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Growth and Tissue Elemental Composition Response of Butterhead Lettuce (*Lactuca sativa*, cv. Flandria) to Hydroponic Conditions at Different pH and Alkalinity

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Received: 28 April 2017; Accepted: 29 June 2017; Published: 13 July 2017

Abstract: Biomass and tissue elemental differences were quantified for lettuce grown in deep-water conventional hydroponic conditions at two pH and alkalinity conditions. Nutrient solutions were created using inorganic salts and either reverse osmosis (RO) water or municipal water with high alkalinity. Three treatments were evaluated: (a) nutrient solution created with reverse osmosis (RO) water and maintained at pH 5.8 (H5); (b) same as H5 but maintained at pH 7.0 (H7); and (c) nutrient solution created using municipal water and maintained at pH 7.0, referred to as HA7. Averaged across three trials, the HA7 and H7 treatments produced 26% less shoot fresh weight (FW) than the H5 treatment with an 18% reduction in dry weight (DW). The H5 treatment had the least biomass in root FW and DW. In tissue elemental analyses, both the pH 7.0 treatments showed lower concentrations than H5 in Cu, N, Mo, and Sr, and increased concentrations in Ba, Mg, Na, and Zn. There were no differences in Al, C, Ca, Fe, K, Mn, Ni, P, S, and Si concentrations among treatments ($p = 0.05$). The results from this experiment can be used to isolate the effects of pH and alkalinity in aquaponic conditions where pH and alkalinity will mimic HA7 conditions.

Keywords: hydroponics; aquaponics; pH; biomass; nutrient analysis; lettuce; tissue analysis; elemental composition

1. 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 media 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 via nutrient film techniques [1,2]. In recirculating aquaculture systems (RAS), aquatic organisms are fed and raised in carefully controlled tank systems employing biological filtration to oxidize toxic nitrogenous wastes to nitrate [3]. Aquaponics combines hydroponics and RAS, enabling multiple uses of resources such as water and nutrients, while sharing infrastructure, management, and labor costs [3–6].

The macronutrients and micronutrients required by hydroponically grown plants are formulated to meet the needs of particular crops. In this study with lettuce, the solution formula employed was derived for lettuce by Sonneveld and Straver [7]. In earlier research, the original lettuce formula was equally as effective at half the concentration recommended by Sonneveld and Straver [8], and consequently was used at half-strength in the present work (electro conductivity ca. 1300 µS/cm).

We further modified the nutrient solution by eliminating silicon, which is specified at 0.5 mM in the original Sonneveld and Straver recipe, since silicon is not an element essential to yield in the absence of stress [9]. All references to the nutrient solutions in this paper refer to Table 1, which gives elemental concentrations for both starting and ending concentrations by trial and treatment.

Table 1. Nutrient solutions starting, ending, and target concentrations, averaged by treatment (H5 = hydroponic conditions at pH 6.8; H7 = hydroponic conditions at pH 7.0; HA7 = hydroponic conditions at pH 7.0 and higher alkalinity).

Element, mg/L	H5		H7		HA7		Nutrient Solution
	Start	End	Start	End	Start	End	Target
Macronutrients							
K	215	227	232	296	222	292	215
Ca	87	109	87	79	74	71	90
N: NO3-N	144	130	143	134	105	113	133
N: TAN	10	0.25	10	0.11	12	0.26	8.75
P	30	31	30	18	30	19	31
Mg	12	13	12	12	8	9	12
S	16	18	16	18	16	17	18
Micronutrients							
Fe	1.10	1.19	1.05	0.94	0.95	0.82	1.12
Mn	0.13	0.03	0.12	0.03	0.13	0.02	0.14
B	0.15	0.20	0.20	0.19	0.20	0.18	0.16
Cu	0.030	0.035	0.031	0.034	0.038	0.050	0.024
Zn	0.15	0.14	0.15	0.17	0.18	0.17	0.13
Mo	0.021	0.023	0.021	0.026	0.029	0.028	0.024
Other Elements							
Na	3.5	4.7	3.6	4.7	23.5	28.0	0.0

The pH of the nutrient solution affects the solubility and thus availability of certain elements, particularly iron and phosphorus [1,10]. Moderately low pH (e.g., 5.8) keeps most ions available in a solution while a higher pH (e.g., >6.5) can cause nutrient deprivation due to nutrient precipitation and depletion [10]. Additionally, there may be an electrochemical burden in moving excess ions across membranes as discussed by Barber [11]. The modified Sonneveld solution used in this research was originally designed to be maintained between pH 5.0 and 6.0 [7]. This range overlaps the "ideal" target pH of 5.8 recommended by Bugbee [10] for hydroponic solutions. Even within this range, iron needs to be chelated to avoid oxidation, becoming insoluble, and precipitating. The pH of the solution also affects how much energy must be expended by the plant to import ions across the cell membrane and the tonoplast against electrochemical gradients. In many cases, protons (H^+ ions) are used in active co-transport of ions across membranes. The concentration of H^+ ions in the nutrient solution affects energy requirements for nutrient uptake and transport [12].

