Effect of Hydroponic System Type on Growth and Nutrient Uptake of Lettuce (*Lactuca sativa* 'Rex') Irrigated with Aquaculture Effluent

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, AL

December 10th, 2021

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Abstract

Aquaponics (AP) combines the use of traditional hydroponic (HP) practices with aquaculture effluent which is used to irrigate and provide necessary nutrients to the plants. Current methods of HP production include Nutrient Film Technique (NFT), deep water culture (DWC) and substrate culture (SC). Currently there are few papers detailing differences in production based on system type. Thus, there is a need for research to help bolster and expand the knowledge base for aquaponic lettuce production.

The objective of the first experiment was to assess the effect of HP system type on the growth and nutrient uptake potential of lettuce irrigated with sole aquaculture effluent. It was hypothesized that a system type utilizing a substrate could possibly allow for increased foliar nutrients when compared to other HP systems in low stocking conditions. Three different hydroponic system types (NFT, DWC and SC) were arranged in a randomized complete block design. The trial lasted a total of 45 days from start of germination to harvest. Analyses of foliar quality, nutritional content, yield, and water nutritional content were conducted at the end of the growth cycle at harvest. Results from foliar analyses showed SC had higher uptake of nitrogen, magnesium and copper when compared to DWC and NFT systems. Substrate culture and NFT reported higher fresh head weight than DWC in the first trial, while SC reported the highest fresh weights in contrast to the other system types in the second trial.

The objective of the second experiment was to assess the effect of HP system type on the growth and nutrient uptake potential of lettuce irrigated with aquaculture effluent supplemented with fertilizer. It was hypothesized that a system type utilizing a substrate could possibly allow for increased plant foliar quality when compared to other HP system types at recommended nutrient levels in irrigation solution. The three different hydroponic system types were arranged in a randomized complete block design. This trial lasted a total of 45 days from seeding to harvest. Analyses of foliar quality, nutritional content, yield, and water nutritional content were conducted at the end of the growth cycle at harvest. Fresh head weight was highest in SC and NFT systems and lowest in DWC. Leaf chlorophyll estimates were highest in SC and DWC systems and lowest in NFT. Stomatal conductance to water vapor (gsw), vapor pressure deficit (vpd), leaf apparent transpiration (E), photosystem II efficiency, and electron transport rate (ETR) were shown to be similar between all system types. Lettuce cultivated utilizing SC reported the highest foliar accumulation of magnesium and calcium. Plants grown in DWC systems reported increased foliar accumulation of potassium when compared to SC yet was similar to NFT levels. All system types were similar in foliar concentrations of nitrogen, phosphorous, sulfur, boron, iron and copper.

Acknowledgements

I would like to begin by thanking my major professor and advisor for my thesis, Dr. Daniel Wells for the opportunity and following guidance throughout the completion of this research. I would also like to thank my committee members Dr. Glenn Fain and Dr. Brendan Higgins for their expertise and guidance on my thesis. This research would not have been possible without Dr. Emmanuel Ayipio, Emelia Larson, Mackenzie Pennington, and Mollie Smith, who all provided help and support ensuring the success of this research. For providing a welcoming and support filled environment, I would like to thank my fellow graduate students and colleagues in the Auburn University Horticulture Department. Finally, I would like to thank my parents and family who mean the world to me and blessed me with a wonderful support system throughout the entirety of my studies.

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Chapter 1: General Introduction

Introduction to Aquaponics

Aquaponics is a rapidly developing sector in greenhouse production of fruit and vegetable crops. This combines the use of traditional hydroponic practices with waste effluent from aquaculture production which is used as irrigation water (Goddek et al., 2015). The ecosystem in an aquaponics system is comprised of the crop being produced, fish in the aquaculture production tank/pond as well as bacteria in the microbiome which help convert macro- and micronutrients into plant available forms (Somerville et al., 2014). Plant essential nutrients enter the system through fish feed which is digested and metabolized by the fish and excreted as a nutrient rich manure. It is estimated that ~18% of feed provided to fish is not consumed becomes homogenized with the aquaculture environment (Montanhini and Ostrensky, 2014). Fish excrement combined with leftover feed particles and other microorganisms in the aquaculture tank/pond biome create an aquaculture effluent (Holliman et al., 2008). Aquaculture effluent generally contains high levels of nitrates (NO_3^-), nitrites (NO_2), unionized ammonia (NH_3) and ammonium (NH_4^+) (Moya et al., 2016). The main source of plant available nitrogen comes from the conversion of NH_3 to NO_3^- completed by nitrifying bacteria (Love et al, 2014). Excessive accumulation of compounds such as NH_3 and NO_2 can be toxic and detrimental to aquatic animal health (Mateus, 2009). The plants in an aquaponic system can act as a bio-screen filter; thus, attenuating excess microbe and nutrient accumulations (Rakocy et al., 2006). Aquaculture effluent can provide most of the necessary nutrients plants require for metabolic processes (Holliman et al., 2008). After the plants, effluent can be recirculated back to the aquaculture production area or discharged into the environment.

Substantial research in the field of aquaponics began in the mid 1970s with difficulties in viable solutions for novice systems (Al-Hafedh et al., 2008). Through trials and progression over a decade, the first reported closed-loop or coupled aquaponic system was developed which delivered aquaponic effluent from tilapia aquaculture to sand-based media tomato cultivation beds and ultimately recirculated the water back to the aquaculture tank (McMurtry et al., 1990). Different innovations since the beginnings of AP have developed the technology and practices into a feasible and viable method of producing food crops (Diver, 2006). AP is rapidly transforming into commercial-scale production where the developing technologies and strategies are allowing for increased efficiency and product output (Goddek et al., 2019). Aquaponics is unique in its ability to produce not just a plant crop but also a protein product in the form of fish. This method of farming has the advantage of operation cost reduction by producing an efficient source of protein as well as fruits and vegetables in one system (Diver, 2006). Having a method of farming producing two commodities in one system and reducing costs can be an efficient means for farmers to diversify.

There are two classifications of systems in aquaponics: recirculating or coupled systems and non-recirculating or decoupled systems (Yeo et al., 2004). With combining hydroponic plant production and a recirculated aquaculture system (RAS) you have a symbiotic relationship between two agricultural products within a single system. Plants utilize and remove nutrients within the effluent water which reduces the nutrient loading of effluent returning to the aquaculture tank in recirculating systems (Seawright et. al., 1998). Effluent nutrients can reduce synthetic nutrient input requirements for the plant producing system and reduce the amount of waste discharge from aquaculture production (Tyson et al., 2012). Conversely, a non-recirculating or de-coupled aquaponic system delivers the effluent from the fish tank to the plant production area and ultimately to a runoff or dump site. Aquaculture feed and resulting effluent is known to contain high amounts of phosphorous and dumping this waste into the environment can lead to eutrophication of creeks, rivers, ponds, and lakes ultimately leading to the decimation of aquatic inhabitants (Mustapha and Bakali, 2021). The reuse of this waste and withdrawal of potentially toxic nutrients through plant production would be a beneficial and sustainable means of processing.

One study reported hydroponic and recirculating aquaponic systems used up to 80% less water than traditional field production of the same crops (Sayara et al., 2016). This reduction of water usage and resulting discharge into the environment can be crucial in the adaptation of aquaponic production to more urban areas where production space and access to inexpensive water is limited. Aquaponic production has the benefits of reduced land area, reduced water usage, plant growth acceleration as well as virtually continuous production throughout the year in a controlled environment (Patillo, 2017). While conserving water, AP can also be sustainable by limiting the discharge of aquaculture production waste into the surrounding environment (Boxman et al., 2017). With a rapidly growing population, more food will be required with less production land available. In cities with reducing populations, unused properties can be converted for agricultural use (Schilling and Logan, 2008). This method of production has the benefit of using miniscule sections of land in urban settings to bolster the selection of local food sources for populous cities (Goddek et al., 2019). Farmers in urban agricultural settings could potentially greatly benefit from sustainable systems like aquaponics.

Economics

Economic feasibility data on aquaponic farming is limited due to the commercial interest in this production method having started so recently that few studies focus on this topic. In one review of studies only 13% focused on AP producer profitability (Greenfeld et al., 2018). AP has many initial costs for materials as well as operational costs throughout production. High initial capital investment is one of the leading obstacles hampering the wide-spread adaptation of aquaponic technology (Dediu et al., 2012). Recirculating aquaculture systems can be quite expensive to build and operate and the profitability of an operation largely depends on the market demand of fish products (Rackoy, 2012). Some studies have shown that less than one third of aquaponics operations can be considered profitable (Eguibar et al, 2020). Even solely indoor recirculating catfish and tilapia production systems have been shown to net negative returns annually without the added component of plant production which can financially benefit an operation long term (Holliman et al., 2008). Construction of systems and production areas can be extremely costly and make up a large portion of the initial investment cost for aquaponics. Electricity and maintenance of the production areas can generate another substantial production cost that questions the sustainability and efficiency of these systems (Forchino et al., 2017). Smaller farms in general seem to net lower returns on investments than larger operations (Quagrainie et al., 2018). Differing crops yield different revenue per unit and careful consideration must be taken by the producer to select profitable cultivars that have the highest returns to cover associated costs with start up as well as production (Bailey and Ferrarezi, 2017). Small farms may not be a viable investment in aquaponic production due to the large initial input costs for materials especially when considering the risks associated with aquaponic production (Bailey et al., 1997). Based on operations surveyed across the U.S. and U.S. territories, small scale operations experienced annual net returns ranging from $4,222 to $30,761 whereas large scale operations ranged from net losses greater than -$11,500 to net returns of $278,038 (Engle, 2016). In another international survey conducted by Love et al. (2015) sales revenue averages for small-scale producers fell between $1000 and $4,999. Another survey of small-scale aquaponic producers in Hawai'i saw producers selling directly to supermarkets with an average modified internal rate of return at 10% (Tokunaga et al., 2015). This shows promise of profitability with new and starting aquaponic businesses. However, previous studies of other small-scale producers have shown that many times the operation is not profitable due to high start-up costs and low rate of return for fish production (Tokunaga et al., 2013). Larger scale producers generally achieve higher and more financially viable returns for the initial investment (Bailey et al., 1997). More studies are needed to conclusively show economic feasibility for accurate insight to the true economic potential of aquaponic production in either large- or small-scale settings.

Aquaponic Production

There is much debate on whether aquaponic production levels and quality can compare to those seen with conventional hydroponics. Generally, hydroponic nutrient formulations are specifically engineered for a certain crop's needs. Levels of nutrients in AP can vary widely system to system depending on the health, diet, and stocking density of the aquaculture production area (Seawright et. al., 1998). Without proper management of the ratio of fish production density to plants, nitrogen uptake will be limited within the plant production area (Wongkiew et al., 2017). Generally, higher fish stocking density leads to an increase in the available nutrients within an aquaponic system. In a study by Pantanella et al. (2012), high fish density aquaponic production using deep water floating rafts was similar when compared to hydroponics which produced 2.8 kg m^{-2} of lettuce while aquaponics produced 2.7 kg m^{-2} in the first trial and 6.0 kg m^{-2} hydroponic lettuce with 5.8 kg m^{-2} aquaponic lettuce in the second trial. As far as production goes, both hydroponic and aquaponic lettuce production were similar with one another providing some evidence that aquaponic production can be comparable to traditional hydroponic production. Contrastingly, in an alternate study by Sayara et al. (2016), hydroponic production was found to be significantly more efficient than aquaponic production in achieving faster growth rates and requiring the least amount of input.

The idea behind AP is to negate the need for adding synthetic fertilizers while using a waste product from aquaculture production. Commercial hydroponics producers face the added costs of fertilizers and AP could provide nutrients more sustainably (Suhl et al., 2016). A significant finding by Pantanella et al., (2012) was in the comparison of nutrient compositions of the hydroponic lettuce plants to their aquaponic counterparts. Hydroponic plants contained higher amounts of phosphorous in the foliage whereas the aquaponic plants contained higher levels of sodium, potassium, magnesium and calcium (Pantanella et al., 2012). Higher concentrations of carotene, antioxidants, phenolic compounds, chlorophyll a, and total chlorophyll have been found in AP when compared to hydroponics (Ahmed et al., 2021). Additionally, studies by Delaide et al. (2016) using an NFT system have shown that adding supplemental nutrients into aquaponic effluent has the potential to increase production to outperforming conventional hydroponics. The addition of supplemental nutrients into an AP production system is supported in a study by Ako and Baker (2009) where supplemental nutrients were needed to reach sufficient concentrations as is found in hydroponic production. When analyzed, aquaponic effluent nutrient levels within a high density stocked media-based system have been found to be lacking in some essential nutrients when compared to recommended levels for hydroponic solutions (Blanchard et al., 2020). Two of the main lacking nutrients in the study done by Blanchard et al., 2020 (Dutch bucket SC using 100% perlite media) were found to be iron and magnesium. Although nutrients within the aquaponic effluent were found to be well below recommended levels, foliar analyses reported leaves contained above satisfactory levels of macro- and micro-nutrients (Blanchard et al., 2020). These findings in the supported literature lead toward a SC system possibly allowing for better acquisition of nutrients given a greater chance for accumulation of solids.

