BIOLOGICAL RESPONSES OF LETTUCE TO HYDROPONIC AND AQUAPONIC CONDITIONS

This Report

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Master of Engineering

by

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ABSTRACT

Aquaponics is the combination of aquaculture with hydroponics where nutrients from a recirculating fish rearing system support a plant culture system. There has been significant discussion, in particular within the hydroponic community, on how aquaponics quantifiably compares to current, commercially practiced, hydroponics and how the nutritional aspects change when not in the inorganically optimized solution known to hydroponic growers.

Our objective was to provide foundational data quantifying biomass and tissue elemental concentrations for hydroponics, aquaponics, and intermediary water quality conditions that may provide further insight into any plant and rhizosphere dynamics. The first two papers are divided into part Part I and Part II. Part I investigates the solely inorganic hydroponic comparisons of the water quality aspects impact upon the key response variables, while Part II compares the conventional hydroponics to a recirculating aquaponics system. The third paper provides key metrics and design variables that will be of use to the rapidly developing aquaponics industry including sizing ratios of the fish to plant area and nitrification rates on the natural root hairs in comparison to inert surfaces. Lettuce was used as the model crop throughout due to its consistency at all life stages and its significant presence in hydroponic operations while Koi were chosen for their hardiness in a large range of water quality parameters, which provided flexibility and security to current and future planned experiments.

BIOGRAPHICAL SKETCH

Tyler Anderson was born in San Diego, CA in 1989 to Barry and Lesley Anderson. He graduated from Westview High School in 2007. He then completed his undergraduate studied at the University of California, Davis in the department of Biological Systems Engineering, and graduated with a B.S. and minor in Technology Management in 2012.

Tyler's has designed and prototyped an Automated Processing Tomato Inspection Station for the Processing Tomato Advisory Board of California that was successfully put to use at one of the 11 processing tomato grading station in California measuring pH, soluble solids (sugar), and color. At a Global 500 company's business development department, he redesigned a major food product processing design significantly improving upon the designs efficiency and throughput and received recognition from the North American Executives and publication in the company's international, intra-publication on new and exciting developments in the pipeline.

Tyler has spent the last 3 years as project and lab manager for Dr. Timmons and the Cornell Controlled Environment Agriculture group for Dr. Albright. While at Cornell, Tyler has contributed to over eight different projects, developing the foundational base upon which the Cornell Aquaponic group and others internationally can continue to build and use quantitative metrics and design variables to develop this rapidly growing industry. He has advised and supported the newly develop Cornell Ponics club, as well as multiple international visiting scholars, post-docs, venture capitalists, commercial entities, as well as masters and undergraduate students of various departments. Tyler has identified and tested a means to overcome Pythium infection in hydroponic spinach production, a particularly virulent and ubiquitous pathogen to spinach that has devastated and disallowed commercial production

worldwide. The cultural practices methodology allows completely natural, pesticide and chemical-free means to overcome the last major obstacle to global production of hydroponic baby leaf spinach. Tyler has subsequently used his expertise in overcoming spinach disease to consult and enable a multi-million dollar hydroponic baby leaf greens company with multiple greenhouses currently and several more being built to overcome their disease issues and enable production of hydroponic baby leaf spinach in the United States.

In 2015, Tyler was accepted into the Masters of Engineering program in the department of Biological and Environmental Engineering at Cornell University. He has built upon his experience in the hydroponics and aquaponics industry with additional knowledge in water chemistry, continued to build upon his experience in Technology and business management, and developed a new area of expertise in the integration and optimization of renewable energy systems.

His hobbies include whitewater rafting, hiking, reading, and traveling.

This project is dedicated to my family and my wife,

whose unquestioning love and support have made everything I've achieved possible.

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Paper 1:

Growth and Tissue Elemental Composition Response of Butterhead Lettuce (*Lactuca sativa* , cv. Flandria) to water quality conditions: Part I Hydroponic (water quality) conditions

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Abstract

Our objective was to provide foundational data quantifying biomass and tissue elemental analysis for lettuce grown using deep water hydroponics in standard hydroponic conditions or aquaponics-like conditions. The three treatments included two pH 7.0 conditions, one using tap water with initial high alkalinity to represent aquaponic-like conditions (HA7) and one using reverse osmosis water (H7), against a standard lettuce nutrient solution with pH 5.8 (H5). Inorganic chemicals were used to match initial elemental concentrations of all three nutrient solutions. The biomass results were greater than H5 ($p<0.01$) from H7 in all biomass response categories (fresh weight (FW), dry weight (DW), and dry weight to fresh weight ratio (DW/FW) for both shoots and roots. The pH 7.0 treatments (H7 and HA7) were not different from each other ($p>0.05$) for all biomass response categories except DW root. The H5 versus HA7 biomass

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response categories were different ($p < 0.05$) for shoot FW and DW, and root FW. Lettuce shoot FW and DW were highest under the standard hydroponics treatment (H5). The HA7 and H7 treatments produced 24% less FW growth than the H5 treatment with an 18% reduction in DWs. Some of the FW differences seen in the pH 7 conditions can be attributed to their lower water content. Conversely to the shoots, the H5 condition had the least biomass in root FWs and DWs. Comparatively, the H7 condition produced an additional 22% in root FW and 50% in DW, while the HA7's condition produced 15% more FW and 17% more DW (the HA7 and H7 DW responses were not different, $p=0.2216$). The H7 root DW/FW was larger than H5 and HA7. The tissue elemental results showed decreased concentration from H5 for ($p<0.05$ unless stated): N:-8.5% for H7 and -4.6% for HA7 (alpha=0.10); Mo: -34% for H7 and -35% for HA7; Cu: -50% for HA7 only; and St: -33% for HA7 only. Elements with increased concentration from H5 were ($p<0.05$ unless stated): Zn: +50% for H7 and +21% for HA7; Mg: +39% for H7 and +22% for HA7 (alpha=0.10); Na: +153% for HA7 only; and Ba: +1350% for HA7 only. No differences in elemental concentration ($p>0.05$) among treatments were found for C (Carbon), P, K, Ca, S, Fe, Mn, Si, Al, and Ni. Tissue contents for As, Cd, Co, Cr, Se, and V are presented but not discussed. Part II will compare the effects of aquaculture water from a fish recirculating system integrated directly with the hydroponics system.

Introduction

Hydroponics is the soilless culture of plants in a nutrient solution that contains ions of all the necessary elements for good plant growth. Inert mediums such as perlite or rockwool are frequently utilized for support purposes and plants may be grown in a variety of ways such as deep water culture or nutrient film techniques (Jones 1982). In recirculating aquaculture systems (RASs), which feature minimal daily water exchange, aquatic organisms are fed and raised in carefully controlled tanks employing biological filtration to oxidize toxic nitrogenous wastes to nitrate (Timmons and Ebeling 2013). Aquaponics combines hydroponics and aquaculture, making multiple uses of resources such as water and nutrients, while sharing infrastructure, management, and labor costs (Rakocy 1999; Timmons et al. 2002; Diver and Rinehart 2010; Tyson et al. 2011).

The macronutrients and micronutrients required by hydroponically grown plants are formulated to contain proportions and concentrations according to particular crops. In this experiment, the crop was lettuce, and the solution formula employed was derived for lettuce by Sonneveld and Straver (1994). In earlier research, the original lettuce formula was found to be equally as effective at half the concentration recommended by Sonneveld and Straver (Both et al. 1997), and was used consequently at half-strength in this experiment (electro conductivity ca. 1300µS/cm). We further modified the nutrient solution by eliminating silicon, which is specified at 0.5mM in the original Sonneveld and Straver recipe, since silicon is not an element essential to yields in the absence of stress (Danoff, 2011) and our previous work with this modified nutrient solution showed no differences in yield. In the remainder of this paper, nutrient solution refers to the concentrations listed in Table 1.

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Bugbee (2003) implies that plants can grow equally well at pHs between 4.0 and 7.0 if the required nutrients are available in the solution. The pH of the nutrient solution affects the availability of certain elements, particularly micronutrients (Jones, 1982, Bugbee, 2003). Moderately low pH (e.g. 5.8) keeps most ions available in solution while higher pH (e.g. >6.5) can cause eventual nutrient deprivation due to nutrient precipitation (Bugbee, 2003). The modified Sonneveld solution used in this research was originally designed to be maintained between pH 5.0 and 6.0 (Sonneveld and Straver, 1994), which overlaps the "ideal" target pH of 5.8 that Bugbee (2003) and Both et al. (1997) recommended for hydroponic solutions. A rough idea of solubility of different ions under a pH range of 4.0-8.0 is depicted in Figure 1 (Bailey, 1996); however this chart is specifically developed and designed for medium based growing techniques. Furthermore, the deep water culture does not experience the same periodic drying and concentrating as many medium based growing techniques.

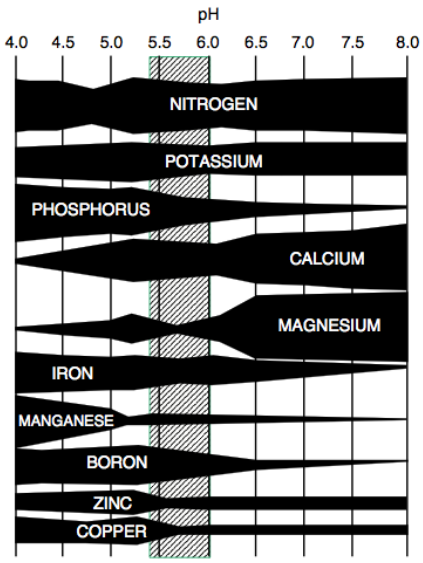

Figure 1. Solubility of elements vs. pH (Bailey 1996).

Alkalinity is an important water quality parameter that needs to be closely monitored in aquaculture systems since alkalinity is consumed during the nitrification process (Timmons and Ebeling, 2013). Alkalinity is a measure of the pH buffering capacity of aqueous solutions; it is the sum of soluble alkaline species in water capable of being neutralized by hydrogen ions (Valdez-Aguilar and Reed, 2006). Bicarbonate ions (HCO_3^-) make up the bulk of alkalinity in hydroponic or aquaponics solutions, which aid to buffer pH shifts due to acid addition or generation (Timmons and Ebeling, 2013). In hydroponic systems, if the makeup water added to replace evapotranspiration is high in alkalinity (different than alkaline), the added carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-) may exceed the systems needs or usage. When this is the case, pH will rise and may reduce availability of elements such as iron (Resh, 2013; Bertoni et al., 1992).

RAS conditions are usually quite different from those of hydroponic systems. Fish are grown in a pH range of 6.5-8.5, and, in freshwater systems, an even narrower range around pH 7.0 (Timmons and Ebeling, 2013). Alkalinity in a RAS is continuously consumed by nitrifying bacteria and thus needs to be replaced (1g of ammonia-nitrogen converted to nitrate-nitrogen requires a total of 3.57g of alkalinity (Timmons and Ebeling, 2013). Safe target levels for alkalinity, pH, and dissolved carbon dioxide in RAS systems are 70 to 190mg/L for alkalinity, to provide an adequate buffering capability to the system, 7.0 for pH, and 15mg/L for dissolved carbon dioxide. The high carbon dioxide levels, compared to hydroponic systems, are partially responsible for maintaining pH values around 7.0.

Fish also excrete organic nutrients into the water column that are waste products from feed ingestion and digestion. Waste produced by the fish is primarily N, P and C (carbon) along with feed micronutrients that are not fully absorbed by the fish. The composition of fish

excretions after biological processing by the fish of what they are normally fed commercially is 78-80% of ingested C, 66-86% of ingested N, and 50% of ingested P (d'Orbcastel and Blancheton 2006; Timmons and Ebeling 2013); it is a reasonable assumption is that micronutrient elements in the feed are discharged into the water column at similar fractions as the P macro element, since this was the minimum discharge for the macro-elements.

A merger of hydroponic and RAS fish systems appears to face a challenge in that the normal water quality conditions for these two types of growing systems, particularly for pH and alkalinity, are quite different, as described above. In view of this difficulty, the objective of this research was to quantify the response of a commonly grown hydroponic crop, lettuce (*Lactuca sativa*, cv. Flandria – a butterhead type), to typical hydroponic conditions (pH 5.8 and low alkalinity) as compared to aquaponics-like conditions (pH 7.0 and moderate alkalinity).

Materials and Methods

An experiment consisting of three trials was conducted in a conventional glass greenhouse to investigate the effects of pH upon the growth and nutrient content responses of butterhead lettuce. The pH conditions investigated included a pH of 5.8 and low alkalinity (H5) to represent conventional hydroponic conditions compared with two pH 7.0 conditions where the source water was either reverse osmosis (RO) water (H7) or tap water that was initially high in alkalinity (HA7). Growing conditions mimicked conventional deep trough grow tanks and plants were grown in a conventional nutrient solution (

Table **1**). Industry norms were used for plant spacing and for a target harvest weight of ~ 150 grams per head fresh weight. Details of the experiment are provided below.

Greenhouse Description. Experiments were conducted in a section of a glass greenhouse range, with section dimensions 7m x 10m x 7m to the ridge, oriented east west (Figure 2). An Argus monitoring and control system logged CO_2, humidity, aerial temperature, and light level. The system controlled aerial temperature and daily light integral (DLI: amount of photosynthetically active radiation, PAR, in units of moles/m^2/day) by controlled use of a supplementary lighting array. The environmental parameters were sampled approximately every two seconds and data queues averaged and logged every two minutes. DLI was controlled to its target value by supplementing natural light using an array of twenty high pressure sodium (HPS) lights (General Electric, 400W clear S51/O, Mogul Base rated ED18 HSP, LU 400/H/ECO). The light fixtures were arranged to provide consistent light within the growing area. The DLI was recorded using a LiCor quantum sensor. The period when lights were on was recorded, and used to calculate what part of the DLI was natural versus supplemental. A target DLI of 21mol/m^2/day was used for trial 1 and was reduced to 17mol/m^2/day for trials 2 and 3 to prevent tipburn. The Argus system controlled a negative pressure ventilation system with evaporative cooling pads to cool the greenhouse airspace as necessary. Two identical hot water to forced air heaters rated at 115,000kJ/h were used to provide air mixing and to raise air temperatures to target values. Carbon dioxide was at uncontrolled ambient levels.

***Growing System and Procedure*.** Six HDPE growing tubs of dimensions 1.82m x 0.91m, depth of 0.30m, and a holding volume of 425L were elevated such that the tops of the floating rafts were 1.31m above the floor and 1.26m below the light fixtures to maximize light uniformity (natural and supplemental) among all tubs. Fifty plants (5 rows of 10 plants per row, 30 plants

per m^2) were placed per tub using Styrofoam rafts 25mm in thickness with 25mm round holes for plant plugs spaced at 200mm on center; rows were staggered to maximize uniformity of light to all sides of each plant. Recirculating pumps and air stones were operated continuously within each tub to ensure vigorous water mixing and to maintain dissolved oxygen (DO) near saturation in each tub. The circulating pumps (24Lpm) mixed the water in the tubs at a rate equal to a hydraulic retention time of 18 minutes. To take advantage of the bilateral symmetry of the greenhouse and minimize any effects due to any airflow differences within the greenhouse, the tubs were numbered 1 through 6 and were paired into two blocks as follows: tubs 1-3 were block 1, and tubs 4-6 were block 2 (Figure 2Figure 3).

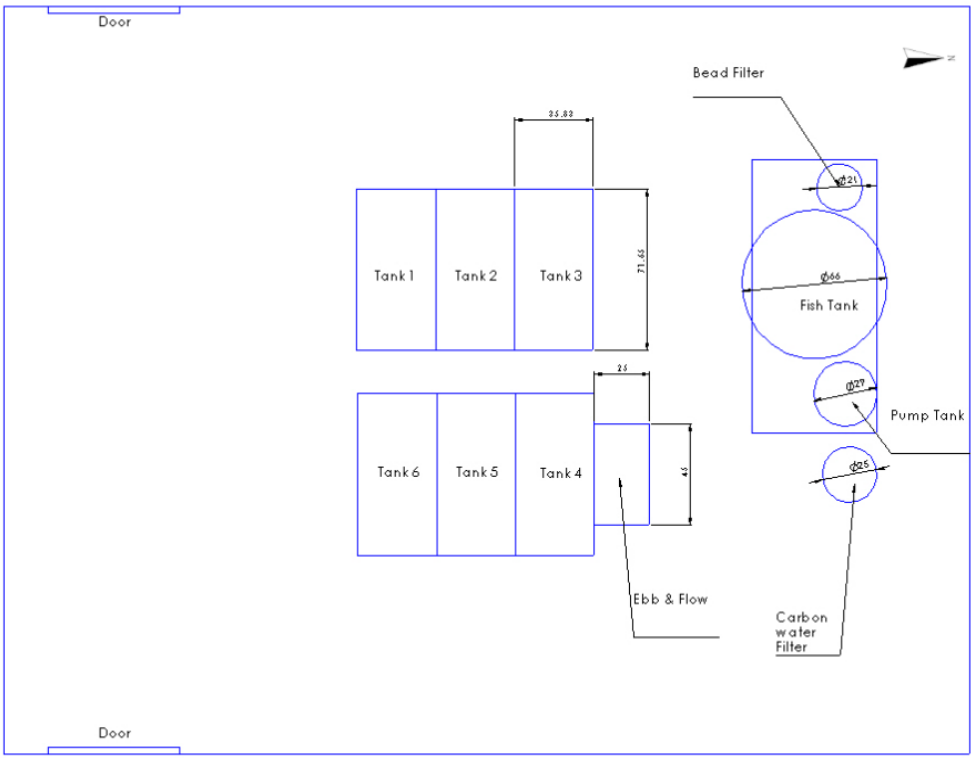

Figure 2. Greenhouse experiment floorplan (to scale).

A single pelleted seed of butterhead lettuce (*Lactuca sativa*, cv. Flandria) was placed into individual rockwool plugs (Grodan AO25/40, 25mm). Care was taken to place the seeds

horizontally at the same depth in the rockwool cavity after which the planted seeds were misted with RO water in several passes so that all pellets were equally saturated. Two standard perforated 1020 trays were used for holding the two 200-cell rockwool sheets. The trays were germinated in the same greenhouse space as the growing experiment (see Figure 2). For the first 24hrs, the trays were covered with clear rigid plastic germination covers and shielded from light to control for temperature and humidity. After 24hrs as seedling emergence occurred, the plastic covers were removed and the trays were placed in an ebb and flood system where they were grown for the next 10 days. The ebb and flood system cycled four times per day using 15 minute flood cycles (7 am, 11 am, 3 pm and 7 pm). The overflow height from the flood bench was set at two-thirds the height of the rockwool cubes. The ebb and flood bench used the same H5 nutrient solution for all flats.

Plants were compared for uniformity using their first true leaf for comparison. At day 7, when the first true leaf was approximately 10mm in length, large and small seedlings were marked, but left in place. On the 11th day, 300 plants (50 per tub) were selected for consistency from the unmarked seedlings. The plants were then randomly placed on the Styrofoam rafts inside the tubs. After transplanting, the plants were grown in the tubs for an additional 24 days (35 days from seeding). The seeding, transplanting, and growing procedures were the same for all trials and treatments including a preliminary trial used to test for uniformity of greenhouse conditions that is described later.

***Water Quality Treatments*.** Three different treatments were evaluated: a) nutrient solution using RO water with a target water pH of 5.8 (this is a standard hydroponics control condition for lettuce and is referred to as H5), b) same as H5 but with the target pH raised to pH 7.0 (referred to as H7), and c) nutrient solution using tap water and a target pH of 7.0 (referred to

as HA7). Adjustments to pH were made daily using 1M KOH or 1M HNO_3. Alkalinity for the three trials was estimated based upon an independent bench-scale test using three-liter containers in triplicate for each of the three treatment conditions. Nutrient water was vigorously aerated to strip off excess CO_2 while 1M HNO_3 was added to achieve target pH levels. Equilibrium of pH was rapidly achieved in approximately one hour due to the vigorous air stripping of the water and then reconfirmed after 24 hours of continuous additional air stripping without any need for further addition of acid or base.

Alkalinity was measured by titrating to an endpoint pH of 4.5 using 0.02N (0.01M) sulfuric acid to determine alkalinity to an accuracy of ±4mg/L as $CaCO_3$. The resulting alkalinities at equilibrium were approximately 20, 40, and 40mg/L $CaCO_3$ for H5, H7, and HA7 respectively. During the three lettuce trials, the RO water treatments, H5 and H7, used approximately equal quantities of base and acid over the course of the trials and experiment. The adjustments to pH were therefore assumed to have caused minimal change to these low initial alkalinity values. In the HA7 water conditions, the tap water typically had an initial pH of 7.0 and a starting alkalinity of 120±8mg/L $CaCO_3$. This alkalinity was consumed over the course of each trial to a final measured value of 40mg/L $CaCO_3$ as acid was added to maintain the target pH of 7.0.

Electroconductivity (EC) was monitored but not controlled and was 1300-1500µS/cm in H5, and was at the higher end of this range in H7 and HA7. Compared to H5, the H7 condition started 100-150µS/cm higher due to the addition of the KOH in the pH adjustment, while the HA7 started at the higher pH due to the additional dissolved ions present in the tap water. Typically the nutrient solution EC did not drift during a trial in our pH 5.8 control, while additional water chemical equilibrium processes that occurred in the pH 7 conditions resulted in

the EC stabilizing at higher values. A 50-100µS/cm drop occurred during the last week from each tub's respective stabilized values and was attributed to the rapid plant growth and usage of nutrients during that period.

***Nutrient Conditions*.** For all trials, the six tubs were each filled to 90% of their capacity with 425 liters of lettuce nutrient solution (Table 1) that was created by adding 2.125L each of two concentrates henceforth referred to as Stock A and Stock B. Stock A contained calcium nitrate ($Ca(NO_3)_2 \cdot 3H_2O$), chelated iron (Sprint 330, Fe-DTPA), ammonium nitrate (NH_4NO_3), and 23% of the total required potassium nitrate (KNO_3). Stock B contained the remaining required potassium nitrate (KNO_3), potassium phosphate monobasic (KH_2PO_4), 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 ($CuSO_4 \cdot 5H_2O$), and potassium sulfate (K_2SO_4). Procedurally, 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. The combined stocks were at a 100x concentration level of their diluted end point concentrations in the nutrient solution. Additional nutrient solution for replenishment was prepared in 200L quantities for H5, H7, and HA7, and used as necessary over the course of each trial.

The H5 and H7 treatment nutrient solutions were prepared using RO water to dilute the Stock A and B solutions, which produced initial concentrations very similar to our target concentrations (Table 1). The HA7 treatment used carbon filtered tap water that had average macro-elemental values of 50mg/L Ca, 13mg/L Mg, 5.5mg/L S, and an EC of 450µS/cm. As a result of these macro elements being present, we adjusted the concentrations of the stock solutions to achieve the targeted final nutrient concentration values in each trial. Sequentially, we first added the micro-elements and chelated iron to the target nutrient solution concentrations.

Then, macro elements were adjusted to attain as close to target concentrations as possible, given the uneven charge balance of existing ions that could require excess concentration in one element to minimize below target concentrations in another, e.g., potassium concentrations were made 10% higher in a particular trial so that the nitrate concentration was only 10% below our target nutrient solution concentrations instead of 16% lower.

Table 1. Nutrient solutions starting, ending, and target concentrations, averaged by treatment.