Alkalinity is an important water quality parameter that needs to be closely monitored in aquaculture systems since alkalinity is "consumed" during the nitrification process [3]. Alkalinity is a measure of the pH buffering and acid neutralizing capacity of aqueous solutions; it is the sum of soluble alkaline species in water capable of being neutralized by hydrogen ions [13]. Bicarbonate ions (HCO_3^-) make up the bulk of alkalinity in hydroponic or aquaponics solutions, buffering pH shifts due to acid addition or generation [3]. In hydroponic systems, if the makeup water added to replace evapotranspiration is highly alkaline, the added carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) may exceed the system's usage. When this is the case, pH will rise and may reduce availability of elements such as iron, potassium, and phosphorus [14].

RAS conditions are usually quite different from those of hydroponic systems, since fish will be grown in a fairly narrow range around pH 7.0 for freshwater systems [3]. Alkalinity in a RAS is continuously consumed by nitrifying bacteria and thus needs to be replaced (1 g of ammonia-nitrogen

converted to nitrate-nitrogen requires a total of 3.57 g equivalents of $CaCO_3$ of alkalinity [3]). Safe target levels for alkalinity in RAS are 70 to 190 mg/L to provide an adequate buffering capability for systems that are typically managed for target values of pH 7.0 and 15 mg/L for dissolved carbon dioxide. Fish respiration and heterotrophic bacterial metabolism are primary sources of elevated aqueous carbon dioxide concentrations in RAS systems, which lead to higher concentrations of dissolved carbonate species. The pH 7.0 setpoint in turn shifts much of the non-contributory carbonic acid to the bicarbonate form, and the increased concentrations of carbonate species from the increased aqueous carbon dioxide further increases the alkaline buffering capacity of these systems. The alkaline buffering capacity of a system can be further increased at the pH 7.0 set point as non-contributory carbonic acid shifts to the bicarbonate form. Alkalinity can continue to an increase as the concentration of carbonate species from carbon dioxide is continually replaced by respiration or atmospheric carbon dioxide (again assuming base is added to maintain a constant pH).

A coupling of hydroponic and RAS fish systems appears to face a challenge in that the normal water quality conditions are quite different, particularly for pH and alkalinity. The objective of this research was to quantify the response of a commonly grown hydroponic crop, lettuce (*Lactuca sativa*, cv. Flandria), in typical hydroponic conditions (pH 5.8 and low alkalinity) and aquaponic-like conditions (pH 7.0 and moderate alkalinity) using only inorganic nutrients. In future research, conventional lettuce hydroponics will be compared to lettuce grown in a fish system coupled to a hydroponic system using organically supplied nutrients at a pH of 7.0 (aquaponics).

2. Materials and Methods

An experiment consisting of three trials was conducted in a conventional glass greenhouse to investigate the effects of pH and alkalinity upon the growth and tissue elemental content responses of butterhead lettuce (*Lactuca sativa*, cv. Flandria). The pH treatments 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 municipal 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 g per head fresh weight. Details of the experiment are provided below.

2.1. The Greenhouse Description

Experiments were conducted in a middle section of a glass greenhouse range, with section dimensions of 7 m × 10 m × 7 m to the ridge, oriented east west (Figure 1). 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 mol/m^2/day) by controlled use of a supplementary lighting array. The total PAR received was recorded by a LiCor quantum sensor placed at a representative place at plant canopy level. 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, 400 W clear S51/O, Mogul Base rated ED18 HSP, LU 400/H/ECO). The light fixtures were arranged to provide reasonably consistent light within the growing area. Whether supplemental lights were on or not was recorded, and was used to calculate what part of the DLI was natural versus supplemental. A target DLI of 21 mol/m^2/day was used for Trial 1 and was reduced to 17 mol/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 115,000 kJ/h were used to provide air mixing and to raise air temperatures when necessary to target values. Carbon dioxide was at uncontrolled ambient levels.

2.2. Growing System and Procedure

Six high-density polyethylene (HDPE) growing tubs with dimensions of 1.82 m × 0.91 m, a depth of 0.30 m, and a holding volume of 425 L were centered within the greenhouse under the light array. Tubs were elevated such that the tops of the floating rafts were 1.31 m above the floor and 1.26 m 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 25 mm in thickness with 25 mm round holes for plant plugs spaced at 200 mm 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 (24 L/min) mixed the water in the tubs at a rate equal to a hydraulic retention time of 18 min. To take advantage of the bilateral symmetry of the greenhouse and minimize any effects due to the airflow within the greenhouse, the tubs were numbered and paired into two blocks as follows: tubs 1–3 were block 1, and tubs 4–6 were block 2 (Figure 1 and Table 2). Treatments were rotated through the two blocks during the 3 trials.

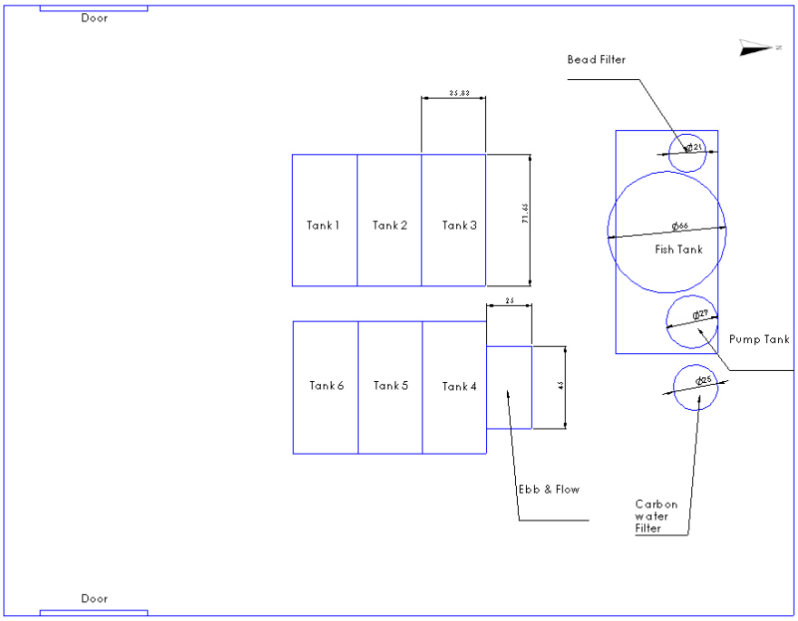

Figure 1. Greenhouse experiment floor plan (drawn to scale).