There are three common types of hydroponic systems used in aquaponic and hydroponic production (Tyson et al., 2011). These are the Nutrient Film Technique (NFT), deep water culture implementing a floating raft for cultivation and substrate (SC)/ media-based trough, reservoir or bag generally filled with clay pebbles, perlite, coco coir and other soilless medias (Patillo, 2017). Some of the most common hydroponically produced crops are leafy greens, especially lettuce (Kaiser and Ernst, 2016). Lettuce is gaining popularity in the aquaponic marketplace especially due to its relatively low nutritional requirements, rapid growth and ease of adaptation to aquaponic systems (Diver, 2006; Sala and Costa, 2012). Lettuce production throughout the industry seems to largely rely on DWC systems and NFT systems with SC systems seemingly not widely utilized for cultivating leafy greens.

In terms of production, some research has shown that one system versus another could possibly be better for nutrient acquisition in aquaponic production. A study conducted by Lennard and Leonard (2006), reported slightly better performance concerning growth and biomass yield in a SC system utilizing a gravel media compared with DWC and NFT production systems. During a trial by Zou et al. (2016), AP utilizing SC and DWC to produce pak choi showed higher nitrogen accumulation in the SC system plants samples when compared to the DWC (Zou et al., 2016). Another study conducted using tomatoes in three differing hydroponic system types showed that the substrate-based production system also performed slightly better when compared to the others (Schmautz et al., 2016). These findings were consistent with findings in review of 122 published works by Maucieri et al. (2018) where in several cases NFT production was shown to be less efficient at plant growth and resulting nutrients than SC or DWC. Through reports by Pinho et al. (2017) aquaponic effluent water containing most of the organic solids showed better production results when compared with aquaponic water that had been screened and clarified. Analyses of effluent solids completed by Blanchard et al. (2020) reported solids consisted of mainly phosphorous and calcium with high amounts of iron and manganese also detected. Observed results between effluent water containing solids and clarified effluent water in conjunction with solid analyses results suggest that solids play a crucial role in the nutrient supply. Increased interaction between plants and effluent solids could increase availability to nutrients like calcium and iron which can ultimately enhance foliar structure and quality.

Conclusion

Lettuce is a valuable hydroponic crop that has minimal nutritional requirement when compared to other crops. The implementation of AP could potentially lessen the input cost of fertilizers for hydroponic lettuce while sustainably using a waste product of the aquaculture industry. Accumulation of effluent solids around plant roots could possibly lead to more available nutrients that are easily accessible to the plant. Each hydroponic system type allows for these effluent solids to interact with plant roots differently. One system type could possibly enhance the potential quality of the lettuce grown and outperform other system types in attributes like fresh head weight. Thus, further research is needed to assess the effect of hydroponic system type on growth and nutrient uptake of lettuce irrigated with aquaculture effluent. The purpose of our research will be to examine any differences in plant quality between the three system types (NFT, DWC, SC) to better discern the interactions between aquaculture effluent and hydroponic system type. Knowing which system type is best for aquaponic lettuce and other crop production can lead to more efficient means of production, more sustainable waste use, higher quality produce and potentially increased revenue for existing and beginning small- or large-scale operations.

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Chapter 2

Effect of Hydroponic System Type on Growth and Nutrient Uptake of Lettuce (*Lactuca sativa* 'Rex') Irrigated with Sole Aquaculture Effluent

Abstract

An experiment was conducted at Auburn University to assess the effect of hydroponic system type on the growth and nutrient uptake of lettuce irrigated with aquaculture effluent. The main objective of this experiment was to analyze differences in aquaponic lettuce performance in connection with system type provided with solely aquaculture effluent. It was hypothesized that a system type utilizing a substrate could possibly allow for increased nutrient uptake and plant growth when compared to other hydroponic systems even with low fish stocking conditions. Three different hydroponic system types, nutrient film technique, deep water culture and substrate. culture) were arranged in a randomized complete block design. Seedlings were germinated for 15 days before transplanting into the systems where they were then grown for another 30 days. Analyses of foliar quality, nutritional content, yield, and water nutritional content were conducted at the end of each growth cycle at harvest. Substrate culture showed increased foliar uptake of nitrogen (N), magnesium (Mg) and copper (Cu) in both trials. Nutrient film technique and SC were shown to have the highest fresh head weights within the first trial however, SC reported the highest fresh head weight in the second trial. Resulting dry matter accumulation was highest in NFT and SC when compared to DWC in both trials. Chlorophyll estimates showed similar concentrations when comparing all system types throughout trial one and two.

Introduction

Aquaponics (AP) is a developing food production technology that seeks to be a more sustainable alternative to conventional hydroponics. The combination of aquaculture with hydroponics (Goddek et al., 2015) allows for multiple uses of water and nutrients, hence improved efficiencies. The ecosystem in an aquaponics system consists of the fish in the aquaculture production unit, plant crops in the hydroponic unit, and bacteria which filter aquaculture water and convert macro- and micronutrients into plant available forms (Somerville et al., 2014). Plant essential nutrients enter the system through fish feed which is digested and metabolized by the fish and excreted as a nutrient rich manure. Fish excrement combined with leftover feed particles and other microorganisms in the aquaculture tank/pond biome create aquaculture effluent (Holliman et al., 2008). Aquaculture effluent generally contains high levels of nitrates (NO_3^-), nitrites (NO_2), unionized ammonia (NH_3) and ammonium (NH_4^+) (Moya et al., 2016). The main source of plant available nitrogen comes from the conversion of NH_3 to NO_3^- completed by nitrifying bacteria (Love et al, 2014). Excessive accumulation of compounds such as NH_3 and NO_2 can be toxic and detrimental to aquatic animal health (Mateus, 2009). Plants in an aquaponic system can act as an additional biofilter further reducing, nutrient accumulations (Rakocy et al., 2006). Aquaculture effluent can provide most of the nutrients (N, P, K, Ca, Mg, S) plants require for metabolic processes (Holliman et al., 2008). Using aquaculture waste in aquaponics can be considered a sustainable symbiosis between two production systems.

There is much debate on whether aquaponic production levels and quality can compare to those attained with conventional hydroponics. Generally, hydroponic nutrient formulations are engineered to meet crop needs. Levels of nutrients in AP can vary widely system-to-system depending on the health, diet, and stocking density of aquaculture production fish (Seawright et.

al., 1998). Without proper management of the ratio of fish production density to plants, especially when aquaculture and hydroponic units are coupled in a recirculatory design, nitrogen availability will be limited within the plant production area (Wongkiew et al., 2017). Despite generally lower nutrient levels, plant yields in aquaponics systems compare well to yields in hydroponic systems in many cases (Ayipio et al. 2019). For example, Pantanella et al. (2012) reported similar lettuce yields between hydroponics and aquaponics when using AE from an aquaculture unit with a high stocking density.

Plant yields in aquaponics systems are also affected by hydroponic system type. There are three common types of hydroponic systems used in aquaponic and hydroponic production: deep water culture (DWC) which uses a floating raft for cultivation, Nutrient Film Technique (NFT), and substrate culture (SC) which could be media-based troughs, reservoirs, and/or bags generally filled with clay pebbles, perlite, peatmoss, coconut coir or other soilless medias (Tyson et al., 2011; Patillo, 2017). In a meta-analysis summarizing experiments that compared plant yields from hydroponic and aquaponic systems, Ayipio et al. (2019) reported that yield differences could be attributed to hydroponic system type. Similarly, some research has reported that hydroponic system type influenced plant yields in aquaponics systems in general. A study conducted by Lennard and Leonard (2006), reported higher biomass in a SC system utilizing a gravel media compared with DWC and NFT production systems. During a trial by Zou et al. (2016), AP utilizing SC and DWC to produce pak choi showed higher nitrogen uptake in plants grown in a SC system compared to those grown in DWC. Similarly, Schmautz et al. 2016 reported that tomatoes grown in SC performed better than those grown in other hydroponic systems. In a review of 122 published works Maucieri et al. (2018) reported that in several cases NFT production performed worse than SC or DWC.

Differences in plant production between system types in hydroponic production are not often reported in the literature so it is likely that plant production differences in aquaponics due to HP system type are related to production factors that differ between aquaponics and hydroponics. Other than relatively low nutrient concentrations a major difference between aquaponics and hydroponics is the presence of dissolved and solid organics in the former. Pinho et al. (2017) reported aquaculture effluent a higher organic solids load produced higher plant biomass compared to aquaculture effluent in which solids were mostly removed via clarification and screening. Differing HP system types may allow plants to interact with effluent solids more efficiently and this could help explain the differences in production others have reported.

The objective of this research was to compare three common HP system types for biomass production and nutrient uptake of 'Rex' butterhead lettuce when using low nutrient aquaculture effluent.

Materials and Methods

Location and materials

This study was conducted in two-bay, gutter-connected greenhouse measuring 18.2 m by 36.5 m oriented north-south at the Auburn University Aquaponics Research System located at the E.W. Shell Fisheries Center in Auburn, Alabama (lat. 32.648932 N, long. -85.487103). These greenhouses were constructed with double polycarbonate end walls and covered in a double, inflated layer of 3-mil polyethylene on the sidewalls and roof. An environmental control computer (Bartlett GHK12X2GH; Bartlett Controllers, Fort Madison, Iowa) regulated temperature which was maintained between 20 and 32 C using gable and endwall exhaust fans on the north gable and endwall coupled with an evaporative cooling pad along the southern wall.

Experimental Design

The experiment was conducted twice and was arranged as a randomized complete block design with the three different hydroponic system types (NFT, DWC, and substrate culture) in three blocks arranged from south to north on the greenhouse floor. Each system in each block held 16 butterhead lettuce plants (Lactuca sativa 'Rex'), for a total of 48 plants per block and 144 plants total. The Lactuca sativa 'Rex' variety of lettuce was chosen for its reported heat tolerance and suitability for production in greenhouses.

System Construction

Construction of the systems started by sanitizing all equipment with ZeroTol 2.0 (BioSafe Systems E. Hartford, Conn.). The design of the systems included one 189.25L trough with a lid made of 1.9-cm thick polystyrene insulation board used as effluent water reservoirs. Aquaculture effluent was circulated through all systems using an individual TotalPond® fountain pump that pumped 1135.63 L hr-1. Each NFT system replicate contained two 11.75cm x 243.84cm CropKing® NFT channels with 8 plants spaced at 20.5cm intervals per channel, 16 plants total in the system. Two NFT channels per replicate were suspended over one reservoir using a table fabricated from 2.54-cm aluminum fencing piping and metal joint clamps. Effluent was pumped through 1.27-cm poly irrigation tubing, into micro-poly tubing, and distributed into the higher end of the NFT channel. Drainage pipes constructed of 2.54-cm diameter schedule 40 PVC piping were attached to the ends to facilitate a no-spill return of water to the reservoir. The deep-water culture consisted of one aerated 189.25-L reservoir with a cut-to-shape polystyrene insulation board as a floating raft on the surface containing 16 plants spaced at 20.5 cm intervals. Aeration was provided via an aeration pump pushing air through an air stone at the bottom of the reservoir once per hour for a period of 3 minutes. With the media-based culture, two 20.32-cm by 243.84-cm (10.2-cm deep) metal troughs fabricated out of sheet metal were filled to 10 cm deep with 100% Sungro® (Agawarm, MA) premium grade horticultural perlite and sloped at 3° to allow drainage from the substrate to return to the effluent reservoir positioned directly underneath the lower end of the troughs. A 20.32-cm space was left around the drainage holes to avoid clogging and emptying of perlite. Screens were made with Phifer® galvanized grey steel mesh and fastened in the trough to hold the perlite. This effluent traveled through 1.27-cm poly irrigation tubing, into micro-poly tubing positioned at each plant. Irrigation was recirculated constantly throughout the experimental run.

Plant culture

Two weeks prior to each experimental run 200 pelleted lettuce seeds (Lactuca sativa L. 'Rex'; Johnny's Selected Seeds, Winslow, ME) were each sown into one 2.54-cm Grodan A-OK rockwool cube in a 200-cell sheet that was placed in a 1020 propagation tray. Seeds were irrigated with municipal water for seven days followed by irrigation with a nutrient solution for eight days. Seedling fertilizer solution was comprised of 2.272g Gramp's Original hydroponic lettuce fertilizer (8-15-36), 1.704g 15.5-0-0 YaraLiva® calcium nitrate fertilizer and 1.136g Magriculture® magnesium sulfate heptahydrate per gallon. At experiment initiation lettuce transplants were randomly-selected and transplanted into the systems and grown for the remainder of the 30-d experimental periods. Aquaculture effluent was supplied from a biofloc-type recirculating aquaculture system producing Nile tilapia (*Oreochromis niloticus* L.) at 0.1 lbs/ gallon stocking density and 1% total body weight feeding ratio.

