor does it consider the economics of either. Economically, aquaponics has potential for reducing the initial capital investment, since part of the RAS filtration and waste removal components can be

eliminated or reduced in size. Aquaponic management appears to be simpler in complexity than what is required in a hydroponic system, since the aquaponic system only requires supplementation of iron, with the rest of the nutrients being adequately supplied from the fish operation.

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