Table 2. Experimental physical arrangement by trial and block.

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

For each trial, two 200-plug rockwool sheets (Grodan AO25/40, 25 mm) were seeded. A single pelleted seed of butterhead lettuce was placed into each plug. Care was taken to place the seeds horizontally at the same depth in the rockwool cavity after which the planted seeds were misted with reverse osmosis (RO) water in several passes so that all pellets were equally saturated. Two standard thermoformed perforated 1020 trays were used for holding the two 200-plug rockwool sheets. The trays were germinated in the same greenhouse space as the growth experiment (Figure 1). For the first 24 h, the trays were covered with clear rigid plastic germination covers and shielded from light to control temperature and humidity. After 24 h, as seedling emergence occurred, the plastic covers were removed and the trays were placed in an ebb and flood system under the supplementary light array where they were grown for the next 10 d (Figure 1). The ebb and flood system cycled four times per day using 15 min 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 on day 7, when the first true leaf was approximately 10 mm 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 d (35 d from seeding). The seeding, transplanting, and growing procedures were the same for all trials and treatments. A preliminary trial was used to test for uniformity of greenhouse conditions that is described later.

2.3. Water Quality Treatments

Three treatments were tested: (a) nutrient solution using reverse osmosis (RO) water with a target 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 municipal water of high alkalinity and a target pH of 7.0 (referred to as HA7). Adjustments to pH were made daily using 1 mol/L KOH or 1 mol/L HNO_3.

Alkalinity for the trials was estimated based upon an independent bench-scale test using three-liters of respective solutions in triplicate for each of the treatment conditions. Alkalinity was measured by titrating to a pH endpoint of 4.5 using 0.02 N (0.01 M) sulfuric acid to an accuracy of $\pm$4 mg/L as $CaCO_3$. In the bench scale testing, prepared nutrient solutions were vigorously aerated to strip or eliminate any excess CO_2 while 0.1 mol/L HNO_3 was added concurrently to achieve respective experimental target pH levels. Equilibrium of pH was rapidly achieved in approximately one hour due to the vigorous air stripping of the water. Target pH values were reconfirmed after 24 h of continuous additional air stripping; there was no evident need for further addition of acid or base.

The resulting alkalinities at equilibrium were approximately 20, 40, and 40 mg/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 their low initial alkalinity values. In the HA7 water conditions, the municipal water typically had an initial pH of 7.0 and a starting alkalinity of 120 $\pm$ 8 mg/L $CaCO_3$. Based upon the level of aeration employed in the tubs, the excess alkalinity in HA7 was probably consumed over the first few days of the experiment to reach its final equilibrium value, which was measured as 40 mg/L $CaCO_3$.

Electroconductivity (EC) was monitored but not controlled and was 1300–1500 µS/cm. Compared to H5, the H7 condition started 100–150 µS/cm higher due to the addition of the KOH in the pH adjustment, while the HA7 started at 100–200 µS/cm higher EC due to the additional dissolved ions present in the municipal water. Typically, the nutrient solution EC did not drift during a trial the pH 5.8 treatment, while additional water chemical equilibrium processes that occurred in pH 7.0 treatments resulted in the EC stabilizing at higher values. A 50–100 µS/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.

2.4. Nutrient Conditions

For all trials, the six tubs were each filled to 90% of their capacity with 425 L of lettuce nutrient solution (Table 1; starting values based upon average of Trials 2 and 3 only; average ending values are based on all 3 trials). Solutions were prepared by adding 2.125 L 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 100× concentration level of their diluted end point concentrations in the nutrient solution. Additional nutrient solution for replenishment was prepared in 200 L 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 the target concentrations (Table 1). The HA7 treatment used carbon filtered municipal water that had average macro-elemental values of 50 mg/L Ca, 13 mg/L Mg, 5.5 mg/L S, and an EC of 450 µS/cm. As a result of these macro elements being present, the concentrations of the stock solutions were adjusted to achieve the targeted final nutrient concentration values in each trial. Sequentially, the micro-elements and chelated iron were added first 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. For example, potassium concentrations were made 10% higher in a particular trial so that the nitrate concentration was only 10% below the target nutrient solution concentrations instead of 16% lower.

2.5. Water Environment and Greenhouse Conditions

Root zone temperature (RZT, °C) and pH data are given in Table 3 by trial and treatment (data collection methods in Section 2.6). Average RZTs (SD, N = 45) 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 values (SD, N = 45) 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 3. 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	5.77 (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, °C) by trial are presented in Table 4. RH and air temperature 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), which would address these changes over the trials in NL, SL and DLI.