Data Collection and analysis

At experiment initiation, water samples from each reservoir were collected and analyzed for plant nutrient concentrations using ICP-MS (Waters Agricultural Laboratory; Camilla, GA). Solution pH and electrical conductivity (using a HANNA® instruments HI 9813-6 portable pH/EC/TDS/ºC meter) and [NO_3^-] (Horiba® LAQUAtwin nitrate meter) were measured once every other day throughout the experiment. At 15 and 30 days after transplanting (DAT) leaf greenness was quantified (Konica Minolta SPAD-502Plus chlorophyll meter) by averaging three readings from middle leaves of plants. At experiment termination (30 DAT) lettuce heads were harvested and fresh weight was measured. Lettuce heads were then dried in a forced-air drier and weighed to determine biomass. Foliar samples were then analyzed for nutrient concentrations using ICP-MS as digestion (Waters Agricultural Laboratory; Camilla, GA).

All plant and water data were subjected to analysis of variance (ANOVA) using PROC GLIMMIX in SAS (SAS Institute, Cary, North Carolina, USA). Two-way ANOVA of hydroponics system versus experimental run was conducted. For data measured over several days, repeated measures design procedure was adopted. In the repeated measures design approach used for SPAD and size index, compound symmetry (cs) was adopted for the covariance structure. In the case of SPAD and size Index, analysis was separated for mid-season and end of season measurements. In all cases, block was included in the 'random' function of the GLIMMIX procedure. Where several plant samples were taken for the same trait, as in the case of plant mass, plant samples were treated as random nested in each experimental unit to avoid pseudo replication. Posthoc mean comparison was done whenever p value was less than

5%. The Tukey Honest Significant Difference (HSD, a=0.05) was adopted to separate significant means. Where there was a significant interaction between hydroponic system type and experimental run, simple effects of experimental run were presented.

Dates

Experimental dates were 23 Jun. 2021 ending 22 Jul. 2021 (seeding on 9 Jun. 2021) for trial one, 10 Aug. 2021 ending 8 Sept. 2020 (seeding on 26 Jul. 2020) for trial two. Each experimental repetition lasted for a total of 45 days from germination to termination.

Results and Discussion

Aquaculture effluent pH was generally lower for all system types in trial one, ranging between 6.4 and 7.2, while Trial 2 pH values ranged from 7.0 to. 7.7 (Fig 2.1, 2.2). Substrate culture generally favored higher pH within the first trial compared to DWC and NFT whereas all system types had similar a pH trend in the second trial. The higher pH and more consistent pH trends in Trial 2 were likely the result of higher buffering capacity conferred by higher liming rates in the aquaculture unit at the time of Trial 2. Electrical conductivity (EC) trends showed SC EC decreasing the most throughout trial 1 (Fig. 2.3) whereas EC decreased most for DWC in Trial 2 (Fig. 2.4). Holliman et al. (2008) reported that aquaculture effluent contains most nutrients for plant biological processes and water analyses taken at the beginning of both trials reported similar concentrations of necessary nutrients such as nitrate-nitrogen (NO_3^{-}–N), phosphorous (P), potassium (K), calcium (Ca), sulfate ($SO^{2-}{}_4$), magnesium (Mg), iron (Fe), boron (B) and manganese (Mn) throughout all reservoirs (Table 2.1, 2.2). Phosphorus depletion was highest in SC in both trials (Table 2.3) likely due to the perlite substrate retaining solids which are known to adsorb P (Blanchard et al., 2020). Phosphorus tends to be adsorbed tightly by most soils and plants have evolved strategies to take up P at extremely low concentrations in soil solution. The P in the aquaculture effluent was likely adsorbed to organic solids that either accumulated in the substrate or that circulated through the system in suspension. It is likely that accumulation in the substrate would both improve uptake of P in extremely limiting conditions and reduce the potential for P to leave the system. The increased reduction by SC of P from effluent discharge could be beneficial by reducing chances for eutrophication of surrounding aquatic environments which is a solution for the issue proposed by Mustapha and Bakali (2021) where environmental contamination by aquaculture P is a main concern.

Foliar analyses from both trials showed a significant difference in N uptake of lettuce grown in a SC system when compared to NFT and DWC (Table 2.5) which coincides with findings from Zou et al. (2016) where SC crops also reported higher N uptake. Foliar concentrations (%) of P and S in lettuce were not significantly different between system types in either trial (Table 2.5). Uptake of foliar K was shown to be non-significant in the first trial but significant in the second trial with NFT having the highest percentage (9.07%) when compared to DWC (7.19%) or SC (7.11%) (Table 2.5). Concentrations (%) of foliar Mg in lettuce grown utilizing SC was shown to be significantly higher when compared to DWC and NFT systems in both trials (Table 2.5). Effluent solids contain high amounts of calcium and phosphorous (Blanchard et al., 2020) and accumulation of solids in substrates could increase uptake potential yet Ca concentrations (%) in foliar samples did not differ by system type in Trial 1 (Table 2.5). However, in Trial 2 foliar Ca was highest in SC which may have been assisted by the accumulation of organic solids.

Foliar B (ppm) was highest in DWC systems when compared to NFT or SC in the first trial and similar to concentrations in NFT but still significantly higher than SC in the second trial

(Table 2.6). Foliar Zn (ppm) was highest in SC and NFT compared to DWC in trial when compared to comparing to DWC in trial one and highest in SC when compared to NFT and DWC in Trial 2 (Table 2.6). Foliar Fe concentrations were similar in all system types in both trials (Table 2.6). Foliar copper (Cu) was highest in SC. (Table 2.6). There were no differences in leaf greenness at either 15 or 30 DAT in either Trial (Fig 2.5, 2.6).

With some exceptions, foliar nutrients were generally highest in SC plants. Nutrient uptake was generally either at the low end of sufficiency or below sufficiency for all nutrients except for Ca which was well above reported sufficiency ranges. This was likely due to the very high Ca concentrations in the AE resulting from frequent liming during fish production to keep pH at or near 7 to facilitate nitrifying bacteria. Foliar Mg concentrations were just below a reported sufficiency range (Mills and Jones, 1996) for NFT and DWC which may have resulted from high Ca uptake. However, foliar Mg was higher and within the reported sufficiency range for SC plants. Foliar Fe concentrations were the lowest compared to reported sufficiency ranges of any foliar nutrient. This was a result of Fe being very scarce in aquaculture effluent (Table 2.1, 2.2). It was hypothesized that providing a substrate for solids accumulation would increase Fe uptake in lettuce because most Fe excreted in fish waste is adsorbed to organic solids (Blanchard et al. 2020). It is likely that Fe was so low in the system that even solids accumulation in the substrate was not helpful. The generally low leaf greenness values are reflective of the low foliar Mg, and especially the low foliar Fe observed. Although plants were able to hyperaccumulate Fe, it is likely that Fe supplementation is necessary to reach maximum yield potentials in aquaponics as reported by others (Ayipio et al., 2019). Ultimately, even though AE was generally low in nutrient concentrations, SC plants were able to accumulate sufficient levels of several necessary macro and micronutrients, a trend also seen in the study conducted by Blanchard et al. (2020).

Size index was highest for NFT and SC in Trial 1 and for SC in Trial 2 at both 15 and 30 DAT (Fig. 2.7, 2.8). Head fresh weight was highest for NFT and SC in Trial 1 and for SC in Trial 2 (Fig 2.9). Head fresh weights were much higher in Trial 1 than in Trial 2 regardless of HP system type (Fig. 2.9). It is possible that this was due to limiting nutrients in Trial 2, particularly P. For example, P in aquaculture effluent sampled from the DWC, NFT, and SC systems at the end of trial 1 were 13.6, 18.8, and 1.2 mg L^{-1}, respectively, but were only 4.8, 2.8, and 0.9 mg L^{-1}, respectively, at the end of Trial 2 (Table 2.3). Recommendations for soluble P in hydroponic solutions vary but are generally around 60 mg L-1 for lettuce. It is likely that the extremely limiting P in Trial 2 led to the decreased marketable fresh weights. Biomass was highest in NFT and SC (Fig. 2.10, 2.11). Lennard and Leonard (2006) reported higher yields when comparing SC to NFT or DWC which is similar to our results in Trial 2 (Fig. 2.9). Deep water culture is the most popular HP system type used in aquaponics for lettuce production, yet performed worse than NFT and SC in both trials. In most cases, AE is continually refreshed since most aquaponics systems link aquaculture and HP systems in a recirculatory fashion. Our results suggest that DWC is not an ideal HP system however, especially when nutrients are limited and AE is not replenished. NFT and SC systems performed much better than DWC in a nutrient-limited scenario indicating that these system types may add resiliency to aquaponics system designs. However, NFT and SC systems are typically more expensive to construct and maintain than DWC.

Conclusion

Systems utilizing substrate culture showed increased foliar accumulation of certain nutrients such as N, Mg and Cu in both trials when compared to DWC and NFT system types. Although SC had higher concentrations of N and Mg, SPAD 502 Chlorophyll estimates were shown to be similar in all system types. Fresh head weight was similarly highest in SC and NFT when compared to DWC in the first trial. However, SC reported the highest fresh weights for trial two when compared to NFT or DWC. Dry weight trends saw SC and NFT having similar yet higher averages than DWC throughout both trials. Phosphorous depletion from SC effluent reservoirs shows the potential for increased removal of environmentally toxic nutrients within aquaponic systems. With increased rates of foliar N, Mg and Cu in SC, it supports the hypothesis of one system type having increased potential for nutrient uptake while These experiments were run under suboptimal conditions within the aquaculture environment resulting in low nutrient concentrations for starting effluent solutions. In order to address a major challenge in aquaponics, which is fluctuating and often limiting nutrient concentrations, future research needs to examine effects of HP system type on aquaponic lettuce under optimum starting nutrient concentrations with fertilizer complemented aquaponics.

Figures and Tables

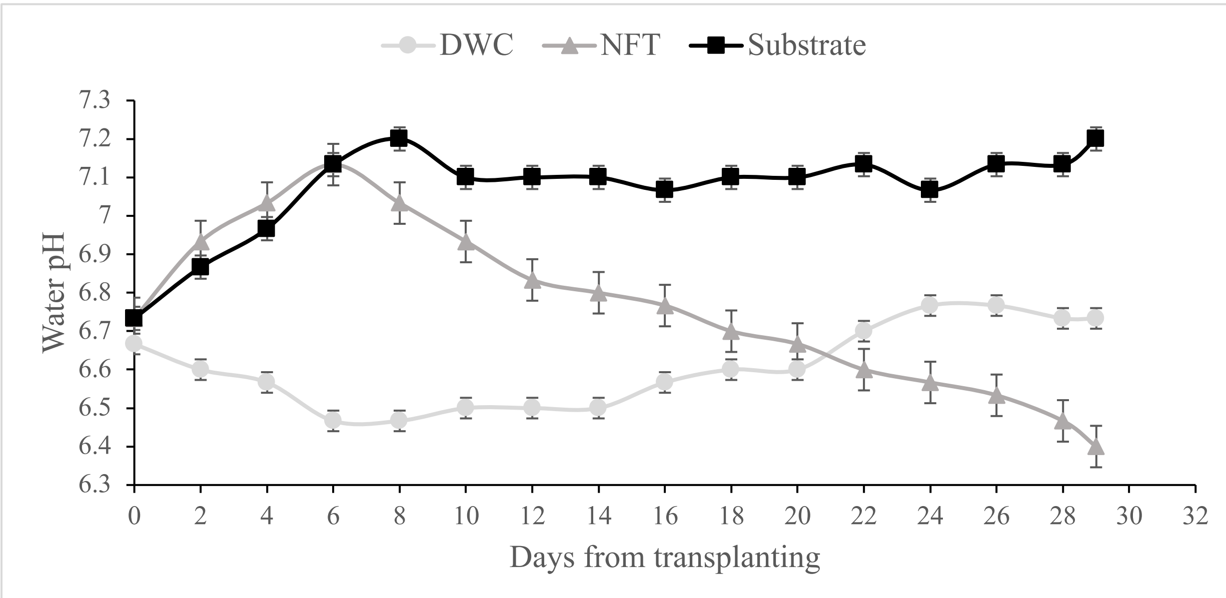

Figure 2.1- Trial 1 pH trends by system type. Repetitions from each block were combined by system type to give an average trend. DWC- deep water culture, NFT- nutrient film technique Substrate- substrate culture. Data taken bi-daily.