Element, mg/l	H5		H7		HA7		Nutrient Solution
Macronutrients	start	end	start	end	start	end	Target
Potassium	215	227	232	296	222	292	215
Calcium	87	109	87	79	74	71	90
Nitrogen - NO3-N	144	130	143	134	105	113	133
- TAN-N	10	0.25	10	0.11	12	0.26	8.75
Phosphorus	30	31	30	18	30	19	31
Magnesium	12	13	12	12	8	9	12
Sulfur	16	18	16	18	16	17	18
Micronutrients							
Iron	1.10	1.19	1.05	0.94	0.95	0.82	1.12
Manganese	0.13	0.03	0.12	0.03	0.13	0.02	0.14
Boron	0.15	0.20	0.20	0.19	0.20	0.18	0.16
Copper	0.030	0.035	0.031	0.034	0.038	0.050	0.024
Zinc	0.15	0.14	0.15	0.17	0.18	0.17	0.13
Molybdenum	0.021	0.023	0.021	0.026	0.029	0.028	0.024
Other Elements							
Sodium	3.5	4.7	3.6	4.7	23.5	28.0	0.0

Note that trial 1 starting samples were not collected so "start" values are the average of trials 2 and 3.

Water Environment and Greenhouse Conditions. Root zone temperature (RZT, °C) and pH data are given in Table 2 by trial and treatment. Average RZT and standard deviation (SD) by treatment were consistent at 25.3 (2.75)°C, 25.2 (2.59)°C and 25.2 (2.80)°C for H5, H7, and HA7 respectively. Average pH and standard deviation by treatment were also consistent at 5.78 (0.47), 6.96 (0.43), and 7.02 (0.35) for H5, H7, and HA7 respectively.

Table 2. Tub averages and standard deviation (SD) of pH and root zone temperature (RZT, ^{0}C) by trial and treatment.

Trial	Treatment	pH (SD)	RZT (SD)
1	H5	5.77 (0.33)	25.2 (1.03)
	H7	6.97 (0.20)	24.7 (0.89)
	HA7	7.05 (0.20)	24.7 (1.13)
2	H5	5.75 (0.24)	25.4 (1.59)
	H7	6.97 (0.25)	25.0 (1.44)
	HA7	7.00 (0.16)	25.4 (2.11)
3	H5	5.84 (0.23)	25.5 (2.00)
	H7	6.95 (0.29)	25.9 (1.97)
	HA7	7.00 (0.23)	25.4 (1.46)
All	H5	5.78 (0.47)	25.3 (2.75)
	H7	6.96 (0.43)	25.2 (2.59)
	HA7	7.02 (0.35)	25.2 (2.80)

Environmental conditions for supplemental light (SL, moles/m^2/day), natural light (NL, moles/m^2/day), daily light integral (DLI, moles/m^2/day), relative humidity (RH, %), and air temperature (AT, ^{0}C) by trial are presented in Table 3. RH and AT over the three trials were both very similar. DLI was very similar for trials 2 and 3, after the DLI was reduced from trial 1 levels to minimize tipburn. The quantity of NL trended upwards through the sequential trials and conversely, the quantity of SL decreased during the experiment. There was no intention to investigate or develop models for environmental effects in this research; however, we did analyze for any trial effect in our statistical analysis (described further below).

Table 3. Greenhouse conditions (mean and standard deviations (SD)) per trial for supplemental light (SL, moles/m2/day), natural light (NL, moles/m2/day), daily light integral (DLI, moles/m2/day), relative humidity (RH, %), and air temperature (AT, ^{0}C).

Trial	**SL**	**NL**	**DLI**	**RH**	**AT**
1	10.8 (4.9)	10.3 (5.7)	21.0 (6.0)	48 (12)	23.9 (0.4)
2	6.1 (4.0)	10.8 (6.8)	17.0 (3.6)	58 (6)	23.9 (0.4)
3	4.7 (3.9)	13.0 (6.4)	17.7 (3.5)	59 (8)	24.3 (0.5)

***Experimental Design, Data Collection, and Analysis*.** Two blocks of three tubs were allocated each of the three treatments in each of the three trials (Figure 3). A preliminary trial was conducted to test for any block effect in the greenhouse rearing space (November 16-December 20, 2013). After establishing no positional, individual tub, or blocking effect in the preliminary trial, three sequential trials were conducted over a period of four months in 2014: trial 1 was February 21-March 28; trial 2 was March 19-April 23; and trial 3 was April 16-May 21. Seeding took place around noon on the first day of each trial and harvesting occurred the last day between mid-morning and noon. The experiments overlap because the plants were seeded and remained in the ebb and flood system while lettuce in the previous trial were in the final days of their production cycle.

Trial	Block	Tub	Treatment
1	1	1	HA7
		2	H7
		3	H5
	2	4	H5
		5	H7
		6	HA7
2	1	1	H7
		2	H5
		3	HA7
	2	4	HA7
		5	H5
		6	H7
3	1	1	H5
		2	HA7
		3	H7
	2	4	H7
		5	HA7
		6	H5

Figure 3. Experimental physical arrangement by block.

During Trial 3 of the experiment, the HA7 tub 2 treatment was discarded due to a bubbler malfunction that caused the DO to drop to anoxic conditions resulting in significantly reduced growth performance. In addition to physiological signs of low DO in the root zone, the DO measurement was ~2.5mg/L, which is close to the 2.1mg/L lower bound value that Goto et al. (1996) found for hydroponic lettuce in similar conditions.

At 35 days-of-age, plants were harvested. Fresh weights (FW) for shoot and root were harvested by cutting the shoots at the top of the rockwool plug and slicing the roots off at the base of the rockwool cube leaving the main root shoot inside the rockwool cube. Only lettuce plants that were from the interior of the tub (24 plants) were used for analysis, i.e., plants along the outside perimeter may have received more light than interior guarded plants and were not included. Dry weights (DW) and tissue analysis data were determined based upon ten individual dried and weighed plants from each treatment and each trial. DWs for root and shoot weights were obtained after four to seven days in a drying oven held at 70^0C.

Tissue analysis data were run by the Cornell Nutrient Analysis Laboratory (CNAL) using hot plate acid digestion plus fully automated inductively coupled plasma-atomic emission spectroscopy (ICP-AES) for the metals analysis and combustion analysis for carbon and nitrogen. For each trial, three samples for shoot data and three samples for root data were submitted from each tub. Each sample contained three random heads such that the three average tissue responses were from 9 random heads per tub.

Some plant biomass samples were contaminated with titanium. A preliminary set of analyses were performed to identify how samples containing titanium influenced the results. Where the titanium contamination affected the interpretation of the data (Fe, Mn, Si, Al, As, Ni,

Co, Cr, and V), samples were removed. A thorough investigation of possible sources of contamination was conducted and is presented in the results section.

***Statistical Analysis*.** Mixed effect models using least squares analyses were conducted using JMP PRO 11[5]. In the final model, shoot (FW, DW, DW/FW), root (FW, DW, DW/FW), and individual elemental (macros, micros, assorted metals, and carbon) data were treated as response variables; treatment and trial were treated as fixed effects; and tub nested within trial was treated as a random effect. A Tukey HSD test was utilized to determine significance of pairwise differences among trials. Blocking effects were analyzed and an F test was used to determine the validity of removing blocking from all models. Trial effects were investigated in the mixed model as an initial step in the overall statistical analysis.

Results and Discussion

Blocking and Trial effects. While the original experiment did include a blocking design (Figure 3), no variance could be assigned to the blocking for shoots and 3% or less of the variance could be assigned in the root statistical analysis. Therefore, the blocking variable was eliminated from the mixed effects models.

The response variables were significantly influenced by trial. The largest difference between trials was the DLI although DLI was similar for two of the three trials. There were substantial differences among trials for SL and NL primarily due to the season change from winter to early summer. There were also differences between trials for AT and RH, but they were relatively small (Table 3) particularly if one was trying to determine their effects given the expected natural variations in biological response. We do not believe our experiments provided

[5] JMP Pro software: http://www.jmp.com/en_us/software/jmp-pro.html

sufficient range in these environmental variables to specifically identify their effects and no formal analysis was pursued. Thus, trial was kept as an independent variable in the mixed effect model to account for changes by trial, but we are not correlating this trial effect with any particular environmental variable that was measured.

Biomass Results Overview. Trial and treatment mean and standard deviation values for shoot FWs, DWs and DW/FWs are presented in Table 4, and the equivalent for root responses is shown in Table 5. Significant differences (at alpha=0.05 level) between the means over all three trials are indicated by letter differences (See bolded averages. H7 was significantly different from the control treatment (H5) in all biomass response categories (FW, DW, and DW/FW for both shoots and roots) with $p<0.01$ (Tables 4 and 5). The HA7 biomass response categories were only significant different from H5 at $p<0.05$ for shoot FW, shoot DW, and root FW. The pH 7.0 treatments (H7 and HA7) were not significantly different at $p<0.05$ for all biomass response categories except DW root.

Table 4. Shoot mean, standard deviation (SD), and percentage comparison to H5 response for fresh weight (FW, g), dry weight (DW, g), and dry weight to fresh weight ratio (DW/FW, g/g) by trial and treatment with multi-model significance.

		FW		DW		DW/FW	
		Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
Trial 1	H5	160 (12)	100%	6.9 (0.61)	100%	0.043 (0.0019)	100%
	H7	135 (12)	85%	6.3 (0.50)	91%	0.047 (0.0011)	109%
	HA7	139 (11)	87%	6.4 (0.49)	93%	0.046 (0.0023)	106%
Trial 2	H5	160 (15)	100%	6.2 (0.52)	100%	0.039 (0.0014)	100%
	H7	100 (15)	63%	4.5 (0.49)	73%	0.045 (0.0021)	114%
	HA7	132 (10)	83%	5.1 (0.30)	82%	0.039 (0.0015)	100%
Trial 3	H5	159 (15)	100%	6.6 (0.49)	100%	0.041 (0.0033)	100%
	H7	108 (10)	68%	5.0 (0.43)	77%	0.047 (0.0016)	114%
	HA7	95 (9)	60%	4.5 (0.49)	68%	0.049 (0.0014)	119%
LSM All Trials	**H5**	**159 (14)^{A}**	**100%**	**6.6 (0.60)^{A}**	**100%**	**0.041 (0.0028)^{A}**	**100%**
	H7	**114 (19)^{B}**	**72%**	**5.3 (0.88)^{B}**	**81%**	**0.046 (0.0019)^{B}**	**112%**
	HA7	**122 (20)^{B}**	**77%**	**5.3 (0.90)^{B}**	**82%**	**0.045 (0.0043)^{AB}**	**109%**

All responses are in grams except %, and DW/FW, which is g/g or dimensionless.
Differing letter within response variables (columns) denote significance at alpha=0.05.
%=Percentage comparison to H5 control; SD=Standard Deviation

Table 5. Root mean, standard deviation (SD), and percentage comparison to H5 response for fresh weight (FW, g), dry weight (DW, g), and dry weight to fresh weight ratio (DW/FW, g/g) by trial and treatment with multi-model significance.

		FW		DW		DW/FW	
		Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
Trial 1	H5	8.3 (1.3)	100%	0.38 (0.05)	100%	0.046 (0.0030)	100%
	H7	10.0 (1.9)	122%	0.57 (0.16)	151%	0.057 (0.0112)	124%
	HA7	9.8 (1.7)	118%	0.44 (0.06)	117%	0.046 (0.0023)	101%
Trial 2	H5	8.0 (1.4)	100%	0.35 (0.06)	100%	0.043 (0.0019)	100%
	H7	8.4 (1.2)	105%	0.44 (0.06)	128%	0.051 (0.0035)	119%
	HA7	8.2 (1.3)	102%	0.35 (0.04)	103%	0.047 (0.0034)	109%
Trial 3	H5	7.5 (1.1)	100%	0.29 (0.05)	100%	0.039 (0.0037)	100%
	H7	10.4 (1.4)	139%	0.51 (0.08)	174%	0.050 (0.0061)	129%
	HA7	9.3 (1.3)	125%	0.41 (0.08)	139%	0.046 (0.0037)	120%
LSM All Trials	**H5**	**7.9 (1.3)^{A}**	**100%**	**0.34 (0.06)^{A}**	**100%**	**0.042 (0.0041)^{A}**	**100%**
	H7	**9.6 (1.7)^{B}**	**122%**	**0.51 (0.12)^{B}**	**150%**	**0.053 (0.0082)^{B}**	**124%**
	HA7	**9.1 (1.6)^{AB}**	**115%**	**0.40 (0.07)^{A}**	**119%**	**0.046 (0.0030)^{AB}**	**109%**

All responses are in grams (g) except %, and DW/FW, which is g/g or dimensionless.
Differing letter within response variables (columns) denote significance at alpha=0.05.
%=Percentage comparison to H5 control; SD=Standard Deviation

Shoot (head) biomass response. Lettuce shoots from H7 and HA7 were smaller than H5 in both FW and DW (Table 4). The FW responses show a reduction from 159g in H5 to 114g in H7 ($p<0.0001$) and 125g in HA7 ($p=0.002$). As both pH 7 conditions were not different from each other at a p-value of 0.3351, the 28 and 21% reduction in mean FW is averaged to give a 24% reduction due to raising the pH from 5.8 to 7 in our experimental setup and conditions.

The DW responses show a reduction from 6.56g in H5 to 5.28g in H7 ($p=0.0003$) and 5.46g in HA7 ($p=0.0019$). As both pH 7 conditions were not different from each other at $p=0.7559$, the 19% and 17% decrease in DW response is averaged to an 18% reduction due to raising the pH from 5.8 to 7.0 in our experimental setup and conditions .

The shoot DW/FW shows the water content of the plant at harvest. The mean DW/FWs were 0.041, 0.046, and 0.044g/g for H5, H7, and HA7 respectively. H5 was different from H7 at $p=0.019$, but only different from HA7 at an alpha of 0.10 ($p=0.0661$). Since the H7 and HA7 responses were both significant at $p<0.10$, the 12% increase for H7 and 7% increase for HA7 are summarized as a 10% increase in dry matter content from the pH difference.

In summary of the biomass head results, pH 7 conditions decreased DW biomass growth by ~18% and FW biomass weights by 24%. The pH 7 effects on water content are more variable between H7 and HA7 treatments but show an increase in dry matter content of 10%.

Root biomass response. Mean root FW increased from 7.9g in H5 to 9.6g in H7 ($p=0.0038$) and 9.1g in HA7 ($p=0.0502$) (Table 5). We consider the comparison of H5 to HA7's p-value of 0.0502 and 15% increase in FW as meaningful, particularly since it is consistent with what we observed in H5 to HA7's shoot response. H7 and HA7 were not different at $p=0.4580$). The 22 and 15% increase in biomass is summarized as an 18% increase in root FW due to the higher pH.

Mean root DWs were 0.34, 0.51, and 0.40g for H5, H7, and HA7 respectively. H5 was different from H7 at p=0.0003, and H7 was different from HA7 at p=0.0122. H5 and HA7 were not different at p=0.2216.

The DW/FW responses show that the H5 response of 0.042 is significantly different from H7's response of 0.053 at p=0.0040. H7 was not different from HA7's 0.046g/g at alpha 0.05, however would have been significant at an alpha of 0.10 (p=0.0617). H5 and HA7 were not different at p=0.4119.

Shoot Tissue Analysis Response. The tissue analysis results of lettuce heads (shoots) mean, standard deviations, and significance are shown in Table 6. All elements reported from the CNAL are included for completion and the interest of readers. All values in the tissue analysis portion are in mg of respective element per kg of total dry material unless otherwise noted.

Table 6. Shoot tissue analysis mean, standard deviation (SD), and multi-model significance; all data is on dry weight basis and in mg/kg unless otherwise noted.

	H5		H7		HA7	
Parameter (mg/kg)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Carbon Content	321352^{A}	(8703)	327128^{A}	(10356)	324888^{A}	(6994)
Macronutrients						
Nitrogen	54166^{A}	(1976)	49537^{B}	(1962)	51663^{AB}	(2709)
Phosphorus	10205^{A}	(978)	11223^{A}	(1508)	11065^{A}	(1657)
Potassium	30123^{A}	(6541)	31190^{A}	(1759)	30345^{A}	(4841)
Calcium	13081^{A}	(1979)	13281^{A}	(1437)	12906^{A}	(1681)
Magnesium	2759^{A}	400)	3891^{B}	(874)	3358^{AB}	(771)
Sulfur	2391^{A}	(237)	2351^{A}	(225)	2373^{A}	(185)
Micronutrients						
Iron*	74^{A}	(10)	67^{A}	(7)	67^{A}	(10)
Manganese*	80^{A}	(20)	67^{A}	(9)	68^{A}	(11)
Copper	5.4^{A}	(0.6)	5.3^{A}	(0.7)	6.5^{B}	(0.9)
Zinc	42.2^{A}	(6)	21.3^{B}	(4)	33.4^{A}	(12)
Molybdenum	0.95^{A}	(0.18)	1.43^{B}	(0.23)	0.93^{A}	(0.23)
Other elements						
Sodium	480^{A}	(105)	613^{A}	(56)	1213$^{\mathbf{B}}$	(285)
Aluminum*	38.5^{A}	(30)	36.5^{A}	(22)	22.6^{A}	(12)
Nickel*	0.17^{A}	(0.38)	0.34^{A}	(.18)	1.78^{A}	(1.78)
Silicon*	3.3^{A}	(1.1)	5.2^{A}	(0.8)	4.6^{A}	(1.5)
Lead	3.8^{A}	(2.7)	4.0^{A}	(1.7)	3.9^{A}	(3.3)
Strontium	66^{A}	(10)	73^{A}	(9)	44^{B}	(4)
Arsenic*	-^{A}	-	-^{A}	-	-^{A}	-
Barium	0.13^{A}	(0.26)	0.89^{A}	(0.69)	1.87^{B}	(0.83)
Cadmium (µg/kg)	-^{A}	-	58^{B}	(6)	-^{A}	-
Cobalt* (µg/kg)	0.002^{A}	-	4.113^{B}	(3.42)	0.005^{A}	-
Chromium* (µg/kg)	26^{A}	(52)	345^{B}	(217)	6^{A}	(20)
Selenium (µg/kg)	-^{A}	-	22^{B}	(20)	-^{A}	-
Vanadium* (µg/kg)	0.002^{A}	-	160.000^{B}	(140)	0.004^{A}	-

Columns with differing letters are significantly different at alpha=0.05.
Sample size is 18, 15, and 15 for H5, H7, and HA7, except HA7-copper, which has an n of 14 due to an outlier concentration.
* Analysis was run with zero titanium samples only; n = 13, 5 and 6 for H5, H7 and HA7 respectively.
"-" = below detection

Carbon, P, K, Ca, S, Fe, Mn, Al, Si, Pb, Ni, and As shoot tissue responses were not different among treatments. K, Ca, and S were particularly consistent in their response. No Si was added to the tubs so Si likely entered the system as an impurity in the salts used to create the nutrient solutions, the tap water, or direct uptake from the rockwool cubes (Table 6). As mentioned above, lettuce shoot carbon contents were not significantly different between treatments (Table 6). The smallest p-values were 0.2837 for H5 vs. H7, and 0.6347 for H5 vs. HA7. An interesting comparison is the total fixed carbon, a measure of fixed carbon (cumulating any difference in photosynthesis efficiencies, intercepted light, and duration of photosynthesis) minus lost energy (culminating the varying respiration rates) by treatment. In a companion paper, we identified that 27% of the total root mass was contained in the rockwool for H5 FW and 38% for DW, while H7 contained 24% for FW and 32% for DW (Anderson et al., submitted). Using these percentages, H5 is estimated to have 0.21g DW of root mass in the rockwool cube, while H7 and HA7 (assumed to respond with the same 32% DW) had 0.24g in H7 and 0.19g in HA7. By adding these values to the root masses given in Table 5, this gives us cumulative root DW total of 0.55, 0.75, and 0.59g for H5, H7, and HA7 respectively. Cumulating the root, shoot, and root mass within the rockwool cube, the average DWs for H5, H7, and HA7 were 7.11, 6.03, and 6.05g respectively. The average H5 total dry matter content of 7.11g was 18% larger than that of the pH 7 conditions. Applying the respective average carbon contents to these values, we can look at total fixed carbon contents by treatment, which were 2.28g for H5, 1.97g for H7, and 1.97g for HA7, e.g., for H5, 7.11g DW * 32.1352% carbon/DW = 2.28g C. Shoot average carbon contents were used for shoot, root, and root mass within the rockwool cube, however note that the roots are only 8, 12 and 10% of the total plant mass for H5, H7, and HA7 respectively, so our estimates are being extrapolated minimally. The carbon content is a measure of the fixed minus

respired carbon in the plant throughout its life. Neither photosynthesis nor respiration measurements were tested in this experiment.

N contents of H5 shoots, 54166mg/kg (5.42% of total dry matter), was significantly different from H7 shoots, 49537mg/kg (4.95%), at p=0.0013. H5 and HA7's, 51663mg/kg (5.17%), were not different at alpha=0.05, however were significant at alpha 0.10 (p=0.0744). The N content decreases by 8.5% in the H7 condition in comparison to H5 and by 4.6% for the HA7 condition.

Mg tissue concentrations were 2759, 3891, and 3358mg/kg for H5, H7, and HA7 respectively. H7 was different from H5 p=0.0009 (a 39% increase). HA7 was not different from H5 at alpha =0.05, but was at an alpha of 0.10 (p=0.0701, 22% increase). The two pH 7 conditions were not different at p=0.1110.

Cu contents were 5.4, 5.3, and 6.5mg/kg for H5, H7, and HA7 respectively. HA7 was different from H5 (p=0.0366) and H7 (0.0221). H5 and H7 were not different at p=0.9516.
The average values of copper in the HA7 nutrient solutions were 0.044mg/L, 35% larger than the H5 and H7 concentrations of 0.033mg/L (Table 1), which correlates with the both the use of copper piping in the greenhouse tap water lines and the 19-22% increase in the HA7 tissue concentration. Furthermore, increasing the pH from 5.8 to 7 did not appear to influence the accumulation in the tissues as is seen comparing the H5 and H7 tissue and nutrient solution concentrations.

Zn content was one of the most different elements analyzed. H5's 42.4mg/kg was different from H7's 21.3mg/kg (p= 0.0005), and H7 was different from HA7's 33.4mg/kg (p=0.0310). H5 and HA7 were not different at p=0.1222. These differences in Zn correspond to a 50% reduction in Zn tissue content from H5 to H7 and a 21% reduction from H5 to HA7. Zn is

an important micronutrient that influences many aspects of plant growth and physiological functions. While the Zn content is lower in the two pH 7 conditions, Hafeez (2013) states that tissue contents greater than 20ppm, which we observed in all three treatments, are unlikely to negatively affect plant growth. It is possible that control (H5) plants are more readily able to absorb and utilize Zn at the lower pH and that the higher pH is negatively influencing Zn uptake. Given how similar the H7 and HA7 solutions ultimately were, the magnitude of the difference between the Zn tissue concentrations is surprising.

While soil nutrient availability charts are a little dubious to use for hydroponic availability, they may provide general clues of what to expect and Figure 1 shows that Zn availability drops off significantly with pH values rising above 5.5. We recognize this as a weak correlation, since the same logic would predict a decrease in Cu, B, Mn, and P where we did not see consistent tissue concentration decreases, e.g., Cu and P was unaffected, while manganese decreased similarly to zinc. Development of conceptual or similar availability charts for hydroponic and water based growth mediums is an area of potential research that would be valuable to the development and optimization of nutrient culture techniques.