Table 4. Greenhouse conditions (mean and standard deviations (SD)) per 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, %), and air temperature (AT, °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)

2.6. Experimental Design, Data Collection, and Analysis

Two blocks of three tubs were allocated to each of the three treatments in each of the three trials (Table 2). A preliminary trial, 16 November–20 December 2013, was conducted to test for any block effect in the intended tub layout for this experiment. After establishing that no positional, individual tub, or blocking effects occurred in the preliminary trial, three sequential trials were conducted over a period of four months in 2014: Trial 1 was between 21 February and 28 March; Trial 2 was between 19 March and 23 April; and Trial 3 was between 16 April and 21 May. The end result was that over the course of the 3 trials, each of the six tubs was used for each of the three treatments two times.

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 overlapped because the plants were seeded and remained in the ebb and flood system while plants in the previous trial were in the final days of their production cycle.

During Trial 3 of the experiment, bubblers in the HA7 tub 2 treatment became fouled. That tub data was discarded due to anoxic DO levels resulting in visibly reduced growth. Physiological signs of low DO were observed in the root zone, e.g., significant growth of roots in the rockwool cube including roots showing at the top of the cube, smaller plants as well as thinner and oak-like new growth of leaves, and thicker roots with less branching. The DO measurement was ~2.5 mg/L, which is close to the 2.1 mg/L lower bound value that Goto et al. [15] found for hydroponic lettuce in similar conditions.

All plants were harvested at 35 days-of-age. Fresh weights (FW) for shoot and root were measured by cutting the shoots at the top of the rockwool plug through the hypocotyl and slicing the roots off at the base of the rockwool cube leaving the main root shoot inside the rockwool cube. Surface water was removed from the roots via gentle palm pressure on a stack of paper towels above and below a rootball. The intention of the stacked paper towels was to avoid lysing the root membranes and to allow simple wicking from the root surfaces. Only lettuce plants that were from the interior of the tub (24 guarded plants) were used for analysis; plants along the outside perimeter may have received more light than interior guarded plants and were therefore not included. Dry weights (DW) and tissue analysis data were determined based upon 10 randomly selected plants from each treatment and each trial. DWs for the root and shoot weights were obtained after four to seven days in a drying oven held at 70 °C.

Water quality data for all elements except pH, EC, RZT, nitrate and TAN were run by the Cornell Nutrient Analysis Lab (CNAL) in an automated inductively coupled plasma-atomic emission spectroscopy (ICP-AES) system (Vulcan 84 digestion unit manufactured by Questron Technologies, Mississauga Canada). The pH, EC, and RZT were collected by hand (pH Testr 30 and EC Testr11+,

Oakton Instruments, Vernon Hills, IL, USA) twice daily except the final day where data was collected once after harvest at approximately noon. The pH was calibrated daily, EC was calibrated with a 1413 µS/cm standard and confirmed to zero in deionized water, and temperature was confirmed against a laboratory thermometer.

Tissue analysis data were run by CNAL using hot plate acid digestion plus an ICP-AES for the plant elemental analysis, and combustion analysis was used for carbon and nitrogen. For each trial, three samples for shoot data and three samples for root data from the same plants were submitted from each tub. Each sample contained three random heads such that the three average tissue responses were from nine 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. Samples were removed where the titanium contamination affected the interpretation of the data via significant statistical differences and based upon our own analysis of elemental concentrations from rockwool cubes via CNAL digestion and literature [16,17].

2.7. Statistical Analysis

Mixed effect models using least squares analyses were conducted using JMP PRO 11 (JMP Pro software: http://www.jmp.com/en_us/software/jmp-pro.html; SAS, Cary, NC, USA). 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. Data was analyzed as one data set with three trials. However, the trial was controlled in the model as a fixed effect. Residuals were thoroughly checked for normality and constant variance, not only versus the predicted value, but across trials. Treatment was also addressed as a fixed effect. Conclusions regarding treatments were made after controlling or removing for any trial-to-trial variability. 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.

3. Results

3.1. Blocking and Trial Effects

While the original experiment included a blocking design (Table 2), no variance was attributed to the blocking for shoots and 3% or less of the variance was attributed for 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 Trials 2 and 3. There were substantial differences among trials for SL and NL, primarily due to the seasonal change from winter to early summer. There were also differences between trials for air temperature and RH, but they were relatively small (Table 4), particularly if trying to determine their effects given the expected natural variations in biological response. We do not believe our experiments provided sufficient range in these environmental variables to specifically identify their effects; 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 this trial effect was not correlated with any particular environmental variable that was measured.

3.2. Biomass Results Overview

H7 was significantly different from H5 in all biomass response categories (FW, DW, and DW/FW for both shoots (Table 5) and roots (Table 6)) at $p < 0.01$. The HA7 biomass response categories were only significantly 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 for all biomass response categories, except DW root.

Table 5. Least squares means (LSM) for 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) with multi-model significance. Differing superscript letters within response variables (columns) denote significance at $\alpha = 0.05$.

		FW		DW		DW/FW	
		Mean (SD)	**%**	**Mean (SD)**	**%**	**Mean (SD)**	**%**
LSM All Trials	H5	159 (14) ^{A}	100%	6.6 (0.60) ^{A}	100%	0.041 (0.0028) ^{B}	100%
	H7	114 (19) ^{B}	72%	5.3 (0.88) ^{B}	81%	0.046 (0.0019) ^{A}	112%
	HA7	122 (20) ^{B}	77%	5.5 (0.90) ^{B}	82%	0.045 (0.0043) ^{A,B}	109%

Table 6. Least squares means (LSM) for 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) with multi-model significance. Root mass does not include root mass within the rockwool cube. Differing superscript letters within response variables (columns) denote significance at $\alpha = 0.05$.