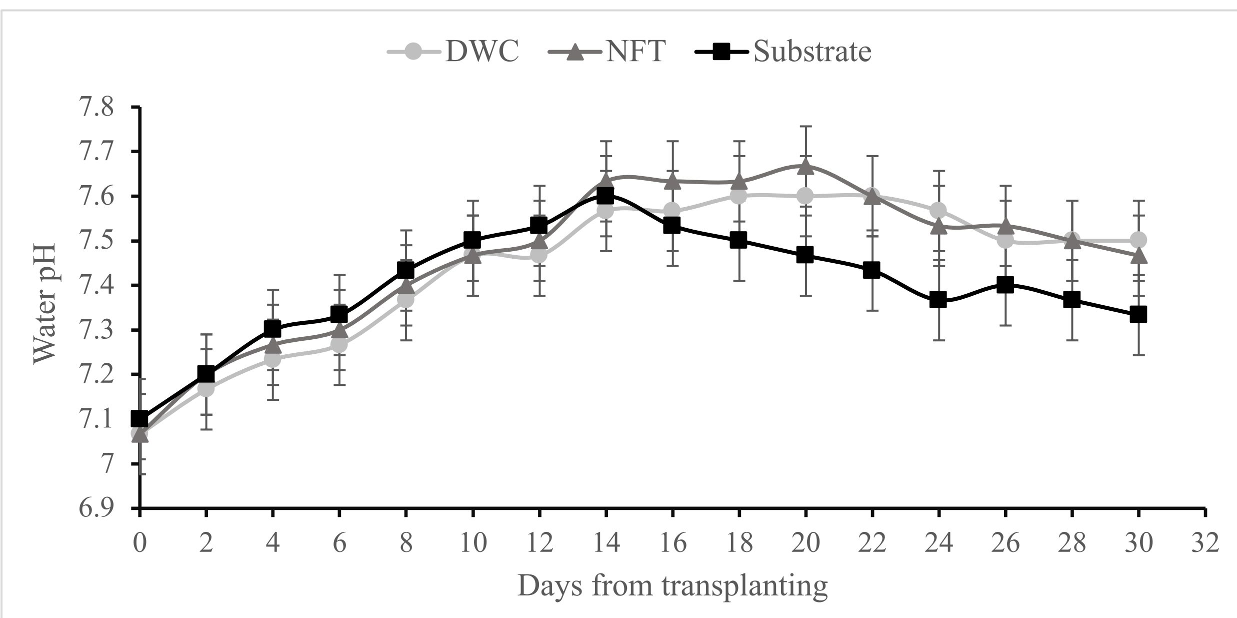

Figure 2.2- Trial 2 pH trends by system type. Repetitions from each block were combined by system type to give an average trend. DWC- deep water culture, NFT- nutrient film technique Substrate- substrate culture. Data taken bi-daily

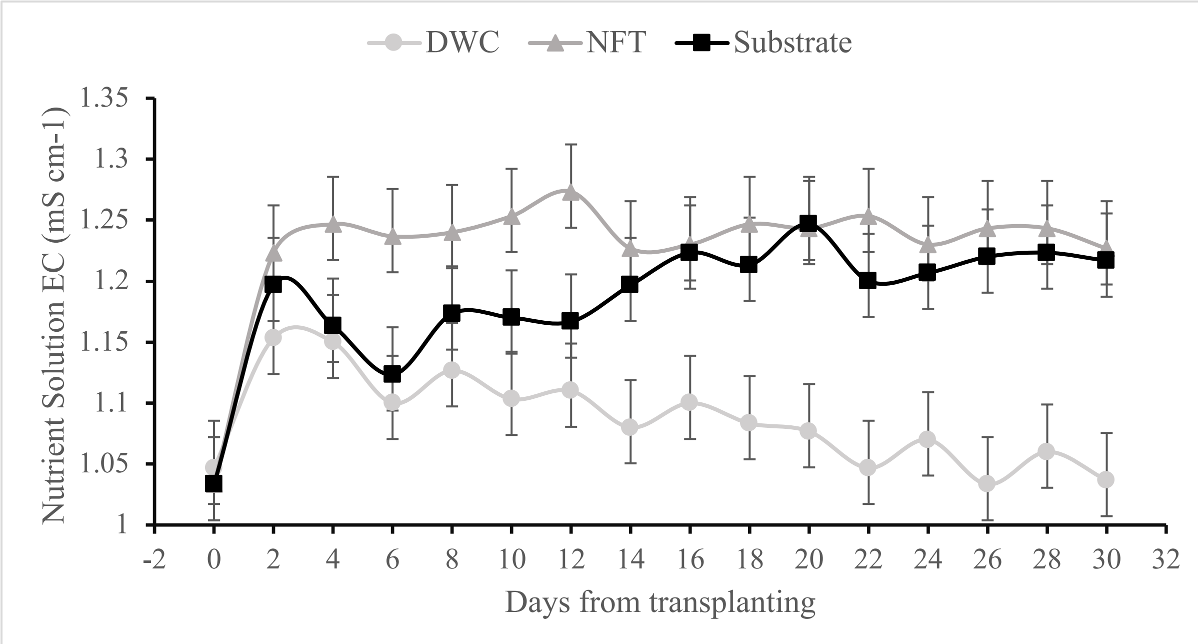

Figure 2.3- Trial 1 electrical conductivity (EC) trends by system type. Repetitions from each block were combined by system type to give an average trend. DWC- deep water culture, NFT- nutrient film technique Substrate- substrate culture. Data taken bi-daily.

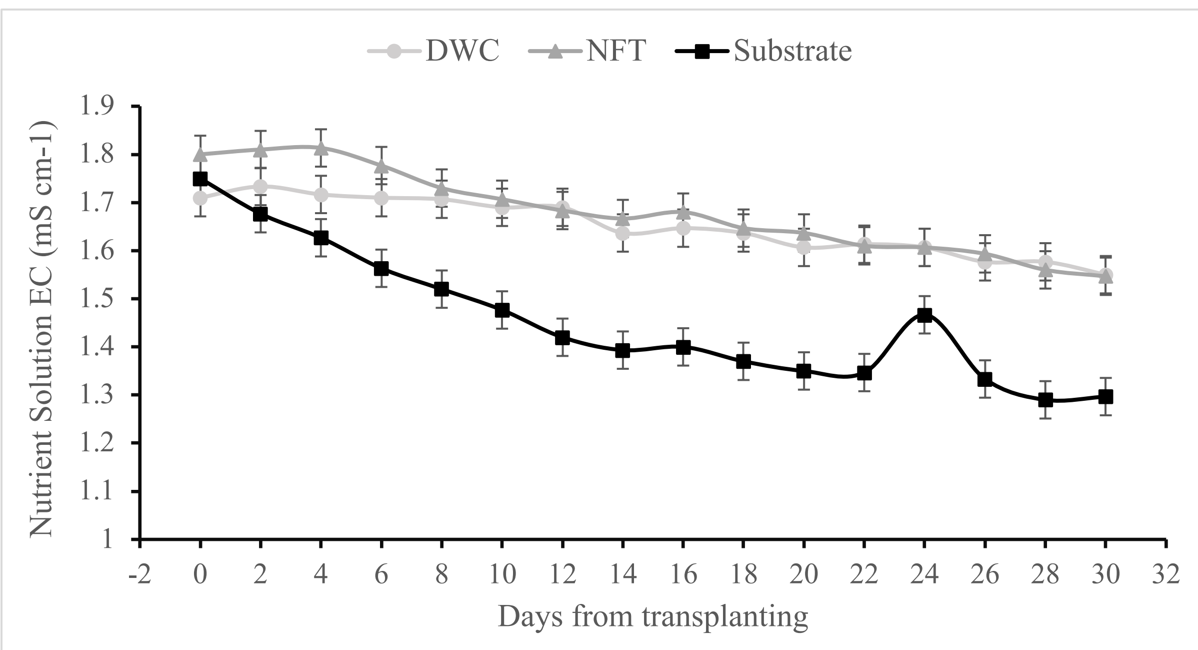

Figure 2.4- Trial 2 electrical conductivity (EC) trends by system type. Repetitions from each block were combined by system type to give an average trend. DWC- deep water culture, NFT- nutrient film technique Substrate- substrate culture. Data taken bi-daily.

Table 2.1. Effect of hydroponic system type on starting and ending nutrient concentration of aquaculture effluent in a 30 d experiment starting on 23 June 2021.

Nutrient	Nutrient Film Technique		Deep Water Culture		Substrate Culture		Hydroponic Recommended
	Start	End	Start	End	Start	End	
NO_3^--N	117.63[Z]	86.96	119.30	85.23	117.43	94.23	150
P	13.07	18.79	13.18	13.59	12.82	1.16	62
K	203.27	61.03	203.61	92.73	203.41	83.30	300
Ca	119.98	173.09	120.3	102.04	119.56	105.96	210
Mg	14.26	24.11	14.25	14.28	14.22	16.54	40
SO_4^{2-}	30.53	50.39	30.43	87.69	30.5	35.36	70
Fe	0.02	<0.01	0.017	<0.01	0.017	<0.01	2.5
B	0.057	0.087	0.057	0.057	0.053	0.073	0.4
Mn	0.02	<0.01	0.03	<0.01	0.01	<0.01	0.6

[Z]All data are reported in mg L^{-1} and are means from experimental blocks (n = 3).

Table 2.2. Effect of hydroponic system type on starting and ending nutrient concentration of aquaculture effluent in a 30 d experiment starting on 10 August 2021.

Nutrient	Nutrient Film Technique		Deep Water Culture		Substrate Culture		Hydroponic Recommended
	Start	End	Start	End	Start	End	
NO_3^--N	74.45[Z]	121.80	74.46	102.57	77.42	128.2	150
P	9.79	2.76	9.94	4.81	9.87	0.95	62
K	167.03	265.33	168.17	232.1	168.43	110.04	300
Ca	103.37	144.9	103.9	113.4	103.63	120.7	210
Mg	14.33	20.6	14.35	16.56	14.35	16.15	40
SO_4^{2-}	32.38	46.2	32.1	37.54	32.15	40.73	70
Fe	0.03	0.02	0.04	0.02	0.04	0.01	2.5
B	0.08	0.09	0.08	0.08	0.08	0.1	0.4
Mn	0.05	<0.01	0.06	<0.01	0.06	<0.01	0.6

[Z]All data are reported in mg L^{-1} and are means from experimental blocks (n = 3).

Table 2.3. Effect of hydroponic system type on macronutrient concentration (%) of aquaponic effluent solutions after 30 d.

	N	P		K		S		Mg		Ca	
System type[Z]	Pooled[Y]	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
DWC	93.9b[X]	13.6b	4.8	92.7	232.1a	29.2c	37.5	37.5b	16.6b	102.0b	116.7b
NFT	108.1ab	18.8a	2.8	61	265.3a	50.4a	46.2	46.2a	20.6a	173.1a	144.9a
Substrate	111.1a	1.2c	0.9	83.3	110.0b	35.4b	40.7	40.7b	16.1b	106.0b	120.7b
S.E.D.	4.69	0.58	1.05NS	12.28NS	10.12	1.49	2.03NS	0.86	0.45	5.87	2.9
P-Values											
System	0.0305	<.0001	0.1051	0.2508	0.0005	0.0004	0.0749	0.002	7.00E-04	0.0009	0.0038
Run	0.0002	<.0001		<.0001			0.0319		0.3071		0.9119
System x Run	0.3805	<.0001		<.0001			0.0061		0.0029		0.0008

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).
[Y]Data were pooled if experimental run was nonsignificant or were analyzed separately for Run 1 and Run 2 if experimental run was a significant effect according to an analysis of variance. Analysis of variance was performed using PROC GLIMMIX in SAS.
[X]Means followed by different letters were different according to Tukey's Honest Significant Different Test ($\alpha = 0.05$).

Table 2.4. Effect of hydroponic system type on micronutrient concentration (mg L^{-1}) of aquaponics solutions after 30 d.

System[Z]	B	Mn	Fe
DWC	0.067b[Y]	0.1	0.01333
NFT	0.088a	0.1	0.015
Substrate	0.085a	0.1	0.01167
S.E.	0.0041	NA	0.0029NS
P-Value			
System	0.0057	NA	0.6702
Run	0.0059	NA	0.0493
System x Run	0.1978	NA	0.6702

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).
[Y]Means followed by different letters were different according to Tukey's Honest Significant Different Test ($\alpha = 0.05$).

Table 2.5. Effect of hydroponic system type on foliar macronutrient concentration (%) of 'Rex' butterhead lettuce after a 30 d experimental period.

System type[Z]	N	P	K		Mg	Ca		S
	Pooled[Y]	Pooled	Run 1	Run 2	Pooled	Run 1	Run 2	Pooled
DWC	4.04b[X]	0.57	6.69	7.19b	0.28b	1.72	1.88c	0.24
NFT	4.40ab	0.62	6.99	9.07a	0.33b	2.47	2.43b	0.24
Substrate	4.78a	0.65	6.4	7.11b	0.43a	2.65	3.50a	0.24
Sufficiency Range[W]	4.2-5.6	0.62-0.77	7.82-13.68		0.24-0.73	0.8-1.2		0.25-0.32
SE	0.17	0.027NS	0.18NS	0.16	0.014	0.22NS	0.062	0.0052NS
P-Value								
System	0.0296	0.1403	0.1149	<.0001	<.0001	0.0528	<.0001	0.7893
Run	0.0375	<.0001	<.0001		0.0222		0.0304	0.1205
System x Run	0.4869	0.1465		0.0015	0.0832		0.043	0.7389

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).