Mo concentrations were 0.95, 1.43, and 0.93mg/kg for H5, H7, and HA7 respectively. H7 was different from H5 ($p=0.0056$) and HA7 (0.0066). H5 and HA7 were not different ($p= 0.9934$). Mo interpretation suffers from a similar issue as zinc. A raw comparison from H5 to H7 suggests a possible pH effect for Mo, however the HA7 condition, which was very similar to H7, had non-significant impacts on the Mo concentrations and resulted in values similar to the control.

Na contents were 480, 613, and 1213mg/kg for H5, H7, and HA7 respectively. HA7 was different from H5 ($p=0.0001$) and H7 ($p=0.0002$). H5 and H7 were not different at $p=0.3854$,

which is reasonable given the same source water and measured sodium concentrations. The elevated Na concentration in the HA7 tissue correlates with ~6.7x elevated Na in the nutrient solution.

St concentrations in the HA7 treatment were significantly different (lower) than either of the two Hydro treatments (66, 73, and 44 mg/kg for H5, H7, and HA7, respectively), while the two Hydro conditions were not different. As St may be used as a less ideal and effective Ca substitute within the plant, the Ca to St ratio may be important if the St concentration in solution started becoming very large. As our St to Ca ratios were incredibly large and unchanging, the small differences seen in the tissue analysis between conditions are very likely insignificant.

Ba did show some significance between treatments. H5's 0.127mg/kg values were different from HA7's 1.865mg/kg with a p-value of 0.0012. H7's 0.887mg/kg was also different from HA7 with a p-value of 0.0476 while H5 and H7 were not different at p=0.1095.

Cd values were only detected in the H7 condition at 0.06mg/kg and were quite consistent among all samples with a standard deviation of 0.006. H7 was significantly different at $p<0.0001$ in comparing H5 and HA7.

Co followed the same trend as cadmium in that it was only detected in the H7 condition except for two samples that had 0 values after removing samples containing any trace of titanium. H7's 0.004mg/kg was significantly different from the 0 values of H5 and HA7 at p-values of 0.0475 and 0.00342.

Cr contents were significant between H5's 0.026mg/kg and H7's 0.3454mg/kg at p=0.0395 and almost significant between H7 and HA7's 0.00618mg/kg at p=0.0512. H5 and H7 were not different at p=0.9762.

Se contents were significantly different among treatments with only H7 samples registering Se contents at 0.022mg/kg. This findings was highly significant at p-values of <0.0001 in both comparisons.

V concentration of H7's 0.16µg/kg was significantly different from H5's 0.0017µg/kg at a p-value of 0.0348 and almost statistically different from HA7's 0.0044µg/kg with a p-value of 0.0590. The V analysis was run on samples without Ti.

No data is reported on B due to the hot plate acid digestion not being an EPA certified method (the high ramping temperatures necessary for heavy metal extraction begin to volatize boron). A number of cellular functions are affected by B that was well covered by Brown et al. (2002). Typical dicots have B tissue concentrations in the 20-100ppm range (Brown et al. 2002). As B is acquired passively and B concentrations were controlled and the same in all conditions, pH is not expected to have affected tissue accumulation of B; our samples, despite possible losses during volatization of the samples, were in excess of 20ppm in the vast majority of all samples suggesting no negative impact was to be expected.

H7 vs. HA7 water quality conditions. We assumed that our H7 conditions could be maintained at different alkalinity levels than our aquaponic-like (HA7) conditions, which we knew would be at moderately low levels of alkalinity, but certainly much higher than a zero level. In both conditions we maintained pH 7.0 so that we would be able to isolate the effects of pH 5.8 (H5 condition, normal hydroponics) vs. pH 7. However, once we equilibrated the H7 and HA7 conditions to the pH 7 target, the alkalinity values were the same. This is the result of the complex carbonate balance that exists.

A pH rise will be seen whenever carbon dioxide is stripped by aeration from solution. As carbon dioxide is in balance in solution with carbonic acid, bicarbonate, and carbonate, loss of

carbon dioxide will shift the equilibrium such that more carbonic acid is formed, binding the hydrogen ions and raising the pH. The opposite effect can be seen if dissolved carbon dioxide concentrations increase. Reverse osmosis (RO) or deionized water can be used to avoid these issues with alkalinity in hydroponics. Very low alkalinity on the other hand, has the disadvantage that small additions of acid, base, or pH altering processes such as changing plant or fish respiration (change in dissolve carbon dioxide equilibrium) can alter pH significantly, which may then change nutrient availability (Bailey 1996). Nelson (1998) provides a recommendation for alkalinity to be in a range of 0 to 120 mg/L as $CaCO_3$ equivalents, which is not particularly useful due to its wide breadth.

Based on our own experience and experimental setup, if pH is maintained at circa 6.0, the nutrient solution concentrations are controlled, and stripping of air is large in comparison to respiration and carbon dioxide contributing sources, then only low levels of alkalinity can be maintained, e.g. ~ 20 mg/L as $CaCO_3$ equivalents. This is due to low levels of carbon dioxide production in hydroponic systems compared to fish systems and the low pH that is maintained in conventional hydroponic systems that maintains the majority of inorganic carbon dioxide species in non-ionized forms. In circumstances with a low ratio of stripping to dissolved carbon dioxide generation, such as higher density aquaculture systems or hydroponic and aquaponic systems that utilize pure oxygen and thus have very low mass fluxes of air passing through the nutrient solution, carbon dioxide and carbonate species will form equilibriums at much higher concentrating opening the possibility of significant storage depending on the maintained pH level. It may be worth recognizing here that changing dissolved carbon dioxide concentrations does not change alkalinity levels; changing dissolved carbonate species concentrations in solutions changes the pH, and it is the user's correction of the pH with acid or base that changes

the alkalinity of the solution. In our particular experimental arrangement, both the H7 and HA7 conditions resulted in similar alkalinity values of 40 mg/L as $CaCO_3$, due to their near identical elemental compositions and nearly identical respiration loading in the root zone. Different responses between H7 and HA7 cannot be attributed to alkalinity differences but other factors not specifically identified in our research findings.

Titanium investigation. During the analysis of the tissue data, we found three sources of contamination due primarily to the presence of titanium that resulted in some samples being discarded from the final analysis. Titanium is unavailable to plant roots without titanium specific chelation and thus should return concentrations of zero in root and shoot data (Pais et al. 1977). Possible sources of titanium contamination were identified as being from: Grodan rockwool cubes; paint flecks from an aging greenhouse; and whitewash used to spray the greenhouse glass. Each source was investigated. Small quantities of rockwool left with shoots and roots from the top and bottom of the rockwool cubes may have remained on samples. When the tissue analysis samples were sent to be ground and analyzed by the CNAL, these small quantities of residual rockwool may have influenced calcium, aluminum, iron, and magnesium, with smaller effects on other elements (Tables 7 and 8). Our results are consistent with the findings of Zuang and Mustard (1986) shown in Table 8 who also found approximately 1% Titanium component in their Rockwool analysis. Note that the acid digestion method is ineffective at solubilizing silicon and thus gives a false "zero" reading, however the results from our hot acid digestion are reasonably in line with those published by Zuang and Mustard if our percentages in Table 7 are approximately halved to account for the 46% silicon content by weight measured by Zuang and Mustard's work (Table 8). In contrast with both our and Zuang and Musard results, the Material Safety Data Sheet (MSDS) for Grodan rockwool states 95-100% of Rockwool is composed of

silicate fibers bound with 18% alkaline and alkali earth oxides such as Na_2O + K_2O + CaO + MgO + BaO (Rockwool B.V. – Grodan, 2011). No mention of Titanium was included in the MSDS.

Table 7. Grodan rockwool elemental analysis by hot plate acid digestion and ICP/AES metal analysis. Percentages are of all included elements sans silicon such that the sum is 1.

Element	Content (mg/g)	Percentage
Silicon	0.12*	*
Calcium	38.9	34%
Aluminum	27.2	24%
Iron	23.3	20%
Magnesium	13.7	12%
Sodium	3.70	3%
Potassium	2.96	3%
Manganese	1.44	1%
Titanium	1.38	1%
Phosphorus	0.41	0%
Sulfur	0.34	0%
Chromium	0.33	0%
Barium	0.23	0%
Zinc	0.18	0%
Copper	0.06	0%
Nickel	0.03	0%
Boron	0.03	0%
Lead	0.02	0%
Cobalt	0.01	0%
Cadmium	0	0%
Arsenic	0	0%
Molybdenum	0	0%
Selenium	0	0%

*** This method of acid digestion is not effective at solubilizing silicon and provides a false 0, although silicon is undoubtedly the main constituent of a silicate fiber material**

Table 8. Grodan rockwool elemental analysis, modified from its original form in Zuang and Musard, 1986.

Rockwool Elemental Analysis		
Element	Percentage	Cumulative
Silicon	46%	46%
Calcium	16%	62%
Aluminum	14%	76%
Iron	8%	84%
Magnesium	1%	85%
Sodium	2%	87%
Potassium	1%	88%
Manganese	1%	89%
Titanium	1%	90%

Table 7 shows that rockwool contamination could be detected by the presence of titanium and, assuming only rockwool had contaminated a sample and rockwool makeup is consistent within each batch of samples, each part of titanium contamination would have concurrently contributed an additional ~28 parts calcium, ~20 parts aluminum, ~17 parts iron, et cetera[6] to our samples. An original goal was to identify samples contaminated from rockwool, however no sample micronutrient contents were sufficiently different to identify specific samples with this method.

The next potential source of titanium contamination was the whitewash used for shading the greenhouse glass, Kool Ray Classic White Shade – White. This was also investigated as a source of contamination due to the dripping and splashing that may have occurred from our older glass greenhouse that dripped during rain or the single yearly application of whitewash shading. The whitewash MSDS (sans water) stated the Kool Ray shade is composed of 60% rutile titanium dioxide, 27% calcium carbonate, 13% calcined china clay, and 0.001% Formaldehyde (Continental Products Company, 2008). The whitewash material showed significant quantities of rutile titanium dioxide. The data was not extrapolated further to elemental concentrations

[6] Calculated directly from Table 5 via dividing the element of interests mg/g composition by titanium's mg/g concentration

because we are unsure of the grade or quantity of impurities contained in each raw material. Raw rutile, primarily titanium dioxide, may have varying impurities from iron, tantalum, niobium, vanadium, and tin, as well as a large array of other elements. Any samples that were contaminated by whitewash would have spikes in titanium and calcium, and may have smaller contributions from the noted rutile impurities and the calcined china clay. No samples could effectively be identified by calcium spikes that would have indicated this form of contamination.

Condensate and roof leaking rain could have been alternative sources of continual and more widespread contamination of whitewash, and thus were investigated as well. The purpose was to identify the magnitude of any contamination from hydrophobic whitewash solubilizing or sloughing off and continually contaminating samples through direct drip and indirect splash onto plant leaves or into the nutrient solution reservoirs. The leak runoff sample showed 92% of the detectable elements were chlorine, calcium, and sodium, and that titanium was undetectable. Given this analysis, the continual drips from rain or condensation were deemed to be an unlikely source of contamination where no mechanical removal of whitewash was occurring, such as winter snowfall or human induced.

The last potential source of contamination was paint flecks falling from the old and cracking white paint used on the greenhouse structure. Experiments in a similarly aged and painted greenhouse space in the same complex had found titanium contamination from paint flecks and paint dust on their samples. Since the greenhouses were painted by a contractor, information on the specific paint used is unavailable, however titanium is a common ingredient for glossy white paints. While the occasional paint fleck was observed on the rafts, paint dust or paint flecks were not seen as ubiquitously as would be necessary to explain the number of samples or concentrations of titanium seen in this experiment. Conversely, the paint flecks may

explain the few samples with larger spikes of titanium if a paint fleck fell out of sight within a head.

Lead analysis. Non-zero lead concentrations were surprising to find in the tissue analysis, in particular because we were unaware of any significant source or concentrations of lead in any of our materials. Concentrations of Pb were not different among treatments and were 3.9mg/kg for shoots (Table 6). Neither the rockwool nor condensate dripping from the roof had a significant enough concentration or quantity to explain these results. With help from the CNAL, we did identify the source of the lead as the approximately five foot portion of 5/8" garden hose used with the tub recirculation pumps for mixing. We later identified low levels of lead in the nutrient solutions and that the garden hoses themselves were the source of the contamination (Anderson et al., submitted). From a plant growing basis, the lead contents seemed to have had no effect on the biomass growth of the plants or conditions.

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Paper 2:

Growth and Tissue Elemental Composition Response of Butterhead Lettuce (*Lactuca sativa* , cv. Flandria) to water quality conditions: Part II Aquaponic conditions

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Keywords: Hydroponics, aquaponics, pH, biomass, lettuce, elemental analysis, elemental composition, tissue analysis, leaf surface area, leaf count, rockwool cubes, nutrient analysis, garden hose

Abstract

Aquaponics is the combination of aquaculture with hydroponics where nutrients from a fish rearing system support a plant culture system. There has been significant discussion, in particular within the hydroponic community, on how aquaponics quantifiably compares to current, commercially practiced hydroponics and how the nutritional aspects or uptake within the aquaponic plants changes when not available in the ratios and concentrations of optimized inorganic nutrient solutions.

A three trial experiment was conducted in a conventional glass greenhouse to investigate the effects of recirculation aquaculture waste upon the growth and nutrient responses of butterhead lettuce. In particular this paper looks to quantify the biomass (shoot, root), leaf count, surface area, shelf life, and tissue composition differences between a conventional hydroponic nutrient solution (H5) and an aquaponics system (A7). Koi were raised within the aquaculture system as a model species with the assumption that many aspects of warm water aquaculture

would be similar between warm water fish species that suit a large range of hydroponic crop water temperature preferences. The conventional hydroponic solution was increased from pH 5.8 to pH 7.0 (H7) as an additional treatment to build upon previous work within our group and to provide a comparison that isolates a pH only comparison within inorganic hydroponics.

The aquaponics (A7) biomass response was not different from conventional hydroponics (H5) ($p>0.05$) in all biomass response categories (FW, DW, DW/FW for shoots, roots, leaf count, leaf surface area, and leaf surface area to FW ratio). H7 was different from H5 ($p<0.05$) for shoot FW, DW, and DW/FW, as well as root FW and DW. The H7 was different from the A7 for shoot FW, DW/FW, and root DW. Roots contained within the rockwool cube, primarily tap root for our lettuce, had approximately 67% more DW/FW then that of the shoots or roots, and were not different among treatments.

Tissue analysis comparison was different ($p<0.05$) between treatments for calcium, magnesium, manganese, copper, zinc, molybdenum, sodium, silicon, strontium, barium, cobalt, and chloride. Tissue contents were not different ($p>0.05$) among treatments for carbon content, nitrogen, phosphorus, potassium, sulfur, iron, boron, aluminum, nickel, silicon, lead, arsenic, cadmium, or chromium.

Lead was found entering system from the recirculation pump garden hoses of all six tubs. The nutrient solution concentrations were below 15ppb (EPA drinking water limit), however the tissues accumulated to circa 1ppm lead levels by DW. The hydroponics solution maintained at pH 7 (H7) solutions had significant precipitation that occurred over the course of each trial that was identified to be calcium phosphate.

No differences ($p>0.05$) between treatments were identified for shelf life comparisons within this experiment, as reflected by seeing no differences in moisture loss up to the time of

spoilage. Furthermore, moisture content losses from clamshells were borderline negligible suggesting that leafy greens properly stored lose minimal water and are unlikely to gain benefits post-harvest given current storage and packaging techniques. Furthermore, no differences were observed between treatments for length of time before spoilage.

Introduction

Hydroponics is the soilless culture of plants in nutrient solution that contains ions of all the necessary elements for good plant growth and includes the aid of an inert medium such as perlite or rockwool is employed for support purposes. The method of application of the nutrient solution to the roots varies widely and may include rafts such as is employed in this experiment (Jensen & Collins, 1985). Hydroponics is an increasingly important field as the demand increases for more food and sustainably produced products (Resh, 2012; Love et al., 2015). Another form of sustainable food production is from recirculating aquaculture systems (RAS) that produce aquatic and/or marine organisms. In a RAS, fish are fed and raised in carefully controlled tanks employing biological filtration to oxidize toxic nitrogenous wastes to nitrate while requiring minimal water in the process (Timmons and Ebeling 2013). Aquaponics combines hydroponics and aquaculture. Such systems make multiple uses of resources such as water and nutrients, and share infrastructure, management, and labor costs (Rakocy 1999; Timmons et al. 2002; Diver and Rinehart 2010; Tyson et al. 2011). Coupling an aquaculture system with a plant system can be an effective means to remove and maintain nutrient levels while significantly reducing nutrient discharge and pollution. Furthermore, plant roots can provide significant surface area to increase the removal rate of total ammonia nitrogen (TAN-N) and nitrate while also acting as a biological filter for adsorption and bacterial consumption of fine particulates. As the costs of filtering are inversely proportional to the particulate size being addressed, the addition of plants may remove a capital expense of the aquaculture system.

Hydroponic systems operate best around a pH of 5.8 to keep all nutrients in solution and using RO water, while fish RASs operate best around a pH of 7.0 to balance issues of carbon

dioxide toxicity (problematic at low pH) and ammonia toxicity (problematic at high pH). Water management issues are constrained when combining the systems for aquaponics due to aquaculture system in water quality requirements.

Claims that aquaponically grown lettuce have longer shelf lives have been made in the popular press and by advocates of aquaponic systems. Two major aspects to leafy green shelf life are physiological and microbiological spoiling. Physiological spoiling can occur through water loss (turgid leaves vs wilted leaves), discoloration, or enzymatic browning, as well as through significantly diminished quality such as decreased sugar content or nutritional/vitamin losses, etc. Controlled experiments need to be conducted to investigate these types of claims.

The research reported in this paper is Part II to our previous study (Anderson et al., submitted – Part I) where we investigated lettuce growth response to the impact of pH and water source. In Part I, using the same target nutrient conditions supplied by inorganic fertilizers, we compared conventional hydroponic conditions at pH 5.8 using RO water to hydroponic conditions using RO water at pH 7.0 and to aquaponic-like conditions using pH 7.0 and tap water with an initial high alkalinity. The major finding was significant decreases in lettuce production (both fresh weights and dry weights) for both pH 7.0 conditions and that the elevated pH limits the maximum concentrations of calcium and phosphate in solution. The research reported here is an extension of that work where we changed the aquaponic-like conditions to actual aquaponic conditions with the nutrients being supplied by continuously recirculating water between a fish rearing system and the lettuce growing system (sole addition being chelated iron).

Materials and Methods

A three trial experiment was conducted in a conventional glass greenhouse to investigate the effects of recirculating aquaculture waste upon the growth and nutrient responses of butterhead lettuce. The conditions investigated included a conventional hydroponics nutrient solution and pH control at pH 5.8 (referred to as H5), the conventional hydroponics nutrient solution maintained at pH 7.0 (referred to as H7), and a continuously recirculating aquaponics system maintained at pH 7.0 (referred to as A7). The Aquaponic system provided nutrients to the plants solely from the fish waste generated by feeding a commercial fish feed to koi fish (*Cyprinus carpio)* along with the addition of chelated iron to the water environment. Growing conditions mimicked industry norms for deep water hydroponics, spacing, and a target fresh harvest weight of ~150g per head. In depth details are provided below.

Greenhouse Description. Experiments were conducted in Ithaca, NY (42°26'56.2"N 76°28'08.3"W), in a section of glass greenhouse range with dimensions 7m x 10m x 7m to the ridge. The greenhouse was built in 1953 and was oriented east west. A scaled floor plan is shown in Figure 4. Carbon dioxide, humidity, aerial temperature, and light intensity were logged by an Argus monitoring and control system. The Argus system controlled aerial temperature and daily light integral (DLI: the amount of radiation received in the photosynthetically active area, PAR, in units of moles per meter squared per day). Environmental parameters were sampled every 2 seconds, averaged every 2 minute, and logged. Twenty high pressure sodium (HPS) lights (General Electric, 400W clear S51/O, Mogul Base rated ED18HSP, LU 400/H/ECO), arranged for most consistent light at the crop level, were used for supplementing natural light to a consistent DLI. A LiCor quantum sensor (LI-190R) was synced into the Argus control system

for more accurate tracking of the target 15mol/m^2/day from cumulative natural and supplemental light. The greenhouse was equipped with evaporative pads on the north side for use as necessary. Two heaters (forced air via hot water rated at 115,000kJ/h) provided air mixing at all times at the crop height and heating as necessary. The heaters were located in opposite corners, with one located near the south west door facing north-north east while the other heater was located approximately equi-distance from the crop on the north east side and was facing south-south west. Carbon dioxide was not calibrated, controlled or investigated in any way in these experiments and is assumed to be ambient.

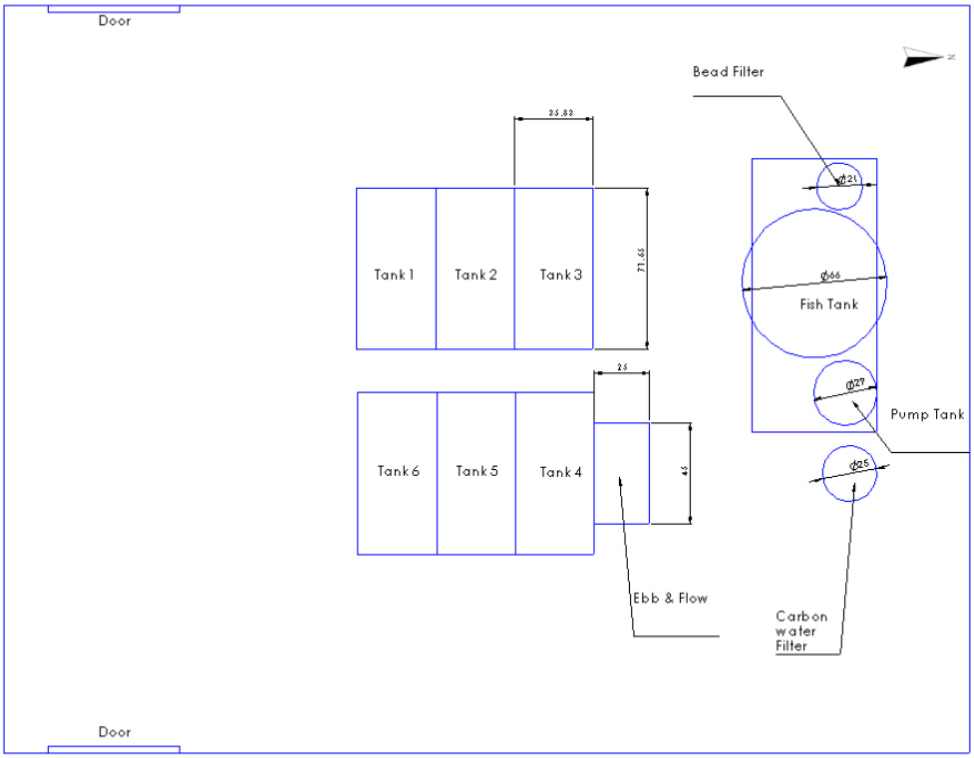

Figure 4. Greenhouse experiment floor plan (to scale).