		FW		DW		DW/FW	
		Mean (SD)	**%**	**Mean (SD)**	**%**	**Mean (SD)**	**%**
LSM All Trials	H5	7.9 (1.3) ^{B}	100%	0.34 (0.06) ^{B}	100%	0.042 (0.0041) ^{B}	100%
	H7	9.6 (1.7) ^{A}	122%	0.51 (0.12) ^{A}	150%	0.053 (0.0082) ^{A}	124%
	HA7	9.1 (1.6) ^{A,B}	115%	0.40 (0.07) ^{B}	119%	0.046 (0.0030) ^{A,B}	109%

3.3. Shoot (Head) Biomass Response

Lettuce shoots from H7 and HA7 were smaller than from H5 in both FW and DW (Table 5). The FW responses showed a reduction from 159 g in H5 to 114 g in H7 ($p < 0.0001$) and 122 g in HA7 ($p = 0.002$). As both pH 7 treatments did not significantly differ, the 28% and 23% reduction in mean FW was averaged to give a 26% reduction due to raising the pH from 5.8 to 7.0 in our experimental setup and conditions.

The DW responses showed a reduction from 6.6 g in H5 to 5.3 g in H7 ($p = 0.0003$) and 5.5 g in HA7 ($p = 0.0019$). As both pH 7 treatments did not differ, the 19% and 18% 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 0.045 g/g for H5, H7, and HA7, respectively. H5 was different from H7 at $p = 0.019$, but only differed from HA7 at $p = 0.0661$). Since the H7 and HA7 responses were both significant at $p < 0.10$, the 12% increase for H7 and 7% increase for HA7 can be averaged as a 10% increase in dry matter content due to the pH difference.

In summary, pH 7 conditions decreased DW biomass by ~18% and FW biomass by 26%. The pH 7 effects on water content were more variable between H7 and HA7 treatments but showed an increase in dry matter content of 10% compared to the H5 response.

3.4. Root Biomass Response

The root biomass data does not include any root mass that was contained in the rockwool cube. Mean root FW increased from 7.9 g in H5 to 9.6 g in H7 ($p = 0.0038$) and 9.1 g in HA7 ($p = 0.0502$) (Table 6). We consider the p-value of 0.0502 and the 15% increase in FW of the H5 to HA7 comparison as meaningful, particularly since it paralleled the inverse with what was observed in the shoot response. H7 and HA7 were not different at $p = 0.4580$. The 22 and 15% increases in root biomass can be averaged as an 18% increase in root FW due to the higher pH.

Mean root DWs were 0.34, 0.51, and 0.40 g 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.

The DW/FW responses showed that the H5 ratio of 0.042 was significantly different from the H7 ratio at 0.053 at $p = 0.0040$. H7 was not different from HA7, however it would have been significant at an alpha of 0.10 ($p = 0.0617$). H5 and HA7 were not different.

3.5. Shoot Tissue Analysis Response

We did not detect a difference in shoot tissue responses among treatments at $p = 0.05$ for C, P, K, Ca, and S, amongst macronutrients. Particularly consistent in their responses were K, Ca, and S. Amongst micronutrients, Fe, Mn, Al, Si, Pb, and Ni showed no differences at $p = 0.05$. No Si, Al, or Pb were added to the tubs. Each likely entered the system as impurities in the salts used to create the nutrient solutions, in the municipal water, or by direct uptake from the rockwool cubes (Table 7). We determined Pb leached out of the plumbing (see below). The most striking differences in tissue contents of mineral nutrients were for N and Mg between the pH 5.8 and the higher pH treatments. Mg tissue concentrations were 2759, 3891, and 3358 mg/kg for H5, H7, and HA7, respectively. H7 differed from H5 $p = 0.0009$ (a 39% increase). HA7 did not differ from H5, except at $p = 0.0701$ (a 22% increase). The two pH 7.0 conditions did not differ.

Table 7. 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. Columns with differing superscript letters are significantly different at alpha = 0.05.