[Y]Data were pooled if experimental run was nonsignificant or were analyzed separately for Run 1 and Run 2 if experimental run was a significant effect according to an analysis of variance. Analysis of variance was performed using PROC GLIMMIX in SAS.

[X]Means followed by different letters were different according to Tukey's Honest Significant Different Test (α = 0.05).

[W]Foliar sufficiency ranges from Mills and Jones (1996).

Table 2.6. Effect of hydroponic system type on foliar micronutrient concentration in 'Rex' butterhead lettuce after a 30 d experimental period.

System type[Z]	B		Zn		Fe	Cu
	Run 1[Y]	Run 2	Run 1	Run 2	Pooled	Pooled
DWC	27.3a[X]	33.0a	63.3b	29.3c	47.8	2.3c
NFT	23.0b	29.7a	104.7a	80.7b	45	3.7b
Substrate	22.0b	22.3b	102.0a	125.7a	41.8	5.0a
Sufficiency Range[W]	32-43		33-196		158-223	6-16
Standard error	0.69	1.62	12.04	6.68	4.67NS	0.22
P-Value						
System	0.0035	0.0058	0.0041	0.0008	0.6701	<.0001
Run		0.0007		0.1775	0.2546	0.0989
System x Run		0.0293		0.03	0.3077	0.4719

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).

[Y]Data were pooled if experimental run was nonsignificant or were analyzed separately for Run 1 and Run 2 if experimental run was a significant effect according to an analysis of variance. Analysis of variance was performed using PROC GLIMMIX in SAS.

[X]Means followed by different letters were different according to Tukey's Honest Significant Different Test (α = 0.05).

[W]Foliar sufficiency ranges from Mills and Jones (1996).

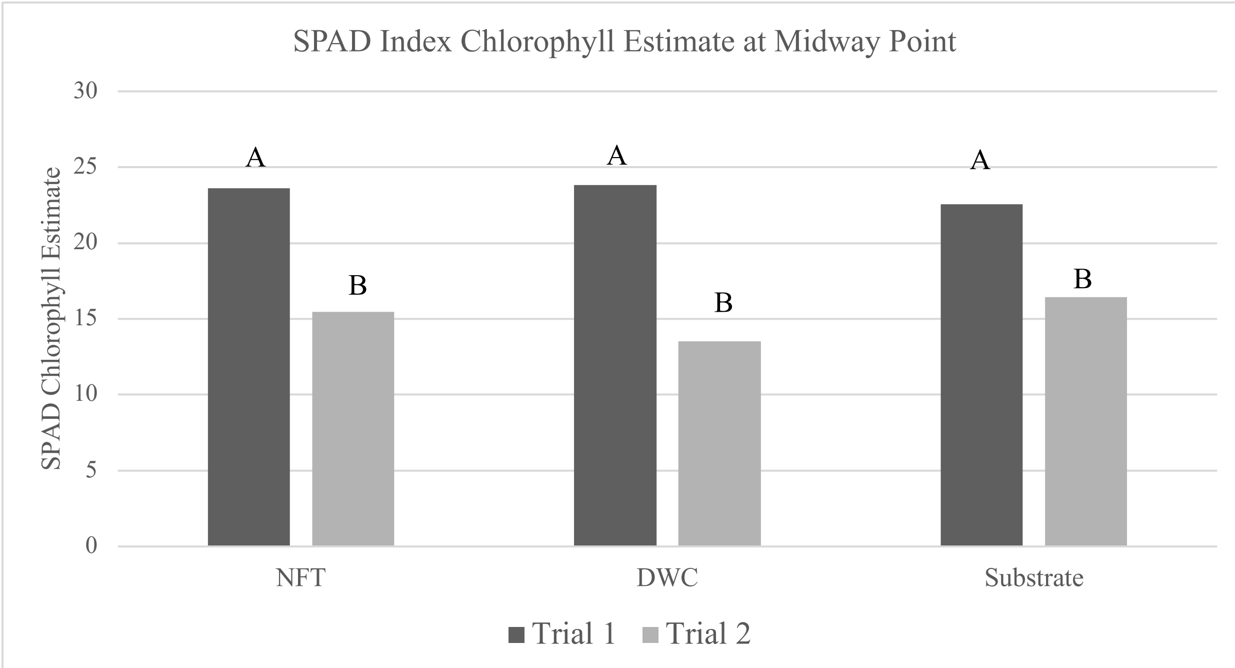

Figure 2.5- SPAD 502 Chlorophyll content estimates at midway points of trial (day 15). Reported as system averages of replicates. Trials were separated due to significant differences between trial results. NFT- nutrient film technique, DWC- deep water culture, Substrate- substrate culture

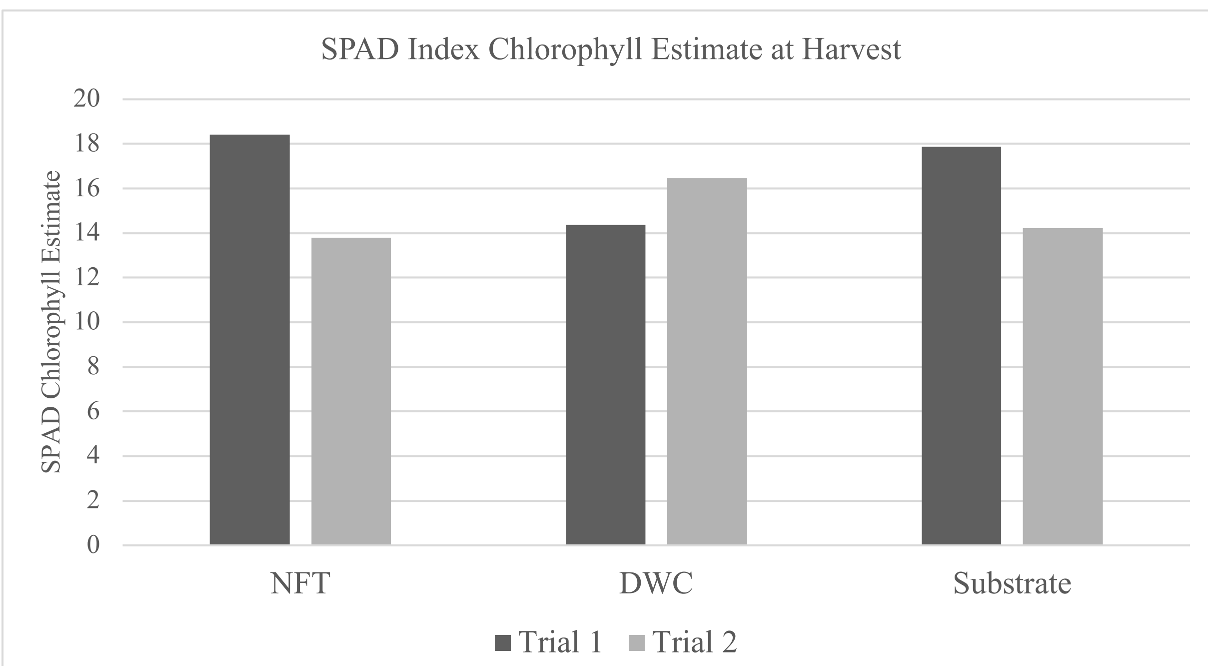

Figure 2.6- SPAD 502 Chlorophyll content estimates on day of harvest (day 30). Reported as system averages of replicates. Trials were separated due to significant differences between the trials.

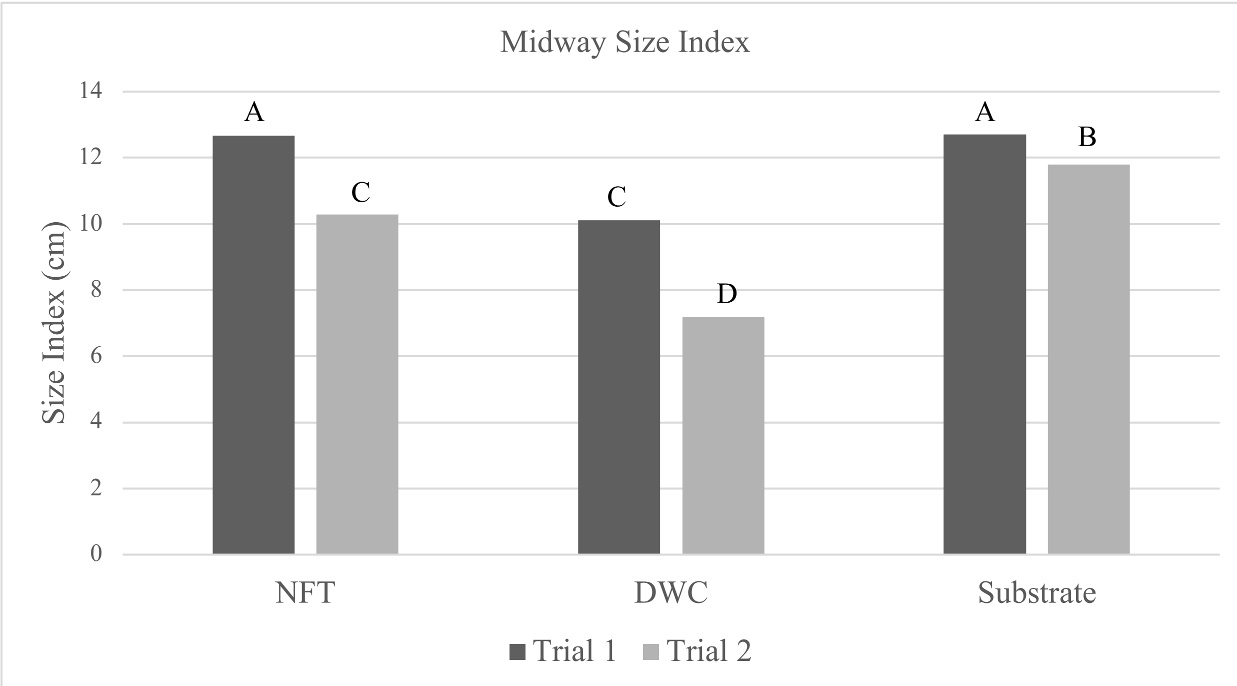

Figure 2.7- Size index reported on day 15 of the 30-day trials. Reported as averages of system replicates within each trial. Trials were separated due to significant differences between the trials.

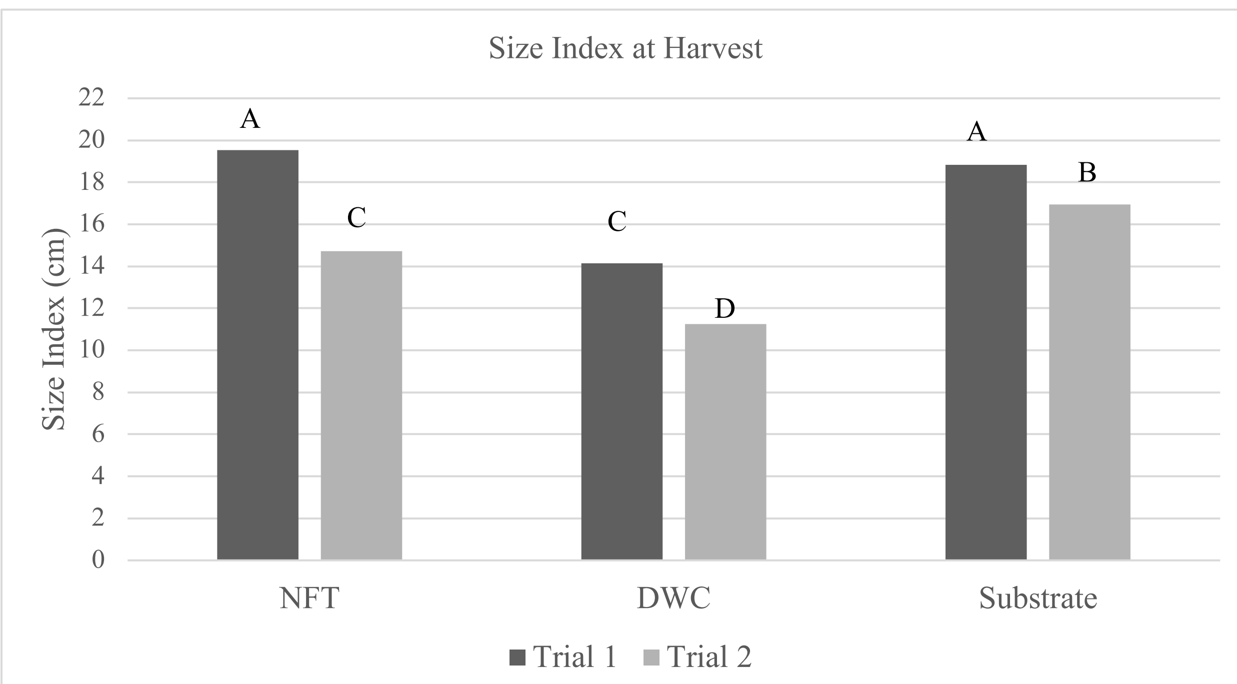

Figure 2.8- Size index reported on day 30 of the 30-day trials at harvest. Reported as averages of system replicates within each trial. Trials were separated due to significant differences between the trials. Size index- (l+w+h/3)