Growing System and Procedure. Six HDPE growing tubs (1.82m x 0.91m x 0.3m, 0.425 m^3) were elevated to the most uniform lighting for all tubs from the light array. The growing rafts were 1.31m above the floors and 1.26m below the light fixtures. Fifty lettuce plants (5 rows

of 10 plants per row) were grown at 30 plants per m^2 in rigid Styrofoam rafts of 25mm thickness. 25mm diameter holes for rockwool plant plugs were spaced at 200mm on center and rows were staggered for optimal uniformity of light on all sides. Recirculating pumps and two coarse air stones per tub (Sweetwater AS-2s rated at 0.1 CFM/stone, 1.9cm x 1.9cm x 3.8cm) were operated continuously within each tub to ensure strong mixing and to maintain dissolved oxygen (DO). Diffusing air stones were confirmed to be flowing vigorously multiple times weekly. The tub recirculation pumps (24Lpm) mixed the water in the tubs at a rate equal to a hydraulic retention time of 18 minutes. Tubs 4 and 5 (Figure 4) were set up as the aquaponic tubs. The aquaponic tubs were modified to include a 38mm passive water height equilibrating pipe attaching to each tub at the bottom of each tub. The mixing of these two tubs was modified slightly such that water from tub 4 was transferred and mixed the water in tub 5 and vice versa with tub 5's pump taking water from tub 5 and transferring/mixing it with tub 4 water to ensure rapid equilibrating of nutrients in both tubs. Water from the aquaponic setup was adjusted to be delivered to the aquaponic tubs at approximately 10L/min while a standpipe in tub 4 maintained the water levels in both tubs. The specifics of the fish system are described below.

Aquaculture System. The koi rearing tank was a round HDPE tank of upper inner diameter (I.D.) of 1.5m, lower I.D. of 1.35m, and a depth of 0.76m with the water depth maintained at 0.51m. The water inlet was a horizontal 5cm PVC pipe with two rows of 10mm drilled holes and adjusted to create clockwise circulation rates of approximately 0.2m/second near the exterior wall. The outlet drain was a two stage, centrally located system. A 76mm x 0.76m tall PVC pipe with 13mm tall cut outs on the base and six vertical slots of <10mm aligned at or below 0.3m from the base of the tank. This outside layer pulled waste from the bottom of the rotating water column up and over a 50mm x 0.3m tall PVC interior standpipe that

functioned as a backup contingency to maintain at least 0.3m of water in the case of water loss emergencies and to keep the otherwise unanchored 76mm outer intake pipe centered in the tank. A plastic mesh was zip tied to the top of the outer intake pipe to ensure no fish could jump into the standpipe assembly. In addition to the natural aeration from the horizontal sprayer bar, four air diffusors were placed inside the rearing tank for redundant DO supply and as primary DO source during system cleanings (Sweetwater ASI-15, 0.15m x 38mm x 38mm, rated at 14.2Lpm). The diffusors were supplied by an 8W, diaphragm pump rated at 20Lpm and standard 5mm aquarium tubing.

The rearing tank was covered by two layers of standard greenhouse blackout curtain hung at ~7m high from the east side of the rearing tank to the bead filter (Figure 4) to minimize algae production and heat load. The surface of the sump tank was covered by 25mm sheet insulation to also block direction light, UV penetration, and excessive algae generation. Water from the rearing tank traveled through the 50mm PVC pipe, entered vertically through the bottom of a cylinder HDPE tank of upper I.D. 0.69m, lower I.D. 0.53m, and depth 0.71m (termed the “sump tank”). The sump tank (dual function of pumping and settling) had a redundant 0.3m tall standpipe for maintaining water levels in the case of emergency loss of water and the system’s water pump (115V, 1 phase, 249W submerged utility pump rated at 113/57Lpm). Water left the sump tank through a 32mm smooth walled and flexible pipe and entered an hourglass shaped bubble bead mechanical and biofilter made externally of HDPE with maximum I.D. of 0.53m, height of 1.35m, and volume of 164L (Figure 4).

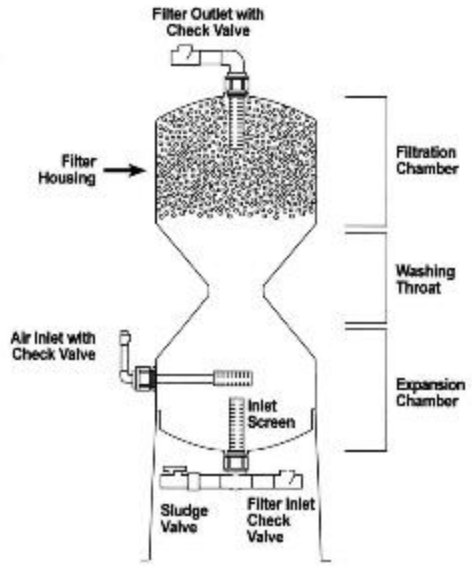

Figure 4. Cut away view of bubble bead filter. Here you can see the shape, setup, and bead orientation during normal operation (Aquatica International, 2004).

Tap water (tempered during winter and supplied as necessary) entered the fish system through an adapted, cone bottomed settling tank (Upper I.D 0.53m, lower I.D. 0.15m, depth 0.71m). Greenhouse tap water from a 15m length of 16mm garden hose entered vertically through the bottom of the tank through a fine metal mesh, passed through 50kg activated charcoal (Aquatic Ecosystems, ProLine® AC55s), and left the system from an outlet at 61 cm height into the sump tank.

Koi feed was purchased from Blackwater Creek Farms, small floating extruded pellet, Max Growth Diet and all food was used within 6 months of the mill date. The guaranteed analysis provided from Blackwater Creek farm is min 38% crude protein, min 8% crude fat, max 4% crude fiber, min 1% phosphorus, and max 10% moisture. The ingredients list was: Fish Meal, Rice Bran, Wheat Flour, Shrimp Meal, Poultry By-Products Meal, Wheat Middlings, Ground Wheat, Propionic Acid (a preservative), Ascorbic Acid, Iron Oxide, Vitamin A Supplement, Vitamin D3 Supplement, Vitamin E Supplement, Vitamin B12 Supplement, Riboflavin Supplement, Niacin Supplement, Calcium Pantothenate, Menadione Sodium Bisulfite

(source of Vitamin K activity), Folic Acid, Thiamine Mononitrate, Pyridoxine Hydrochloride, Biotin, Calcium Carbonate, Zinc Sulfate, Ferrous Sulfate, Magnesium Oxide, Manganese Sulfate, Copper Sulfate, Ethylenediamine Dihydriodide, Cobalt Sulfate, Sodium Selenite. Our feed's in depth nutritional analysis from CNAL hot plate acid digestion with ICP-AES is provided in Table 9.

Table 9. 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 aquaculture tank carried 53 koi fish and a system biomass of 8-10 kg of fish within the 2200L system including hydroponic tubs. The fish were supplied as 1g fingerlings by Blackwater Creek Farms in February of 2014 and were continually culled to maintain a system biomass of ≤ 10kg. Fish were fed 90g of feed a day on weekdays (~1% of the system mass/day)

in two feedings, and 60g/day in one feeding on weekends. Bead filter cleanings were performed weekly by backflushing. In the months prior to this experiment, backflushed water was collected in a separate tank and allowed to settle for 1 to 2 hours before pumping 90% of the water back into the RAS system.

Crop Seedling Preparation. Butterhead lettuce (*Lactuca sativa,* cv. Flandria, pelleted) was grown in individual rockwool plugs (Grodan AO25/40, 25mm). The rockwool cubes were prepped with thorough soakings and rinses in reverse osmosis (RO) water and H5 nutrient solution to remove any areas of excess lime. The rockwool sheets were cut into 2 cubes by 5 cubes sections for ease of removal and handling during the transplant stage. Tweezers were used to equilibrate the depths of the rockwool cavities and the pelleted seeds were laid horizontally, with good contact with the rockwool. RO water was misted over the trays to ensure equal saturation of the pellets, with the thought to minimize any differences that may have occurred in the initial water wicking rate from the rockwool. Two sheets of rockwool (400 seeds planted, 25% contingency planting) were placed in standard perforated 1020 trays. Clear germination covers were placed on the trays and the flats were placed in the greenhouse space, covered from direct light to ensure saturated relative humidity and as constant as possible temperature.

After 24 hours, confirmation of greater than 95% signs of germination was noted (cracked pellets counted as germinating seedlings for this purpose) with greater than 99% after 30 hours. Seedlings trays were placed in an ebb and flood system (Figure 4), where they were grown for the next 11 days. The ebb and flood system cycled four, 15 minute flood cycles (7am, 11am, 3pm, and 7pm) of prepared nutrient solution (same H5 control nutrient solution as described below). Flood height reached approximately 2/3^{rds} of the height of the rockwool cubes. During the first 24hrs in the ebb and flood, the clear germination covers were left on and rotated

approximately 30° to balance maintaining higher humidity while the radicals finished penetrating the rockwool. The rotated covers allowed some air movement to avoid any temperature spikes from being exposed to full sunlight. Plants were inspected for uniformity when the first true leaves were approximately 1cm in length (day 7 for our methods and setup); large, small, and abnormal plants were marked with toothpicks and left in place. When the plants began to compete for light, (day 12 for our methods and setup), the seedlings were transplanted into the tubs to begin the experimental treatments. Pre-marked seedlings were set aside and seedlings were placed randomly on the Styrofoam rafts inside the tubs using a random dice rolling program to designate tub placement (50 plants/tub). After transplanting, the plants were grown in the tubs until estimated 150g lettuce average head size in the H5 control was reached. All trials and treatments were seeded, transplanted, and grown the same except as noted and required by treatments.

Water Quality Treatments. Three treatments were investigated: a) nutrient solution using RO water with a target pH of 5.8 (this is our standard hydroponics control and hereafter referred to as H5); b) the same nutrient solution as H5 but raised to a target pH of 7.0 (referred to as H7); and c) continuously recirculating aquaculture water housing ornamental koi fed with commercial fish feed as described above, chelated iron, topped off with tap water run through an activated carbon filter as necessary, with a target pH of 7.0 (referred to as A7). Adjustments to pH for the H5 and H7 conditions were made with 1M HNO_3 and 1M KOH while adjustments to the A7 condition were made with 1M K_2CO_3 (two normal solution) and no acid was required.

Potassium carbonate was used for our aquaculture system because it gave more consistent control of the pH by contributing one normal base to raise the pH and one additional normal buffering compound to better maintain the pH through the continual nitrification occurring in the

system (circa one normal contribution to pH change and two normal contribution to alkalinity). Addition of potassium carbonate to the aquaponics setup was calculated via titration of a 3L sample and added by accounting for water volumes in the various tanks and tubs and flow rates between them. The pH between all 3 bodies of water within the A7 system were within 0.1pH of each other within 10 minutes (the time it took to slowly add the base to the system while allowing full mixing) despite the two hour hydraulic retention time between the aquaculture tubs and the two A7 lettuce tubs.

Electroconductivity (EC) was monitored but not controlled within experiments. H5 and H7 were solutions made from RO water and supplemented to target concentrations so the EC values are fairly similar for them. Since this formulation is specifically for lettuce based upon a mass calculation, most nutrients and subsequently the EC does not change significantly during the experiment (1300-1500µS/cm). EC measurements were taken using an Oakton "ECTestr 11," pin style conductivity tester that was calibrated to 1413µS/cm and validated with DI water and a reference thermometer. The aquaponic system was diluted several weeks prior to the start of the experiment to bring the EC closer to the 1300µS/cm starting concentration. The starting, ending, average, and standard error of the EC values by trial and tub are shown in Table 10. As shown, tub 4 and 5 and the fish tank returned very similar EC readings as expected due to them all being the same water constantly recirculating. A circa 50-100µS/cm decrease in EC occurred during the last week and was attributed to the rapid plant growth and usage of nutrient elements during that period.

Table 10. EC starting, ending, average and standard error (s.e.) for each trial and tub.

		Tub 1	Tub 2	Tub 3	Tub 4	Tub 5	Tub 6	Fish
Trial 1	Start	1370	1370	1350	1070	1060	1380	1080
	End	1380	1300	1260	1200	1200	1260	1200
	Average	1430	1363	1331	1161	1166	1349	1164
	s.e.	6.6	6.2	7.1	8.4	8.5	8.9	7.8
Trial 2	Start	1450	1450	1450	1370	1380	1440	1390
	End	1330	1440	1360	1470	1470	1330	1460
	Average	1418	1485	1429	1466	1469	1418	1462
	s.e.	9.3	5.1	6.7	10.2	10.6	8.7	10.6
Trial 3	Start	1340	1350	1360	1390	1390	1390	1390
	End	1310	1320	1300	1540	1530	1320	1540
	Average	1355	1360	1359	1490	1490	1382	1482
	s.e.	4.8	3.9	5.9	10.3	9.9	6.1	10.2

Nutrient Conditions. For all trials, the four tubs associated with H5 and H7 were filled to where the top of the 2.54cm floats were flush with the ridge of the plastic lining (90% of capacity, 425L) with the modified half Sonneveld and Straver lettuce solution (Table 11). The solution was created by diluting prepared 100x concentrates that we refer to as Stock A and Stock B. Stock A contained calcium nitrate ($Ca(NO_3)_2 \cdot 3H_2O$), chelated iron (Sprint 330, Fe-DTPA), ammonium nitrate (NH_4NO_3), and 23% of the total required potassium nitrate (KNO_3). Stock B contained the remaining required potassium nitrate (KNO_3), potassium phosphate monobasic (KH_2PO_4), 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 ($CuSO_4 \cdot 5H_2O$), and potassium sulfate (K_2SO_4). Tubs were pre-filled with the majority of the required water, concentrates were added slowly and sequentially added at a ratio of 1:1 with vigorous mixing and running circulation and air pumps. Additional nutrient solution for replenishment of water for these tubs were prepared in 200L HDPE barrels and covered from light penetration when not in use.

Table 11. Nutrient solutions starting, ending, and target concentrations, averaged by treatment.

Element (mg/L)	H5		H7		A7		Nutrient Solution
Macronutrients	Start	End	Start	End	Start	End	Target
Potassium	233	210	253	273	217	222	215
Calcium	101	115	90	75	73	80	90
Nitrogen: NO3-N	153	128	141	129	112	122	133
TAN-N	8.6	0.1	8.3	0.0	0.6	0.3	8.8
Phosphorus	35	34	29	12	9	9	31
Magnesium	14	15	14	14	19	20	12
Sulfur	21	26	21	26	17	21	18
Micronutrients							
Iron*	1.1	1.0	0.9	0.9	2.7	2.0	1.1
Manganese	0.148	0.025	0.099	0.006	0.046	0.004	0.140
Boron	0.20	0.24	0.20	0.23	0.02	0.02	0.16
Copper	0.036	0.040	0.032	0.037	0.028	0.029	0.024
Zinc	0.13	0.15	0.14	0.17	0.32	0.36	0.13
Molybdenum	0.03	0.03	0.03	0.03	0	0	0.02
Other elements							
Silicon	0.05	0.12	0.10	0.30	1.27	1.55	-
Lead	0.0005	0.0005	0.0008	0.0009	0.0109	0.0006	-
Sodium	6	7	6	7	61	61	-
Aluminum	0.025	0.038	0.022	0.038	0.026	0.044	-
Strontium	0.7545	0.8389	0.6906	0.6244	0.4868	0.5296	-
Barium	0.0114	0.0061	0.0074	0.0035	0.0193	0.0098	-

H5 are average of 3 tubs; H7 is a single tub-single sample; and A7 is a single sample from the rapidly exchanging tubs
*A7 had 2mg/L chelated iron added for each new trial
TAN = Total Ammonia Nitrogen

A week prior to transplanting during trial 1, the two aquaponic tubs were filled with carbon filtered water and the flow control valves were adjusted for continual recirculation at ~10L/min from the aquaculture fish tank system. This allowed the combined systems to come into equilibrium prior to the start of the experiment. The day of transplant, chelated iron (Sprint 330, Fe-DTPA) was added at 2mg/L as equivalent elemental iron. Starting and ending concentrations of primary and incidental elements are shown in Table 11. The aquaponics tubs (tubs 4 and 5) were not drained or cleaned between trials except a surface cleaning of the floats.

Blackout curtains were laid over the tubs (on top of the floats) between trials to minimize light and algae growth. The A7 treatment's 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. No adjustments or additions were made to the A7 water except for the addition of chelated iron and the daily adjustments of pH with potassium carbonate. The iron was assumed to have been UV degraded by the start of the next trial and thus chelated iron was added at 2mg/L for every trial (Fe-DTPA). This assumption and protocol decision was made to ensure sufficient chelator for iron availability in a system that was constantly exposed to indirect and diffuse light.

Note that while molybdenum (Mo) was not detectable in the majority of samples taken from A7 tubs, the seedling phase did include the standard H5 nutrient solution and thus all plants had equally available Mo until transplant into their respective treatment tubs.

Water Environment and Greenhouse Conditions. The root zone temperature (RZT) and pH are given in Table 12 for each treatment and trial. RZTs were recorded daily with the pH adjustments but the tubs were not equipped for continual control. Due to the large exposed surface area of the piping and the evaporative cooling from the aquaponics system, the aquaponics system had two submersible 300W aquarium heaters added into the sump tank. The submersible heaters' set points were manually adjusted to the average temperature of the non-controllable tubs. An additional submersible heater was added to both tub 4 and tub 5 during trial 3 due to the colder seasonal temperatures.

The entire experiment average pHs were 5.79 (0.16), 6.96 (0.22), and 6.95 (0.40) for H5, H7, and A7 respectively. Average pHs per trial were generally on target except trial 1, where A7 was ~0.1 below target, and trial 2, where H7 was ~0.1 below target. The H5 condition was quite

predictable and consistent in all trials. The overall average RZTs were 25.6 (1.20)°C, 25.6 (1.44)°C, and 24.8 (1.18)°C for H5, H7, and A7 respectively, which showed a general 1°C decrease in RZT trend across the entire experiment.

Table 12. Tub averages and standard deviation (SD) of pH and root zone temperature (RZT, °C) by trial and treatment.

Trial	Treatment	pH (SD)	RZT (SD)
1	H5	5.79 (0.18)	26.3 (1.20)
	H7	6.97 (0.23)	26.1 (1.24)
	A7	6.90 (0.66)	25.1 (0.88)
2	H5	5.81 (0.15)	25.7 (1.64)
	H7	6.92 (0.29)	25.6 (1.75)
	A7	6.99 (0.11)	24.0 (1.49)
3	H5	5.79 (0.14)	24.9 (1.28)
	H7	6.97 (0.13)	25.2 (1.21)
	A7	6.95 (0.19)	25.2 (0.75)
All	H5	5.79 (0.16)	25.6 (1.49)
	H7	6.96 (0.22)	25.6 (1.44)
	A7	6.95 (0.40)	24.8 (1.18)

The environmental conditions by trial are presented in Table 5 and show supplemental light integral contribution (SL), natural light integral contribution (NL), total daily light integral (DLI), relative humidity (RH), and air temperature (AT). Overhead fans for vertical air flow to avoid tipburn were turned on at approximately day 20. One fan was located 2 meters above tubs 1-3, and one fan was located 2 meters above tubs 4-6.

The average daily supplemental light integrals (SL) were 3.3 (SD=3.1)mol/m^2/day, 5.6 (2.2)mol/m^2/day, and 10.6 (2.2) mol/m^2/day for trial 1, 2, and 3 respectively. This trend matches the expected decrease in natural light as the experiment was started in a whitewashed greenhouse during summer and ended at the beginning of winter having not yet had a heavy enough storm to significantly shear off the whitewash. The average natural light integrals (NL) were 10.9

(3.7)mol/m^2/day, 8.5 (3.8)mol/m^2/day, and 3.8 (2.4)mol/m^2/day for trial 1, 2, and 3 respectively. The average daily light integral (DLI) was fairly consistent between trial as 14.2 (3.3)mol/m^2/day, 14.2 (3.8)mol/m^2/day, and 14.4 (0.6)mol/m^2/day for trials 1, 2, and 3 respectively. The daily average relative humidity (RH) percentages were 75% (SD=10.4), 75% (9), and 52% (7.4) for trials 1, 2, and 3 respectively. The drop in relative humidity during the last trial fits with the expected loss of relative humidity from condensation on the inside of the cold greenhouse glass and lower absolute humidity of the outside air. The greenhouse air temperature settings were split between day/night settings. The day period was between 07:30 and 17:30 (military time) with a heating set point of 24°C, and a cooling set point of 25°C. The night period was between 20:00 and 05:00 with a heating set point of 19°C and a cooling set point of 20°C. Two and a half hour ramping periods bridged the day and night period set points.

Table 13. Greenhouse conditions (mean and standard deviation (SD)) by trial for supplemental light (SL, moles/m^2/day), natural light (NL, moles/m^2/day), daily light integral (DLI, moles/m^2/day), relative humidity (RH, %), day air temperature (AT-Day, °C), and night air temperature (AT-Night, °C).

Trial	SL	NL	DLI	RH	AT-Day	AT-Night
1	3.3 (3.1)	10.9 (3.7)	14.2 (3.3)	75 (10.4)	28.8 (2.7)	26.7 (2.4)
2	5.6 (2.2)	8.5 (3.8)	14.2 (2.9)	75 (9.0)	23.9 (2.8)	23.9 (1.0)
3	10.6 (2.2)	3.8 (2.4)	14.4 (0.6)	52 (7.4)	22.4 (2.2)	21.0 (2.2)

Experimental Design, Data Collection, and Analysis. Three tubs were assigned to H5, one tub to H7, and two tubs to A7 within each trial. We chose tubs 4 and 5 to sync to the aquaponic system (Figure 1) to simplify the transfer of water to and from the aquaculture system. H7 was randomly selected from the four remaining tubs and we rotated its position for each trial. H5 conditions filled the remaining positions. A preliminary trial was conducted in this greenhouse space and setup previously to identify any positional effects (November 16, December 20, 2013). Our previous work (Anderson et al., submitted) was a three trial

experiment used to isolate pH effects of changing from a conventional hydroponics nutrient solution and low pH conditions (pH 5.8) to a pH more similar to those used in a commercial aquaponics facility (pH 7.0) and occurred from February 21st through May 21st, 2014. Our current experiment also consisted of three sequential trials in 2015: trial 1 June 30th – August 12th; trial 2 August 14th – September 18th; and trial 3 October 30th – November 24th. Seeding occurred at noon of the first date and harvests started at approximately 8:30am on the last day until complete. Transplant into the tubs and treatments occurred on July 16th, August 27th, and October 30th for trial 1, 2, and 3 respectively.

Data collection included fresh weight (FW) heads (24 samples per tub per trial), dry weight (DW) heads (12/tub/trial), FW roots (24/tub/trial), DW roots (12/tub/trial), root contained within the rockwool plug FW (Trial 1: 5/tub, Trial 3: 4/tub, henceforth referred to as "rootball"), rootball DW (Trial 1: 5/tub, T3: 4/tub), leaf count (Trial 3 only, 5/tub), leaf surface area (Trial 3 only, 5/tub), root surface area (Trial 3 only, 4/tub), and rootball surface area (Trial 3 only, 4/tub). Nutritional analyses included tissue analysis for heads (3 samples/tub/trial where each sample consisted of 3 heads), and the nutrient solution analyses presented earlier in the paper (starting and ending values of each tub and trial). Miscellaneous tests included a shelf life comparison focused on hydration (Trial 3 only, sample size: H5=8, H7=4, A7=8), tub precipitate analyses, and ICP analyses of garden hose inner lining with particular focus on lead content. The previous paper that this work builds upon includes Grodan rockwool cube elemental analysis (Anderson et al., submitted). Each analysis performed is described in more detail below.