Parameter	H5		H7		HA7	
	Mean	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**
Carbon Content (%)	32.1 ^{A}	(0.87)	32.7 ^{A}	(1.04)	32.4 ^{A}	(0.70)
Macronutrients (%)						
N	5.42 ^{A}	(0.20)	4.95 ^{B}	(0.20)	5.17 ^{A,B}	(0.27)
P	1.02 ^{A}	(0.098)	1.12 ^{A}	(0.015)	1.11 ^{A}	(0.017)
K	3.01 ^{A}	(0.065)	3.12 ^{A}	(0.018)	3.03 ^{A}	(0.048)
Ca	1.31 ^{A}	(0.020)	1.33 ^{A}	(0.014)	1.29 ^{A}	(0.017)
Mg	0.280 ^{B}	(0.004)	0.389 ^{A}	(0.009)	0.336 ^{A,B}	(0.007)
S	0.239 ^{A}	(0.002)	0.235 ^{A}	(0.002)	0.237 ^{A}	(0.002)
Micronutrients (mg/kg)						
Fe *	74 ^{A}	(10)	67 ^{A}	(7)	67 ^{A}	(10)
Mn *	80 ^{A}	(20)	67 ^{A}	(9)	68 ^{A}	(11)
Cu	5.4 ^{A}	(0.6)	5.3 ^{A}	(0.7)	6.5 ^{B}	(0.9)
Zn	42.2 ^{A}	(6)	21.3 ^{B}	(4)	33.4 ^{A}	(12)
Mo	0.95 ^{B}	(0.18)	1.43 ^{A}	(0.23)	0.93 ^{B}	(0.23)
Other elements (mg/kg unless stated)						
Na	480 ^{B}	(105)	613 ^{B}	(56)	1213 ^{A}	(285)
Al *	38.5 ^{A}	(30)	36.5 ^{A}	(22)	22.6 ^{A}	(12)
Ni *	0.17 ^{A}	(0.38)	0.34 ^{A}	(0.18)	0.85 ^{A}	(1.78)
Si *	3.3 ^{A}	(1.1)	5.2 ^{A}	(0.8)	4.6 ^{A}	(1.5)
Pb	3.8 ^{A}	(2.7)	4.0 ^{A}	(1.7)	3.9 ^{A}	(3.3)
Sr	66 ^{A}	(10)	73 ^{A}	(9)	44 ^{B}	(4)
As *	- ^{A}	-	- ^{A}	-	- ^{A}	-
Ba	0.13 ^{B}	(0.26)	0.89 ^{B}	(0.69)	1.87 ^{A}	(0.83)
Cd (µg/kg)	- ^{A}	-	58 ^{B}	(6)	- ^{A}	-
Co * (µg/kg)	0.002 ^{B}	-	4.113 ^{A}	(3.42)	0.005 ^{B}	-
Cr * (µg/kg)	26 ^{B}	(52)	345 ^{A}	(217)	6 ^{B}	(20)
Se (µg/kg)	- ^{B}	-	22 ^{A}	(20)	- ^{B}	-
V * (µg/kg)	0.002 ^{B}	-	160.000 ^{A}	(140)	0.004 ^{B}	-

Sample size was 18, 15, and 15 for H5, H7, and HA7, respectively, except HA7-copper, which had an $n = 14$ due to an outlier concentration that was eliminated. * Analysis with zero titanium samples only; $n = 13$, 5 and 6 for H5, H7 and HA7, respectively. A dash (-) means the level was below detection limits and was recorded as 0 for data analysis.

There are several interesting observations that can be made from what happened to elemental concentrations in the nutrient solutions from start to finish (Table 1). For macronutrients, K in the nutrient solution accumulated from start to finish in the high pH conditions (ca. 30%), but stayed much the same in the H5 treatment (Table 1). Tissue contents were not different. Ca accumulated in the pH 5.8 treatment (25%), but stayed the same in the pH 7 treatments. Tissue contents were not different. N stayed much the same throughout, but it started 21% lower than target in the HA7 condition. The pH 5.8 tissue had a higher N content than the other treatments. P was extremely stable and consistent at pH 5.8 but fell by 40% in both pH 7 treatments by the end, as expected. Tissue contents were not different despite the decline in concentration over the course of the trials in the high pH conditions. Mg was steady, as is typical. The HA7 condition was only 2/3 of the target value at the start and 3/4 at the end. Tissue content of Mg was significantly lower in the H5 treatment than the high pH treatments. S was very steady in all conditions and not different among treatments.

Six micronutrient elements were intentionally included in the nutrient formulation used in this study, namely Fe, Mn, Cu, Zn, B, and Mo. Of these, there was no difference among treatments in levels of Fe and Mn. B was not reported since a non-approved method was used for its determination.

Cu contents were 5.4, 5.3, and 6.5 mg/kg for H5, H7, and HA7, respectively. HA7 was different from H5 (p = 0.0366) and H7 (p = 0.0221). H5 and H7 were not different. The average values of copper in the HA7 nutrient solutions were 0.044 mg/L, 35% larger than the H5 and H7 concentrations of 0.033 mg/L (Table 1), which correlates with the both the use of copper piping in the greenhouse municipal water lines and the 19–22% increase in the HA7 tissue concentration. Furthermore, increasing the pH from 5.8 to 7.0 did not appear to influence the Cu accumulation in the hydroponic tissues.

Zn tissue content was one of the most varied elements between treatments. H5 at 42.4 mg/kg was different from H7 at 21.3 mg/kg (p = 0.0005), and H7 was different from HA7 at 33.4 mg/kg (p = 0.0310). H5 and HA7 were not different. These differences in Zn corresponded 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 treatments, Hafeez [18] stated that tissue contents greater than 20 ppm, which we observed in all three treatments, were unlikely to negatively affect plant growth. It is possible that H5 plants were more readily able to absorb and utilize Zn at the lower pH, and that the higher pH negatively influenced Zn uptake. Given how similar the H7 and HA7 Zn solution concentrations ultimately were (Table 1), the magnitude of the differences between the Zn tissue concentrations is surprising.

Mo concentrations were 0.95, 1.43, and 0.93 mg/kg for H5, H7, and HA7, respectively. H7 was different from H5 (p = 0.0056) and HA7 (p = 0.0066). H5 and HA7 did not differ. A comparison of H5 to H7 suggests a possible pH effect on Mo; however, the HA7 treatment, which was very similar to H7, had no significant impacts on the Mo concentrations and resulted in values similar to H5.