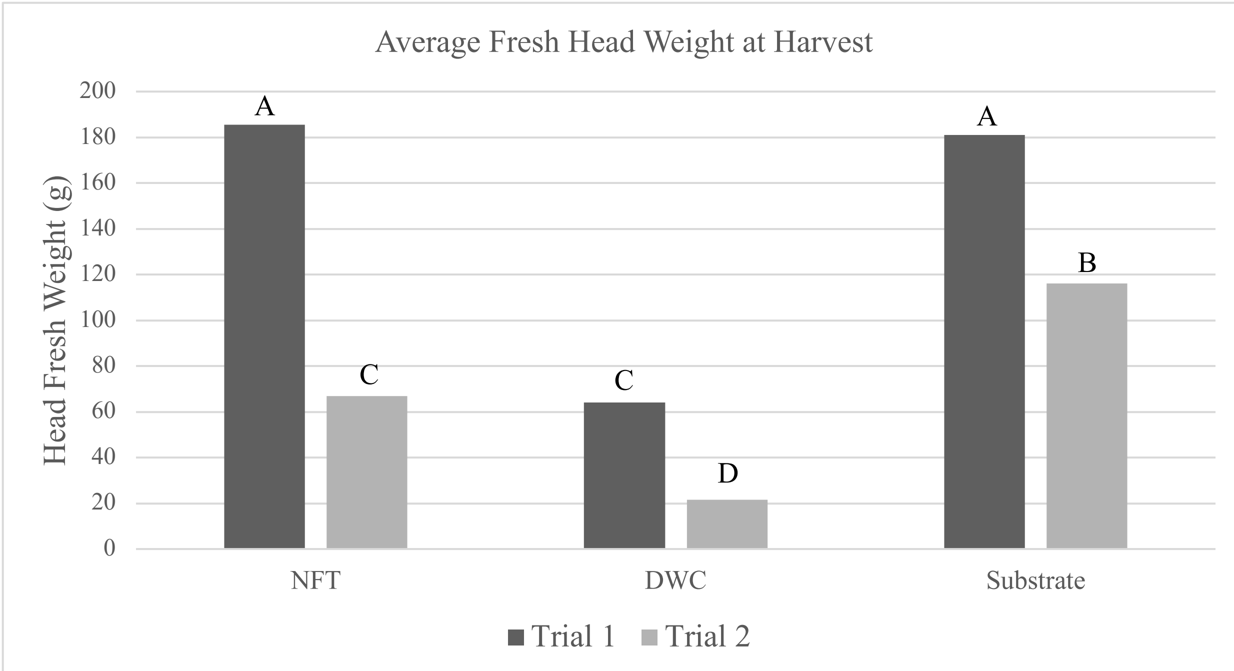

Figure 2.9- Effect of HP system type on resulting fresh head weights at harvest (day 30). Trials were separated due to significant differences between the trials.

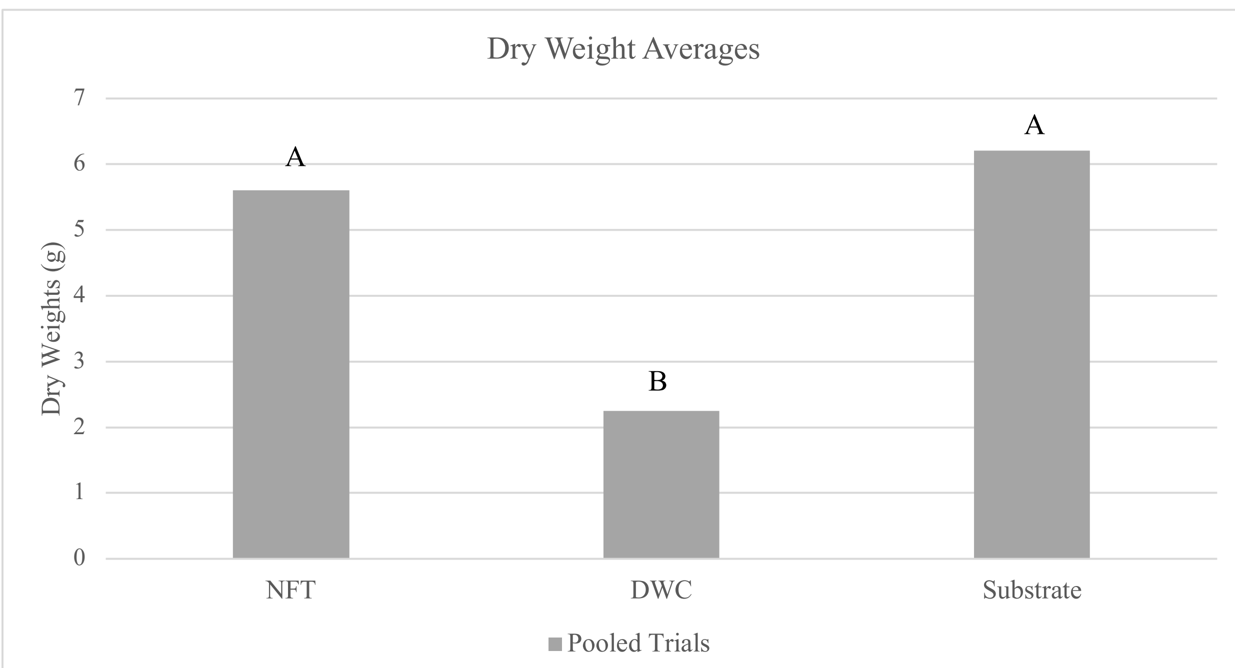

Figure 2.10- Effect of HP system type on ending dry weight/ biomass averages. Experimental trials were pooled due to no significant differences between the trials.

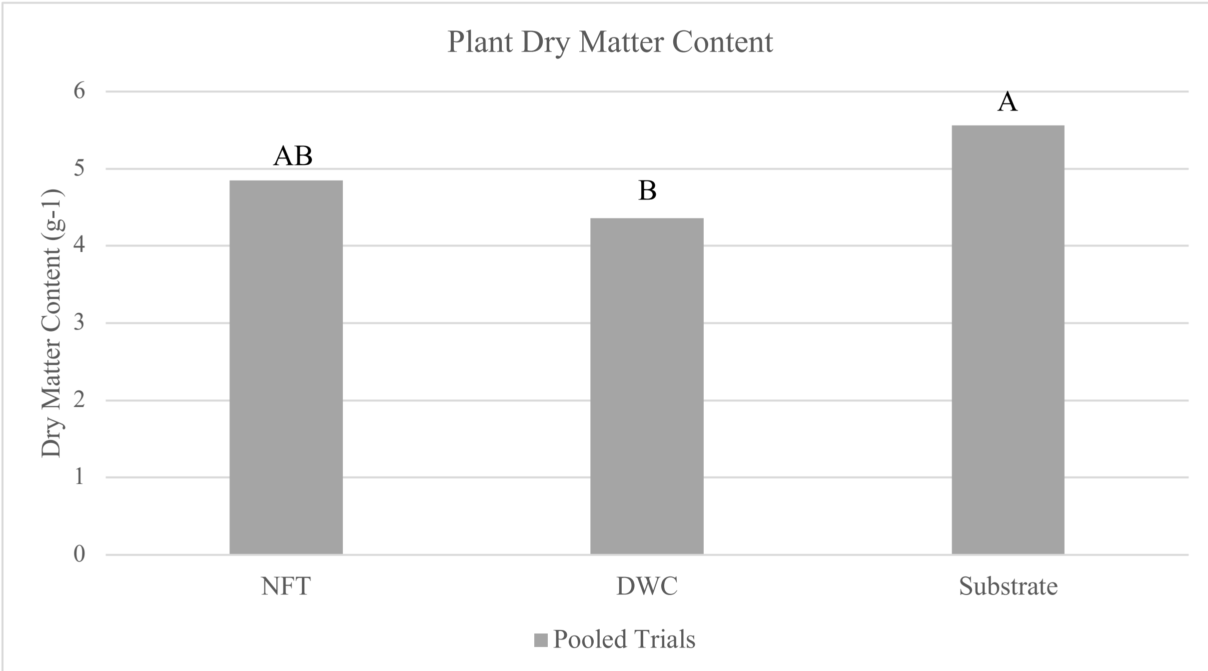

Figure 2.11- Plant dry matter content percentage averages by system type. Experimental trials were pooled due to no significant differences between the trials.

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Chapter 3

Effect of Hydroponic System Type on Growth and Nutrient Uptake of Lettuce (*Lactuca sativa* 'Rex') Irrigated with Supplemented Aquaculture Effluent

Abstract

A study was conducted at Auburn University with the objective to evaluate the effect of hydroponic system type on the growth and nutrient uptake potential of lettuce irrigated with aquaculture effluent supplemented with fertilizer. It was hypothesized that a system type utilizing a substrate would allow for increased foliar nutrient uptake when compared to other HP system types. This experiment implemented a randomized complete block design consisting of three blocks. Plants were grown for a total of 45 days from seeding to harvest. Analyses of foliar nutritional content and quality, yield, stomatal conductance, chlorophyll fluorescence, and effluent solution nutritional content were conducted to assess any differences.

Size index recorded at harvest reported substrate culture (SC) and nutrient film technique (NFT) as being alike yet were both considerably higher in contrast to deep water culture (DWC). Fresh head weight was highest in SC and NFT systems and lowest in DWC. Leaf chlorophyll estimates were highest in SC and DWC systems and lowest in NFT. Stomatal conductance (gsw), vapor pressure deficit (vpd), leaf apparent transpiration (E), photosystem II efficiency, and electron transport rate (etr) were shown to be similar between all system types. Lettuce cultivated utilizing SC reported the highest foliar accumulation of magnesium and calcium. Plants grown in DWC systems reported increased foliar accumulation of potassium when compared to SC yet was similar to NFT levels. All system types were similar in foliar concentrations of nitrogen, phosphorous, sulfur, boron, iron and copper. Similar trends in pH as well as electrical conductivity were observed throughout all system types.

Introduction

Aquaponics is an emerging means of food production that is often considered a more sustainable approach to traditional hydroponics. This method of production implements hydroponic practices with aquaculture waste effluent as irrigation water (Goddek et al., 2015). An aquaponics ecosystem is comprised of the plants, aquatic species in an aquaculture production pond/tank as well as nutrient converting bacteria in the microbiome (Somerville et al., 2014). Feed, after metabolization of aquatic organisms, is excreted as waste. Fish excrement and undigested feed mix with other microorganisms in the aquaculture environment creating a nutrient rich aquacultural effluent (Holliman et al., 2008; Montanhini and Ostrensky, 2015). Analyses of aquaculture effluent have shown high levels of nitrates (NO_3^-), nitrites (NO_2), unionized ammonia (NH_3) and ammonium (NH_4^+) can be found in solution (Moya et al., 2016). The main source of plant available nitrogen comes from the conversion of NH_3 to NO_3^- completed by nitrifying bacteria (Love et al, 2014). Excessive accumulation of compounds such as NH_3 and NO_2 can be toxic and detrimental to aquatic animal health (Mateus, 2009). Excess microbe and nutrient buildups in AP systems can be filtered out by plants (Rakocy et al., 2006). Using aquaculture waste in aquaponics can be considered a sustainable symbiotic between two production systems.

In terms of production, some research has shown that one system versus another could possibly be better for nutrient acquisition in aquaponic production. A study conducted by Lennard and Leonard (2006), reported slightly better performance concerning growth and biomass yield in a SC system utilizing a gravel media compared with DWC and NFT production systems. During a trial by Zou et al. (2016), AP utilizing SC and DWC to produce pak choi showed higher nitrogen accumulation in the SC system plants samples when compared to the DWC. Another study conducted using tomatoes in three differing hydroponic system types showed that the substrate-based production system also performed slightly better when compared to the others (Schmautz et al., 2016). These findings were consistent with findings in review of 122 published works by Maucieri et al. (2018) where in several cases NFT production was shown to be less efficient than SC or DWC. Through reports by Pinho et al. (2017) lettuce grown with aquaculture effluent containing organic solids resulted in better production when compared with effluent that had been screened and clarified. Observed results between lettuce grown in effluent containing solids and lettuce grown in clarified effluent as well as solids nutritional content analyses from Blanchard et al., (2020) show that solids play a crucial role in the nutrient supply.

When analyzed, aquaponic effluent nutrient levels within a high-density aquaculture and media-based AP system have been found to be lacking in some essential nutrients when compared to recommended levels for nutrient solutions (Blanchard et al., 2020). Two of the main lacking nutrients reported by Blanchard et al., 2020 (Dutch bucket SC using 100% perlite media) were found to be iron and magnesium. Although nutrients within the aquaponic effluent were found to be well below recommended levels, foliar analyses reported leaves contained above satisfactory levels of macro- and micro-nutrients (Blanchard et al., 2020). These findings in the literature lead toward a SC system possibly allowing for higher acquisition of effluent solids around the root biome.