Biomass data, head and root data, was collected immediately in the greenhouse on an Ohaus NV511 scale (510g capacity, accurate to 0.1g). Heads were removed by slicing the hypocotyl at the level of the rockwool plug. Root data was collected by removing individual

plugs and slicing roots off at the rockwool interface. Heads pre-selected randomly for dry weight and tissue analysis were rinsed around the hypocotyl interface with RO water and a gentle tactile brush to remove any rockwool fiber, aged algae, and salts that may have transferred during harvest. The head and root portions were placed in individual paper bags. At the end of the harvest day, bagged samples were transferred to 70 °C drying ovens for 6-8 days. Dry weights were taken on a Mettler Toledo NewClassic MF, model MS1003S /03 scale to 0.001g accuracy. In trial 3, some missing data points occurred due to either failed plants or root tangling. From trial 3, root surface areas were collected and reported in a separate paper (Schwartz et al., 2016).

Plants randomly selected for leaf count, leaf surface area, and shelf life tests were immediately pre-treated for analysis upon removal from root. Pre-treatment consisted of dissecting heads apart to separate leaves and counting any leaves that were greater than 1cm. “Dissected” heads were then passed through a continual measuring leaf surface area machine that was carefully cleaned and calibrated immediately prior to running the samples. Heads were bagged for DW analysis.

The randomly selected lettuce heads for the shelf life tests were immediately moved from the greenhouse into the headhouse for manual preparation. The clamshells were filled with individual leaves snapped off from the stalk of the lettuce head until the clamshell was full, starting with the largest marketable leaves and proceeding inwards on the lettuce head (marketable is defined as a leaf with no browning, discoloration, damage, or other physiological blemishes). Twenty clamshells (Inline Plastics, Part #TS64) were filled with treatments: 8 clamshells from H5 tubs, 4 from H7 tubs, and 8 from A7 tubs. As the main comparison was between traditional hydroponics and aquaponics heads, additional samples were collected from

those tubs. Clamshells were transferred to a 4°C refrigerator immediately after preparation and stacked 4 high.

All clamshells were weighed on Mondays and Thursdays from 11/27/2015 through 12/10/2015, and then one final measurement on 12/29/2015. Lettuce clamshells remained closed throughout the entirety of the shelf life trial and were randomly shuffled after each weighing day before being returned to the refrigerator. Relative humidity readings of the refrigerator were 80-84% RH during the trial. A qualitative observation on whether the person weighing would consume the observable lettuce was also included in the protocol.

Nutrient analysis was done as a dry tissue analysis by the Cornell Nutrient Analysis Lab (CNAL). Three samples were submitted to CNAL per tub, per trial. Each sample represented three randomly chosen heads such that each tissue analysis is an averaged sample from the corresponding tub (9 heads per each trials' tub). For dry tissue analysis, CNAL first performed a hot plate assisted acid digestion (CNAL methods incorporate EPA Method No. 3050, 3051, 3052, and ELAP Method No. 4084). Then, digested tissue samples were analyzed using a fully automated inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Dry tissue samples were analyzed using a combustion analysis for total carbon and total nitrogen content. Additionally, a dry ash extraction methods with ICP-AES was run on trial 3 plants primarily to provide accurate data on boron tissue concentrations. Boron was analyzed by CNAL using their dry ash extraction methods since it is an approved method and the tissue hot plate assisted method is not. The hot plate assisted digestion method will under predict concentrations for boron due to material volatization.

Precipitate was found in the H7 tubs between trial cleanings. The precipitate samples were collected and analyzed by the CNAL with hot plate acid digestion plus ICP-AES. The results and findings are presented in the discussion.

Two hose samples with sheathing removed were submitted to CNAL for hot plate acid digestion with ICP-AES (as used in other portion of this experiment). The first hose was a piece of 5/8" garden hose sample that had been used for multiple years and no further identifying information was retained from its purchase; this hose sample represented the approximately 2m of hose used in all 6 tubs. The second hose was a piece of new and unused Flexxon Medium Duty, 5/8, "lead-free" garden hose (Model#FHR58150, made in the USA). This new hose stock was not used in this experiment but data was collected for safety evaluation.

Statistical Analysis. Mixed effect models using least squares analyses were applied using JMP PRO 11[7]. Shoot (FW, DW, and DW/FW), root (FW, DW, and DW/FW), elemental content, and leaf count and surface area (trial 3 only) data were treated as response variables. Treatment and trial were fixed effects; tub nested within trial was treated as a random effect. For the leaf count, leaf surface area, and dry ash extraction, the trial effect was dropped from the mixed model and tub was entered as a random effect due to these response variables only being run in trial 3. A Tukey HSD with pairwise differences among treatments and trials was utilized on the respective mixed effect models for comparison.

Differences with 2014 Experiment. The intention of the current experiment was to accompany and expand upon Part I of our earlier work where we investigated butterhead lettuce response to hydroponic conditions (Anderson et al., submitted). However, there were significant differences in the DLI used in the current experiment (14.2, 14.2 and 14.4moles/m^2/day for trials 1, 2, and 3 respectively) versus average values of DLI of 21, 17, and 17.7 moles/m^2/day for trial

[7] JMP Pro software: http://www.jmp.com/en_us/software/jmp-pro.html

1, 2 and 3 respectively in the 2014 experiment. Although, in both experiments we targeted the same cumulative light load and lettuce harvest size, the rate of growth in the 2014 experiment was higher since the DLI was higher. This difference may have caused some differences in plant response that we do not attempt to address. Further, in the current experiment, the sample size for H7 was always smaller than H5 and H7, which would require larger effects for the H7 treatment to see statistical significance between the comparisons to H5 or A7. The main comparison was intended to be between H5 and A7, and hence our larger sampler sizes for these two treatments.

Results and Discussion

Biomass. Shoot (head) data including mean and standard errors for shoot FW, DW, DW/FW ratios are presented in Table 14. The equivalent information for roots and rootballs is presented in Table 15 and Table 16 respectively.

The aquaponics treatment (A7) was not different ($p>0.05$) from the hydroponics control (H5) in all biomass responses (FW, DW, and DW/FW ratio for shoot, root, rootball, leaf count, leaf SA, and leaf SA/FW). A difference between A7 and H5 is detected at an alpha of 0.10 for the 5% smaller FW shoot. H7 was different from H5 ($p<0.05$) for shoot FW, DW, and DW/FW, as well as root FW and DW. The two pH treatments (H7 and A7) were different for shoot FW, DW/FW, and root DW at $p<0.05$. H7 and A7 were also different from shoot DW at $p<0.10$. The dry matter contents of the roots contained within the rockwool, primarily the lettuce taproot, had dry matter contents (DW/FW) 67% larger than those of the shoots or roots.

Table 14. Shoot mean, standard error (s.e.), and percentage comparison to H5 response for fresh weight (FW, g), dry weight (DW, g), and dry weight to fresh weight ratio (DW/FW, g/g) by trial and treatment with multi-model significance.

		FW			DW			DW/FW		
	Tmt	N	Avg±s.e.	%	N	Avg±s.e.	%	N	Avg±s.e.	%
Trial 1	H5	72	186±3	100%	36	6.8±0.11	100%	36	0.036±0.0004	100%
	H7	24	152±3	81%	12	6.3±0.11	93%	12	0.040±0.0004	113%
	A7	48	187±4	101%	24	6.9±0.13	101%	24	0.035±0.0003	99%
Trial 2	H5	72	160±1	100%	36	6.3±0.06	100%	36	0.041±0.0003	100%
	H7	24	113±2	71%	12	5.7±0.12	90%	12	0.048±0.0005	117%
	A7	48	155±2	97%	24	6.7±0.43	106%	24	0.042±0.0022	104%
Trial 3	H5	71	187±3	100%	36	7.6±0.12	100%	36	0.040±0.0004	100%
	H7	24	147±4	78%	12	6.8±0.14	89%	12	0.045±0.0007	111%
	A7	48	166±3	88%	24	7.0±0.15	92%	24	0.041±0.0004	103%
All Trials	H5	215	178±2$^{\mathbf{A}}$	100%	108	6.9±0.08$^{\mathbf{A}}$	100%	108	0.039±0.0003$^{\mathbf{A}}$	100%
	H7	72	137±3$^{\mathbf{B}}$	77%	36	6.3±0.11$^{\mathbf{B}}$	90%	36	0.044±0.0006$^{\mathbf{B}}$	114%
	A7	144	169±2$^{\mathbf{A}}$	95%	72	6.9±0.16$^{\mathbf{AB}}$	99%	72	0.040±0.0008$^{\mathbf{A}}$	102%

All average responses are in grams except %, and DW/FW which is g/g or dimensionless
%=Percentage comparison to H5 control; Tmt = Treatment; N=sample size
Differing letters within response variables (columns) denote significance at alpha=0.05 for all trials only

Table 15. Root mean, standard error (s.e.), and percentage comparison to H5 response for fresh weight (FW, g), dry weight (DW, g), and dry weight to fresh weight ratio (DW/FW, g/g) by trial and treatment with multi-model significance.

		FW			DW			DW/FW		
	Tmt	N	Avg±s.e.	%	N	Avg±s.e.	%	N	Avg±s.e.	%
Trial 1	H5	72	7.8±0.18	100%	36	0.31±0.008	100%	36	0.039±0.0005	100%
	H7	24	9.0±0.26	115%	12	0.40±0.012	130%	12	0.043±0.0009	112%
	A7	48	9.5±0.28	122%	24	0.30±0.009	96%	24	0.031±0.0008	80%
Trial 2	H5	72	7.6±0.15	100%	36	0.29±0.007	100%	36	0.039±0.0003	100%
	H7	24	8.6±0.31	114%	12	0.41±0.021	139%	12	0.046±0.0005	118%
	A7	48	7.9±016	105%	24	0.27±0.008	91%	24	0.033±0.0006	85%
Trial 3	H5	70	8.1±0.23	100%	34	0.36±0.013	100%	34	0.045±0.0045	100%
	H7	24	11.2±0.71	137%	10	0.46±0.046	127%	10	0.046±0.0016	103%
	A7	47	7.1±0.25	87%	24	0.39±0.026	107%	24	0.054±0.0017	120%
All Trials	H5	214	7.8±0.11^{A}	100%	106	0.32±0.006^{A}	100%	106	0.041±0.0006^{A}	100%
	H7	72	9.6±0.30^{B}	122%	34	0.42±0.016^{B}	131%	34	0.045±0.0006^{A}	111%
	A7	143	8.2±0.16^{AB}	105%	72	0.32±0.011^{A}	98%	72	0.039±0.0014^{A}	97%

All average responses are in grams except %, and DW/FW which is g/g or dimensionless
%=Percentage comparison to H5 control; Tmt = Treatment; N=sample size
Differing letters within response variables (columns) denote significance at alpha=0.05 for all trials only

Shoot (head) biomass response. Shoot FWs were 178, 137, and 169g for H5, H7, and A7 respectively (Table 14). H5 was different from H7 ($p<0.0001$), H5 was not different from A7 at alpha =0.05, but was at alpha=0.10 ($p=0.0974$), and H7 was different from A7 ($p<0.0001$). Compared to H5, the average FW heads were 23% smaller for H7 and 5% smaller for A7.

The shoot DW responses were 6.9, 6.3, and 6.8g/head for H5, H7, and A7 respectively. H5 and A7 were not different at $p=0.9563$. H5 and H7 were different at $p=0.0336$. H7 and A7 were not different at alpha=0.05, however they were different at alpha=0.10 ($p=0.0664$). H7 shoot DWs were 9% smaller than H5.

Head DW/FW is a measure of water content of the plants at harvest. The average DW/FWs were 0.039, 0.044, and 0.040g/g for H5, H7, and A7 respectively. H7 was different from the other two responses from both H5 and A7 at $p<0.0001$. H5 and A7 were not different at $p=0.5324$. H7 was 14% larger than H5.

Root biomass response. Average root FWs, the root portions removed from below the rockwool, were 7.8, 9.6, and 8.2g for H5, H7, and A7 respectively (Table 15). A7 was not different from H5 ($p=0.7519$) and H7 was not different from A7 ($p=0.1391$). The 22% decrease in root FW for H7 compared to H5 was significant at $p=0.0408$.

Root DW average response was 0.32, 0.42, and 0.32g for H5, H7, and A7 respectively. H7 was different from both H5 and A7 at $p<0.0001$ for both pairwise comparisons. H5 and A7 were not different at $p=0.9733$. H7 root DWs were 33% larger on average.

Root DW/FW average response was 0.041, 0.045, and 0.039g/g for H5, H7, and A7 respectively. No pairwise comparisons were significant. P-value comparisons were 0.3595, 0.9904, and 0.2547 for H5-H7, H5-A7, and H7-A7 respectively.

The average rootball FWs, the root portions contained within the rockwool, were 3.0, 3.2, and 2.7g/rockwool cube for H5, H7, and A7 (Table 16). No pairwise comparisons were significant with the smallest p-values equal to 0.3138 for H7-A7, and 0.3650 for H5-A7. Rootball DWs were 0.21, 0.21, and 0.18g/rockwool cube for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values equal to 0.3515 for H5-A7 and 0.3709 for H7-A7. Rootball DW/FWs were 0.068, 0.067, and 0.070g/g for H5, H7 and A7 respectively. No pairwise comparisons were significant with the smallest p-values equal to 0.7356 for H5-A7 and 0.7364 for H7-A7.

Table 16. Rootball mean response to the treatments for fresh weight (FW, g), dry weight (DW, g), and dry weight to fresh weight ratio (DW/FW, g/g or dimensionless).

	FW	%	DW	%	DW/FW	%
H5	3.0^{A}	100%	0.21^{A}	100%	0.068^{A}	100%
H7	3.2^{A}	105%	0.21^{A}	103%	0.067^{A}	99%
A7	2.7^{A}	89%	0.18^{A}	90%	0.070^{A}	103%

Trial 1 and 3 only

A possible explanation for the equivalence of the H5 and A7 conditions is the cumulative effect in the A7 treatment of the organic molecules, such as the natural chelators, enzymes, hormones, and microflora in the root zone. Humic acids, a byproduct of organic decomposition, increases root size, branching, and the uptake of micronutrients (Canellas and Olivares 2014). Humic acids are shown to increase cell membrane permeability and stimulate growth beyond that of mineral only nutrients (Chen and Aviad, 1990). The role of humic acids on the primary and secondary metabolism as well as changes to hormonal and gene expression is a current area of active research and may further explain our results in future years.

Hydroponics is still evolving as an alternative growing system in comparison to other forms of agriculture and many current hydroponic protocols and methodology attempt to mimic

an inorganic water environment. While the mostly "sterile" inorganic environment can provide positives such as predictability of results and reproducibility, it is plausible that these systems lose benefits of an ecosystem of flora within the nutrient solution that can reduce stress and better prepare the plants for unforeseen nutrient and environmental stresses.

Leaves. The average leaf counts were 39.5, 38.4, and 37.2 leaves greater than one cm per plant for H5, H7, and A7 respectively (Table 17). No pairwise comparisons were significant with the smallest p-values equal to 0.3838 for H5-A7 and 0.8301 for H5-H7. The average leaf surface areas per plant were 2721, 2731, and 2363cm^2 for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values equal to 0.1894 for H5-A7, and 0.3668 for H7-A7. The average leaf surface areas per gram of head FW were 14.5, 17.0, and 14.1cm^2/g for H5, H7, and A7 respectively. No pairwise comparisons were significant at alpha=0.05. No pairwise comparisons were significant with the smallest p-values equal to 0.1773 for H7-A7, and 0.2403 for H5-H7. The average FWs for the randomly selected heads used for leaf SA were 188, 161, and 168g for H5, H7, and A7 respectively.

Comparing across the three leaf surface area tests there are two trends that were marginally significant (alpha=0.10). The first was that the H7 treatment plants maintained the same leaf SA per plant as the H5 treatment despite the significantly smaller head weights (Table 14). Along a similar observation, lettuce under the A7 treatment had ~13% decreased leaf surface area but achieved non-statistically different head FW from the H5 lettuce heads. The results were seen with the data flipping such that leaf SA/head FW for the H7 treatments were 17% larger while the A7 treatment's was within 3% of the H5 response. This effect may easily have been accounted for in the leaf thickness or by differential storage of water or other weight in the hypocotyls. No tests were performed to identify if leaf thicknesses were different by this

~15% difference for H7 and A7 plants. More significance may have been attained with additional trials and a larger sample size.

Table 17. Leaf count, leaf surface area (cm^2), and leaf surface area/head fresh weight (cm^2/g) response by treatment.

	Leaf Count*	%	Leaf SA	%	Leaf SA/Head FW	%
H5	39.5^A	100%	2721^A	100%	14.5^A	100%
H7	38.4^A	97%	2731^A	100%	17.0^A	117%
A7	37.2^A	94%	2363^A	87%	14.1^A	97%

* Leaf count defined as leaves >1cm

**Leaf count and leaf surface area were run in trial 3 only with five samples per tub

Elemental Analysis. The elemental analysis for lettuce shoots are given in Table 18. Nutrient solution analysis are given in Table 11, which also includes target concentrations. This paper is a follow up to Part I (our foundational paper) that investigated responses of butterhead lettuce to hydroponic conditions only (two of the treatment conditions repeated in this paper, H5 and H7; see Anderson et al., submitted). We will reference the earlier work as 2014 data where deemed interesting or valuable to a reader.

Table 18. Shoot elemental analysis by treatment for mean, standard error (se), and significance among treatments; all data is on dry weight basis and in mg/kg unless otherwise noted.

	H5		H7		A7	
Parameter (mg/kg)	Mean	(se)	Mean	(se)	Mean	(se)
Carbon Content	335225^{A}	(2677)	334924^{A}	(4637)	337312^{A}	(3279)
Macronutrients						
Nitrogen	58164^{A}	(523)	56840^{A}	(907)	58373^{A}	(641)
Phosphorus	10360^{A}	(207)	11070^{A}	(359)	10425^{A}	(254)
Potassium	37879^{A}	(561)	38706^{A}	(972)	39032^{A}	(688)
Calcium	13744^{A}	(282)	13304^{AB}	(489)	12170^{B}	(346)
Magnesium	3238^{A}	(109)	3563^{A}	(189)	3568^{A}	(133)
Sulfur	2470^{A}	(25)	2430^{A}	(44)	2534^{A}	(31)
Micronutrients						
Iron	61^{A}	(1)	60^{A}	(2)	60^{A}	(1)
Manganese	77^{A}	(3.2)	65^{A}	(5.6)	45^{B}	(3.9)
Boron*	28.0^{A}	(1.7)	38.3^{A}	(2.9)	30.6^{A}	(2.1)
Copper	7.0^{A}	(0.6)	9.9^{B}	(1.0)	10.7^{B}	(0.7)
Zinc	34^{A}	(2.2)	31^{A}	(3.9)	64^{B}	(2.7)
Molybdenum	0.86^{A}	(0.09)	0.83^{A}	(0.16)	0.20^{B}	(0.12)
Other elements						
Sodium	720^{A}	(128)	717^{A}	(222)	2027$^{\mathbf{B}}$	(157)
Aluminum	15^{A}	(1)	13^{A}	(1)	14^{A}	(1)
Nickel	0.06^{A}	(0.03)	0.03^{A}	(0.05)	0.09^{A}	(0.04)
Silicon	28.3^{A}	(0.4)	27.3^{A}	(0.7)	28.2^{A}	(0.5)
Lead	1.4^{A}	(0.5)	0.7^{A}	(0.9)	0.8^{A}	(0.6)
Strontium	94^{A}	(2)	98^{A}	(3)	75^{B}	(2)
Arsenic	0.36^{A}	(0.02)	0.35^{A}	(0.03)	0.35^{A}	(0.02)
Barium	1.2^{A}	(0.1)	1.0^{A}	(0.2)	3.0^{B}	(0.2)
Cadmium	0.13^{A}	(0.00)	0.12^{A}	(0.01)	0.13^{A}	(0.00)
Cobalt	0.011^{A}	(0.001)	0.010^{A}	(0.002)	0.012^{A}	(0.001)
Chromium	0.26^{A}	(0.03)	0.31^{A}	(0.05)	0.31^{A}	(0.04)

Columns with differing letters are statistically significant at alpha=0.05.
*Boron analysis data from ash extraction on trial 3 samples only.

Carbon (C) average tissue contents were 335225 (33.5%), 334,924 (33.5%), and 337,312 (33.7%) mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-value equal to 0.8750 for H5-A7.

Nitrogen (N) average tissue content results from this experiment were 58164, 56840, and 58373mg/kg for H5, H7, and A7 respectively. No pairwise comparison was significant with the smallest p-values as 0.3790 and 0.4387 for H7-A7 and H5-H7 respectively. The 2014 N tissue analysis data, 54166 and 49537 for H5 and H7 respectively and were significantly different at p=0.0013, was not replicated in this experimental comparison of H5 and H7. It is possible that this experiment's lower DLI of ~14 instead of 17moles/m^2/day was sufficiently decreased to allow equivalent uptake and fixing on nitrogen in the plant.

Phosphorus (P) average tissue content via acid digestion of all trials were 10360, 11070, and 10425mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values as 0.2367 and 0.3376 for H5-H7 and H7-A7 respectively. The lack of difference in P was surprising considering the significant differences and supposed ratios of availability in the nutrient solution. H7 started at target concentrations but dropped in availability to a third of the starting concentrations while the aquaponics condition remained fairly constant at a third of the target nutrient solution concentration.

Potassium (K) average tissue content results were 37879, 38706, and 39032mg/kg for H5, H7, and A7 respectively. There were no significant pairwise comparisons with the smallest p-values as 0.4206 and 0.7465 for H5-A7 and H5-H7 respectively. The 2014 K tissue analysis results were 30123 and 30190mg/kg for H5 and H7 respectively. The pairwise comparison for potassium within the 2014 experiments were highly non-significant with the smallest p-value as

0.8868. While the results and differences were the same, the variation in total potassium contents was different between experiments.

Calcium (Ca) average tissue content results were 13744, 13304, and 12170mg/kg for H5, H7, and A7 respectively. The pairwise comparison of H5-A7 was significant at p=0.0097. H5-H7 and H7-A7 were not significant at p-values of 0.7217 and 0.1793. The A7 calcium tissue content was ~11% lower than H5. The A7 plants had 11-12% less Ca tissue concentrations then H5 plants. The nutrient solution in both H7 and A7 were below the nutrient solution targets but pH alone did not look to influence the availability of calcium to the lettuce, since the H7 value was not different from H5 where the only difference was pH.

Magnesium (Mg) average tissue concentrations were 3238, 3563, and 3568 mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the lowest p-values as 0.1734 and 0.3262 for H5-A7 and H5-H7 respectively. The 2014 Mg tissue analysis were 2759 and 3891mg/kg for H5 and H7 respectively and were significantly different. The H7 Mg concentration was larger in both experiments than H5, but the H5 tissue Mg was much lower in the 2014 data versus the current experiment (2015 data).