Of the remaining elements detected in the tissue, only Na was reported for the start and finish values for the nutrient solutions (Table 1). The remaining elements were all at very low levels and presumably entered the tissue as contaminants. Na contents were 480, 613, and 1213 mg/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, which is reasonable given the same source water and measured sodium concentrations. The elevated Na concentration in the HA7 tissue correlates with seven-fold elevated Na concentration in the nutrient solution.

Sr tissue concentration in the HA7 treatment was significantly lower than the other treatments (66, 73, and 44 mg/kg for H5, H7, and HA7, respectively), while H5 and H7 were not different. Sr may be used as a Ca substitute within the plant, but is less ideal and effective. The Ca and Sr ratio may become important if the Sr concentration in the solution was very large. As our Sr to Ca ratios were relatively small and not very different between conditions, the small differences seen in the tissue analysis among treatments are very likely not physiologically meaningful.

Ba content did show some significant differences between treatments. H5 at 0.127 mg/kg were different from HA7 at 1.865 mg/kg (p = 0.0012). H7 at 0.887 mg/kg was also different from HA7 (p = 0.0476) while H5 and H7 did not differ.

Cd was only detected in the H7 treatment at 0.06 mg/kg and was quite consistent among all samples with a standard deviation of 0.006. H7 significantly differed from H5 and HA7 (p = 0.001).

Co followed the same trend as cadmium in that it was only detected in the H7 treatment. H7 at 0.004 mg/kg for Co was significantly different from the 0 values of H5 and HA7 (p = 0.0475 and 0.00342, respectively).

Cr contents significantly differed between H5 at 0.026 mg/kg and H7 at 0.3454 mg/kg (p = 0.0395), H7 and HA7 at 0.00618 mg/kg differed at p = 0.10 but not at p = 0.05. H5 and H7 were not different.

Se contents were significantly different among treatments with only H7 samples exhibiting Se contents at 0.022 mg/kg.

V content of H7 at 0.16 µg/kg was significantly different from H5 at 0.0017 µg/kg (p = 0.0348) and was statistically different from HA7 at 0.0044 µg/kg at p = 0.10 but not p = 0.05. The V analysis was run on samples without Ti.

A number of cellular functions are affected by B [19]. Typical dicots have B tissue concentrations in the 20–100 ppm range [19]. As B is acquired passively and B concentrations were controlled and the same in all conditions, pH was not expected to have affected tissue accumulation of B. Our samples, despite possible losses during volatilization of the samples, were in excess of 20 ppm (data not shown) in the vast majority of all samples suggesting no negative impact upon growth. No data was reported on B due to the hot plate acid digestion method used not being an EPA certified method (the high ramping temperatures necessary for heavy metal extraction starts to volatize B).

3.6. Lead Analysis

Low concentrations of lead (Pb) were surprising to find in the tissues, particularly because we were unaware of any significant source or concentrations of Pb in any of our materials. Concentrations of Pb were not different among treatments and were 3.9 mg/kg for dried shoot matter. Neither the rockwool nor condensate dripping from the roof had a significant Pb concentration to explain these results. With help from the CNAL, we identified the source of lead as the approximately two meter portion of 16 mm garden hose used with the tub recirculation pumps for mixing. We also found detectably low levels of Pb in the nutrient solutions and that the garden hoses themselves were the source of the contamination. From a plant growth basis, the lead contents seemed to have had no effect on the biomass production of the plants.

4. Discussion

4.1. Biomass Response

In all three trials, high pH and elevated alkalinity resulted in a substantial difference in shoot FW and DW. Taking the results at face value and expressed as LSM values, the high pH conditions showed a deficit in shoot FW of ca. 26%. On a trial-to-trial basis, there was a range of 14 to 36% in shoot FW, which is a substantial effect that would have negative commercial consequences for aquaponics if the pH effect were unchanged for a coupled RAS system with 'real' fish water.

4.2. Nutrient Analysis

There were no notable lack of nutrients in either the nutrient solutions at the end of the trials or in the plant tissues. Any significant differences were unlikely to have influenced biomass from deficiency or toxicity symptoms. P in the solution did trend downwards over the duration of the trials at high pH conditions, as expected, considering its ionic form and solubility at pH 7. The depletion occurred slowly and P remained available for plant growth after stabilization of the solution. Tissue concentrations of P were neither deficient nor different.

The large difference in shoot FW among treatments may have been due to the fact that root weights were consistently greater in the high pH treatments than in the 5.8 pH treatment. Roots were 5.0% of shoot FW in the low pH treatment, but 8.4% or 7.5% in the high pH treatments. Production of extra root biomass under high pH conditions does not fully explain the large differences in shoot biomass, but it is suggestive of other possible explanations. Larger roots and smaller shoots suggested plants in the high pH conditions were adjusting to difficulties in nutrient uptake by enlarging roots, a familiar plant response [20].

Very considerable amounts of the total energy produced in photosynthesis are expended in nutrient uptake, especially during the early stages of plant growth. Consumption of available ATP has been measured as 36% for ion uptake during rapid vegetative growth for *Carex diandra* [21]. These resources are then not available for growth, which may have cascading effects on seedling leaf expansion rates and, ultimately, total intercepted light during the life cycle.