Aquaponic production can be a sustainable alternative to hydroponics that mitigates waste discharge from an aquaculture operation (Mustapha and Bakali, 2021). Knowing which system type more efficiently utilizes effluent will benefit aquaponic producers. Therefore, the aim of this study will be to assess the differences in yield and foliar nutrient uptake of butterhead lettuce (*Lactuca sativa* 'Rex') between the SC, NFT and DWC utilizing fertilizer complemented aquaculture effluent from low-density Nile tilapia (*Oreochromis niloticus* L.) production. We hypothesize that SC systems would possibly allow for increased nutrient uptake potential and resulting growth when compared to DWC or NFT with initial optimum nutrient levels.

Materials and Methods

Location

This study took place in a dual-bay, gutter-connected greenhouse with dimensions of 18.2 m by 36.5 m with a north-south orientation at the Auburn University Aquaponics Research System located within the E.W. Shell Fisheries Center in Auburn, Alabama (lat. 32.648932 N, long. -85.487103). Research greenhouses were constructed with double polycarbonate end walls and layered in an inflated, double layer of 3-mil polyethylene on the sidewalls and roof. A controlled environment computer (Bartlett GHK12X2GH; Bartlett Controllers, Fort Madison, Iowa) governed temperature which was maintained between 20 and 32 C using gable and end-wall exhaust fans on the north gable and end-wall coupled with an evaporative cooling pad along the southern wall.

Experimental Design

The study was conducted once and was arranged as a randomized complete block design with three differing hydroponic system types (DWC, substrate culture and NFT) in three blocks arranged from south to north on the greenhouse floor. Each individual system in each block held 16 butterhead lettuce plants (Lactuca sativa 'Rex'), for a total of 48 plants per block and 144 plants total. The Lactuca sativa 'Rex' variety of lettuce was chosen for its reported heat tolerance and suitability for production in southern greenhouses.

System Construction

Construction of the systems started by sanitizing all equipment with ZeroTol 2.0 (BioSafe Systems E. Hartford, Conn.). The design of the systems included an individual 189.25L tank with a lid made of 1.9cm thick polystyrene insulation board used to contain the effluent. Aquaculture effluent was circulated through all systems via an individual TotalPond® fountain pump that pumped 1135.63 L hr-1. Each NFT system replicate contained two 11.75cm x 243.84cm CropKing® NFT channels with 8 plants spaced at 20.5cm intervals per channel, 16 plants total in the system. Two NFT channels per replicate were suspended over one reservoir using a table fabricated from 2.54cm aluminum fencing piping and metal joint clamps. This effluent traveled through 1.27cm poly irrigation tubing, into micro-poly tubing, and distributed into the higher end of the NFT channel. Drainage pipes constructed of 2.54cm diameter schedule 40 PVC piping was attached to the ends to facilitate a no-spill return of water to the reservoir. The deep-water culture consisted of one aerated 189.25L reservoir with a cut-to-shape polystyrene insulation board as a floating raft on the surface containing 16 plants spaced at 20.5 cm intervals. Aeration was provided via an aeration pump pushing air through an air stone at the bottom of the reservoir once an hour for a period of 3 minutes. With the media-based culture, two 20.32cm by 243.84cm (10.2cm deep) metal troughs fabricated out of sheet metal were filled to 10cm deep with 100% Sungro® premium grade horticultural perlite and sloped at 3° to allow drainage from the substrate to return to the effluent reservoir positioned directly underneath the lower end of the troughs. A 20.32cm space was left around the drainage holes to avoid clogging and loss of perlite. Drainage screens were fabricated out of Phifer® (Tuscaloosa, Al.) galvanized grey steel mesh and fastened in the trough to hold the perlite. This effluent traveled through

1.27cm poly irrigation tubing, into micro-poly tubing positioned at each plant. Irrigation was recirculated constantly throughout the experimental run.

Plant Culture

Fifteen days prior to beginning this experimental run 200 pelleted lettuce seeds (Lactuca sativa L. 'Rex'; Johnny's Selected Seeds, Winslow, ME) were each sown into one 2.54-cm Grodan A-OK rockwool cube in a 200-cell sheet which was placed in a 1020 propagation tray. Seeds were irrigated with municipal water for seven days followed by irrigation with a seedling oriented nutrient solution for eight days. Seedling fertilizer solution was comprised of 2.272g Gramp's Original hydroponic lettuce fertilizer (8-15-36), 1.704g 15.5-0-0 YaraLiva® calcium nitrate fertilizer and 1.136g Magriculture® magnesium sulfate heptahydrate per gallon. Upon initiating the experiment lettuce transplants were randomly-selected and transplanted into the systems and grown for the remainder of the 30-d experimental period. Aquaculture effluent was supplied from a biofloc-type recirculating aquaculture system producing
Nile tilapia (*Oreochromis niloticus* L.) at 0.1 lbs/ gallon stocking density and 1% total body weight feeding ratio.

Fertilizer for supplementation of effluent reservoirs was Jack's® Nutrients Part A, 5-12-26. Based on water analyses conducted (Waters Diagnostics Laboratory located in Camilla, Ga.) it was determined to add 416g of fertilizer to each water reservoir meet recommended nutrient levels for hydroponic greenhouse lettuce. Nutrient sufficiency targets were as follows:150ppm-N, 62ppm-P, 300ppm-K, 210ppm-Ca, 40ppm-Mg, 70ppm-S, 2.5%-Fe, 0.4%-B, 0.05%-Cu, 0.6%-Mn, and 0.1%-Zn. The fertilizer was added on the fourth experimental day due to the time needed for completing water analyses.

Data and analysis

Data measured included effluent water samples for each reservoir being taken at the beginning and end of the experiment and sent to Waters Diagnostics Laboratory located in Camilla, Ga. for nutrient composition analysis. Water data such as pH and electrical conductivity (using a HANNA® instruments HI 9813-6 portable pH/EC/TDS/ºC meter) were taken once every other day from the transplant date throughout the study until termination. Nitrate readings of each reservoir were taken every other day alongside pH and EC using a Horiba® LAQUAtwin NO_3^- water quality meter (Ann Arbor, Mi.). An estimation of chlorophyll content in the plants was conducted using an average of three readings from the middle leaves of the plants from a Konica Minolta SPAD-502Plus chlorophyll meter at the 15-day mark (midpoint of trial) and at the termination of the experiment (day 30). Measurements of active transpiration, stomatal conductance, electron transport rate, and photosystem 2 activity (chlorophyll fluorescence) were taken using the LI-COR® (Lincoln, Ne, USA) LI 600 porometer/ fluorometer at the time of harvest (day 30). Readings for the LI 600 were taken on four sub-samples per system type, with two readings per plant on mid-stage growth. Harvest fresh head weight was measured in grams on each of the plant sub-samples. Whole plant mass excluding root mass was dried for three days in a commercial drier and ultimately a measurement of total biomass accumulated per sample was collected.

All plant and water data were subjected to analysis of variance (ANOVA) using PROC GLIMMIX in SAS (SAS Institute, Cary, North Carolina, USA). Two-way ANOVA of hydroponics system versus experimental run was conducted. For data gathered over a period of several days, repeated measures design procedure was utilized. In the repeated measures design approach used for SPAD and size index, compound symmetry (cs) was used for the covariance structure. With SPAD and size Index, analysis was separated for mid-season and termination of season measurements. In all cases, Block was included in the 'random' function of the GLIMMIX procedure. Where several plant samples were taken for the same trait, as in the case of plant mass, plant samples were treated as random nested in each experimental unit to avoid pseudo replication. Posthoc mean comparison was done whenever p value was less than 5%. The Tukey Honest Significant Difference (HSD, a=0.05) was adopted to separate significant means. Where there was a significant interaction between hydroponic system type and experimental run, simple effects of experimental run were presented.

Dates

This study was conducted from 24 Sept. 2021 ending 24 Oct. 2021 (seeding on 9 Sept. 2021). This experiment ran for a total of 45 days from seeding to termination.

Results and Discussion

Analyses from effluent solutions at the beginning of the trial reported similar nutrient composition for all system replicates (Table 3.1). Final nutrient concentrations of the effluent showed an increase in nutrients such as P, K, Mg, SO^{2-}_4, Fe and B throughout all system types most likely due to a combination of the addition of fertilizer and decrease in dilution from evaporation and water loss (Table 3.1). Each system type showed similar trends of pH throughout the trial with SC sustaining the highest pH and DWC sustaining the lowest (Fig. 3a). Electrical conductivity (EC) followed similar trends in all system types with SC reporting the lowest EC at the end of the trial (Fig. 3.2). System trends for NO_3^- concentrations (ppm) followed similar patterns for each system type with SC consistently reporting the lowest amounts of NO_3^- while NFT consistently reported the highest NO_3^- concentrations throughout the trial (Fig. 3.3). Stomatal conductance (gsw), vapor pressure deficit (vpd), leaf apparent transpiration (E), photosystem II efficiency, and electron transport rate (ETR) were shown to be similar between all system types (Table 3.2).

Substrate culture lettuce accumulated the most foliar Mg and Ca (%) when compared to NFT or DWC (Table 3.3). Magnesium is a central element in chlorophyll (Vojnich et al., 2017) as well as necessary element for plant metabolism, an increase in Mg helps support foliar quality and chlorophyll presence. Increased calcium in lettuce helps bolster cellular structure as calcium is a key element in plant cell walls (White and Broadley, 2003). Potassium foliar concentrations (%) were highest in DWC compared to SC yet similar when compared to NFT (Table 3.3). Plant accumulation of zinc was higher in NFT systems when compared to DWC however, found to be similar when compared to SC (Table 3.4). All system types were similar in foliar concentrations of N, P, S, B, Fe and Cu.

Leaf chlorophyll readings at the end of the trial showed SC and DWC system types were similar and the highest when compared plants grown in NFT (Table 3.5). Size index recorded at harvest reported SC and NFT as being alike yet were both considerably higher in contrast to DWC (Table 3.5). Fresh head weight was highest in SC and NFT when compared to DWC plants (Table 3.5). Findings from dry weight comparisons reported similar trends to fresh weights with SC and NFT having the highest averages in contrast to DWC samples (Table 3.5). Dry matter percentage data showed DWC systems with increased averages when compared to NFT or SC which were similar in their reports (Table 3.5).

Conclusion

Uptake of some nutrients and their assimilation within plant tissues can be affected by system type as was seen with foliar uptake of Mg and Ca in SC systems. However, one system type was not shown to significantly outperform the rest with ending harvest weights. In this trial at this specific location, SC and NFT performed similarly in many aspects of growth. All system types reported similar concentrations of N, P, S, B, Fe and Cu within foliar content. Calcium additions to the aquaculture area in the form of hydrated lime ($Ca(OH)_2$) would most likely be the factor causing an overabundance of Ca within foliar samples. This is one example of how any changes within the aquaculture environment supplying the effluent can ultimately affect plant cultivation. Within an aquaculture system with low stocking density, as observed in this study, an increased addition of fertilizer is needed to reach sufficient nutrient concentration ranges. Under optimum stocking density and feeding ratios it is expected that less fertilizer would need to be supplemented for adequate supply.

Calcium increase observed in SC could be a result of deposits of Ca rich effluent solids trapped within the perlite substrate. Solids accumulation within the substrate could have allowed the roots to better interact with nutrients trapped within the particles as was the trend observed by Blanchard et al. (2020) as well at Monsees et al. (2017) working with aquaculture sludge. An interesting observation was the pH trend within the SC systems where it remained between 6.6 and 7.3 opposed to DWC which remained in a range of 6.2 to 6.7. The interaction of pH and relative nutrient availability was not the purpose of this study yet resulting available nutrients could have been affected by higher or lower pH trends. Further research should investigate the nutrient accumulation within differing hydroponic substrates under optimum aquaculture production density and nutrient concentrations.

Figures and Tables

Table 2.1. Effect of hydroponic system type on starting and ending nutrient concentration of aquaculture effluent in a 30 d experiment starting on 24 September 2021.

Nutrient	Nutrient Film Technique		Deep Water Culture		Substrate Culture		Hydroponic Recommended
	Start	End	Start	End	Start	End	
NO_3^--N	155.56	116.67	153.54	108.76	156.56	152.02	150
P	12.57	40.61	12.59	45.61	12.63	60.93	62
K	258.55	295.55	263.86	279.32	264.54	354.23	300
Ca	154.59	63.05	157.21	61.69	156.87	75.13	210
Mg	16.7	116.08	16.81	107.72	16.85	128.06	40
$SO^{2-}{}_4$	45.18	240.23	46.1	232.21	45.42	278.09	70
Fe	0.15	1.06	0.12	0.84	0.13	1.34	2.5
B	0.06	0.46	0.06	0.44	0.06	0.55	0.4
Mn	0.01	<0.01	0.01	0.02	0.01	<0.01	0.6

[Z]All data are reported in mg L^{-1} and are means from experimental blocks (n = 3).