The dry ash extraction method was also used to determine Mg levels using samples from trial 3; both analysis methods used are reportable for Mg. The Mg values from ash extraction were 2639, 3865, and 3234 mg/kg for H5, H7, and A7 respectively. All pairwise comparisons were significant with p-values of <0.0001 for H5-H7, 0.0003 for H5-A7, and 0.0026 for H7-A7. The ash extraction data follows the 2014 acid digestion data more closely both in trends and actual concentration values. Since the 2014 Mg data for H5 and H7 were the same as the current dry extraction method, we believe them to be a more accurate reading of the Mg in all treatments. Although, the A7 nutrient solution Mg concentrations were about 25% higher at 19-

20mg/L for starting and ending concentrations then either the H5 or H7 conditions (Table 11), it appears to have had no effect on tissue Mg concentration.

Sulfur (S) average tissue concentrations were 2470, 2430, and 2534 mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values as 0.1637 and 0.2825 for H7-A7 and H5-A7 respectively. Nutrient S content has been consistent in all trials from both experiments from slightly below the 18ppm target to now slightly above giving us confidence that S is not affected by pH and has some flexibility in nutrient solution concentrations without affecting tissue concentrations.

Iron (Fe) average tissue concentrations were 61, 60, and 60 mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values as 0.4836 and 0.8027 for H5-A7 and H5-H7 respectively. Tissue Fe was consistent despite the higher initial and fluctuating Fe concentrations in the A7 nutrient solution.

S and Fe tissues concentrations were very consistent for all treatments and trials despite increased concentration of both for the A7 treatment. Figure 5 shows the chelated percentage of Fe at differing pH. Within the typical pH range used in conventional hydroponics, the DTPA chelator, as used in this experiment, is at or very nearly 100% selective for Fe. As the solution approaches pH 7.0, as is the case for aquaponics systems, the DTPA chelator is still very effective at $\geq$ 97% selective for Fe. Given that a chelator may bind to other elements, the 3% of chelator not bound to iron may be aiding transport of other elements into the plant.

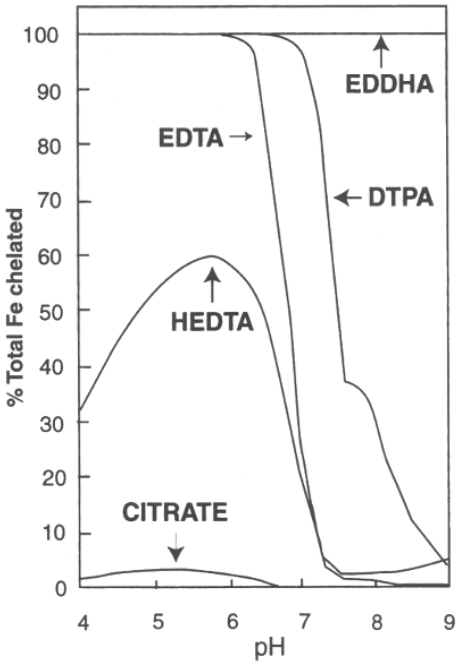

Figure 5. A pH comparison of chelators and their proportion of Fe chelated (Reed, 1996).

Manganese (Mn) average tissue concentrations were 77, 65, and 45 mg/kg for H5, H7, and A7 respectively. A7 plants were significantly different from both H5 and H7 plants (p=<0.0001 and 0.0328 respectively). H5 and H7 plants were not different at p=0.1694. The 2014 Mn were replicated in this experiment with the same significance and magnitude for H5, 80mg/kg, H7, 67mg/kg (p-value=0.3634).

The Mn nutrient solution concentrations (Table 11) showed a downward trend in all treatments. The average starting concentrations were 0.148, 0.099, and 0.046mg/L for H5, H7, and A7 while the average ending Mn concentrations were 0.025, 0.006, and 0.004mg/L. The target was 0.140mg/L. Evaluating between Mn data sources, A7 was different in tissue concentration from both H5 and H7. The nutrient solution Mn average starting concentrations were surprisingly approximately a third smaller than target for H7 (despite using the same starting solution and simply raising the pH) and approximately two thirds below target for the A7 treatment. Mn nutrient solution starting and ending concentrations generally correlated with the

average tissue analysis trends, namely that the H5 nutrient solution and tissue concentrations were the highest, the H7 responses were reduced below the H5 levels, and the A7 responses for nutrient solution and tissue content were further reduced below H7 levels. Sonneveld and Voogt 1980) saw similar responses in nutrient solution Mn responses in similar pH ranges (6.5-7, and 5-5.5) suggesting that both the changing nutrient solution concentrations and management of any long term hydroponic system will want to consider non-plant Mn consumption for their respective pH. While H7 received the exact nutrient solution concentrates from the exact same well mixed buckets, the Mn concentrations were lower including starting values that should have been the same for H5 and H7. Zafrin et al. (2015) states that Mn concentrations sharply increased below pH 5.5 suggesting that lowering pH is less favorable to Mn oxidizing bacteria.

Boron (B) average tissue concentrations were 28.0, 38.3, and 30.6mg/kg for H5, H7, and A7 respectively from the trial 3 ash extraction method. No pairwise comparisons were significant with the smallest p-values as 0.1087 and 0.2267 for H5-H7 and H7-A7 respectively. Brown et al. (2002), states that dicots have typical B concentrations in the 20-100ppm range and that B is uptaken primarily by passive absorption. A7 B nutrient solution concentrations were an order of magnitude smaller than the H5 and H7 conditions, which still caused no differences in tissue concentrations. If B is passively uptook, as is stated in the Brown et al. (2002) white paper, then our hypothesis is that the B plant usage is low enough and B in surplus enough in the nutrient solutions such that equilibrium is reached between the plants and solution. This would explain the order of magnitude lower nutrient solution concentrations resulting in the same plant tissue concentrations.

Copper (Cu) average tissue concentrations were 7.0, 9.9, and 10.7mg/kg for H5, H7, and A7 respectively. H5 was different from both H7 and A7 at p=0.0331 and 0.0003 respectively. H7

and A7 were not different at p=0.4434. The Cu nutrient solution concentrations were close to equivalent or slightly smaller in H7 and A7 then in H5. Despite the less than or equivalent Cu in solution, the evaluated tissue Cu concentrations suggests that pH has some effect on the uptake of Cu in hydroponic solutions.

Tissue Cu contents were increased for H7 and A7 lettuce. The increased Cu content in A7 was seen despite decreased Cu concentrations in the nutrient solution. Davis and Beckett (1978) found that lettuce do not exhibit toxic effects that decrease biomass yields until the tissue concentrations exceed 21ppm. Since our Cu concentrations in the H7 and A7 treatments were between 7-11ppm, this would indicate that these higher accumulations of Cu did not cause the decreased yields in H7 lettuce.

Zinc (Zn) average tissue concentrations were 34, 31, and 64mg/kg for H5, H7, and A7 respectively. A7 was different from H5 and H7, both at p<0.0001. H5 and H7 were not different at p=0.7258. The 2014 tissue analysis data concentrations for Zn were 42mg/kg for H5 and 32mg/kg for H7 (p=0.0005). This data agrees almost identically for H7, but the H5 treatment was higher for the 2014 data; however in all cases the Zn concentrations were >20mg/kg, which Hafeez et al. (2013) state is the lower bound value before expecting deficiency symptoms such as decreased biomass yields. Additionally, the average nutrient solution concentrations in A7 were 2x higher than H5 and H7 treatment, which correlates with the 2x higher tissue concentration in A7. The A7 Zn tissue concentration is likely not to cause toxicity issues from zinc (Davis and Beckett, 1978).

Molybdenum (Mo) average tissue concentrations were 0.86, 0.83, and 0.20 for H5, H7, and A7 respectively. A7 was different from H5 at p<0.0001 and H7 at p=0.0009. Mo was not detected in any of the samples for Trial 1, even though nutrient solution concentrations were

consistent with other trials. We attribute the non-detectable Mo values in Trial 1 to a Type II error (false negative). The lower values of Mo in the A7 tissue is expected given there was no detectable Mo in the A7 nutrient solution. Mo tissue concentrations from the 2014 data (Anderson et al., submitted) were 0.95mg/kg for H5 and 1.43mg/kg for H7 and were different at $p=0.0056$. These 2014 results are reasonably consistent with our current work if the current Trial 1 data is ignored because of the false negative. Mo nutrient solution concentrations were 0.03mg/L for both starting and ending conditions in both H5 and H7 treatments. Mo was only slightly detectable in the A7 tubs at the start of trial 2, 0.4µg/L, and trial 3, 1.06µg/L.

Sodium (Na) average tissue concentrations were 720, 717, and 2027mg/kg for H5, H7, and A7 respectively. A7 was different from H5 at $p<0.0001$ and H7 at $p=0.0009$. H5 and H7 were not different at $p=0.9999$. The Na nutrient solution concentrations and tissue concentrations for H5 and H7 were consistent suggesting no pH effect. The increased Na concentration in the A7 nutrient solution correlated with the increased tissue concentration. The 2014 tissue data included a condition (HA7) using tap water and pH 7 conditions that also had higher Na concentrations than the RO water conditions (H5 and H7). In that 2014 data, the HA7 tissue contents were also elevated in a similar manner to the current A7. The nutrient solution Na concentrations are higher in the tap water conditions due to the existing Na in our tap water source. The aquaculture system can elevate this Na concentration even higher due to the recycling of water and concentration from evaporation. The data does not suggest a pH effect on sodium uptake.

Aluminum (Al) average tissue concentrations were 15, 13, and 14 mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values as 0.6299 for H5-H7 and 0.7894 for H7-A7. Al was not added to the nutrient solutions as it is not a

necessary microelement according to the Sonneveld and Straver formula. Reported nutrient concentrations (see Table 5) are all very low and show a similar increase from start to end of the trials.

Nickel (Ni) average tissue concentrations were 0.06, 0.03, and 0.09 mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values as 0.2367 for H5-H7 and 0.3376 for H7-A7. Nickel was not detectable in the nutrient solution analyses.

Silicon (Si) average tissue concentrations were 28.3, 27.3, and 28.2 mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values as 0.3867 for H5-H7 and 0.5462 for H7-A7. The 2014 tissue analysis results were 3.3 mg/kg for H5 and 5.2mg/kg for H7 and were not different at p=0.1399. We do not have sufficient data to explain the large increase in Si tissue concentration between the 2014 data and the current experiment. Si starting nutrient solution concentrations were 0.05, 0.10, and 1.27mg/L for H5, H7, and A7 while ending nutrient solution concentrations were 0.12, 0.30, and 1.55mg/L for H5, H7, and A7 respectively. The very low concentrations in the nutrient solution appear to still accumulate significant quantities in the shoots. Furthermore, the order of magnitude larger nutrient solution concentration for A7 did not increase the tissue concentration.

Lead (Pb) average tissue concentrations were 1.4, 0.7, and 0.8mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-values as 0.1637 for H7-A7 and 0.2825 for H5-A7.

Following detectable lead contents in the plant tissues after tissue analysis, an in depth investigation into the source within our system was initiated. All RO, tap water, and nutrient solution samples that had interacted for less than a day with the tubs had no detectable

concentrations of Pb. Tubs were pre-filled up to a week in advance due to the size and limitations of the RO water system. Starting nutrient solutions from the tubs that had been filled one to seven days in advance of the nutrient solution concentrates and elemental nutrient analysis all had detectable concentrations of lead. The ending nutrient solutions also presented detectable concentrations of lead in all treatments. This identified the Pb source to be part of the system that interacted with the water or nutrient solution. We considered all parts exposed to nutrient solution as being capable of contributing lead, but the only feasible component was identified as the garden hose part.

The 2m lengths of garden hose in each tub, used for recirculating the solution, had a 136mg/kg Pb content while the new "lead-free" hoses had 52mg/kg Pb content. A quick calculation showed that a 2m length of hose weighing 200g is sufficient (27.2mg Pb) to supply Pb at 1ppm to 50 full size heads of lettuce for more than fifty additional harvests. Furthermore, the 136mg/kg value was after >10 harvests and over 4 years in use. A7 tubs' nutrient solutions had higher Pb concentrations than H5 and H7, which correlated with the protocol differences for these two treatments. The A7 system was run continuously from the experiment start through to the end and thus there was significant time for low level accumulation between experiments. As water was added to the H5 and H7 tubs only a few days before the experiment start, significant accumulation would not have been expected. The 10.9ppb lead concentration is below the EPA limits of 15ppb in drinking water and the low 0.6ppb final concentration suggests fairly low level leaching from the hosing.

Strontium (St) average tissue concentrations were 94, 98, and 75 mg/kg for H5, H7, and A7 respectively. A7 was different from H5 at $p=0.0001$ and from H7 at $p=0.0003$. H5 and H7 were not different at $p=0.5268$. The A7 treatment's decreased concentration of St in the tissue

analysis correlated with the decreased concentration in the nutrient solutions. St was statistically lower than H5 or H7, however St may be used as a less effective calcium substitute within the plant, and thus the ratio of St to Ca is important, particularly if this ratio starts to increase significantly. Since our Ca to St ratios for all treatments were incredibly large, we viewed this difference in strontium to be incidental and not causal to other effects.

Arsenic (As) average tissue concentrations were 0.36, 0.35, and 0.34 mg/kg for H5, H7, and A7 respectively. No pairwise comparisons were significant with the smallest p-value as 0.8892. Nutrient solution concentrations for As starting and ending concentrations were not consistently detected between trials. The largest detected concentration was 13 ppb.

Barium (Ba) average tissue concentrations were 1.2, 1.0, and 3.0mg/kg for H5, H7, and A7 respectively. A7 was different from both H5 and H7 with $p<0.0001$. H5 and H7 were not different at $p=0.6704$. The A7 tissue content of Ba is consistent with the slightly elevated nutrient solution concentration in A7 tubs.

Cadmium (Cd), cobalt (Co), and chromium (Cr) were all at very low concentrations and were not significantly different among treatments. Tissue average values are provided for interest and completion but are not further discussed.

Shelf Life. Water losses for the first 17days were 0.08g for H5, not detectable for H7, and 0.12g for A7. The extended period of an additional 19 days did not include any mixing of the clamshell order or position. The five clamshells at the top of each stack lost on average 6.15g (SD=0.12), while the remaining 15 clamshells lost an average of 0.54g (SD=0.12). By treatment, the total water loss from harvest through 10/29 by treatment (36 days) and excluding the top clamshells were 0.53g (SD=0.08, n=8) for H5, 0.40 (n=1) for H7, and 0.58g (SD=0.16, n=6) for A7.

Ultimately, in either scenario of placement and exposure to increased air movement, the water loss was insignificant from the clamshells. Furthermore, commercial methods and systems for storage of leafy greens are expected to be more optimal and decrease the effect further. While there may be differences in open air such as when a consumer opens the package, it is anticipated the majority of water loss from the system is the saturated humidity and condensation drips from the container, aspects that are not treatment dependent. As such, we found shelf life aspects related to leafy greens were not influenced once in the clamshell.

The visual evaluation for whether the lettuce package was consumable was not different among treatments. All clamshells were considered consumable at the end of the 17 day biweekly observation, but after an extra 19 days of storage, no lettuce from any of the clamshells was deemed consumable. Also, no clear trends between treatment and physiological or microbial discoloration were identified.

Our observation on whether the lettuce was consumable at 17 and 36 days was not different between treatments. It is possible that the physiological and microbial aspects were more strongly influenced by the constant saturated humidity in the clamshells plus condensation formed from re-cooling after brief but likely notable warming during the biweekly weighing in a warm headhouse. This comparison was not intended to be rigorous, but merely to identify and quantify the impact of treatment to a general consumer.

Aquaponic System A7 Turbidity. Following addition of the chelated iron and connection to the hydroponic tubs, the turbidity rose in the aquaponic system and visibility decreased rapidly. The 0.2μm filtration of the A7 solution had no meaningful impact on elemental concentrations in the solution (

Table **19**). Lab microscopy on the samples showed green rod algae and suspended white debris in the water and was attributed to the turbidity and low visibility in the ponds.

Table 19. Comparison A7 solution before and after 0.2µm filter.

Element, mg/L	A7	A7 Filtered	% Diff from A7
K	250	248	99%
NO3-N	126	127	101%
Ca	77	78	101%
Na	55	55	99%
Mg	19	19	102%
S	18	19	102%
P	10	10	101%
Si	1.43	1.45	101%
Fe	1.20	1.22	102%
TAN-N	0.87	0.86	99%
Sr	0.55	0.54	99%
Zn	0.29	0.29	99%
Al	0.04	0.04	89%
Cu	0.02	0.02	95%
Ba	0.02	0.02	102%
Mn	0.02	0.01	69%
NO2-N	0.018	0.020	115%
B	0.01	0.01	73%
As	0.00	0.00	-
Ni	0.00	0.00	-
Mo	0.00	0.00	-
Se	0.00	0.00	-
Cd	0.00	0.00	-
Co	0.00	0.00	-
Cr	0.00	0.00	-
Pb	0.00	0.00	-
Ti	0.00	0.00	-
V	0.00	0.00	-

A7 sample is collected and unaltered per standard methods
A7 filtered was a portion of the A7 solution, run through a 0.2um filter and submitted alongside

H5 vs. H7. The control hydroponics (H5) and aquaponics (A7) were not different in FW or DW root; however the H7 condition, simply a difference in pH and shift in the concentration

of Ca and PO_4^{3-}, caused significant changes in growth response. Compared to the average FW root mass of H5 and A7, 8g/plant, the H7 root mass in the rock wool was 24% of the total root mass, compared to 27% for the H5 and A7 treatment.[89]

A DW comparison of the root contained in the rootball versus the total root mass showed that 38% was in the rootball (rockwool) for H5 and A7 plants with 32% for the H7 comparison. This differences was due to the rootball roots' dry matter content (DW/FW) being 63% higher than the roots below the rockwool.

An interesting comparison is total fixed C; a measure of the fixed C (cumulating any difference in photosynthesis efficiencies, intercepted light, and duration of photosynthesis) minus lost energy (varying respiration rates), at harvest, by treatment. The total C contents at harvest were 2.49g in H5, 2.30g in H7, and 2.48g in A7 (Table 14Table 15,Table **16**Table 18).[10] In comparison to the H5 treatment, the H7 treatment was 8% reduced in total C content while the A7 treatment was reduced by 0.5%. Note that the comparison makes the assumption that the roots and rootball contained the same concentration of C per dry gram and that the concentrations in the roots were not different between treatments (the H5 and A7 were likely to be quite similar comparatively either way as the A7 was 0.05g smaller in shoot DW, not different in root DW, and 0.03g smaller in rootball DW). Neither photosynthesis nor respiration measurements were tested in this experiment so no causation claims are made regarding differences in fixed carbon assimilation or respiration between conditions.

Precipitation from system. We observed significant precipitate as granular sand in the H7 HDPE tubs and formation of a precipitate "skin" on their sides. White precipitate was also

[8] 3g in the rockwool divided by total root mass (3 from rootball + 8 from FW root)
[9] 3g in the rockwool divided by total root mass (3 from rootball + 8 from FW root)
[10] H5=(6.91+.32+.21)g*335225mg/kg=2.494g, H7=(6.26+0.42+0.21)g*334924mg/kg=2.298g, and A7=(6.86+0.32+0.18)g*337312mg/kg=2.483g carbon content calculations

observed on the roots of the H7 condition (Figure 7) using a dissecting scope plus camera. The precipitate was collected from the H7 tubs and submitted to CNAL for hot plate acid digestion. At pH 7, slow addition of 1M KOH did occasionally cause white "plumes." The protocols dictated slow addition of acid and base with vigorous mixing, but this phenomenon still occurred, but only in the H7 conditions. At pH 7, the unbound phosphate in solution is close to half $H_2PO_4^{1-}$, and half HPO_4^{2-} (Figure 6, Asadi et al, 2014). The other observations were that the H7 condition required daily pH adjustment including the first week when the plants are proportionally having very little influence on the nutrient solution. The H5 condition required little to no adjustments the first week and typically much smaller adjustments compared to H7 as the trials proceeded.

The precipitate digestion results were consistent in all four tub samples analyzed and ranged between 97-98% as Ca and PO_4^{3-}. Furthermore, the average Ca to P molar ratio of the precipitate was 1.39:1 (SD=0.06). Since precipitation was unique to H7 and correlated to a daily requirement to raise the pH with KOH, we believe the precipitated form of calcium phosphate donated (a) hydrogen ion(s) to solution.

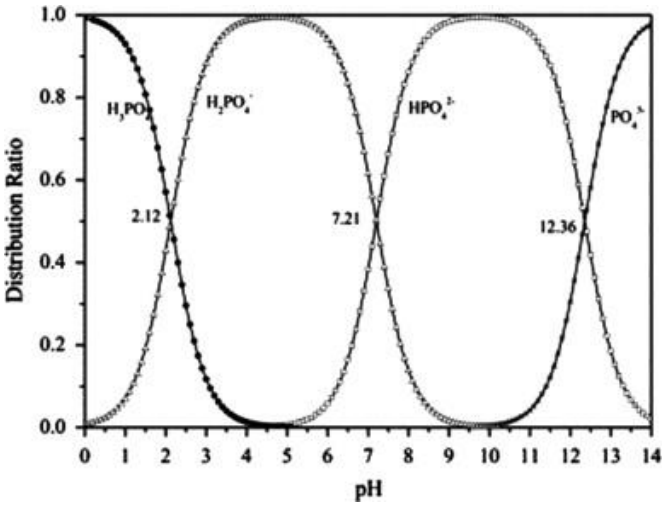

Figure 6. Ratios of phosphate ion forms in solution at differing pH values (Asadi et al 2014).

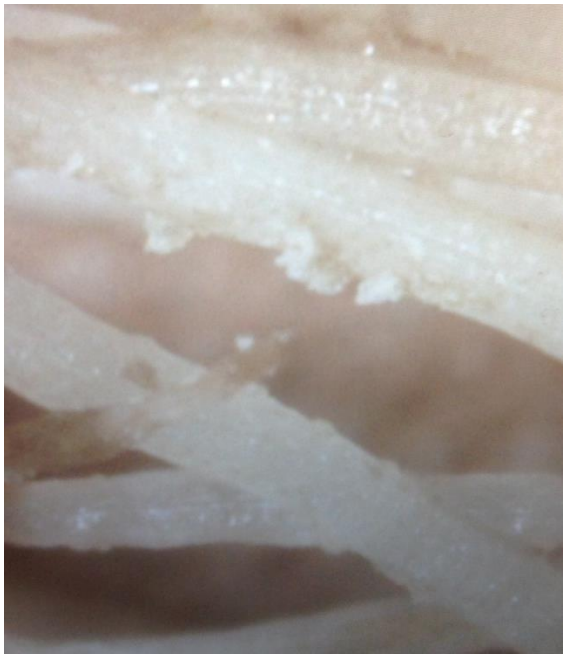

Figure 7. White precipitate observed on the roots of H7 condition from a dissecting scope inspection with the Cornell Pathology Lab, 2014.10.21.

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Paper 3:

Quantification of root nitrification capacity of Bibb lettuce plants for use in a recirculating aquaculture system (RAS)

Anderson, T.S., Goldstein, L.T., Timmons, M.B.

Abstract

Aquaculture and hydroponics are both experiencing rapid growth and expanding presence in the commercial world. While aquaponics, the combination of these fish and plant culture systems, is beginning to experience the same exponential growth and interest that hydroponics did many years ago, very little information is available on sizing and design of the systems are available. We believe the further information available to current hydroponic and aquaculture facilities may spur growth in this field of organic, pesticide free produce on the hydroponics side. Since nitrogenous toxins produced by the fish can be an issue and filtration costs are inversely proportional to the size of the particulates, incorporation of the hydroponic plants and aid in removal of ammonia/ammonium based wastes, thus eliminating the need for water discharge, while also reducing capital costs associated with filtration that can occur by adsorption to the plant roots or mineralized in the additional area of the plant system.