It would also follow that that some pH levels require more use of plant energy resources for transporting ions across the cell and vacuole membranes than others. With knowledge of the concentration of different ions in the environment external to the cell, the cell interior, and vacuole and quantitative evidence of import and export of ions to and from these locations, one could attempt an energy analysis for different pH environments for the root system. This type of analysis is not possible with the present data. However, we conclude that a strong depressing effect on saleable biomass in lettuce occurs when using pH 7 in an inorganic hydroponic nutrient solution simulating aquaponic solution conditions.

4.3. H7 vs. HA7 Water Quality Conditions

It was assumed that the H7 treatment could be maintained at a different alkalinity level than the aquaponic-like (HA7) treatment. It was expected that H7 would be at moderately low levels of alkalinity, but certainly much higher than a zero level. However, once the H7 and HA7 treatments equilibrated to the pH 7.0 target, the alkalinity values were the same. Differing responses between H7 and HA7 cannot be attributed to alkalinity differences but rather to other factors not specifically identified in our research findings.

Our H7 and HA7 equilibrated to the same alkalinity values as the result of the carbonate balance coming to an equilibrium, which is affected by air stripping (elimination). Solution pH will rise when carbon dioxide is lost from the solution via active aeration or passive exchange. As carbon dioxide is in balance in the solution with carbonic acid, bicarbonate, and carbonate, a 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 is seen if dissolved carbon dioxide concentrations increase. Reverse osmosis (RO) or deionized water can be used in nutrient solution creation to avoid these issues with alkalinity in hydroponics. On the other hand, very low alkalinity has the disadvantage that small additions of acid, base, or pH altering processes can alter pH significantly. This may happen with changing plant or fish respiration (change in dissolved carbon dioxide equilibrium), which may then change nutrient availability [21]. Nelson [22] provided 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 experience, if pH is maintained at circa 6.0, nutrient solution concentrations are controlled, and potential carbon dioxide stripping 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 concentrations. Such conditions open the possibility of significant

carbonate species storage. Carbon species contribution to alkalinity is dependent upon the pH level, and thus the ratio of carbonate species, where low pH has much higher carbonic acid. It should be recognized that changing dissolved carbon dioxide concentrations does not change alkalinity levels but 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, acid addition was used to reduce pH increases each day from carbon dioxide stripping. We believe we reached the alkalinity equilibrium within 3–7 days of the start of each trial start due to the small ratio of bubbler flow rate and the tub volumes, compared to one hour in the bench scale tests previously described. Both of the H7 and HA7 treatments resulted in similar alkalinity values of 40 mg/L as $CaCO_3$, due to their near identical elemental compositions and nearly identical respiration loading with shared ambient CO_2 atmosphere in the root zone.

In future research, the effects of a pH 7 target combined with organic nutrients from fish water in continuous circulation will be studied, i.e., under actual aquaponic conditions as would be practiced using a coupled system. The present work was intended to identify if there are bioavailability impacts for nutrient uptake provided by the fish system component being coupled to the hydroponic system component. We reported on such an experiment using baby leaf spinach where production performance was compared between hydroponic and aquaponic water nutrient conditions [23].

5. Conclusions

Biomass and tissue elemental differences were quantified for butterleaf lettuce grown in deep-water conventional hydroponic conditions at two pH and alkalinity conditions. This research was motivated by our parallel research in aquaponic production of lettuce where the water quality conditions are similar in pH and alkalinity to the HA7 conditions in this experiment. To isolate the effects of pH and alkalinity apart from the effect of aquaponics where nutrients are supplied to the plants from fish feed, we conducted the current research as an experiment consistently of three consecutive trials under different hydroponic treatments. We created three treatment conditions with all nutrients supplied from inorganic salts at concentrations of macro and micro elements that followed recommended levels for hydroponic production of leaf lettuce. The treatments 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 municipal water that was initially high in alkalinity (HA7). All treatments were harvested at approximately 35 days when the conventionally grown lettuce (H5) was at an average weight of 159 g FW. Averaged across three trials, the HA7 and H7 treatments produced 26% less shoot fresh weight (FW) than the H5 treatment with an 18% reduction in dry weight (DW). The H5 treatment had the least biomass in root FW and DW. In tissue elemental analyses, both the pH 7.0 treatments (HA7 and H7) showed lower concentrations than H5 in Cu, N, Mo, and Sr, and increased concentrations in Ba, Mg, Na, and Zn. There were no differences in Al, C, Ca, Fe, K, Mn, Ni, P, S, and Si concentrations among treatments ($p = 0.05$). The results from this experiment provide a basis to isolate the effects of pH and alkalinity in aquaponic conditions where pH and alkalinity will mimic HA7 conditions.

Acknowledgments: This research was supported entirely by the Cornell University Agricultural Experiment Station federal formula funds, Project No. 1237650 and NYC-123421 received from Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. We would like to thank Pamela Schwartz, Erica Cartusciello, Zach Wielgosz, and Haydn Lenz for their assistance in data collection and daily maintenance of the production systems. We would like to thank Francoise Vermeylen from the Cornell Statistical Consulting Unit for her assistance and guidance in the statistical models and analysis.

Author Contributions: Tyler S. Anderson and Miguel Martini conceived and designed the experiment; Tyler S. Anderson performed the experiment; Tyler S. Anderson and Miguel Martini analyzed the data; Tyler S. Anderson, Michael B. Timmons and David De Villiers contributed reagents/materials/analysis; all authors contributed significant portions to the writing of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. Sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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