Table 3.2. Effect of hydroponic system type on lettuce leaf physiology in a 30 d trial starting on 24 September 2021.

`	gsw[a]	Leaf vpd[b]	Leaf apparent E[c]	PSII[d] efficiency	ETR[e]
DWC	0.2032	0.8977	1.7398	0.7417	68.1778
NFT	0.2337	0.8898	1.9428	0.7288	77.1194
Substrate	0.1492	0.9204	1.3322	0.707	81.003
S.E.D	0.024NS	0.017NS	0.16NS	0.013NS	6.4NS
P-Value	0.0892	0.4673	0.0575	0.2718	0.2737

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).

[Y]stomatal conductance to water vapor (gsw)

[X]vapor pressure deficit (vpd)

[W]leaf apparent transpiration (E)

[V]photosystem 2 (PSII)

[U]electron transport rate (ETR)

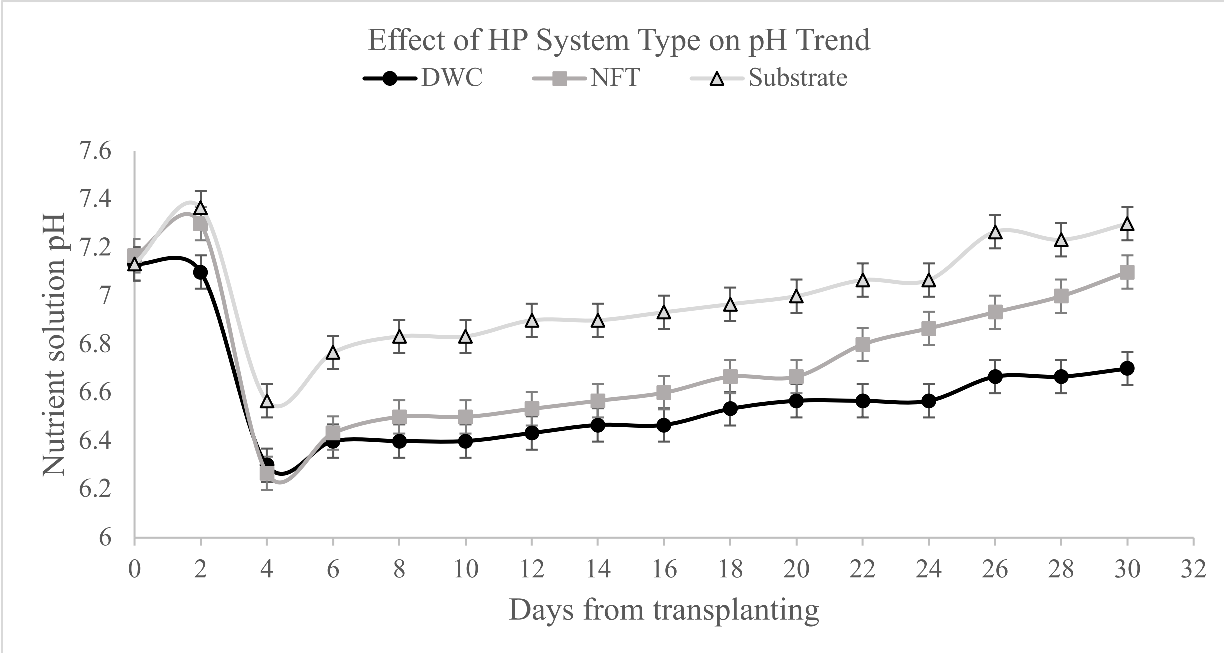

Figure 3.1- Effect of hydroponic system type on pH trends of effluent solution throughout trial (30 days).

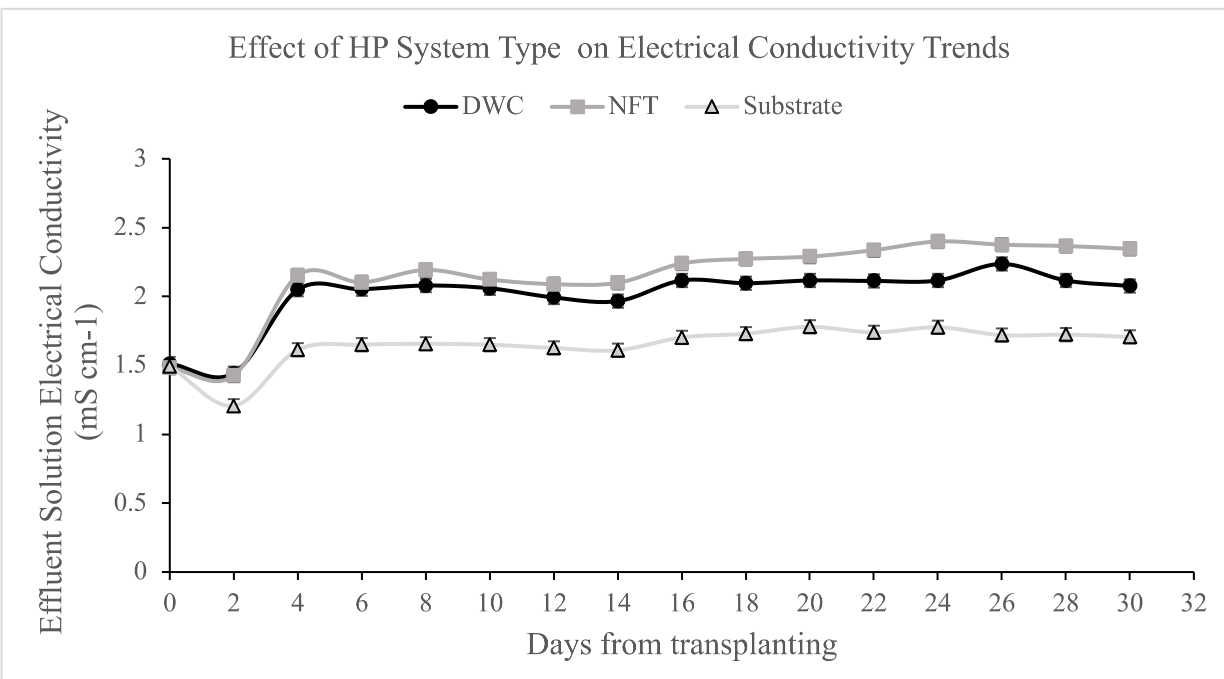

Figure 3.2- Effect of HP system type on electrical conductivity trends of effluent solution throughout trial (30 days).

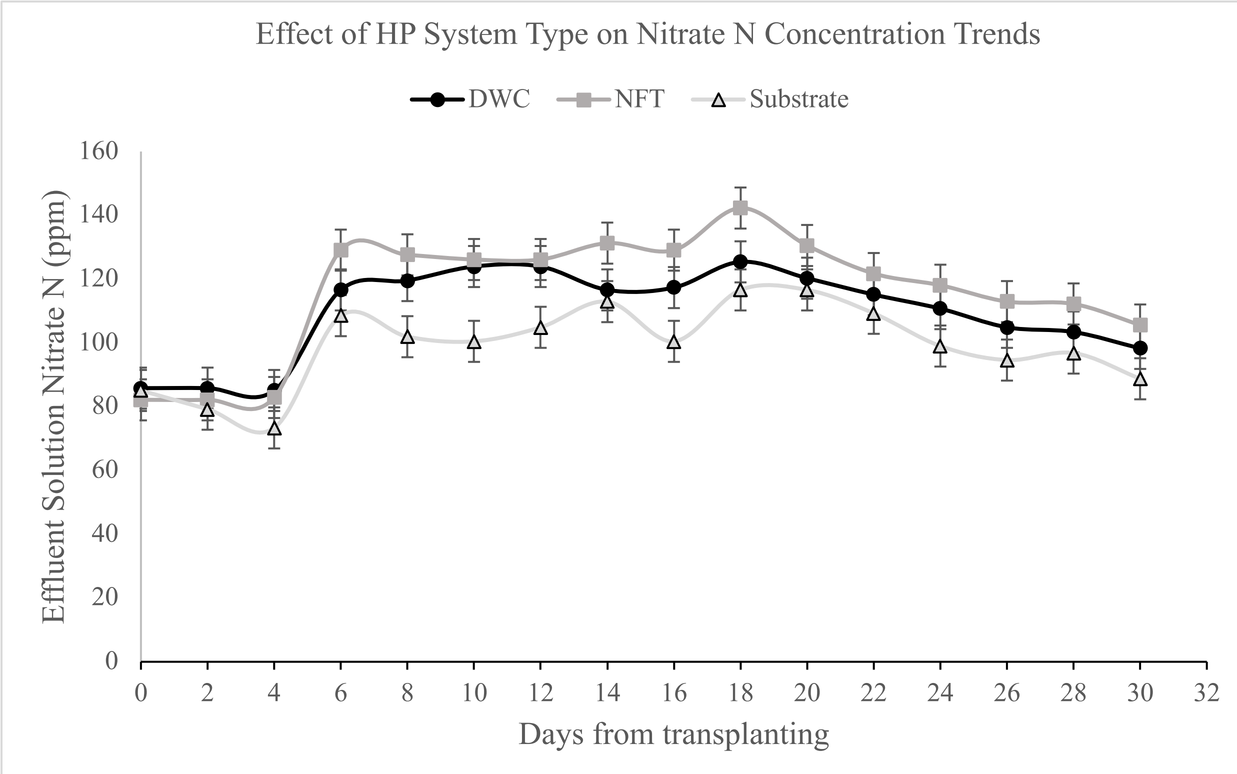

Figure 3.3- Effect of hydroponic system type on Nitrate N concentrations throughout 30-day growth cycle.

Table. 3.3. Effect of hydroponic system type on foliar macronutrient uptake from aquaculture effluent in an experiment lasting 30 d.

System Type[Z]	N	P	K	Mg	Ca	S
DWC	5.01	0.85	9.1a[Y]	0.43b	1.06b	0.26
NFT	5.33	0.89	8.69ab	0.48ab	1.28ab	0.25
Substrate	5.51	0.93	7.81b	0.55a	1.66a	0.25
Sufficiency Range[X]	4.2-5.6	0.62-0.77	7.82-13.68	0.24-0.73	0.8-1.2	0.25-0.32
Standard error	0.1911NS	0.02906NS	0.3552	0.02028	0.09856	0.008819NS
P-Value	0.0974	0.1322	0.0282	0.0157	0.0139	0.3338

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).

[Y]Means followed by different letters were different according to Tukey's Honest Significant Different Test ($\alpha = 0.05$).

[X]Foliar sufficiency ranges from Mills and Jones (1996).

Table. 3.4. Effect of hydroponic system type on foliar micronutrient uptake from aquaculture effluent in an experiment lasting 30 d.

System Type[Z]	Fe	B	Cu	Zn
DWC	71	27	6.33	31b[Y]
NFT	44	28	7	65a
Substrate	115	22	9.67	55ab
Sufficiency Range[X]	158-223	32-43	6-16	33-196
Standard error	24.2449NS	1.8954NS	1.0364NS	6.2923
P-Value	0.1949	0.0832	0.1317	0.0245

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).

[Y]Means followed by different letters were different according to Tukey's Honest Significant Different Test (α = 0.05).

[X]Foliar sufficiency ranges from Mills and Jones (1996).

Table 3.5. Effect of hydroponic system type on leaf greenness, size index, fresh weight, dry weight, and dry matter content of 'Rex' butterhead lettuce in a 30 d experiment.

System Type[Z]	Leaf Chlorophyll Index (SPAD)		Size Index[Y]		Fresh Weight (g)	Dry Weight (g)	Dry Matter Content (%)
	15 DAP[X]	30 DAP	15 DAP	30 DAP			
DWC	21.1a[W]	23.0a	9.5b	18.2b	99.4b	5.15b	5.26a
NFT	19.2ab	19.5b	11.4ab	21.8a	181.1a	8.21a	4.55b
Substrate	18.4b	23.6a	12.2a	23.4a	221.3a	9.57a	4.33b
SE	0.39	0.64	0.46	0.48	10.93	0.41	0.14
P-Value	0.02	<.0001	0.0207	0.0007	0.0006	0.0007	0.0069

[Z]DWC = deep water culture; NFT = nutrient film technique; Substrate = trough filled with perlite. All systems contained 189.25L of effluent from an aquaculture unit that was either constantly recirculated (NFT and Substrate) or served as the nutrient solution on which a raft was floated which contained all plants (DWC).
[Y]Size index was measured as [(Widest width + perpendicular width + height)/3]
[X]DAP = days after planting.
[W]Means followed by different letters were different according to Tukey's Honest Significant Different Test (α = 0.05).

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Figure 4.1- Diagram of Auburn University aquaponics effluent flow courtesy of Dr. Emmanuel Ayipio, Auburn University Aquaponics. FT- tilapia fish tank, ST- settling tank, CL- solids clarifier, PGH- plant greenhouse, Sumps- storage tanks for used effluent.