The objectives of this paper were to quantify ammonia removal rates provided by plant roots in hydroponic systems and to define plan growing areas using Bibb lettuce that are required to support fish feeding rates. Raw nitrification rates were identified to be 0.79-0.88g/m^2/day on inert surface areas while root surface only nitrification rates were identified to be 0.11-0.21g/m^2/day. We identified that a 1000kg carrying capacity fish system would require 900m^2 of

lettuce production area to support the nitrogen removal requirements. This would shift the currently recommend 60g of fish feed by Rakocy per m^2 of lettuce area closer to 11g of fish feed per m^2 of plant area. The hydroponic lettuce production side of such an operation would account for 97.5% of the yearly gross sales given reasonable assumptions for market values of $1.5/head for lettuce and $6/kg for live sale of tilapia.

Introduction

Aquaponics is the combined culture of fish and plants into a common system where the culture water is recirculated on a continuous basis between the fish system and the plant system (Rakocy 1997; Adler et al. 2000; Al-Hafedh et al. 2008; Graber and Junge 2009; Danaher et al., 2011). The vegetable crop is responsible for the direct assimilation of dissolved fish wastes and products of microbial breakdown in the recirculating aquaponic system. However, methods to remove solids from the production system are still necessary to sustain fish and plant health and prevent sub-optimal water quality parameters, such as high un-ionized ammonia, nitrite and low dissolved oxygen (Cripps and Bergheim 2000; Piedrahita 2003).

As currently practiced, aquaponics involves large-scale hydroponics in concert with small scale fish systems, e.g., current rule of thumb is that ~ 60grams of fish feed per day will support ~ 1 m^2 of lettuce production, which is approximately 24 harvest sized heads (142g). This type of ratio results in the economic scales being dramatically larger for the plant side, creating farm marketing issues among other things for the fish component. An alternative approach is one where the economic scales of the plant and fish side are more comparable.

Biological filtration systems for RAS use inert materials to provide surface area for fixed film nitrogenous bacteria to provide for the oxidative removal of ammonia and nitrite created from the fish feeding and metabolic processes. Two major groups that are commonly used for this function include Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB), both chemosynthetic autotrophs. AOB and NOB work in conjunction to oxidize ammonia to nitrite, and nitrite to nitrate, respectively. The step contributed by the AOB has a much smaller kinetic rate than the respective NOB reaction, so nitrite rarely has the opportunity to accumulate.

Nitrate is the major end product of the two coupled reactions, and is the least toxic of all the nitrogen compounds (Timmons & Ebeling, 2013).Both the nitrate, as the fully oxidized nitrogenous waste component, and any ammonia present in the plant water column can be used by the plants for their nitrogen growing requirements.

Once a RAS is coupled with a hydroponic system, another metric for consideration is the ammonia removal capacity of the plant system and how much nitrification capacity is added for every square meter of plants. Conceptually, the plant system can provide part of the nitrification capacity that is by required by the RAS. Note that we state ammonia removal capacity in lieu of nitrification capacity, since in addition to surface area for nitrification, the plants remove ammonia and nitrate for plant growth, so not all ammonia removal is through a nitrification step. For the fish system design, it is immaterial how the ammonia is removed from the water column. The objectives of this research paper were to: a) quantify ammonia removal rates provided by plant roots, and b) define the plant growing area using Bibb lettuce that is required to support a particular fish feeding rate.

There are few if any commercial scale aquaculture operation (fish operations producing several hundred ton of product per year) that are making any serious effort at embracing aquaponics. Part of this lack of adaptation is due to a lack of design information on how a hydroponic plant component can be effectively utilized as a biofiltration system for the fish system. This paper is a first step towards providing some of this design data.

Materials and Methods

An experiment was conducted in a conventional glass greenhouse to investigate the effects of growing hydroponic lettuce upon total ammonia nitrogen (TAN) removal rates for use in sizing aquaponics facilities. The experiment was run from May 11 through May 15, 2015 for data collection. Lettuce for the experiment was seeded April 8th and transplanted into the tubs on April 20th.

***Growing System and Procedure*.** Six HDPE tubs were elevated to 1.26m below the supplemental light fixture array for consistency of supplemental lighting between our six tubs. The tubs were 0.425m^3 at the fill height with 1.40m^2 of growing area with top dimensions 1.82m x 0.91m, 0.29m depth with tapered sides to flat base dimensions of 1.35m x 0.81m.

Fifty plants (10 plants per row and 5 rows, 30 plants per m^2) were placed per tub using Styrofoam rafts 2 mm in thickness with 25mm round holes for plant plugs spaced at 200mm on center; rows were staggered to maximize uniformity of light to all sides of each plant. Recirculating pumps and air stones were operated continuously within each tub to ensure vigorous water mixing and to maintain dissolved oxygen (DO) near saturation in each tub. Diffusors were confirmed to be running vigorously several times weekly and DO saturation levels were spot tested weekly and found to be at saturation for ambient oxygen conditions. The circulating pumps (24 liters per minutes (lpm)) mixed the water in the tubs at a rate equal to a hydraulic retention time of 18 minutes.

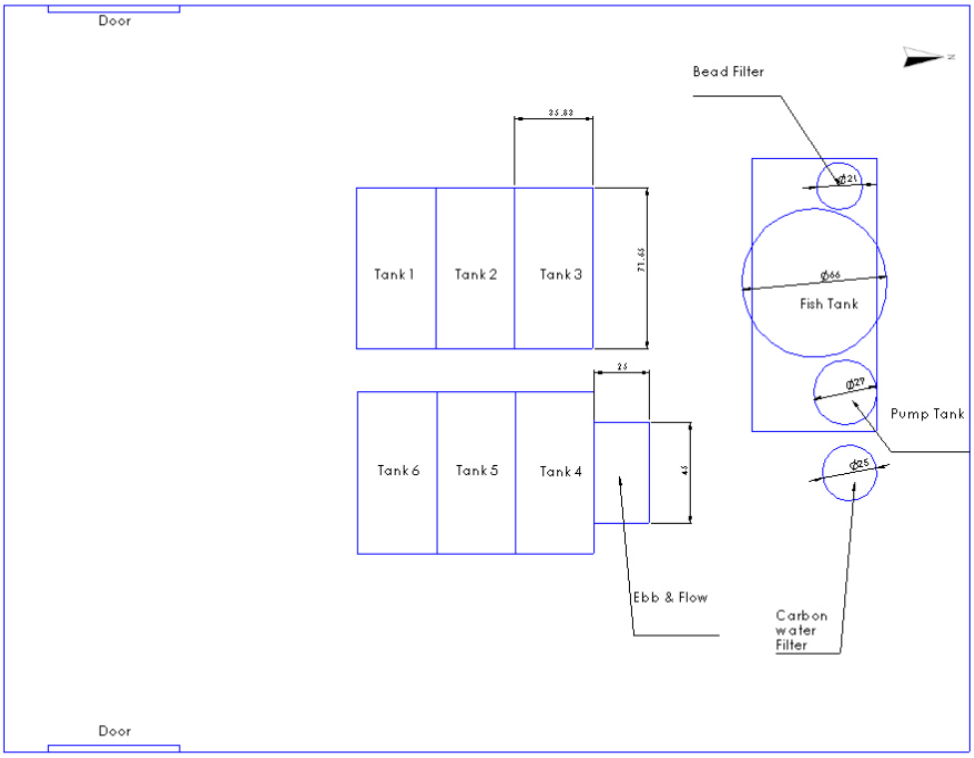

Figure 8. Greenhouse experiment floor plan (drawn to scale). Note the fish system was not utilized in this experiment except to provide seed water to establish the nitrfying community.

A single pelleted seed of butterhead lettuce (*Lactuca sativa*, cv. Flandria) was placed into individual rockwool plugs (Grodan AO25/40, 25mm). Care was taken to place the seeds horizontally at the same depth in the rockwool cavity after which the planted seeds were misted with RO water in several passes so that all pellets were equally saturated. Two standard perforated 1020 trays were used for holding the two 200 cell rockwool sheets. The trays were germinated in the same greenhouse space as the growing experiment (Figure 8). For the first 24 hours, the trays were covered with clear plastic covers and shielded from light to control for temperature and humidity and saturated with a conventional hydroponics water nutrient solution (henceforth referred to as "nutrient solution," details provided later). After 24 hours as seedling emergence occurred, the plastic covers were removed and the trays were placed in an ebb and flood system where they were grown for the next 12 days. The ebb and flood system cycled the

nutrient solution four times per day, using 15 minute flood cycles (7 am, 11 am, 3 pm and 7 pm). The overflow height from the flood bench was set at two-thirds the height of the rockwool cubes.

At day 7, plants were inspected for uniformity using the first true leaf (defined as 10 mm in length to differentiate from an earlier leaf that grew more slowly or not at all) for comparison; large and small plants were marked, but left in place. On the 12th day, 300 plants (50 per tub) were selected for consistency from the unmarked seedlings. The plants were then randomly placed in the described rafts inside the tubs. After transplanting, the plants were grown in the tubs for an additional 22 days (with an average daily light integral of 21moles/m^2/day). Plants remained in the described spacing for the duration of the experiment.

Tubs were cleaned with germicidal concentrations of bleach for several hours, repeatedly rinsed, filled and drained after several hours of running, and then left to air for several days prior to starting the experiment. Sterilization of the tubs was done with hope to address the somewhat smaller lettuce than expected in the system where we had observed leaf spotting (not identified as a particular disease or nutritional deficiency). The sterilization was not successful (as evidenced by smaller than expected lettuce heads at harvest) and no cause was found although the problem disappeared with no changes to protocols or materials months later.

Establishing the nitrifying community. At the beginning of the experiment, the tubs were started at the nutrient solution target concentrations of TAN (8.8ppm) for lettuce and were thereafter given 5ppm spikes of TAN when tub levels dropped below 1ppm. The TAN additions initially came from ammonium nitrate, which worked while the nitrification rates were slow. We switched to ammonium bicarbonate (99.5% from Chem-Impex) to buffer the pH with ammonium additions 2 days prior to the primary data collection, which significantly improved our pH control.

The tubs were seeded with 50L each of the aquaculture system water to more rapidly establish the nitrifying and other biological communities since we had applied a germicidal bleaching immediately preceding this experiment. The aquaculture water was supplemented with inorganic salts to create a lettuce nutrient solution with target concentrations (described later). No further interaction between the aquaculture and hydroponics tubs occurred.

Studies run for nitrification. Nitrification rates were tested in two dosage regimes, a moderate and elevated dosage. The moderate dosages started with an initial TAN concentration of 7ppm with a 5ppm TAN spike for the trial including plants and roots (henceforth referred to as "7/5R"), and a 6ppm initial concentration with a 5ppm TAN dose for the moderate dosage with no roots ("6/5NR"). The elevated dosages were both started at a concentration of 8ppm with a 6ppm TAN spike for both the with-roots ("8/6R") and without-roots trials ("8/6NR"). The spikes occurred consistently 14 hours after the start of the experiment to extend the length of the experiment to include an overnight and day period with the plants, and then matched to the same for the trials without the plants. 7/5R ran for 19.5 hours (5/11 9pm – 5/12 4:30pm), 8/6R ran for 23 hours (5/12 4:45pm – 5/13 5:45pm), 6/5NR ran for 19 hours (5/13 7:30pm – 5/14 2:30pm), and 8/6NR ran for 23 hours (5/14 4:30pm – 5/15 3:30pm).

Data Collection. Lettuce biomass samples, fresh weight (FW) and dry weight (DW), for shoots and roots were collected using two harvests. The first harvest collected five heads and roots from each tub immediately prior to the 7/5R trial while the second harvest removed the remaining 45 heads following the 8/6R trial. Shoots were removed at the top of the rockwool while roots were sliced parallel to and just below the bottom of the rockwool. Nitrogen tests were performed using Hach brand Nessler's reagent for TAN, while nitrate was collected using a Lamotte brand test kit.

Root Zone Conditions. The lettuce was grown in a modified nutrient solution derived from Sonneveld and Straver (1994). The modified nutrient solution has been tested and validated throughout Cornell's extensive 10 years of hydroponic lettuce work (Both et al., 1999). The solution is half concentration of the recommendations from Sonneveld and Straver's lettuce solution sans silicon (Table 20), which was found to perform equally as well as the original recipe while providing other benefits like being able to prepare large batches of concentrates and not requiring daily adjustments of the EC (Both et al., 1997). Further details are provided by Anderson et al. (submitted). Additional nutrient solution for replenishment of water lost by evapotranspiration for the six growing tubs were prepared in 200L HDPE barrels, covered from light penetration when not in use, and transferred to tubs as necessary.

Table 20. Nutrient solution starting concentrations.

Element (mg/L)	Nutrient Solution Target
Macronutrients	
Potassium	215
Calcium	90
Nitrogen: NO3-N	133
TAN	8.8
Phosphorus	31
Magnesium	12
Sulfur	18
Micronutrients	
Iron*	1.1
Manganese	0.140
Boron	0.16
Copper	0.024
Zinc	0.13
Molybdenum	0.02

The pH, EC, and RZT (root zone temperature) were measured daily immediately following the addition of the trial's initial TAN (Table 21Table 22Table 23). The EC was not

adjusted and makeup solution was simply the addition of prepared nutrient solution with pH corrected to 7.1. Table 21 shows the average EC during the four trials and shows the fairly rapidly rising EC from the significant additions of nitrogen from TAN additions, and potassium from the subsequent pH maintenance. Note that the nutrient solution is approximately 1300μS/cm upon creation and does not drift above 1500μS/cm within a typical lettuce growth cycle. As such, the rise in EC is attributed to the additions of nitrogen as ammonium, and potassium, from potassium hydroxide addition to correct the pH.

Table 21. Average EC (μS/cm) during each trial run.

Tub	6/5 w/roots	8/6 w/ roots	6/5 w/o roots	8/6 w/o roots
1	2.3	2.4	2.5	2.5
2	2.4	2.5	2.5	2.6
3	2.4	2.5	2.5	2.6
4	2.4	2.5	2.5	2.6
5	2.4	2.5	2.6	2.7
6	2.4	2.5	2.6	2.6

Likewise, the RZT was not controlled but insulated on the surface with the Styrofoam used for holding the lettuce plants and on all other sides with 5cm sheet insulation covered by 6mm plywood for structure. Table 22 shows the estimated RZT during each of the four trials as an average of the starting and ending temperature and the estimated two to three degrees decrease in root zone temperature during the four day experiment. The decrease in temperature follows the application of whitewash on May 7th following several days of very bright days, and high humidity nights that did not allow the greenhouses to properly cool to night setpoints.

Table 22. Average RZT (°C) during each trail run.

Tub	6/5 w/roots	8/6 w/ roots	6/5 w/o roots	8/6 w/o roots
1	26.8	25.9	24.8	24.3
2	26.9	26.2	24.9	24.4
3	27.1	26.3	25.4	24.9
4	27.4	26.3	25.1	24.6
5	26.2	25.7	24.6	23.8
6	26.7	25.5	24.3	24.2

The pH was corrected daily to 7.0 using 1M KOH (Table 23). Additionally, the use of ammonium bicarbonate for TAN additions functioned to buffer the pH change significantly by increasing the tubs alkalinity in equal proportion to TAN addition.

Table 23. Average pH during each trial run.

Tub	7/5R	8/6R	6/5R	8/6NR
1	6.7	6.6	6.6	6.5
2	6.8	6.7	6.7	6.6
3	6.7	6.6	6.6	6.6
4	6.6	6.6	6.7	6.6
5	6.7	6.6	6.7	6.5
6	6.6	6.6	6.6	6.3

Table 24 displays the average and (standard deviation) for the environmental parameters of all six tubs starting and ending values. Between tubs the environmental conditions were very consistent while the tubs cooling trend from start to finish contributed a significant portion of the variation for the RZT.

Table 24. Average and standard deviation for pH, EC (electrical conductivity, µS/cm), and RZT (root zone temperature, °C) for each trial.

Parameter	6/5 w/roots		8/6 w/ roots		6/5 w/o roots		8/6 w/o roots	
pH	6.7	(0.1)	6.6	(0.1)	6.6	(0.0)	6.5	(0.1)
EC	2.4	(0.0)	2.4	(0.0)	2.5	(0.0)	2.6	(0.1)
RZT	26.8	(0.4)	26.0	(0.4)	24.8	(0.4)	24.3	(0.4)

Analysis. Most analysis was simple algebra and isolation of effects from collected data. However, we also assumed that the nitrification process was a first order reaction with no intercept:

$$\frac{d[A]}{dt} = k[A] \quad (1)$$

Where: k = reaction rate coefficient (RRC)

[A] = TAN concentration

While there is discussion regarding whether the process is a first order or half rate order reaction and whether there are cutoff transitions, the first rate reaction appeared to fit the data well and we do not believe variation would significantly influence the findings.

Results and Discussion

***Nitrification rates by trial*.** The 7/5R and 6/5NR nitrification rates are shown in Table 25, presented on a per tub basis. The raw nitrification results in the system are shown in the second and third columns of Table 25 out of the potential 12ppm for 7/5R and 11ppm for 6/5NR over the 19.5 and 19 hours respectively. The results are absent quantity of any areal quantities.

Table 25. Total TAN removal for the 7/5R and 6/5 NR trial conditions.

	Total TAN removal (mg/L)		Total TAN removal (mg)		Total TAN removal (mg/hr)	
Tub	7/5R	6/5NR	7/5R	6/5NR	7/5R	6/5NR
1	9.5	6.8	4042	2901	207	153
2	7.8	6.6	3334	2815	171	148
3	9.9	7.2	4221	3044	217	160
4	10.3	6.5	4375	2763	224	145
5	9.6	7.2	4085	3074	210	162
6	10.1	7.6	4287	3249	220	171
All Avg	9.5	7.5	4057	3195	208	168

*Conversion to mg/hr used the respective trial times (19.5 hrs for 7/5R and 19 hrs for 6/5NR)

The equivalent results for 8/6R and 8/6NR nitrification rates are shown in Table 26. The raw nitrification of the tubs in the second and third columns is from the total 14mg/L available for nitrification during the 23 hours.

Table 26. Total TAN removal for the 8/6R and 8/6NR trial conditions.

	Total TAN removal (mg/L)		Total TAN removal (mg)		Total TAN removal (mg/hr)	
Tub	8/6R	8/6NR	8/6R	8/6NR	8/6R	8/6NR
1	10.2	8.0	4319	3400	188	148
2	8.8	8.3	3722	3547	162	154
3	10.3	7.9	4371	3360	190	146
4	10.5	7.9	4470	3336	194	145
5	10.1	8.5	4287	3611	186	157
6	9.9	8.5	4192	3630	182	158
All Avg	9.9	8.2	4227	3473	184	151

*Conversion to mg/hr used the respective trial times (23 hrs for both 8/6R and 8/6NR)

Table 27 shows the average response of all four trials. The areal removal rates were 0.61 and 0.53g/m^2/day for 7/5R and 8/6R respectively while the removal rates for 6/5NR and 8/6NR were 0.85 and 0.79g/m^2/day respectively. In both 8/6 trials, the areal removal rates are lower

than the 6/5 trial counterparts, which was surprising given the first order nature of nitrification studies. The lower 8/6 nitrification rates are presented and discussed in more detail later in the paper.

Table 27. A summary of TAN by trial including starting and ending TAN, a time averaged TAN concentration, total areal surface area, total TAN removal, and areal removal rate.

Trial	Starting TAN ppm	Ending TAN ppm	Added TAN ppm	Time Avg TAN ppm	Total SA avail* m^2	TAN removed g	Areal removal rate $g/m^2/day$
7/5R	7.0	2.41	5	3.2	8.4	4.1	0.61
8/6R	8.0	4.05	6	5.4	8.4	4.2	0.53
6/5NR	6.1	3.61	5	3.8	4.6	3.2	0.85
8/6NR	8.0	5.81	6	6.5	4.6	3.5	0.79

*Note the root SA uses average tub SA (Table 11).

The predicted TAN concentrations (using equation 1) were used to identify the average TAN during each experiment (Figure 9. **TAN levels from reaction rate coefficients predictions (calculated from equation 1 and Table 27).**).

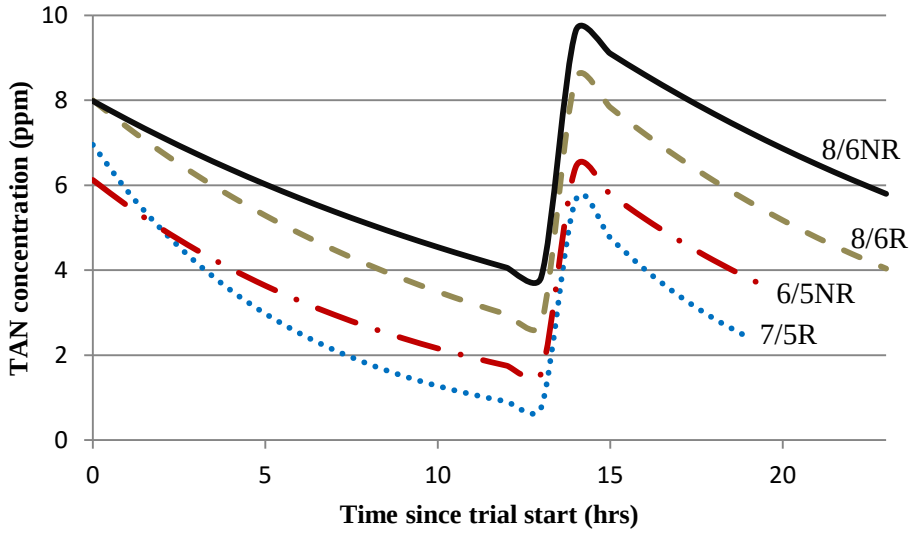

Figure 9. TAN levels from reaction rate coefficients predictions (calculated from equation 1 and Table 27).

Nitrification rates of inert surface trials. From the summary results (Table 27), the nitrification rates of inert surfaces were found to be quite consistent for all tubs within treatments (6/5NR and 8/6NR) (Table 28). The average nitrification rate for 6/5NR was 0.88g/m^2/day and the nitrification rate of the 8/6NR trial was 0.79g/m^2/day.

The results of approximately 0.79-0.88 g/m^2/day for inert surfaces are reasonably in line with the results for a trickling or rotating biological contactor type surface area published by Timmons and Ebeling (2013) of 1-2g/m^2/day for RZTs between 25 and 30°C.

The elevated 8/6 nitrification trials reduced the areal nitrification rate within all tubs by ~10% when we should have seen an increase due the first order reaction nature of nitrification. A decrease in rates may plausibly have been due to protocol differences such as the ~19 vs 23 hours for 7/6R-6/5NR versus 8/6. However our evaluation of the difference in the nitrification period and data suggested this alone could not explain the lower nitrification rates. This can be seen from the data presented in Table 27 by simple observation that the average ending TAN concentration for 8/6NR was 5.8ppm and that the time average TAN concentrations for 8/6 were larger than the 7/5 and 6/5 counterparts. The ending concentration was almost the same as the starting concentration of the 6/5 trials and sufficiently high that the first order nature of nitrification should have seen significantly more nitrification during the 9 hours after the 6ppm spike. If the 8/6 trials were simply spending the additional 3.5-4 hours finishing a low concentration of TAN, we would not see higher average TAN concentrations or final concentrations close to starting concentrations in the lower TAN addition conditions. A final comparative look at the reaction rate coefficients fit to the starting, ending, and 14 hour respective TAN spikes was performed. The reaction coefficients were 0.105/hr for 6/5NR and

0.0548/hr for 8/6NR, showing that the 8/6NR were approximately half the magnitude of the 6/5NR trial.

Table 28. Nitrification rates of inert surfaces from the 6/5NR and 8/6NR trial results.

	6/5NR		8/6NR	
Tub	$mg/m^2/hr$	$g/m^2/day$	$mg/m^2/hr$	$g/m^2/day$
1	35.7	0.86	32.0	0.77
2	34.7	0.83	33.7	0.81
3	37.4	0.90	32.0	0.77
4	34.2	0.82	31.2	0.75
5	37.7	0.90	34.2	0.82
6	39.9	0.96	34.1	0.82
All Avg	36.6	0.88	32.8	0.79

*Total inert SA per tub = $4.6m^2$

Nitrification rates in trials containing the lettuce plants and roots. As shown in Table 27, a raw look at the areal nitrification rates appear to be smaller than the nitrification rates of the inert surfaces trials by up to 33%. This may be due to many reasons including: that the quantity of SA from inert and roots was too large for the protocol TAN additions and did not capture the potential nitrification rates; the delay from new root growth and time required for the existing nitrifying communities to colonize the newly available surface area, which created a pseudo-enlarged root SA; a lower rate due to the larger area; and cumulative effects from dissimilar plant root responses to nitrifying communities then to inert surfaces such as the HDPE tub and sheet styrofoam.

To investigate the 7/5R and 8/6R trial results, we first looked at isolating the inert nitrification results by using the inert reaction coefficients 6/5NR for 7/5R, and 8/6NR for 8/6R. The reaction coefficients of all trials were 0.138, 0.08, 0.105, and 0.0548/hr for 7/5R, 8/6R, 6/5NR, and 8/6NR respectively, which also provided the hourly projected TAN concentration used subsequently. Note the assumption of no significant removal of TAN by the plant was

assumed, which we believe is a fair assumption based upon our calculations that a 0.016ppm reduction in concentration would occur as the result of TAN being assimilated into the plants themselves. The TAN removal assumption can be viewed in more detail in the plant nitrogen removal section. If assimilation and TAN removal from our plant nutrient solution was negligible then nitrification mechanisms on the roots were the next most likely source. We can safely assume the nitrification reactions were not diurnal in nature given an absence of light in the root zone and minor or negligible variations in dissolved oxygen (DO) and RZT such that RRCs and their application were constant through time. Our last assumption was that the inert RRCs were the same between the trials, which we justified given the protocol setup that conducted the inert trials without any time delay following the root trials in the same tubs with the same or nearly identical TAN dosage regimes.

The inert reaction rates were thus used to account for TAN removal by the inert surface areas instead of the hourly projected TAN concentrations for the 7/5R and 8/6R trials. This assumption resulted in an estimate of 88% of the TAN removal in 7/5R being attributed to the inert surfaces while 74% was attributed in the 8/6R trial. Extrapolating the TAN removal associated with the plants only, 0.9ppm and 1.76ppm of the 9.5 and 10.0ppm total nitrification respectively for 7/5R and 8/6R, the areal nitrification rate of lettuce root SA alone was 0.11 and 0.21g/m^2/day for the 7/5R plant only and 8/6 plant respectively.

Biomass and root surface area results. The biomass data for the lettuce is displayed for the lettuce shoots (head) in Table 29 and roots in Table 30. “Start” signifies the average of the 5 lettuce plants per tub harvested immediately prior to the start of the nitrification testing while “End” is the average of the 45 plants (shoots and roots) per tub harvested after the second lettuce head trial just prior to the start of the nitrification trials for the tubs with inert surfaces only.

Given the variability in both starting & ending weights, as well as the tub averages themselves (some of which can be explained by the variable DW/FWs) dry weights were primarily used to compare growth and nitrogen content changes in this experiment. The average starting DW was 2.6g with an average increase of 8% over the two days prior to harvest. Also note that the average dry matter content we see with this cultivar of bibb lettuce and in this system is 0.041g/g, which would equate to a 68g FW average for the lettuce in this experiment (Anderson et al., submitted).

Table 29. Biomass summary data for individual lettuce head (shoots) start, end, and percentage change including fresh weight (FW, g), dry weight (DW, g), and dry weight to fresh weight ratio (DW/FW, g/g or dimensionless) parameters.

	Start			End			% Change		
Tub	FW	DW	DW/FW	FW	DW	DW/FW	FW	DW	DW/FW
1	51	4.0	0.082	70	4.0	0.053	38%	2%	-35%
2	22	2.2	0.103	39	2.4	0.063	76%	9%	-38%
3	34	3.0	0.090	49	2.8	0.060	45%	-7%	-33%
4	21	2.1	0.102	32	2.6	0.068	52%	21%	-33%
5	21	2.3	0.107	34	2.7	0.073	59%	20%	-32%
6	23	2.2	0.096	36	2.5	0.069	56%	13%	-29%
All Avg	29	2.6	0.096	43	2.8	0.065	51%	8%	-33%

We observed some variability in the root data as well attributed to the increased variability in the plants and the small starting sample size and thus the analysis is ultimately focused on the average of all tubs as a measure of growth that occurred during this period. The average starting root FW was 7.3g, with a DW of 0.43g, and a DW/FW of 0.060g/g while the average ending root FW was 8.3g, with a DW of 0.48g, and a DW/FW of 0.058g/g. The percentage increase between the starting and ending root weights was 14% for FW, 10% for DW, and -3% (3% decrease) for DW/FW.

Table 30.Biomass summary data for individual lettuce roots start, end, and percentage change including fresh weight (FW, g), dry weight (DW, g), and dry weight to fresh weight ratio (DW/FW, g/g or dimensionless) parameters.

	Start			End			% Change		
Tub	FW	DW	DW/FW	FW	DW	DW/FW	FW	DW	DW/FW
1	11.1	0.62	0.056	11.3	0.58	0.051	2%	-6%	-8%
2	5.5	0.34	0.061	7.8	0.40	0.051	41%	18%	-17%
3	8.5	0.46	0.054	8.3	0.38	0.046	-1%	-17%	-16%
4	6.2	0.40	0.065	7.3	0.48	0.065	19%	20%	1%
5	6.2	0.36	0.058	8.1	0.58	0.072	29%	61%	24%
6	6.4	0.42	0.065	7.1	0.44	0.062	10%	5%	-4%
All Avg	7.3	0.43	0.060	8.3	0.48	0.058	14%	10%	-3%

Note that the lettuce heads were smaller in proportion to the root mass than what we have typically seen: Shoot DWs ~6.6g, and DW roots ~0.34g (Anderson et al., submitted). The root masses were at the typical size for a full size lettuce head as is the case in Tub 1, or approaching the typical size for a full size lettuce head as is the case in the other 5 tubs. While the shoot masses are atypical, the experimental design captured the primary value of interest, root nitrification rates.

The root surface areas were calculated using predictive equations developed by our research group using WinRHIZO[11] software with the same hydroponics system and on the same *flandria* lettuce cultivar (Schwartz et al., submitted). The SA of the roots are shown in Table 31 using equation 2 below.

$$SA_{root} = 70.8 * FW_{root} + 243 \quad (2)$$

FW_{root} = fresh weight of the root sample (g)

SA_{root} = surface area of the root (cm^2)

[11] WINRhizo (Pro 2007), Regent Instruments, Inc., 2672 Chemin Sainte Foy, Quebec, QC G1V 1V4, Canada

Table 31. Average predicted total root surface area of the lettuce roots by tub (m^2).

Tub	SA_{root}
1	4.8
2	3.7
3	3.7
4	3.6
5	3.7
6	3.3
All Avg	3.8

Nitrogen transport mechanisms and regulation for plants. In consideration of nitrate and TAN uptake form solution, we need to identify critical plant uptake mechanisms and how those mechanisms may influence mass balance or calculations of N flow throughout this system. Plant species adapted to oxygenated root zones appear to perform better when the majority of the nitrogen source in the root zone is nitrate (Masclaux-Daubresse et al., 2010). A review of nitrate and TAN uptake literature showed that nitrate is taken in by the roots by low affinity transport systems (LATSs), which operate continually and are the predominant systems when the nitrate in the root zone is sufficient, and high affinity transport systems (HATSs), which are up-regulated when external nitrate concentrates are low (Masclaux-Daubresse et al., 2010).

Ammonia and ammonium transports (AMTs) are currently less clear than the nitrate transports; however the general understanding currently is that plants may uptake through three mechanisms. The unionized NH_3 form (ammonia) is semi-permeable through membranes, and at pH 7, as is frequently seen in Aquaponics, the circa 1% unionized ammonia in solution may constitute a non-negligible portion of the AMTs total uptake (Ludewig et al., 2007). Similar to the nitrate transporters, the AMTs also include LATSs and HATSs. Under the sufficiency of nitrogen in the root zone and under most conditions, basal productions of AMTs' LATSs are produced, and that both the AMTs' LATSs and HATSs are up-regulated in low and deficient root zone nitrogen concentrations (Ludewig et al., 2007).

Nitrogen uptake assumptions. Given the both high nitrate concentrations in our root zone and high nitrate to TAN ratio, we assumed the following: nitrate was primarily up-took by the LATSs; that the nitrate HATSs was down-regulated and thus a non-significant mechanism; that the AMTs were at ambient expression due to nitrogen sufficiency; and that nitrogen uptake was most likely close to a true non-selective proportional uptake. Finally, we may conjecture that in higher TAN concentrations in the solution such as this experiment, nitrification to nitrate is the predominant TAN removal mechanisms for hydroponic lettuce. There is also the option for nitrate being produced from nitrifiers on the roots is immediately being up taken by the plants but given the 1ppm total N removal from the system,[12] we believe this process to be negligible. We also identified that 74 to 88% of the nitrification was occurring on the inert surfaces and were not assimilated by the plants. Note that many aquaponic systems run at much lower TAN and nitrate concentrations so these assumptions are intended to be for analysis only and not conjecture to aquaponics systems in general; a conversion of the findings will be applied to a aquaponics system sizing in more depth later.

Plant nitrogen removal. The average nitrogen content of the lettuce was 3.83% nitrogen (Table 32). Biomass data from Tables 1 and 2 are worked through equations 3-6 to estimate 0.46g total nitrogen was assimilated into the lettuce during the 7/5R and 8/6R trials, per tub.

[12] $\Delta DW=0.2g*.038\%\ nitrogen*45\ heads/425L/tub$

Table 32. Total nitrogen and carbon content of plants harvested by tub (% w/w from DW).

Tub	Total N (%)	Total C (%)
1	4.29	37.01
2	3.86	37.96
3	3.81	37.72
4	3.75	37.89
5	3.47	38.51
6	3.77	38.51
All Avg	3.83	37.93

$$\Delta DW_{head} = \left(DW_{head\ per\ tub\ start} * (\%\Delta DW_{head} + 1\right) + (DW_{root\ per\ tub\ start} * (\%\Delta DW_{root} + 1) \quad (3)$$

$$\Delta DW_{head} = 0.25 g/head \quad = (2.63 g/head * 8\%) + (0.43 g/head * 10\%) \quad (4)$$

$$Total\ N\ incorporated\ into\ plant = \Delta DW_{tub} * \%N_{avg} * \frac{heads}{tub} \quad (5)$$

$$Total\ N\ incorporated\ per\ tub = \ 0.43 g\ Total - N/tub \ = \frac{0.25\text{g}}{\text{head}} * 3.83\%\ N * 45\ \frac{heads}{tub} \quad (6)$$

If we take the time average TAN concentration from Table 3 and compare it to the nitrate-N concentrations, we see 1.3-2.2% of total nitrogen is TAN (Table 33). Thus, the average percentage TAN concentration during the root portion of the trials was 1.6%.

Table 33. Average TAN and nitrate solution concentrations.

Trial	Time Avg TAN	Avg Nitrate	Avg TAN proportion in solution
	ppm	ppm	ppm/ppm
7/5R	3.2	251	0.013
8/6R	5.4	260	0.020
6/5NR	3.8	270	0.014
8/6NR	6.5	279	0.023

Given the prior assumption of non-selection proportional uptake of nitrogen, 1.6% of the 0.43g per tub total nitrogen is 6.88mg TAN removed per tub in the two days of 7/5R and 8/6R. A total removal of 6.88mg TAN per tub over the two days is a concentration reduction within the tubs of only 0.016ppm, supporting the assumption that TAN removal by the plants is relatively small for the two day testing period and for a tub growing area of 1.4m^2 of moderate sized lettuce.

Identification of the nitrate uptake by plants could not be readily identified from the data due to the small uptake by the plants (~1ppm), the large changes in solution nitrate (19-20ppm over the two days), and the natural variability in the measurements (both from the testing absorbances and the large tub size) (Table 34).

Table 34. Change in nitrate concentration during each trial in ppm.

Tub	7/5R	8/6R	6/5NR	8/6NR	Total Change
1	10	11	10	11	42
2	8	8	8	10	34
3	10	9	11	13	43
4	11	8	12	11	40
5	8	13	6	11	39
6	9	10	7	11	37
Avg	9.4	9.6	9.0	11.1	39

System Sizing

Hypothetical calculation of TAN movement in full-scale hydroponic facility. As mentioned previously, Bibb lettuce is usually grown on a rotational basis. The approximate time elapsed during a single rotation is 35 days assuming an average of 17 moles/m^2/day of natural and supplemental light (Both et al., 1997). This can be broken down into three stages of growth: the seedling phase where germination and initial growth occurs and the beginnings of light

competition dictate economic benefits to transplant into the next spacing (10 days); the nursery phase, where plants are first placed into a raft system (10 days); and the final grow-out phase, which finishes with full sized, harvested lettuce (15 days). Each of the three stages has its own, unique spacing, based on plant size (Table 35).

Table 35. Bibb lettuce stages, timing, and spacing for commercial hydroponic production with supplemental lighting.

Stage	Age at end, days	Mass/plant, g	Spacing, plants/m^2
Seedling	10	2	1550
Nursery	20	20	78
Grow-out	35	150	24

Our previous correlation of plant shoot FW to root SA (Equation 2) will allow us to size a plant system based on fish feed input. One of the assumptions is that the plants at nursery stage comprise a negligible amount of the total surface area (since plant mass is ~1% of the harvest plant mass) resulting in an insignificant amount of nitrification and/or nitrogen uptake when compared to the more mature plants.

We calculate the weighted average plant size in the system by using 10 years of hydroponic lettuce cv. Vivaldi growth curves to predict the size of the head at each day (Figure 10) (Both et al., 1999), averaging the FW prediction within each plant spacing stage, and weighting the FWs to spacing densities (Table 36). The DWs were converted to a FW basis assuming a 4.3% dry matter content, which fit to an average harvested head size of 150g, which provides a margin above the target 5oz FW head such that the vast majority of heads are of sufficient size at harvest. The root FW predictions also reflect a 2x multiplied data set prediction of the cv. *Vivaldi* results to fit more closely with the root FWs seen in our group's cv. *Flandria* work as our cv. *Flandria* roots are twice as large.

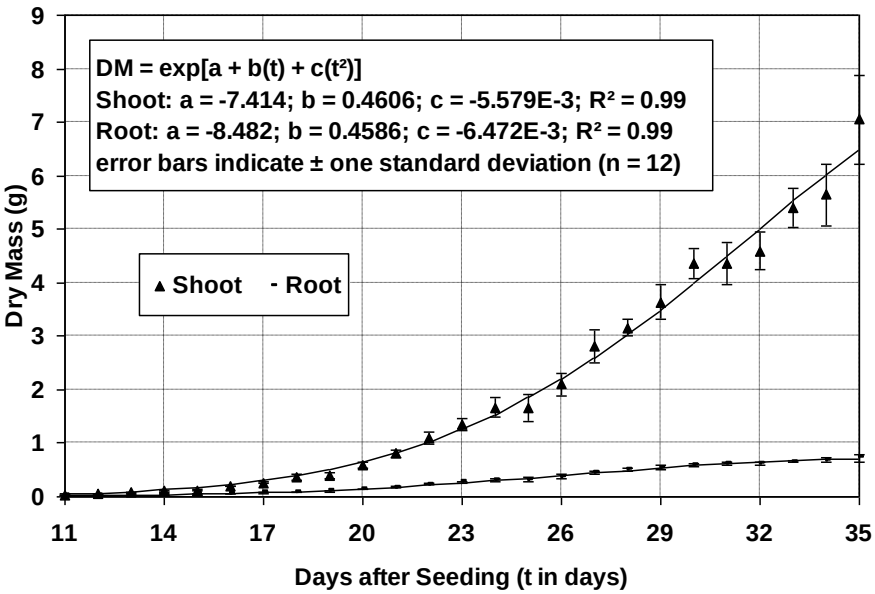

Figure 10. Root and shoot growth of lettuce plants (cultivar Vivaldi) grown in a floating hydroponics system under a daily integrated light level of 16 mol-m-2-d-1. (Both et al. 1999)

Table 36. Average FW shoot and root weight by stage of lettuce production cv. Vivaldi (g).

Stage	Shoot	Root	Root x2 **
Seedling	0.3	0.3	0.5
Nursery	14.1	0.4	0.7
Grow-out	99.8	1.9	3.8

*Assumes 4.3% dry matter content

**Simply root prediction multiplied by 2

Nitrification on root surface. The TAN removal can be from nitrification or assimilation into the plant. We'll first identify the quantity of nitrification on the root surface using example facilities nitrogen concentrations.

Estimating from equation 1, the predicted root SAs from the biomass includes from the root area $70.8 * 0.4 + 243 = 271 \frac{cm^2}{plant} = 0.0271 \frac{m^2\ root\ SA}{plant}$ for the nursery plants and $70.8 * 1.9 + 243 = 378 \frac{cm^2}{plant} = 0.0378 \frac{m^2\ root\ SA}{plant}$ for grow-out plants. The 2x multiple root FWs would predict $70.8 * 0.7 + 243 = 293 \frac{cm^2}{plant} = 0.0293 \frac{m^2\ root\ SA}{plant}$ for the nursery plants and

$70.8 * 3.8 + 243 = 512 \frac{cm^2}{plant} = 0.0512 \frac{m^2\ root\ SA}{plant}$ for grow-out plants. This gives us a range of root SAs from 271-293cm^2/plant for nursery plants and 378-512cm^2/plant for grow-out plants.

To calculate the square meters required for a specific load we need to convert the identified reaction rate information to that more closely represented in aquaponics systems, namely 1ppm TAN (Timmons and Ebeling, 2013). Reaction rate kinetics for the roots only were identified by using the quantity of TAN removal associated with the plants and fitting the reaction coefficients to fit to the final concentrations. [13]

The 7/6R plant removal only reaction coefficient was 0.0097/hr and 0.0113/hr for 8/6R, which we averaged to 0.0105/hr for the root only RRC given the difference between readings was very small. Given the assumption of negligible TAN assimilation and removal as TAN from the solution in our experiment, these reaction coefficients are assumed to be entirely nitrified on the root surface. We'll assume that the TAN concentrations are 1ppm constantly without any hour to hour variation and that the reaction rate coefficients identified are reasonable approximations for a 1ppm solution. Converting the RRCs to a single plant's average root SA instead of the 3.8m^2 currently fit implicitly to the RRC, we see the root only RRC become $7.2 \cdot 10^{-5}$/hr for nursery plants and $1.3 \cdot 10^{-}4$/hr for 8/6R's RRC (implicitly on a per plant basis currently).

Applying the plant spacings (Table 35) and average root surface areas (Table 36), we have 0.0056/hr on an average square meter basis for the nursery tub, and 0.0032/hr on a square meter basis for the grow-out tubs. Given the constant 1ppm TAN assumption, the plant nitrification portions are simply 0.13 mg TAN nitrified/m^2/day for nursery spaced and sized roots and 0.08 mg TAN nitrified/m^2/day for grow-out plant spaced and sized roots. Given the ratio of

[13] Previous RRCs were 0.138, 0.08, 0.105, and 0.0548/hr for 7/5R, 8/6R, 6/5NR, and 8/6NR

3.25m^2 in the grow-out tubs to every m^2 in the nursery tub, assuming every plant is transplanted from the nursery tub, we can establish an average m^2 nitrification for plant roots of 0.09mgTAN/hr/m^2 on average pond sized roots.

Mass balance removal of TAN. In a continuous system where the same quantity of heads are produced every day of the year, the problem may be simplified from a tub basis to a simply proportion of nitrogen source multiplied by the quantity of heads and their average DWs. The quantity of heads becomes the target design variable, the DW content of each harvestable plant was about 7g, calculated from approximately 6.5g/head + 0.5g/root. The total nitrogen content of our flandria lettuce was 3.8%, so we'd project 0.266g total nitrogen assimilated per full size 150g lettuce head.

The TAN concentration for aquaponics systems may frequently be stable around 1ppm and an example nitrate concentration from an aquaponics system is 40ppm (Timmons and Ebeling, 2013). If we maintain the proportional uptake assumption from the discussion before, then 2.5% of the nitrogen uptake is TAN and every lettuce assimilates 6.65mg TAN during its growth. However, the nitrate concentration is fairly low in this case and it is entirely possible that the AMTs are upregulated and additional transports are created on the root surfaces. As such we ran a sensitivity comparison looking at 5 and 10% uptakes for comparison. The uptake of TAN is 13.3mg TAN/plant for 5% uptake and 26.6 mg TAN/plant for 10% uptake from a total uptake of 266mg.

System sizing bringing together mass basis and surface area removals. Initial Assumptions:

1) We would like to support 1000 kg of fish.

2) Required daily fish feed is 1% of total fish weight, or 10 kg/day

3) As a “rule of thumb” in the aquaculture industry, 1 kg of fish feed produces 0.03 kg or 30 g of TAN

4) Assume the removal of TAN by the seedling stage is negligible for sake of footprint calculations

5) TAN concentrations are steady at 1ppm

6) Nitrate-N concentrations are steady at 40ppm.

For a system requiring removal of 300g total nitrogen a day and each head removing an average of 0.266g total nitrogen, we can support a greenhouse producing 1128 heads of lettuce a day. The assumption numbers were intentionally fit to both be simple and round numbers and to fit closely to 1000 heads of lettuce/day, which was the marginal production for a small family producing farm (or 2-3 full time employees) to support themselves. This general lower bound value was identified by the Cornell Controlled Environment Agriculture group (for photos of the 750m^2 demonstration greenhouse that produced 945heads/day, see Both et al., 2014). A 900m^2 facility would be sufficient area for the lettuce production given the demonstration greenhouse ratios. The feed rate per m^2 of lettuce would thus be 10kg per 900m^2 of lettuce or 11g of feed per m^2 of lettuce given the nursery and grow-out spacing described. 11g feed/m^2 lettuce is much smaller than the 60g feed/m^2 lettuce Rakocy recommends in the Recirculating Aquaculture book (Timmons and Ebeling, 2013).

Economically, if we sell each lettuce head at $1.50, our lettuce production accounts for $617k a year gross sales. We might expect to see 2-3x the carrying capacity of the fish system. If we produced and sold tilapia in the RAS system, we might expect to sell 2500kg of tilapia.

Assuming a sale value of $6/kg (live value), we expect the fish system to account for 15k of the gross yearly sales or 2.3% of the yearly sales.

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